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Integrated Ecology: From classroom to alpine summit

by

Stephanie D. Zorio

A dissertation

submitted in partial fulfillment

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To the Graduate Faculty:

The members of the committee appointed to examine the dissertation of STEPHANIE D. ZORIO find it satisfactory and recommend that it be accepted.

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Dedication

To humans, with love.

"One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise."

― Aldo Leopold, A Sand County Almanac

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Integrated Ecology: From classroom to alpine summit

Abstract--Idaho State University (2019)

Alpine regions are critical areas of study because of their rapid response to disturbances, such as climate change. Compelling places provide a point of reference for understanding our obvious and obscure connections to the biosphere. Studies that include multiple biotic components of an alpine ecosystem, such as the plant and bacterial communities described here, are ideal for addressing understanding interactions across time and organismal levels.

Repeat-sampling is essential for detecting the magnitude and direction of alterations within plant communities in varying environments. We revisited a subset of permeant plant transects near the northeastern border of Yellowstone National Park on three mountains (Mineral, Republic, and Wolverine) in four alpine habitats (north-facing, ridgetop, south-facing, and late snowmelt). We detected shifts in species abundances and diversity in plant communities from 2008 to 2017. In general, species were more widespread among habitats, but formerly abundant species retained, or increased in, dominance. Habitats most exposed to wind and UV radiation had the lowest rate of species turnover.

To explore associations between alpine plants and soil bacteria, we collected rhizoplane and rhizosphere soil from three abundant alpine plant species (*Astragalus kentrophyta*, *Carex albonigra*, and *Lupinus argenteus*) in multiple habitats. Bacterial community composition was investigated using high-throughput sequencing of a bacterial 16S rRNA gene fragment. Community composition of bacteria within four alpine habitats appears largely driven by plant species associations. We did not detect significant compositional differences between soil types. Additionally, the patterns of diversity across mountains may be suggestive of microenvironmental effects on the structure of soil bacteria.

Many studies of atmospheric bacteria are focused on high-altitude environments or agricultural landscapes. In our study, we collected bacteria from the near-terrestrial atmosphere in four alpine habitats across three mountains. High-throughput sequencing of a bacterial 16S gene was used to delineate community composition. We detected significant temporal and spatial shifts in composition and highly associated bacterial families. Bacteria were most abundant and diverse in July. Habitat was not significantly correlated to atmospheric bacteria; however, mountains were distinct, which could indicate the influence of local geography.

Key Words: plant ecology, community ecology, microbial diversity, alpine, near-terrestrial atmosphere, 16S, Illumina, repeat measures, undergraduate biology education, inquiry-based learning

UNIT 1: RESEARCH

CHAPTER 1: Temporal comparison of alpine plant communities near Yellowstone National Park

1. Introduction

The highly adapted plant communities of high-elevation ecosystems appear to be particularly sensitive to the effects of a rapidly changing climate and concomitant effects, including the increasing occurrence and severity of wildfires (Swetnsm *et al.*, 1999; Romme *et al.*, 2016). In subalpine and alpine ecosystems, increasing temperatures have been correlated with a global trend of species range expansions to higher elevation, but decreased species richness at mountain summits (Pauli *et al.*, 1996; Currie *et al.*, 2004; Kammer *et al.*, 2007; Sproull *et al.*, 2015; Zorio *et al.*, 2016). Alpine plant communities are important model systems for documenting and predicting the wide-ranging effects of human activities because of the general lack of direct effects of human activities present within such biomes (Harris, 2008).

Long-term observational and experimental studies of plant communities have been crucial to the development of foundational hypotheses about community structure and environmental filtering (particularly in response to disturbance; Venn *et al.*, 2014). For example, the bioclimatic envelope hypothesis (Sykes *et al.*, 1996) proposes that species will shift historical ranges to stay within an ideal climate. Likewise, the environmental filtering hypothesis (Keddy, 1992) predicts that the abiotic environment will select for plant species with similar traits within communities. Our understanding and ability to predict the responses of plant communities to environmental change increases as these concepts are tested across various scales and ecosystems (Bai *et al.*, 2011; Stöckli *et al.*, 2012; Romme *et al.*, 2016).

North America still suffers from a paucity of long-term research concerning alpine plant communities, though important trends are being revealed as studies mature and historical data is analyzed in novel ways (Curtis & McIntosh, 1951; Walther *et al.*, 2005; Zorio *et al.*, 2016). In particular, field research laboratories like the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, and the Niwot Ridge Long-term Ecological Research site on the Colorado Front Range have been critical components in our current understanding of alpine ecosystems in state and federally-managed land within the Rocky Mountains (Dunn *et al.*, 2003; Venn *et al.*, 2014; Sproull *et al.*, 2015).

As public land managers struggle to adapt to budget constraints and ecological instability, research in alpine areas within protected land is increasingly important. As a striking example, Yellowstone National Park and surrounding areas are home to ~1,300 native plant species. A complex assemblage of microbes, invertebrates, and animals find refuge and nourishment from alpine plants (Prem *et al.*, 2014; Donhauser & Frey, 2018).

This project utilized permanent high-altitude vegetation plots in Yellowstone National Park (YNP) and the surrounding Gallatin National Forest to investigate associations between previously established alpine plant community types (Aho 2006, 2012). Aho measured vegetation and abiotic attributes for 152 high altitude (> 3000 m) randomly selected plots, including 110 plots established in 2006 (Aho, 2006; Figure 1) within five topographic types (plateau, acute ridgetop, north-facing, south-facing, and late snow-melt) on eight mountains located in or adjacent to Northeastern YNP. The current project revisited a subset of baseline plots established by Aho (2006, 2012) to both resample vegetation, and describe previously unconsidered microbial characteristics in the region (Zorio, 2019).

Our objectives were to **1)** determine species diversity and plant species composition in the four habitat types on three mountains, **2)** consider potential effects of environmental filtering (temperature, precipitation, limited growing season, high radiation) and soil characteristics (material and chemical composition) on species assemblages across habitat types, and **3)** detect direction and magnitude of change in diversity and species composition since 2008.

2. Methods

2a. Study Area

Comprising 89,030 km^2, YNP and the Greater Yellowstone Ecosystem (GYE) encompass one of the largest nearly intact temperate-zone ecosystems on Earth. The Absaroka Mountain Range is a sub-range on the eastern side of the Northern Rocky Mountains oriented in a north-west to south-east direction for a distance of approximately 241 km. Substrate in the region is primarily composed of andesitic lavas and alluvial volcanoclastics originating from the Absaroka Volcanic Supergroup, (Smedes & Prostka, 1972). Volcanic breccias, a remnant of younger formations within the Supergroup, are found on most mountain summits in the region (Smedes & Prostka, 1972).

The three mountains considered in this study (Wolverine Peak, Mineral Peak, and Republic Peak; >3000 m) are located within or near the northeast boundary of YNP and Cooke City—Silver Gate, MT (45.0160° N, 109.9166° W; elevation 2,319 m; Figure 1). The annual average maximum and minimum temperatures are 9.1°C and -5.9°C, respectively. Average yearly precipitation is 628 mm, and average annual snowfall is 513 cm (NOAA, 2017). The alpine regions here experience a shorter growing season (~ 3 month frost-free) than similar ecosystems in Colorado (Bowman & Seastedt, 2001; Zorio *et al.*, 2016) and are characterized by graminoid turf, cushion plants, and snow-bank vegetation (Bowman & Seastedt, 2001).

Krummholz vegetation found in some lower altitude sites generally consists of *Abies lasiocarpa*, *Picea engelmannii*, and *Pinus albicaulis*. Historic climate data was accessed from the Fisher Creek SNOTEL site in the study area to determine if variation occurred in local temperature and precipitation between survey periods.

2b. Field Methods

I sampled 12 permanent vegetation transects on the three studied mountains during July 2016 and July 2017 (Figure 1). Transects in four habitat types on each of the mountains included in the survey were sampled during peak growing season to deduce effects of local environmental conditions. These habitats are ridge-top, late snow-melt, south-facing, and north-facing sites. The vegetation transects were a small subset of plots established by Aho (2006, 2012) to monitor alpine plant community integrity.

Vegetation sampling was performed during peak growing season, to assess abundance and composition of plant species, and to match previous sampling efforts for comparison. Transect start and end points were located with Trimble Juno™ and Garmin eTrex® GPS units. A meter tape was used to demarcate the 10m transect between endpoints. At every meter, a ten 20 x 50cm frame (total sampling unit = $1m^2$) was used to quantify occurrence of plant species (Daubenmire, 1959). Plant abundance was determined, for each species, by averaging the number of counts of individual species across the 10 frames. Plants detected in previous and current sampling seasons were largely identified to the species level, but the frequency of non-flowering individuals (especially graminoids) at times confounded precise identification. Nomenclature follows Dorn (1992). Voucher specimens are deposited in the Yellowstone National Park herbarium (YELLO) in Gardiner MT.

2c. Data Preparation and Statistical Methods

2c.1. Tests of community diversity and species distributions

The Shannon-Weiner index was used to measure α-diversity within vegetation communities (Magurran, 2013). The Shannon-Weiner index assumes all species are represented and is relatively sensitive to the occurrence of rare species (Magurran, 2013). Beta diversity between paired sites of the vegetation survey periods was measured using average Bray-Curtis dissimilarity (Bray and Curtis, 1957). *P*-values were adjusted, when appropriate, for simultaneous inference using Holm's procedure (Holm, 1979). Univariate split-plot ANOVA (habitat in mountain, paired in time) was applied to all diversity metrics within and across years sampled.

2c.2. Ordination and multivariate hypothesis testing

Non-Metric Multidimensional Scaling (NMDS) ordination was used for indirect gradient analysis (Jongman *et al.*, 1995; Legendre and Legendre, 1998). Environmental variables were overlaid on the species space of each ordination and tested for significance using vector fitting (Oksanen *et al.*, 2013). Bray-Curtis dissimilarity (Bray and Curtis, 1957) was used as the underlying resemblance metric in NMDS. Ordinations of plant counts and cover over time were performed separately. Environmental data, including physical site characteristics and soil chemistry, were taken from raw data collected by Aho (2006, 2012). The correlation of each environmental variable to the orientation of communities within ordinations was tested with vector-fitting (*i.e.*, individual multiple regressions for each environmental variable with ordination dimensions as predictors). Repeated measures PERMANOVA (a permutational analogue of MANOVA; Anderson, 2005) was used to determine whether sites from each survey effort were distinct in multivariate species space, and to test for effects of time. The value α = 0.05 was used as the significance level for all significance tests.

2c.3. Software

The statistical software package R was used for all analyses (R Core Team, 2015). I relied heavily on the package vegan (Oksanen *et al.*, 2013) for ordination, PERMANOVA, and other community level analyses.

3. Results

3a. Richness and Diversity

A total of 86 species within 24 families were recorded on 12 sites on Republic, Mineral, and Wolverine Peaks when considering both 2008 and 2017. Abundance of the most common species in from the four habitats generally remained similar or become more dominant over time. Habitats also became more heterogeneous in species composition as indicated by decreasing evenness and increasing β-diversity between 2017 versus 2008 (Table 2, Tables 6-9). Rank abundances of the species found in 2008 and 2017 are shown in Table 1. Four of the most dominant species in 2008 (*Festuca brachyphylla, Lewisia pygmaea*, *Dryas octopetala, Poa pattersonii*) were markedly less abundant in 2017. Species that increased in dominance in 2017 consisted of: *Achillea millefolium, Draba incerta, Arenaria obtusiloba*, *Elymus scribneri, Phlox multiflora*, *Geum rossii, Salix arctica,* and *Selaginella densa* (Table 1).

Split-plot ANOVAs showed that richness between years increased ($F_{1,3}$ = 8.4, P = 0.02) for all sites except ridgetop sites on Mineral and Republic Peaks (Figure 2). Within habitats, ridgetop ($F_{1,4}$ = 75.53, P = <0.001) and north-facing sites ($F_{1,4}$ = 18.60, P = 0.013) became more heterogeneous over time. Overall Shannon-Weiner diversity by site did not change from 2008 to 2017 ($F_{1,3}$ = 1.64, P = 0.24; Table 2, Figure 3).

3b. Count Data: Ordination and Multivariate Hypothesis Testing

For count data, ordinations the NMDS was robust (two dimensions, stress = 0.14; Kruskal and Wish, 1978). Snowmelt and north-facing habitats showed more movement across the first dimension, whereas most ridge-top and south-facing sites moved in a more across the second dimension. Ridgetop and south-facing sites were generally more associated with significant soil chemistry parameters Cu and Mg, whereas late snowmelt and north-facing sites were positively associated with Mn, OC, TKN, P, Zn, and NH_4. Sites on Wolverine Peak were more associated with Ca than sites on Mineral or Republic (Figure 4). When blocked by time in PERMANOVAs, plant communities in 2008 and 2017 were significantly different by habitat ($F_{3,11}$ = 1.95, P = 0.008), and demonstrated weak trends by mountain ($F_{2,11}$ = 1.67, P = 0.054).

3c. Changes in Species Dominance Between Surveys

The rank order and constancy of the dominant species changed markedly between sampling periods (Table 1 and 2). Notable shifts in the most abundant/dominant species occurred between 2008 and 2017 in each habitat type, as outlined below. North-facing sites saw a dramatic increase in vegetation abundance between surveys (Table 6). Several formerly dominant plant species increased in abundance but decreased in rank abundance. Non-sedge graminoids, *Poa alpina* and *Poa pattersonii*, increased from 17^{th} to 6^{th} and 18^{th} to 7^{th}, respectively, in terms of rank abundance. Sedges found in 2008 were not among the most dominant species in 2017. *Carex phealocepela* decreased in relative abundance (2008 = 1.1, 2017 = 0.7) and dropped from 10th to 22nd in rank. *Juncus parryi* decreased in abundance (2008 = 1.3, 2017 = 0.3) and dropped from 8th to 31st in rank. The majority of species shifted ranks but remained dominant while increasing mean relative abundance. Exceptions were *Lewisia pygmaea* (rank 2008 = 4, 2017 = 37) and *Antennaria media that* both fell from dominance (rank 2008 = 13, 2017 = 20) and decreased in relative abundance. Species that rose to dominance in 2017 include *Sibbaldia procumbens* (rank 2008 = 26, 2017 = 14) and *Salix artica* (rank 2008 = 19, 2017 = 5; Table 6). *Salix artica* was the only dominant shrub species to appear in 2008 and 2017. An additional shrub, *Phyllodoce empetriformis*, not found in 2008 was detected in low abundance in 2017 (mean relative abundance = 0.07, rank = 49).

Sites within the ridge-top habitat generally saw increased mean abundance for most species and retained 13 out of the 15 most abundant species in 2008 (Table 7). The most abundant species, *Astragalus kentrophyta*, became markedly more abundant over time, driving an overall decrease in evenness (Table 1 and 7). Other herbaceous species, *Ceratium arvense* and *Lomatium cous*, remained abundant but decreased in mean relative abundance over time (Table 7). *Smelowskia calycina* was the only herbaceous species to dramatically decrease in rank (2008 = 11, 2017 = 20) and abundance (2008 = 0.43, 2017 = 0.07). *Castilleja pulchella*, was not recorded in 2008, but in 2017 was ranked 11th with an abundance of 0.48. More graminoids were among the most abundant species in ridge-top sites than other habitat types. Of the dominant graminoids in 2008, *Festuca bracyphylla* showed a slight decrease in abundance (2008 = 0.8, 2017 = 0.6) and rank (2008 = 7, 2017 = 10), and *Poa secunda* was not detected in 2017 (2008 = 0.23). *Elymus scribneri* remained the third most abundant species and increased abundance from 1.37 to 2.10. *Poa pattersonii* and *Tristeum spicatum* became more abundant, although *P.*

pattersonii increased in rank over time (2008 = 12, 2017 = 6) while the rank of *T. spicatum* remained stable (mean relative abundance 2008 = 0.60, 2017 = 0.70).

South-facing sites retained 13 of the 15 most abundant species in 2008 although rank abundances shifted (Table 8). Species within this community generally became more abundant, with lower evenness among dominant species (Table 1). *Cerastium arvense* had the greatest abundance in 2008 (3.6) and 2017 (7.4). Abundant graminoids in 2008, *Carex albonigra* (2008 = 1.97) and *Poa pattersonii* (2008 = 1.17), increased in abundance to 6.32 and 1.55, respectively. *Tristeum spicatum*, included within the most abundant species for 2017 but not in 2008, also became more abundant over time (2008 = 0.7, 2017 = 0.95) and increased in rank abundance from 16 to 14 (Table 8). South-facing sites had the greatest number of dominant species in the Fabaceae, including *Astragalus alpinus*, *Astragalus kentrophyta*, and *Lupinus argenteus*. *Astragalus kentrophyta* had the greatest increase in abundance over time from 2008 (abundance = 1.27, rank = 6) to 2017 (abundance = 4.42, rank = 4), outpacing the formerly more abundant *Lupinus argenteus* (2008: abundance = 1.47, rank = 5; 2017: abundance = 2.42, rank = 7).

In snow-melt sites (Table 9), *Carex paysonius* and *Carex albonigra* remained the characteristic dominant species, although at much greater abundances. Notably, *Carex albonigra* became more abundant from 2008 (2.6) to 2017 (12.1), displacing *Carex paysonius* which remained essentially stable over time (2008 = 3.1, 2017 = 2.9). Other dominant graminoids present in 2008 were *Festuca brachyphylla*, and *Deschampsia cespitosa*. *Festuca bracyphylla* increased in abundance across time (2008 = 1.2, 2017 = 1.5), but moved from 7th to 11th most abundant. *Luzula spicata* dropped from 13th most abundant species to the 23rd, although its abundance slightly increased over time (2008 = 0.7, 2017 = 0.9). *Deschamsia ceptisosa* was not recorded at north-facing sites in 2017, instigating the decrease in rank from 14th most abundant to 61st. Two woody species, *Salix artica* and *Phyllodoce glanduliflora* increased in abundance and remained among the dominant species across time. Dominant herbaceous species remained dominant over time, except for *Antennaria lanata* (rank 2008 = 6, rank 2017 = 19), and increased in abundance but shifted ranks. Two species, *Siliene acaulis* and *Geum rossii* were among the most abundant species in 2017, but not in 2008. *Siliene acaulis* increased in rank from 21st to 14th, and *Geum rossii* increased from 22nd most abundant to 15th (Table 9).

4. Discussion

I detected a significant shift in the community composition of alpine plants between 2008 and 2017, although I note that inference is limited by the low number of time frames used. Given that shifts in plant communities generally occur slowly over time (Dirnbock *et al.*, 2003). I did not expect significant changes to have occurred in the composition over a decade, barring a significant catastrophic disturbance. I found substantial changes in diversity and abundance of species that are largely consistent with patterns expected with warmer, wetter weather (Callaway & Brooker, 2002; Whitlock *et al.*, 2017). Precipitation data from the Fisher Creek SNOTEL station located within the study area shows an increase in summer precipitation (Table 5).

The lack of relative change in the proportion of bare ground and increase in average relative abundance in all communities suggest increased productivity over time. Sites within habitats generally had similar trajectories of compositional change (Table 1, Figure1), suggesting that the observed community changes are driven by shared underlying causes. Many species became more wide spread over time, appearing in sites in 2017, but not in 2008. However, significant increases in heterogeneity within individual habitats occurred, suggesting the influence of local topography and substrate on species plant assemblages (Bowman & Seastedt, 2001; Pauli *et al.*, 2003). In contrast to temporal variation, I did expect to see distinctive differences between habitats. Changes in diversity and species' distributions were largely consistent with other studies linking climate change to dramatic alterations in diversity, particularly in water and temperature limited alpine environments (Pauli *et al.*, 2003; Wipf *et al.*, 2013; Sproull *et al.*, 2015).

4a. Changes in richness and diversity

In concordance with other studies (Kammer *et al.*, 2007; Wipf *et al.*, 2013), I did detect an increase in average species richness in alpine plant communities in a subset of permanent transects in Montana (Table 2). In terms of diversity, *α*-diversity was unchanged, and *β*-diversity increased between 2008 and 2017 (Table 2).

Increased species richness was due to the spread of local alpine species across more sites, and not migrants from lower elevation. A lack of lower elevation migrants could be caused by snow and nutrient-limited growing conditions that stall upward species expansion (Kammer *et al.*, 2007). Additionally, earlier snowmelt in the region coupled with changes in precipitation (*i.e*. increase in spring, decrease during summer) could cause exposed alpine sites on perennially snow-free ridges and south-facing slopes to become warmer and more water-limited during the growing season (Aho, 2006; Chersich *et al.*, 2015; Whitlock *et al.*, 2017). Average yearly snowpack in Montana has declined from 1930 to 2015; however, snowpack at lower elevations are decreasing at a faster rate than alpine regions (Whitlock *et al.*, 2017). This differential could play a role in retarding upward expansion of species in our study area. As a result, our findings do not support the prediction that alpine specialists will decline due to loss of habitat or from competition from invasives or plant species common to lower elevations (Pauli *et al.*, 2003; Harrison *et al.*, 2010).

Graminoid species, such as *Carex albonigra* and *Poa pattersonii*, and mat-forming herbaceous species, such as *Astragalus kentrophyta* and *Sedum lanceolatum* assumed greater dominance throughout all habitat types. Vertebrate herbivores, such as the exotic mountain goat (*Oreomnos americanus*) common to YNP may also reduce the abundance and diversity of highly palatable species, such as *Lewisia pygmaea*. (Table 1); thus driving the increase of less palatable species, such as *Sedum lanceolatum* (Doyle, 2011).

Sites within all four habitat types have become more heterogeneous in composition and less distinct over time because of changes in diversity and species' abundance. North-facing sites receive relatively low solar radiation, generally have well-developed soils and are sheltered from the prevailing wind (Aho, 2006), and were unique in that six out of the 15 most abundant dominant plants declined in abundance over time (Table 6). The rise of formerly low-abundance species to dominance, such as *Poa alpina* and *Poa pattersonii*, may parallel other studies that show the expansion of grasses and sedges under climate warming (Pauli *et al.*, 1996; Kelly & Goulden, 2008; Rudgers *et al.*, 2014).

Ridge-top and south-facing sites retained the greatest numbers of dominant species over time. *Astragalus kentrophyta*, the most abundant plant in ridge-top sites, increased dramatically over sampling periods (Table 7 & 8). The constant exposure to wind and solar radiation, especially as these sites stay snow-free for most of the year, is not ideal for many local migrants (Bowman & Seastedt, 2001; Aho, 2006). Additionally, Callaway and Brooker (2002) found that abundance of alpine plant species were higher when other plants were nearby, supporting the notion that facilitation is more important than competition in alpine plant communities under increasing abiotic stress. The dominant plant species on south-facing sites are mat-forming or low-growing species (Table 8). This habitat is generally found on steep slopes that receive high amounts of solar radiation and harbor poorly developed soils (Aho, 2006). Over time, dominant mat-forming species, like *Sedum lanceolatum* and *Astragalus kentrophyta*, have increased in rank abundance (Table 8). Like ridge-top sites, strong environmental filtering may promote stability within the assemblages of plants found there (Bowman & Seastedt, 2001; Callaway & Brooker, 2002). Alternatively, increasing average soil temperatures and expanded vegetative cover has been linked to increasing seed-set of alpine plants, thereby bolstering the competitive ability of alpine specialists against local migrants and those from lower-elevation (Hoyle *et al.*, 2013).

4b. Changes in Community Composition

The observed shifts of sites across time reflect the underlying changes in the species composition of habitats. These changes largely correspond to rank order changes in species abundance. There was an overall increase in the relative abundance and constancy of the most common species in each habitat type (Tables 6-9). This had the effect of decreasing evenness and increasing the heterogeneity among sites within communities

A competitive advantage of graminoid and shrub growth forms is predicted in some climate change scenarios for montane environments (Bahre & Shelton, 1993; Rudgers *et al.*, 2014). While large declines in herbaceous (forb and grass) richness have been documented in other studies (Damschen *et al.*, 2010; Harrison *et al.* 2010), I found that the relative abundance of forbs, and to a lesser extent, graminoids increased in all habitats. Increased shrub abundance was limited to the greater abundance and dominance of *Salix* spp. (Table 1). For example, in north-facing sites *Salix artica* moved from 19th to 5th most abundant species and increased in dominance in late snowmelt sites (Table 9). Graminoid abundance increased for species detected in 2008 and 2017. However, some species present in low abundance in 2008, *Carex haydeniana*, *Agrostis variabilis*, *Festuca idahoensis*, *Poa rupicola*, and *Poa secunda*, were not observed in 2017. Besides north-facing sites, abundant sedges in 2008 increased or remained similar in relative abundance over time (Table 1).

Although plant communities in the study area were surveyed during the same period in July, summer precipitation was significantly higher in 2017 than in 2008 (Table 5), likely driving greater relative abundance for most species. Montana has recorded a state-wide 0.5°C increase in temperature since 1930; however, temperature ranges were similar during all sampling periods. I note that I revisited a small subsample of ~150 permanent transects (Aho, 2006, 2012). Adding more sites to our analysis may resolve some apparent differences in community composition across years. Nevertheless, I observed almost all the species found in the original survey in our resurvey.

In addition to climate change, several alternative causes could underlie changes in community composition over the past decade in our sites. These include fire, herbivory by native and nonnative ungulates, and phenological differences during survey campaigns (Bahre and Shelton, 1993; Coop *et al.*, 2014). Due to their location in protected lands in and around YNP, most peaks in our study site have been protected from mining, domestic grazing, and real estate development. No substantial wildfires have occurred in the study area since the devastating Yellowstone fires in 1988 (Romme *et al.*, 2016), although post-disturbance succession may occur more slowly in high-elevation ecosystems (Pauli *et al.*, 2003).

5. Conclusions

The overall change within each of the habitats here is suggested by increased species richness from 2008 to 2017 and increasing species abundance. If species were encroaching from lower elevations, as reported elsewhere (Pauli *et al.*, 2003; Walther *et al.*, 2005; Chersich *et al.*, 2015), I would have expected to see an overall increase in richness from species outside of the local pool of alpine species, and more turnover among the most abundant species. Most studies that document this trend use data collected over decades (Stöckli *et al.*, 2012; Wipf *et al.*, 2013). In the future we may see similar patterns here; however recorded responses of alpine vegetation vary across continents and at the local scale (Tingley & Beissinger, 2009; Donhauser & Frey, 2018). In the meantime, long-term studies are bolstered by numerous time-point observations for reasons including increased resolution of data and better identification of normal variation (Van de Ven *et al.*, 2007; Wipf *et al.*, 2013). This is particularly important for dynamic high-elevation ecosystem where local climatic factors are important deterministic drivers (Romme *et al.*, 2016). Species composition is also an important component of plant communities. If species turnover becomes more pronounced, then important plant functional traits (*e.g.* nutrient cycling) may be lost, leading to a loss of integrity within alpine plant assemblages (Callaway & Brooker, 2002).

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7. Tables

Table 1. Relative abundance, rank abundance and percent constancy for plant species detected in 2008 and 2017. Species are ranked by their mean relative abundance in 2008.

	2008				2017			
2008	**Rank**	**Const.**	**Rel. Abund.**	**Rel. Cover**	**Rank 2017**	**Const.**	**Rel. Abund.**	**Rel. Cover**
ApiaLOCO	17	58.3	1.5	0.51	26	50.0	1.2	0.33
AsteACMI	33	33.3	0.9	0.35	21	33.3	1.6	0.92
AsteAGGL	37	16.7	0.9	0.75	34	16.7	0.8	0.54
AsteANLA	28	16.7	1.1	0.63	61	8.3	0.2	0.23
AsteANME	35	33.3	0.9	0.43	47	16.7	0.5	0.35
AsteANMI	68	8.3	0.1	0.02	66	8.3	0.1	0.03
AsteARSC	13	25.0	2.0	0.98	9	33.3	2.3	0.97
AsteASAL	11	25.0	2.0	1.10	15	41.7	1.8	0.98
AsteASFO	74	8.3	0.0	0.00	72	0.0	0.0	0.00
AsteERCO	38	33.3	0.9	0.36	29	50.0	1.0	0.48
AsteERSI	5	66.7	3.3	1.88	6	58.3	3.5	0.89
AsteSECA	30	33.3	1.1	0.53	49	16.7	0.5	0.63
AsteSOMU	75	8.3	0.0	0.01	63	16.7	0.2	0.20
AsteTASP	56	25.0	0.3	0.16	59	50.0	0.3	0.05
BoraMEAL	25	41.7	1.2	0.72	27	25.0	1.2	0.19
BoraMYAL	47	25.0	0.4	0.21	24	33.3	1.4	0.30
BrasDRCR	76	8.3	0.0	0.00	41	25.0	0.6	0.18
BrasDRIN	65	33.3	0.1	0.07	56	25.0	0.4	0.41
BrasSMCA	46	41.7	0.4	0.22	53	25.0	0.4	0.33
CaryARCO	58	16.7	0.2	0.18	36	16.7	0.7	0.17
CaryAROB	34	50.0	0.9	0.79	8	58.3	2.4	1.28
CaryARRU	39	41.7	0.9	0.47	39	16.7	0.6	1.60
CaryCEAR	1	58.3	4.3	3.13	5	58.3	4.3	3.12
CarySIAC	44	25.0	0.5	0.67	46	25.0	0.5	0.79
CarySTMO	45	25.0	0.4	0.22	30	25.0	1.0	0.45
CrasSELA	2	75.0	3.7	1.23	1	83.3	4.6	2.91
CyCAMAHA	69	8.3	0.1	0.05	73	0.0	0.0	0.00
CyprCAAL	3	33.3	3.6	6.77	3	33.3	4.4	7.04
CyprCAEL	40	16.7	0.8	0.80	68	8.3	0.1	0.65
CyprCAPA	8	25.0	2.6	3.43	14	33.3	1.9	1.11
CyprCAPH	36	16.7	0.9	0.59	37	33.3	0.7	0.28
CyprCAPY	70	8.3	0.1	0.05	64	8.3	0.2	0.04
EricPHEM	81	0.0	0.0	0.00	74	0.0	0.0	0.00
EricPHGL	42	8.3	0.5	0.50	38	8.3	0.7	0.44
EricVASC	49	8.3	0.4	0.26	35	8.3	0.8	0.29

2008	2008 Rank	2008 Const.	2008 Rel. Abund.	2008 Rel. Cover	2017 Rank 2017	2017 Const.	2017 Rel. Abund.	2017 Rel. Cover
FabaASKE	4	50.0	3.5	2.99	2	66.7	4.5	5.68
FabaLUAR	7	50.0	2.8	3.07	11	50.0	2.1	2.88
JuncJUPA	32	8.3	1.0	1.26	69	8.3	0.1	0.16
JuncLUSP	29	50.0	1.1	0.70	45	16.7	0.5	0.39
LiliLLSE	22	25.0	1.3	0.36	16	25.0	1.8	0.81
OnagEPCL	72	8.3	0.1	0.01	70	8.3	0.1	0.01
PinePIEN	77	8.3	0.0	0.01	67	16.7	0.1	0.16
PoacAGVA	59	8.3	0.2	0.03	75	0.0	0.0	0.00
PoacDAIN	63	8.3	0.2	0.22	60	8.3	0.3	0.22
PoacDECA	31	16.7	1.0	1.97	40	8.3	0.6	0.26
PoacELSC	24	33.3	1.3	0.86	31	33.3	1.0	1.05
PoacFEBR	10	91.7	2.3	1.47	20	58.3	1.6	1.20
PoacFEID	78	8.3	0.0	0.00	76	0.0	0.0	0.00
PoacPOAL	27	50.0	1.1	0.95	17	33.3	1.7	1.74
PoacPOPA	15	58.3	1.8	1.32	4	66.7	4.3	1.77
PoacPORE	64	25.0	0.2	0.04	33	33.3	0.9	0.25
PoacPORU	48	8.3	0.4	0.22	77	0.0	0.0	0.00
PoacPOSE	61	8.3	0.2	0.08	78	0.0	0.0	0.00
PoacTRSP	20	83.3	1.3	0.73	44	25.0	0.5	0.76
PolePHMU	23	25.0	1.3	0.93	22	33.3	1.5	2.14
PolePOVI	16	8.3	1.6	1.72	18	16.7	1.7	0.74
PolyPOBI	6	41.7	3.2	1.03	10	41.7	2.1	1.22
PolyPODO	66	8.3	0.1	0.01	79	0.0	0.0	0.00
PolyRUPA	82	0.0	0.0	0.00	80	0.0	0.0	0.00
PortCLLA	79	8.3	0.0	0.01	54	8.3	0.4	0.08
PortLEPY	12	25.0	2.0	0.93	65	16.7	0.2	0.09
PrimANSE	53	25.0	0.3	0.13	42	41.7	0.6	0.21
PrimDOPU	52	25.0	0.3	0.24	57	16.7	0.4	0.11
RannRAES	43	25.0	0.5	0.22	81	0.0	0.0	0.01
RoseDROC	14	8.3	1.8	4.21	25	8.3	1.3	0.63
RoseGERO	18	25.0	1.5	1.59	13	25.0	1.9	1.56
RosePODI	9	58.3	2.3	1.06	12	50.0	2.0	1.34
RosePOGR	71	8.3	0.1	0.03	32	25.0	1.0	0.32
RosePOOV	80	8.3	0.0	0.01	51	16.7	0.4	0.07
RosePORU	83	0.0	0.0	0.00	82	0.0	0.0	0.00
RoseSIPR	19	25.0	1.4	1.01	28	25.0	1.1	1.26
SaliSAAR	21	25.0	1.3	1.63	23	16.7	1.5	1.68
SaliSARE	51	8.3	0.3	0.34	71	8.3	0.1	0.01
SaxiSACE	67	16.7	0.1	0.02	43	8.3	0.6	0.13
SaxiSARH	54	33.3	0.3	0.16	83	0.0	0.0	0.04
SaxiSASU	73	8.3	0.1	0.04	62	8.3	0.2	0.02
ScroBEWY	50	8.3	0.3	0.27	58	25.0	0.4	0.38

ScroCAPU	57	16.7	0.3	0.18	55	41.7	0.4	0.35
ScroPECY	55	16.7	0.3	0.17	52	16.7	0.4	0.36
ScroPEGR	84	0.0	0.0	0.00	84	0.0	0.0	0.00
ScroPEPR	62	8.3	0.2	0.03	50	8.3	0.5	0.00
ScroSARH	85	0.0	0.0	0.00	48	16.7	0.5	0.05
ScroVEWO	60	16.7	0.2	0.03	85	0.0	0.0	0.00
SelaSEDE	41	16.7	0.7	0.95	7	25.0	3.5	0.63

Table 2. Summary statistics for mean richness, Shannon-Weiner diversity, and Bray-Curtis dissimilarity for vegetation in 2008 and 2016 for habitat types. Standard error is shown in parentheses. Richness and Bray-Curtis varied significantly across time.

Habitat	**Richness**				**Shannon-Weiner**				**Bray-Curtis**			
	2008		*2017*		*2008*		*2017*		*2008*		*2017*	
RT	12	(± 12)	11.9	(± 1.53)	2.06	(± 0.12)	1.8	(± 0.13)	0.42	(± 0.05)	0.68	(± 0.03)
SF	24	(± 3.48)	22.2	(± 1.60)	2.72	(± 0.12)	2.6	(± 0.11)	0.4	(± 0.06)	0.68	(± 0.03)
SM	26	(± 1.20)	25.2	(± 1.09)	2.71	(± 0.16)	2.7	(± 0.10)	0.55	(± 0.11)	0.76	(± 0.02)
NF	18	(± 2.73)	21	(± 1.19)	2.6	(± 0.004)	2.5	(± 0.11)	0.54	(± 0.11)	0.72	(± 0.04)

Table 3. Summary of one-way, split-plot ANOVA results for diversity metrics comparing sampling efforts from 2008 and 2017.

	Variation	df	MS	*F*	*p*
Richness	Between groups	1	63.37	8.4	**0.02**
	Within groups	3	6.60		
Shannon-Weiner	Between groups	1	0.04	4.35	0.24
	Within groups	3	0.05		

Table 4. Multiple regression of environmental parameters fit against plant composition simultaneously for both surveys (2008, 2017). Asterisks denote significance at $\alpha = \leq 0.05$.

Vector	Units	Code	r^2	*p*
Slope	Degree	Slope	0.06	5.18
Aspect	West declination	Aspect	0.4	0.01*
pH		pH	0.79	<0.01*
Organic Carbon	%	OC	0.71	<0.01*
P-Olsen Method	Ppm	P	0.47	<0.01*
Calcium	Ppm	Ca	0.51	<0.01*
Magnesium	Ppm	Mg	0.58	<0.01*
Sulfur	$lbacre^{-1}$	S	0.32	0.02*
Zinc	Ppm	Zinc	0.45	<0.01*
Manganese	Ppm	Mn	0.46	<0.01*
Copper	Ppm	Cu	0.38	0.01*
Ammonium	Ppm	NH4	0.43	0.01*
Total Kjeldahl nitrogen	%	TKN	0.67	<0.01*

Table 5. Summary of one-way ANOVA results for daily temperatures and precipitation in June and July of 2008 and 2017. Raw data originated from the Fisher Creek SNOTEL monitoring site.

	Variation	df	MS	*F*	*p*
Temp	Between groups	1	12.83	3.45	0.68
	Within groups	122	3.72		
Precip	Between groups	1	2785.1	4.35	**>0.001**
	Within groups	122	2.24		

Table 6. The 15 most abundant species for plant communities found in north-facing sites in 2008 and 2017. Species are ranked by their mean relative abundance in 2008.

North-facing					
Species 2008	Rank 2008	Relative Abundance 2008	Species 2017	Rank 2017	Relative Abundance 2008
Antennaria media	13	0.80	*Artemisia scopulorum*	12	1.20
Artemisia scopulorum	12	0.83	*Aster alpigenus*	11	1.22
Aster alpigenus	6	1.40	*Erigeron simplex*	9	1.31
Erigeron simplex	3	1.73	*Arenaria rubella*	15	0.95
Arenaria rubella	15	0.73	*Sedum lanceolatum*	10	1.23
Sedum lanceolatum	11	0.83	*Lupinus argenteus*	2	2.63
Carex phaeocephala	10	1.07	*Lloydia serotina*	8	1.72
Lupinus argenteus	9	1.17	*Poa alpina*	6	2.15
Juncus parryi	7	1.27	*Poa pattersonii*	7	1.95
Lloydia serotina	8	1.27	*Polygonum bistortoides*	13	1.20
Polygonum bistortoides	1	2.50	*Dryas octopetala*	4	2.45
Lewisia pygmaea	4	1.67	*Geum rossii*	1	4.48
Dryas octopetala	2	2.30	*Sibbaldia procumbens*	14	0.97
Geum rossii	5	1.53	*Salix arctica*	5	2.38
Selaginella densa	14	0.73	*Selaginella densa*	3	2.48

Table 7. The 15 most abundant species for plant communities found in ridgetop sites in 2008 and 2017. Species are ranked by their mean relative abundance in 2008.

Ridge-top					
Species 2008	Rank 2008	Relative Abundance 2008	Species 2017	Rank 2017	Relative Abundance 2017
Astragalus kentrophyta	1	3.20	*Astragalus kentrophyta*	1	11.48
Cerastium arvense	2	1.53	*Phlox multiflora*	8	2.43
Elymus scribneri	3	1.37	*Elymus scribneri*	3	2.10
Senecio canus	4	0.93	*Sedum lanceolatum*	5	1.38
Sedum lanceolatum	5	0.90	*Senecio canus*	4	1.17
Lomatium cous	6	0.90	*Poa pattersonii*	12	1.08
Festuca brachyphylla	7	0.77	*Cerastium arvense*	2	0.98
Phlox multiflora	8	0.67	*Erigeron compositus*	10	0.83
Trisetum spicatum	9	0.60	*Trisetum spicatum*	9	0.70
Erigeron compositus	10	0.43	*Festuca brachyphylla*	7	0.60
Smelowskia calycina	11	0.43	*Castilleja pulchella*	20	0.48
Poa pattersonii	12	0.33	*Achillea millefolium*	13	0.40
Achillea millefolium	13	0.30	*Lomatium cous*	6	0.35
Poa secunda	14	0.23	*Androsace septentrionalis*	17	0.35
Silene acaulis	15	0.13	*Silene acaulis*	15	0.24

Table 8. The 15 most abundant species for plant communities found in south-facing sites in 2008 and 2017. Species are ranked by their mean relative abundance in 2008.

South-facing					
Species 2008	Rank 2008	Relative Abundance 2008	Species 2017	Rank 2017	Relative Abundance 2017
Cerastium arvense	1	3.60	*Cerastium arvense*	1	7.40
Sedum lanceolatum	2	2.90	*Carex albonigra*	4	6.32
Polemonium viscosum	3	2.03	*Sedum lanceolatum*	2	5.13
Carex albonigra	4	1.97	*Astragalus kentrophyta*	4	4.42
Lupinus argenteus	5	1.47	*Arenaria obtusiloba*	9	3.62
Astragalus kentrophyta	6	1.27	*Phlox multiflora*	10	2.90
Poa pattersonii	7	1.17	*Lupinus argenteus*	5	2.42
Potentilla diversifolia	8	1.10	*Polemonium viscosum*	3	2.42
Arenaria obtusiloba	9	1.07	*Achillea millefolium*	12	1.67
Phlox multiflora	10	1.00	*Poa pattersonii*	7	1.55
Lomatium cous	11	1.00	*Potentilla diversifolia*	8	1.47
Achillea millefolium	12	0.90	*Astragalus alpinus*	15	1.42
Mertensia alpina	13	0.90	*Draba incerta*	44	1.12
Lewisia pygmaea	14	0.80	*Trisetum spicatum*	16	0.95
Astragalus alpinus	15	0.80	*Lomatium cous*	11	0.82

Table 9. The 15 most abundant species for plant communities found in late snowmelt sites in 2008 and 2017. Species are ranked by their mean relative abundance in 2008.

Late Snowmelt					
Species 2008	Rank 2008	Relative Abundance 2008	Species 2017	Rank 2017	Relative Abundance 2017
Carex paysonis	1	3.10	*Carex albonigra*	1	12.07
Carex albonigra	2	2.60	*Carex paysonis*	2	2.92
Erigeron simplex	3	2.27	*Potentilla diversifolia*	3	2.42
Artemisia scopulorum	4	1.73	*Cerastium arvense*	4	2.35
Sibbaldia procumbens	5	1.57	*Salix arctica*	5	2.08
Antennaria lanata	6	1.43	*Sibbaldia procumbens*	6	1.95
Festuca brachyphylla	7	1.23	*Artemisia scopulorum*	7	1.92
Polygonum bistortoides	8	1.23	*Polygonum bistortoides*	8	1.83
Aster alpigenus	9	1.23	*Lupinus argenteus*	9	1.72
Salix arctica	10	1.20	*Aster alpigenus*	10	1.61
Potentilla diversifolia	11	1.17	*Festuca brachyphylla*	11	1.53
Lupinus argenteus	12	1.03	*Phyllodoce glanduliflora*	12	1.48
Luzula spicata	13	0.70	*Erigeron simplex*	13	1.48
Deschampsia cespitosa	14	0.70	*Silene acaulis*	14	1.37
Phyllodoce glanduliflora	15	0.63	*Geum rossii*	15	1.18

8. Figures

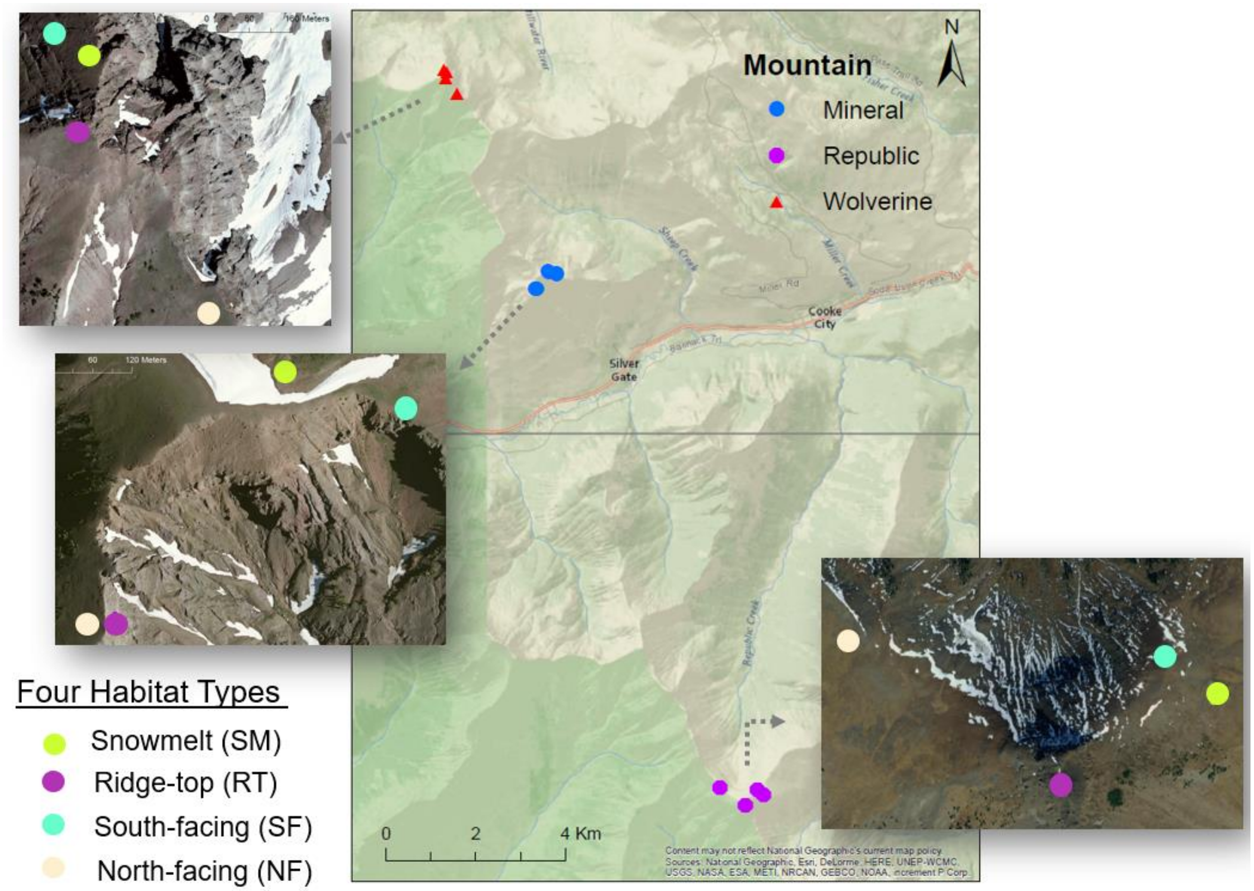

Figure 1. Map of study area near Cooke City, MT. Insets and bottom-left legend show habitat types.

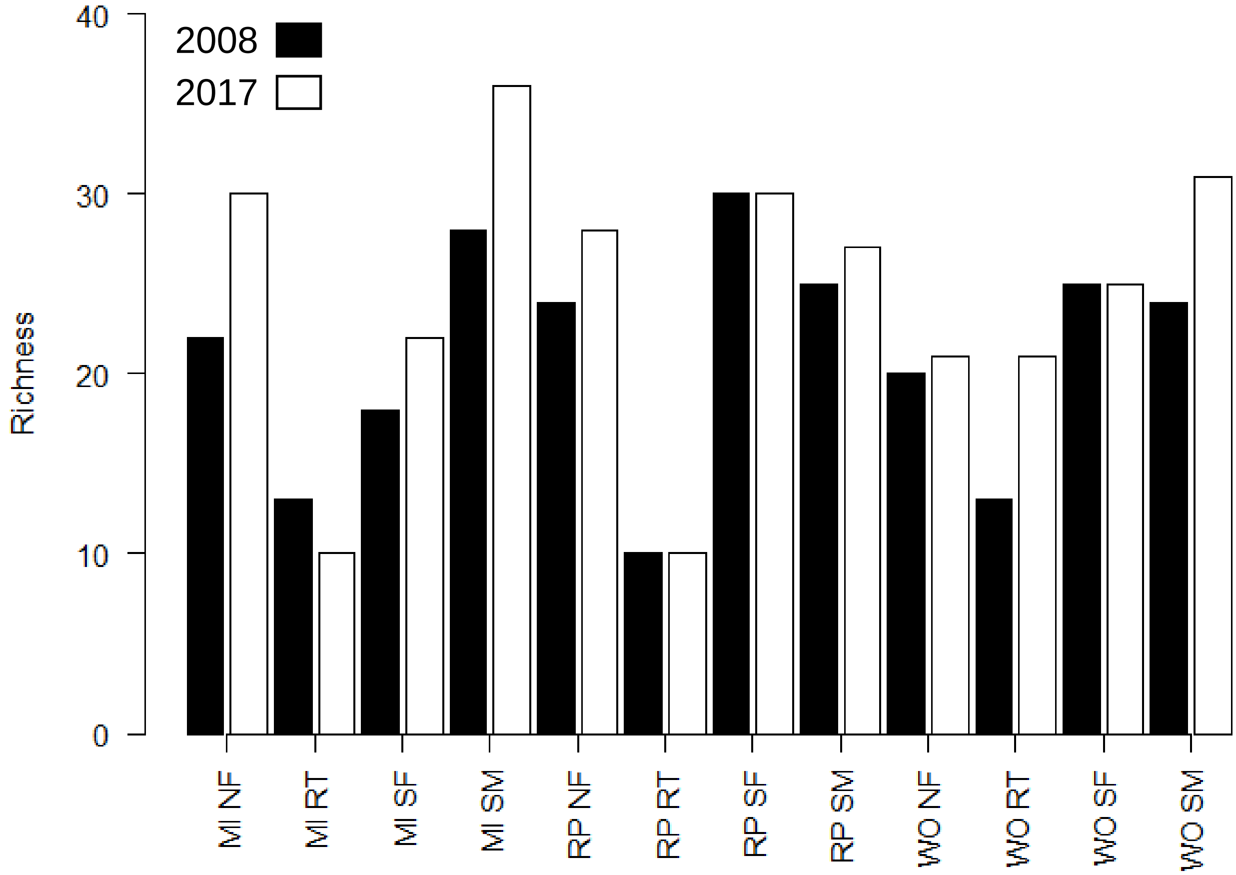

Figure 2. Barplot of richness for 2008 (black bars) and 2017 (white bars). MI, RP, and WO represent Mineral, Republic, and Wolverine Peak, respectively. Habitats are denoted by, NF (north-facing), RT (ridgetop). SF (south-facing), SM (late snowmelt).

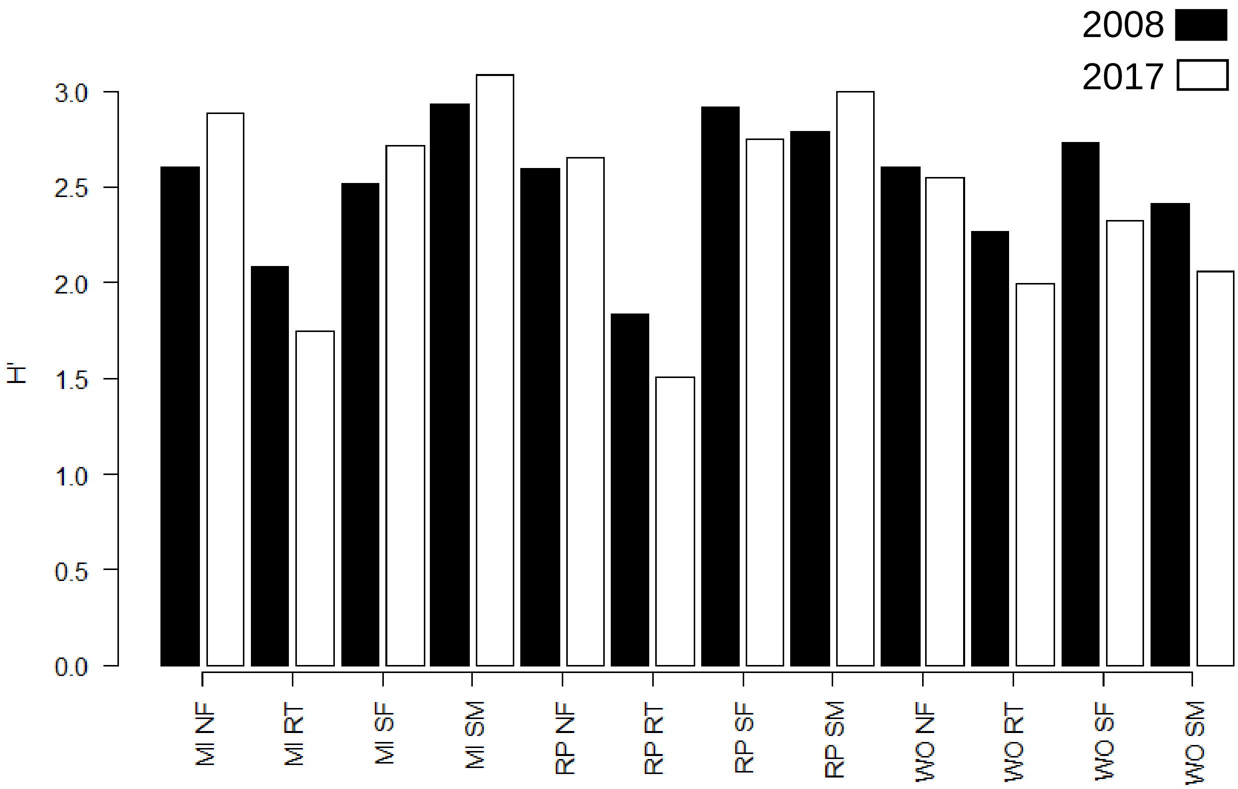

Figure 3. Barplot of Shannon-Weiner (H') values for 2008 (black bars) and 2017 (white bars). MI, RP, and WO represent Mineral, Republic, and Wolverine Peak, respectively. Habitats are denoted by SM (late snowmelt), NF (north-facing), SF (south-facing), and RT (ridgetop).

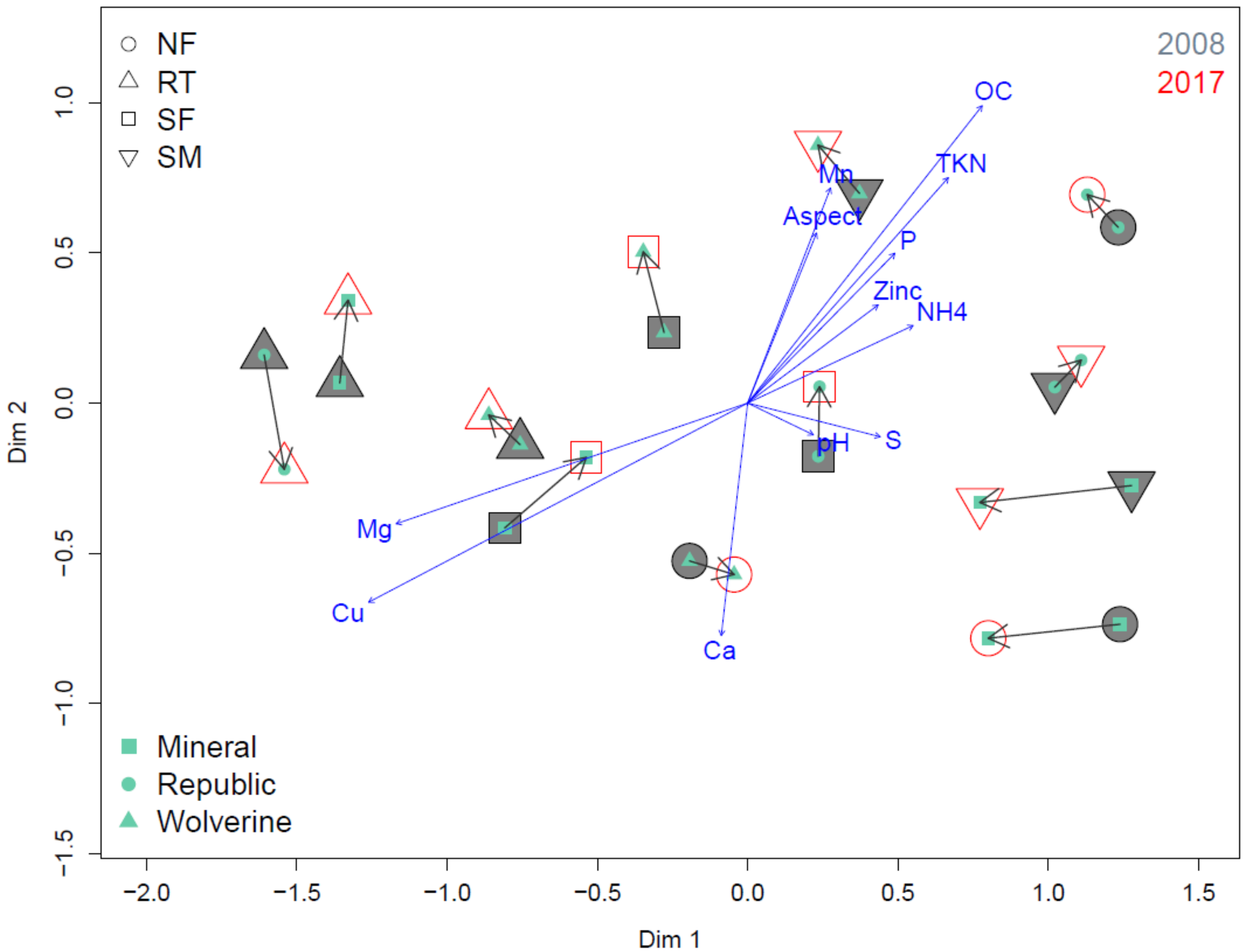

Figure 4. NMDS of vegetation abundance for paired sites on three mountains overlain with significant environmental parameters. Arrows between symbols connect years. Length of vectors indicate the strength of association (R^2) of environmental parameters to the ordination. Direction of vectors indicates direction of increasingly Cu and OC. Stress = 0.14, k = 2.

CHAPTER 2: Plant species are a better predictor than environment for communities of bacteria in rhizoplane and rhizosphere soils

1. Introduction

The biological importance of microorganisms within specific components of the geosphere (*e.g.*, atmosphere, cryosphere, and lithosphere) is well known. As obvious examples, soil microbial activity strongly regulates both the global nitrogen cycle, through the processes of nitrification, ammonification, and denitrification, and carbon cycles via photosynthesis and organic matter mineralization (Lladó *et al.*, 2017; Wang *et al.*, 2017). Further, the presence of particular soil microbial assemblages may have large impacts on plant diversity, composition, and plant-plant interactions (Braga *et al.*, 2016). Little is known, however, about the biodiversity and integrated ecology of plant-microbial systems, particularly in the context of alpine plant communities vulnerable to climate change (Ciccazzo *et al.*, 2015; Prober *et al.*, 2015). To understand alpine ecology more completely, a microbial perspective needs to be integrated into conceptual frameworks of these environments.

Long-term research in alpine areas indicates that significant shifts have occurred in plant community composition due to direct and indirect anthropogenic effects such as the burning of fossil-fuels, land development, and large-scale agriculture (Dirnbock *et al.*, 2003; Harrison *et al.*, 2010; Gottfried *et al.*, 2012; Zorio *et al.*, 2016). Notably, many alpine plant species have shifted their altitudinal range either upward or downward over the last century (Pauli *et al.*, 1996; Zorio *et al.*, 2016). Like plants, the microbes in these regions have adapted to conditions including poorly developed soils, frigid and widely fluctuating temperatures, shorter growing seasons, and high winds (Fuller and del Moral, 2003; Wardle, 2004). It is possible that specialized plant-microbe interactions may buffer or increase the selective impact of these factors on those organisms (Donhauser & Frey, 2018).

Soil bacteria inarguably provide vital ecosystem services (Zak *et al.*, 2003; Allison & Martiny, 2008). Historically, most research has focused on agricultural applications and symbiotic nitrogen-fixing bacteria; therefore, studies situated in natural environments address gaps in our understanding of bacterial ecology (Morris *et al.*, 2013). Soil microbes drive nutrient acquisition and plant productivity in natural ecosystems even as plants lie dormant (Van Der Heijden *et al.*, 2008; Weber *et al.*, 2014). Further, above-ground plant diversity is a strong correlate of below-ground diversity in a wide range of ecosystems, with bacterial assemblages often varying between near-root soils (rhizosphere) and root surface (rhizoplane; Wagg *et al.*, 2014).

The dynamic conditions of alpine areas prompt complex seasonal shifts of soil bacteria For instance, research at the long-term alpine monitoring site at Niwot Ridge, CO suggests that winter may be the time of peak microbial degradation of resilient compounds like polymers and phenolics in plant litter (Lipson *et al.*, 2002; Nemergut *et al.*, 2005). During the spring snow-melt, mineralization and N leaching occurs due to wet soils and the exposure of microbial populations from retreating snow. Available nitrogen is rapidly taken up by emerging plants and decreases during the alpine growing season (Lipson *et al.*, 2002; Nemergut *et al.*, 2005). Snowpack is expected to decrease by 15% by 2100 in the northern hemisphere, with precipitation increasingly occurring from rain (NRC, 2011). A reduction in annual precipitation that falls as snow may affect microbes and add to a cascade of mis-timings, including plants with reduced seed set from frost damaged flowers, and altered plant phenologies that leave pollinators searching for unavailable food sources (Inouye *et al.*, 2002; Aldrige *et al.*, 2011). These changes could spur feedbacks with particularly negative effects to alpine plant communities (Zak *et al.*, 2003; Donhauser & Frey, 2018).

Molecular studies indicate that only approximately 1% of microbial taxa can be cultured (Roh *et al.*, 2010). Thus, the refinement of next-generation (Nextgen) sequencing technology has been vital for the description of potentially hyper-diverse bacterial communities based on operational taxonomic units (OTUs; Nemergut *et al.*, 2005; Roh *et al.*, 2010). Nextgen approaches have addressed a number of well-known drawbacks with older methods, like genotypic fingerprinting, which has a poor ability to provide taxonomic information, and a limited capacity to capture the presence of rare taxa (van Dorst *et al.*, 2014).

Carl Woese's pioneering work on gene sequencing demonstrated the power of the small subunit (SSU) rRNA gene as a target for microbial diversity studies and phylogenetic analysis (Burrows *et al.,* 2009). The ubiquitous presence in all cells of similarly functioning SSU rRNA genes make them useful tools for the detection and taxonomic delineation of organisms, particularly for unculturable microbes. Rapidly evolving SSU rRNA regions help discriminate between closely related species, while slowly evolving regions define more distant phylogenetic relationships. Horizontal gene transfer (HGT) within some domains can obscure evolutionary relationships, but the importance of SSU rRNA genes to protein synthesis, limits, or prohibits, the occurrence of HGT (Burrows *et al.*, 2009). Studies using the SSU rRNA gene have been bolstered by the availability of increasingly large databases of aligned sequences.

In this dissertation chapter, our overall goal was to use Nextgen sequencing to determine the composition of bacteria in rhizoplane and rhizosphere soil of plant assemblages in four distinct alpine habitats in the northern Rocky Mountains, with particular attention to microbial associates of three characteristic plants species. Because certain bacteria have been associated with the soil microbiome of specific plant species (Braga *et al.*, 2016; Lladó *et al.*, 2017) I hypothesize (**H_1**) that distinguishing characteristics of bacterial communities will be observed among the three focal alpine plant species. Additionally, I expect (**H_2**) that bacterial assemblages will correspond to environmental conditions, including edaphic properties. Finally, because functional specificity of microbial organisms increases with proximity to root surfaces (Lladó *et al.*, 2017; Teacon & Or, 2017), I expect (**H_3**) that differences will occur in microbial composition and diversity between rhizosphere and rhizoplane soils.

2. Methods

2a. Study Area

At 89,030 km^2, the Greater Yellowstone Ecosystem (GYE) encompasses one of the largest nearly intact temperate-zone ecosystems on Earth. The Absaroka Mountain Range is a sub-range on the eastern side of the north-central Rocky Mountains that stretches across the states of Montana (MT) and Wyoming (WY) for approximately 241 km. Substrate in the region is primarily composed of andesitic lavas and alluvial volcanoclastics originating from the Absaroka Volcanic Supergroup, (Smedes & Prostka, 1972). Volcanic breccias, a remnant of younger formations within the Supergroup, are found on most mountain summits in the region (Smedes & Prostka, 1972). The three mountains considered in this study (Wolverine Peak, Mineral Peak, and Republic Peak; >3000 m) are located within or near the northeast boundary of Yellowstone National Park (YNP) and are accessible from Cooke City and Silver Gate, MT (45.0160° N, 109.9166° W; elevation 2,319 m; Figure 1). The annual average maximum and minimum temperatures are 9.1°C and -5.9°C, respectively. Average yearly precipitation is 628 mm, and average annual snowfall is 513 cm (NOAA, 2017).

The alpine regions here experience a shorter growing season (~ 3 month frost-free) than similar ecosystems in Colorado (Bowman, 2001; Zorio *et al.*, 2016). This area has been largely spared from anthropogenic impact, such as land development and road-building that can lead to habitat fragmentation and the introduction of invasive species.

2b. Field Methods

I sampled 12 permanent vegetation transects for soil microbes on three mountains (Republic Peak, Wolverine Peak, and Mineral Peak) near the northeastern border of Yellowstone National Park (YNP) during the summer of 2017 (Figure 1). The 10m vegetation transects were a small subset of plots established by Aho (2006, 2008, 2012) to monitor alpine plant community integrity. Methods used for soil microbial sampling largely follow Weber and Werth (2015). To deduce effects of local environmental conditions, transects in four habitat types (ridge-top (RT), late snow-melt (SM), south-facing (SF), and north-facing (NF)) were sampled on each mountain..

Soil samples were taken proximal to three focal species: two nitrogen-fixing legume species *Astragalus kentrophyta* and *Lupinus argentus*, and an abundant sedge, *Carex albonigra*, during the month of July. Nomenclature for plant species follows Dorn (1992). Soil samples from one representative plant per site were collected approximately 5 m away from the transect lines to preserve the integrity of the permanent plots at two locations with respect to the roots of individual plant specimens. Rhizosphere soils (near the roots) were collected by shaking each plant specimen near the mouth of a clean sealable plastic bag. Rhizoplane soil (root surface) was collected by gently wiping roots with Kimwipes™ onto clean plastic sheeting. Samples were transferred into 50 mL Falcon tubes and stored at -20 °C until DNA extraction was performed for bacterial 16S rRNA gene amplification and sequencing. Plants sampled for soil microbes were replanted before leaving each site.

2c. Laboratory Methods

2c.1. Soil chemistry analysis and DNA extractions from soil samples

Soil samples collected from plant roots for use in DNA extraction were insufficient for detailed chemical analysis. As reasonable proxy, I utilized raw soil chemistry data collected by Aho (2008, 2012) for the same transects, to discover possible associations of these variables to soil bacteria assemblages. DNA was extracted from 500 mg of soil from each sample with a FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA). Eluted DNA (~30 µL) was purified using steps 14 to 21 of the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). A blank (no soil or additional water) extraction and an extraction using water instead of soil were added as control groups. Purified extracts were stored at -20°C until pyrosequencing.

2c.2. 16S SSU rRNA gene amplification, Illumina Mi-seq sequencing, and analysis

Bacterial 16S rRNA gene fragments from soil samples were amplified in triplicate via polymerase chain reaction (PCR) with 515F and 806R barcoded primers (Caporaso *et al.*, 2011). Each 25 µL PCR sample included 3.0 µL of template DNA, 0.8 µL of uniquely barcoded reverse primer and 21.4 µL of master mix that contained 10X HotMaster Buffer and HotMaster TAQ 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 Idaho State University Molecular Research Core Facility (ISU-MRCF). The barcoded sequence libraries were trimmed, examined for quality, and analyzed with the software package “MOTHUR” version 1.39.5 (Schloss *et al.*, 2009). 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 (Huse *et al.*, 2010). Sequences were aligned against the SILVA (Pruesse *et al.*, 2007) bacterial 16S rRNA gene reference database. Operational taxonomic units (OTU’s) were clustered at ≥ 97% similarity, using the average neighbor algorithm (Drechsel *et al.*, 1995). The 22-libraries were normalized to contain the same number of sequences (n = 281,290) prior to all comparative analyses among sampling sites and seasons. Two samples that did not meet the minimum sequence size were removed. OTU-based rarefaction curves for each sample were calculated using 1,000 sampling iterations in the MOTHUR software package. All sequences were taxonomically classified using the Ribosomal Database Project (RDP) Classifier (Wang *et al.*, 2007) within MOTHUR with the confidence thresholds set at ≥ 80%.

2d. Data Preparation and Statistical Methods

2d.1. Data preparation: Soil Bacteria Survey Data

The taxonomic classification output from sequencing was organized into multiple matrices for analysis. Indicator Species Analysis (ISA) and stacked bar plots summaries were created using the full dataset. Ordinations and diversity metrics were analyzed via a reduced dataset comprised of OTUs with ≥ 1% of total abundance (across all sites). Metagenome data are currently being processed in the MG-RAST database (https://www.mg-rast.org/).

2d.2. Tests of community diversity and species distributions

The Shannon-Weiner index was used to measure α-diversity within bacteria communities (Magurran, 2013). The Shannon-Weiner index assumes all species are represented and is relatively sensitive to the occurrence of rare species (Magurran, 2013). Beta diversity was measured using average Bray-Curtis dissimilarity (Bray & Curtis, 1957). Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2005) was used to test null hypotheses of identical composition of soil bacteria with respect to soil type (rhizosphere or rhizoplane), habitat (NF,SF, SM, RT), mountain (Wolverine, Republic, and Mineral) and focal plant species (*A. kentrophyta*, *L. argenteus*, and *C. albonigra*). Bray-Curtis dissimilarity was used as the underlying PERMANOVA resemblance metric. Indicator Species Analysis (ISA; Dufrêne & Legendre, 1997) provided a permutational approach to test for the statistical significance of specific microbial taxa association with soil type, habitat, mountain, and focal species. *P*-values were generally adjusted for simultaneous inference families of related tests by controlling for familywise type I error using Holm's procedure (Holm, 1979). The exception were ISA tests. In this case, I report both raw test results and results controlling for false discovery rate (see Benjamini and Hochburg 1995) across taxonomic families under consideration for an associated factor level. The value $\alpha = 0.05$ was used as the significance level for all significance tests. For a more informative view of site characteristics, split-plot ANOVA was used to discern differences in environmental factors among mountains and habitats.

2d.3. Ordination

Non-Metric Multidimensional Scaling (NMDS) ordination was used for indirect gradient analysis (Jongman *et al.*, 1995; Legendre and Legendre, 2012). Environmental variables and dominant bacterial families were overlaid on the species space of each ordination and tested for significance using vector fitting (Oksanen *et al.,* 2013). Bray-Curtis dissimilarity (Bray & Curtis, 1957) was used as the underlying resemblance metric in NMDS. Environmental data, including physical site characteristics and soil chemistry, were taken from raw data collected by Aho (2008, 2012). The correlation of each environmental variable to the orientation of communities within ordinations was tested with vector-fitting (*i.e*., individual multiple regressions for each environmental variable with ordination dimensions as predictors).

2d.4. Software

The statistical environment R was used for all analyses (R Core Team, 2015). I relied on multiple packages, especially vegan (Oksanen *et al.*, 2013) for ordination, PERMANOVA, and other community level analyses. I used the package plottrix (Lemon, 2006) for graphical applications and labdsv (Roberts, 2011) for indicator species analysis which were translated into bar plots using customized functions.

3. Results

3a. Soil Bacteria and soil chemistry

A total of 78,192 operational taxonomic units (OTUs), defined at the 97% nucleotide-sequence identity level, were identified among all sequences. Rarefaction analyses indicated that rhizosphere samples generally had higher total reads but did not as closely approach rarefaction asymptotes, compared to rhizoplane samples. Sites associated with Mineral Peak tended to have the highest read coverage for sampling depth, but lowest number of reads (Figure 2).

Four soil variables frequently cited as important for structuring communities of plants and soil bacteria were used to discern differences between mountains and habitats. Mountains were weakly significant for organic carbon (OC; $F_{2,11}$ = 4.15, P = 0.052), but not for pH, phosphorous (P), or carbon:nitrogen (C:N). Habitat only varied significantly for pH ($F_{3,11}$ = 6.07, P = 0.019).

3b. Community diversity and species distributions

Univariate analyses found that richness (*i.e.* number of unique OTUs) did not vary across mountains ($F_{2,21}$ = 2.54, P = 0.103), habitat ($F_{2,21}$ = 2.54, P = 0.103), soil type ($F_{2,21}$ = 2.54, P = 0.103), or focal plant species ($F_{2,21}$ = 1.45, P = 0.257; Table 1). Alpha-diversity measured through the Shannon-Weiner index was only significant for mountain ($F_{2,21}$ = 10.51, P = >0.001). Wolverine Peak had the highest Shannon-Weiner values followed by Mineral Peak, and Republic Peak. Habitat ($F_{2,21}$ = 0.62, P = 0.611), soil type ($F_{2,21}$ = 0.53, P = 0.474), and plant species ($F_{2,21}$ = 0.62, P = 0.549) did not vary (Table 1).

Similar to α-diversity, β-diversity only varied significantly among mountains ($F_{2,21}$ = 5.57, P = 0.011), and not for habitat ($F_{2,21}$ = 0.16, P = 0.924), soil type ($F_{2,21}$ = 0.18, P = 0.674), or focal plant species ($F_{2,21}$ = 0.78, P = 0.472). Republic Peak had the highest Bray-Curtis index values, followed by Mineral and Republic Peak. Habitat types had nearly identical Bray-Curtis values (Table 1). A lack of replication in habitats occurred due to the unequal numbers of each plant species represented in the dataset. However, I were able to use *A. kentrophyta* to examine the correlation between plant species and soil bacteria because it was collected in two habitats across three mountains. PERMANOVA ($F_{1,2}$ = 1.68, R^2 = 0.33, P = 0.133) indicated that habitat did not significantly influence the composition of soil bacteria and suggests that plant species is a stronger influence. In addition, categorical variables were used to block for site replication (*i.e.* rhizoplane and rhizosphere soil was collected from a single plant per site) to ensure correct degrees of freedom in the model. There was no significant effect of soil type on bacterial composition when controlling for plant species and mountain (PERMANOVA: $F_{1,11}$ = 1.24, R^2 = 0.05, P = 0.312).

3c. Ordination

NMDS ordinations (k = 2, stress = 0.07) of the relationship of sites to bacterial taxa formed a large diffuse cluster along the second dimension, corresponding to C/N ratio trends, with a few sites pulled along the first dimension, likely due to rare OTUs (Figure 3). South-facing sites displayed more within-group similarities than other habitats. In contrast, ridgetop sites are the most scattered.

The correlation of each environmental variable to the spread of communities within ordinations was analyzed using vector fitting (Table 2). The strength of the correlation for vectors is graphically represented by the length of the arrows in the ordination diagrams, whereas the direction of arrows indicates the direction of most rapid increase for a variable among samples (Figure 3). No physical site characteristics (slope and aspect) were correlated with the scatter of microbial communities represented by points in the ordination. Additionally, except for carbon to nitrogen ratios (C:N; r^2 = 0.26, P = 0.033), soil chemistry variables were not closely associated with locations of microbial communities within the ordination (Table 2, Figure 3).

3d. Indicator species analysis

Indicator species analysis (Dufrêne & Legendre, 1997) was used to identify a number of bacterial taxonomic families highly associated with specific mountains (6), with a progressively smaller number of taxa distinguishing habitats (5), plant species (4), and soil types (0) Figures 4-7, respectively. I report and analyze only family-level taxa here, because of accuracy of identification and the relatively low concentration of bacterial DNA extracted from the polycarbonate filters. Barplots show the most abundant taxa (families with the highest number of reads) in our soil microbial dataset (Figures 4-7). Statistically significant indicators in barplots are denoted with asterisks and bold text. Taxa significant at $\alpha = 0.05$ after controlling for false discovery rate are indicated with two asterisks and underlined text. Solirubrobacteraceae was the sole significant indicator for Mineral Peak. Republic Peak was distinguished by Conexibacteraceae and Bradyrhizobiaceae. Wolverine Peak had the highest number of indicator taxa, including the highly associated Chitinophagaceae, as well as Micrococcaceca and Comamonadaceae (Figure 4). Among habitats, north-facing sites had no indicator species. South-facing sites were distinguished by Saprospiraceae (Figure 5). The ridgetop habitat was distinguished by Sphingomonadaceae and Micrococcaceca. Two families, the DA101 soil group and Bradyrhizobiaceae, were indicators for the late snowmelt habitat (Figure 5).

Plant species and soil types had fewer indicator species than site characteristics. For plant species, *A. kentrophyta* (Sphingomonadaceae, Micrococcaceca), and *L. argentus* (DA101 soil group, Conexibacteraceae) had two significantly associated families each (Figure 6). In contrast, *C. albonigra* had none. Neither rhizoplane or rhizosphere soils were significantly associated with any particular bacterial family (Figure 7).

4. Discussion

Advances in molecular biotechnology have released microbial diversity studies from a reliance on culture-dependent techniques (van Dorst *et al.*, 2014; Donhauser & Frey, 2018). In our study, massively parallel sequencing was used to determine patterns of bacterial species composition associated with the soils of three characteristic alpine plant species in the north-central Rocky Mountains. I found that the soils of these plant species harbored unique assemblages of soil bacteria when controlling for habitat type across three mountain locations. Assemblages of bacteria in rhizosphere and rhizoplane soils, however, were more similar than expected. Further, aside from C:N, environmental variables were not useful descriptors for patterns of soil bacteria. Unexpectedly, the three mountains, chosen because of their similarities in elevation and substrate, and proximity, varied more than habitat types and focal plant species with respect to community composition and diversity metrics and were distinguished by the highest number of indicator families.

4a. H_1: Soil bacteria varies by plant species

Our first hypothesis, that community composition of soil bacteria would vary among three focal alpine plant species was partially supported by ISA and PERMANOVA (Table 1, Figure 6). The soil environments of *A. kentrophyta* and *L. argentus* were each distinguished by two families of bacteria. *C. albonigra*, while abundant in north facing and/or late snowmelt habitats that have few occurrences of either *A. kentrophyta* or *L. argenteus*, had no unique microbial associations of its own. The apparent variation of rhizo-associated bacteria among the three plant species here supports evidence that plant species (and their root exudates and root structure) drive differences in rhizo-associated bacteria (Buyer *et al.*, 2002; Beckers *et al.*, 2017; Wang *et al.*, 2017).

As expected, the composition and abundance of soil bacteria between plant species varied (Table 1). Interestingly, the two plant species in the Fabaceae family had soil microbial associations that appeared to diverge more from each other than from the remaining sedge species (family Cyperaceae; Table 1, Figure 6). *L. argenteus* is an upright forb inhabiting a wide variety of ecosystems in western North America, including sagebrush steppe, grasslands, forests, subalpine meadows. *A. kentrophyta*, a mat-forming cushion plant, is generally limited in distribution to rocky mountainous areas, and is a characteristic species of ridgetop environments at the study area (Aho 2006). Like most legumes, *L. argenteus* and *A. kentrophyta* generally possess root nodules containing diazotropic bacteria from the paraphyletic group Rhyzobia, This mutualism allows legumes to increase soil N-availability of the environments they occupy, an outcome clearly demonstrated for *L. argenteus* in a number of distinct ecosystems (Johnson & Rumbaugh, 1981, Kenny & Cuany 1990). The family Bradyrhizobiaceae, a key member of the Rhyzobia group, was one of the most dominant bacterial families at the study site (Figures 4-7), although it was not a significant indicator for either *A. kentrophyta* or *L. argenteus*.

L. argentus soils were distinguished by DA101 (phylum Verrumicrobia), and Conexibacteraceae. Verrumicrobia have been found in soil of various ecosystems and may be useful for disease suppression in plants as exposure to pathogens can activate stress responses such as biofilm formation and the production of secondary metabolites (Weiland *et al.*, 2001; Expósito *et al.*, 2017). Conexibacteraceae have been described as primarily saccharolytic, thereby contributing to carbon cycling, and can reduce nitrate to nitrite, suggestive of a role in nitrification. Both members of Verrumicrobia and Conexibacteraceae were reported to be the dominant taxa in a sample of Alaskan permafrost (Expósito *et al.*, 2017). Their slow-growing habit and cold-tolerance may make them well-adapted to alpine environments.

Indicator families for *A. kentrophyta* were Sphingomonadaceae and Micrococcaceae (Figure 6). Like Verrumicrobia, Sphingomonadaceae was also indicated to respond defensively to soil pathogens (Expósito *et al.*, 2017). Micrococcaceae, within phylum Actinobacteria, contain sphingolipids embedded in the outer membrane (Rosenberg *et al.*, 2014) potentially providing increased resistance to the punishing environmental conditions of ridgetop habitats.

An increasing number of studies report the tendency of plants to create unique assemblages of soil bacteria across species and closely related individuals (Aleklett *et al.*, 2015). Of relevance to this study, Zhao *et al.* (2008) found that *Astragalus* –associated microbes vary more by cultivar than soil type (Zhao *et al.*, 2008). Further, in a study comparing cultivars of *Lupinus angustifolius*, Chen (2013) found significant variation in communities of bacteria when planted in a variety of soil types. This work supports the general hypothesis that plant genotypes, including distinct species, are an important driver of the composition of soil bacteria regardless of soil type or general land use regime (Buyer *et al.*, 2002; Haichar *et al.*, 2008; Zak *et al.*, 2013; Chen, 2014)).

C. albonigra, had no significant indicator taxa and was the dominant plant on some late snowmelt areas and north-facing sites that were not characteristically late snowmelt sites. The relatively steep slopes and high winds of north-facing sites expose vegetation to lower temperatures and thin layers of organic soil. Water from snowmelt quickly evaporates during the growing season, in part driving the difference in plant composition for north-facing and snowmelt sites. In these areas *C. albonigra* performs a similar role to mat-forming herbaceous species, like *A. kentrophyta*, found on exposed peaks and ridgelines; namely as soil stabilizers, nurseries for other plant species, and sources of carbon and other exudates that support a variety of soil biota (Nemergut *et al.*, 2005; Zhao *et al.*, 2008; Jacoby *et al.*, 2017).

4b. H_2: Patterns of soil bacteria will correlate to environmental factors.

Environmental site characteristics (*e.g.* slope, aspect, and soil chemistry factors) were generally weak correlates of soil bacteria assemblages (Tables 2-5). Multiple regression analysis, based on NMDS scores, found that of 15 environmental factors only C:N was a good predictor of microbial composition (Table 2). Additionally, soil nutrients also showed marginal variation across mountains or habitats. Specifically, soil organic carbon (OC) content was weakly significant for mountain, where Republic Peak had the highest OC content of the three mountains and Mineral Peak the lowest (Table 4). Soil pH differentiated habitat type. Seasonal inputs of organic matter from abundant vegetation and removal of leachable bases by meltwater likely drive the low pH of snowmelt sites, while a lack of vegetation biomass retards soil formation in ridgetop sites and resulted in a neutral average pH (Table 4).

Dominant plant species were correlated with indicator taxa for at least two out of four habitat types (Figure 5). Ridgetop and late snowmelt sites share identical indicator taxa as indicated in NMDS ordinations where dominant families are overlaid as vectors (Figure 3). *A. kentrophyta* was collected from ridgetop sites and *L. argenteus* was collected from snowmelt sites (Table 3). The only indicator taxa that described south-facing sites was the copiotrophic Saprospiraceae, although *A. kentrophyta* was also collected from south-facing sites (Figure 5). We note that focal species are confounded with habitat because of their specific growth habits. Previous studies have indicated that this family reaches peak abundance during winter. Snowmelt sites generally stay snow-covered longer into the growing season than south-facing sites. Conversely, snow depth is often much greater on steep to moderately sloped south-facing sites because of wind-blown snow and formation of cornices. This could potentially drive the comparatively high proportion of Saprospiraceae in south-facing sites if those communities are still transitioning into a seasonally competitive community.

Both pH and OC have been shown to affect the structure of bacterial communities at multiple scales (Fierer *et al.*, 2011; Tecon & Or, 2016; Morina *et al.*, 2018), although our data do not support these findings. The association of soil bacteria assemblages to C:N in this study could be a reflection of the combined indirect effects from a variety of soil traits, including pH and OC. Additionally, the high spatial variability inherent in alpine landscapes may obscure some associations, particularly if stronger drivers exist, including plant symbioses (Fierer *et al.*, 2011; Tecon & Or, 2016; Morina *et al.*, 2018). Lastly, we should also consider the possibility that deriving soil chemistry from bulk soil instead of rhizo-associated soil affected the validity of our inferences. For instance, organic acids released as root exudates can alter pH in the surrounding soil (Haichar *et al.*, 2008; Lladó *et al.*, 2017; Morina *et al.*, 2018).

Vectors of the most abundant families were fit to the NMDS ordination as another way to assess patterns of taxa among samples (Figure 3). Less vegetated habitats significantly correlated with bacterial families such as Chitinophagaceae and Comamonadaceae. The copiotrophic actions of Chitinophagaceae help speed the decomposition of organic matter (particularly fungal mycelia). Comamonadaceae has been reported to increase in abundance as decomposition by families like Chitinophagaceae proceeds (Lladó *et al.*, 2017). More densely vegetated habitats were more closely associated with families Bradyrhizobiaceae, including significant correlates of focal species, and Sphingomonadaceae, previously discovered in permafrost (Figure 3; Dworkin, 2006; Shahnavaz, 2009).

4c. H_3: Rhizosphere and rhizoplane soil will harbor unique assemblages of bacteria

I did not detect differences between the composition of bacteria in rhizosphere and rhizoplane of three alpine plant species**.** However, a substantial amount of work has shown that bacterial abundance and composition vary substantially across mineral and organic layers of soil, and across soil depth in general (Fierer *et al.*, 2011; Donhauser & Frey, 2018). Relatedly, studies in natural and artificial environments have reported that communities of bacteria continue to shift with proximity to the root surfaces (Braga *et al.*, 2016; Morina *et al.*, 2018).

The apparent similarities between rhizosphere and rhizoplane soil types, seen here, may have been influenced by sampling methods that could obscure fine-scale differences common in communities of bacteria. However, because many endophytes of plants may be derived from the rhizosphere (Compant *et al.*, 2010; Morina *et al.*, 2018), our results suggest that some bacterial strains migrate between the rhizosphere and rhizoplane (Figure 7). Our results correspond to a recent low-elevation wetland study (Morina *et al.*, 2018) where rhizoplane soil bacteria occurred in greater abundance than rhizosphere associated bacteria. I found total OTU numbers for rhizoplane and rhizosphere soil bacteria were 781,872 and 675,149, respectively.

5. Conclusions

Community composition of bacteria within four distinct alpine habitats at three different mountain locations appears largely driven by plant species associations. However, differences in α and β-diversity among mountains suggest that the environmental heterogeneity common in alpine landscapes in tandem with resource competition among strains of bacteria also shape communities of soil bacteria (Nemergut *et al.*, 2005; Haichar *et al.*, 2008; King *et al.*, 2010). Understanding the dynamic relationships of plant and microbial taxa in alpine ecosystems will become increasingly important as we confront global challenges, like a changing climate, that can have a disproportionate effect on the stability of fragile and unique ecosystems. Climate forecasts for the study site region predict increasing precipitation for every season excluding summer (Whitlock *et al.*, 2017). Concurrently, average yearly temperatures are expected to increase by ~3-5% by the end of the century. Decreases in winter snowfall will likely have a negative effect on summertime water availability. In tandem, the absence or reduction of springtime snowpack could alter plant communities and affect seasonal fluctuations of soil bacteria (Tecon & Or, 2017; Donhauser & Frey; 2018). In addition to increasing the number of metagenomic studies of soil bacteria, future research should focus on their important ecosystem services, such as carbon-cycling and decomposition, that could potentially be impacted by or help ameliorate the effects of anthropomorphic activities. As proteomic and other metabolomic techniques gain optimized protocols and decrease in cost we will soon be able to confidently link function to phylogeny and better predict responses of soil bacteria to biotic and abiotic stimuli (Jacoby *et al.*, 2017; Donhauser & Frey, 2018).

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7. Tables

Table 1. Summary statistics for mean richness, Shannon-Weiner diversity, and Bray-Curtis dissimilarity for soil bacteria on three mountains. Standard errors are shown in parentheses. Bold text denotes significance at α = 0.05.

Mountain	Richness	Shannon	Bray-Curtis
MI	150.88 (± 1.53)	**4.27 (± 0.07)**	**0.44 (± 0.02)**
RP	144.13 (± 4.77)	**4.07 (± 0.09)**	**0.51 (± 0.03)**
WO	153.13 (± 0.77)	**4.5 (± 0.03)**	**0.42 (± 0.02)**
Site			
NF	151 (± 1.21)	4.27 (± 0.07)	0.45 (± 0.01)
RT	154.33 (± 1.43)	4.39 (± 0.06)	0.44 (± 0.02)
SF	148.67 (± 4.22)	4.28 (± 0.13)	0.46 (± 0.04)
SM	143.5 (± 5.13)	4.18 (± 0.14)	0.47 (± 0.04)
Soil			
Rhizoplane	149.92 (± 2.29)	4.24 (± 0.07)	0.46 (± 0.02)
Rhizosphere	148.83 (± 2.89)	4.32 (± 0.08)	0.45 (± 0.02)
Plant Species			
A. kentrophyta	151.29 (± 1.2)	4.32 (± 0.06)	0.45 (± 0.02)
C. albonigra	144.17 (± 4.97)	4.25 (± 0.14)	0.48 (± 0.04)
L. argentus	150.5 (± 3.12)	4.17 (± 0.12)	0.43 (± 0.02)

Table 2. Multiple regression of environmental parameters against soil bacteria abundance. Asterisks denote significance.

Vector	Units	Code	r^2	*P*
Slope	Degree	Slope	0.07	0.475
Aspect	West declination	Aspect	0.16	0.143
pH		pH	0.08	0.443
Organic Carbon	%	OC	0.034	0.689
P-Olsen Method	Ppm	P	0.106	0.325
Calcium	Ppm	Ca	0.021	0.806
Magnesium	Ppm	Mg	0.03	0.696
Sodium	Ppm	Na	0.006	0.95
Sulfur	lb acre^{-1}	S	0.072	0.466
Zinc	Ppm	Zinc	0.57	0.551
Manganese	Ppm	Mn	0.58	0.551
Copper	Ppm	Cu	0.003	0.74
Ammonium	Ppm	NH4	0.03	0.74
Total Kjeldahl Nitrogen	%	TKN	0.046	0.642
C:N		C.N	0.26	0.033*****

Table 3. Site and sample characteristics. Average elevation for the study area is 3,080m.

Mountain	Habitat	Site Name*	UTMN (12 T)	UTME	Plant Species	Slope (°)	Aspect
Mineral	North-facing	MI_NF2_S	579769	4986323	*L. argentus*	10.5	50
		MI_NF2_E	579771	4986313			
	Ridgetop	MI_RT3_S	579459	4986091	*A. kentrophyta*	3.0	160
		MI_RT3_E	579454	4986081			
	South-facing	MI_SF2_S	579436	4986086	*A. kentrophyta*	24.5	255
		MI_SF2_E	579436	4986077			
	Late Snowmelt	MI_SM2_S	579636	4986361	*L. argentus*	16.3	35
		MI_SM2_E	579642	4986355			
Republic	North-facing	RP_NF1_S	583039	4978241	*A. kentrophyta*	14.5	355
		RP_NF1_E	583045	4978248			
	Ridgetop	RP_RT3_S	582859	4977994	*A. kentrophyta*	22.5	330
		RP_RT3_E	582852	4978003			
	South-facing	RP_SF4_S	582446	4978268	*A. kentrophyta*	20.0	212
		RP_SF4_E	582452	4978259			
	Late Snowmelt	RP_SM2_S	583138	4978157	*C. albonigra*	6.0	126
		RP_SM2_E	583145	4978163			
Wolverine	North-facing	WO_NF4_S	583039	4978241	*C. albonigra*	31.0	318
		WO_NF4_E	583045	4978249			
	Ridgetop	WO_RT3_S	577984	4989378	*A. kentrophyta*	20.0	263
		WO_RT3_E	577985	4989366			
	South-facing	WO_SF1_S	578167	4989121	*A. kentrophyta*	26.5	320
		WO_SF1_E	578161	4989130			
	Late Snowmelt	WO_SM2_S	577992	4989471	*C. albonigra*	18.0	225
		WO_SM2_E	578001	4989476			

*consistent with Aho, 2006 & 2012

Table 4. Raw soil chemistry for each site. Soil chemistry was collected by Aho (2008, 2012). For habitat, initials stand for north-facing (NF), ridgetop (RT), south-facing (SF), and late snowmelt (SM).

		Soil Chemistry												
Mountain	Habitat	pH	OC (%)	P (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	S (lb/acre^{-1})	Zinc (ppm)	Mn (ppm)	Cu (ppm)	NH_4 (ppm)	TKN (%)	C:N
Mineral	NF	5.3	4	9	2753	650	19	5	0.62	27.5	1.02	71.1	0.25	15.08
	RT	7.1	1	4	3898	717	16	2	0.32	10.06	0.85	12.5	0.01	120
	SF	7	1	10	3358	1345	14	5	0.39	11.9	1.49	34	0.07	13.43
	SM	4.8	7	14	2117	459	26	8	1.95	56.2	1.08	95	0.36	18.44
Republic	NF	5.7	21	26	2322	472	29	17	3.13	38.8	0.64	87.4	0.82	25.28
	RT	7	1	5	3212	2753	20	2	0.55	7.72	1.94	6.4	0.06	20
	SF	6.3	14	33	2757	868	27	34	5.1	68.99	0.71	212	0.9	15
	SM	5	16	11	1174	178	20	8	1.78	44.9	0.54	134.3	0.85	18.15
Wolverine	NF	6.9	1	9	2349	1211	331	6	0.38	24.65	1.64	48.5	0.1	10
	RT	6.9	2	14	2323	1510	269	5	0.67	36.62	1.5	20.6	0.15	10.67
	SF	5.8	8	22	1921	1340	123	18	1.8	27	1.5	53.3	0.47	16.7
	SM	6.2	4	14	2671	1122	26	11	1.68	7.5	1.31	12.5	0.29	14.43

8. Figures

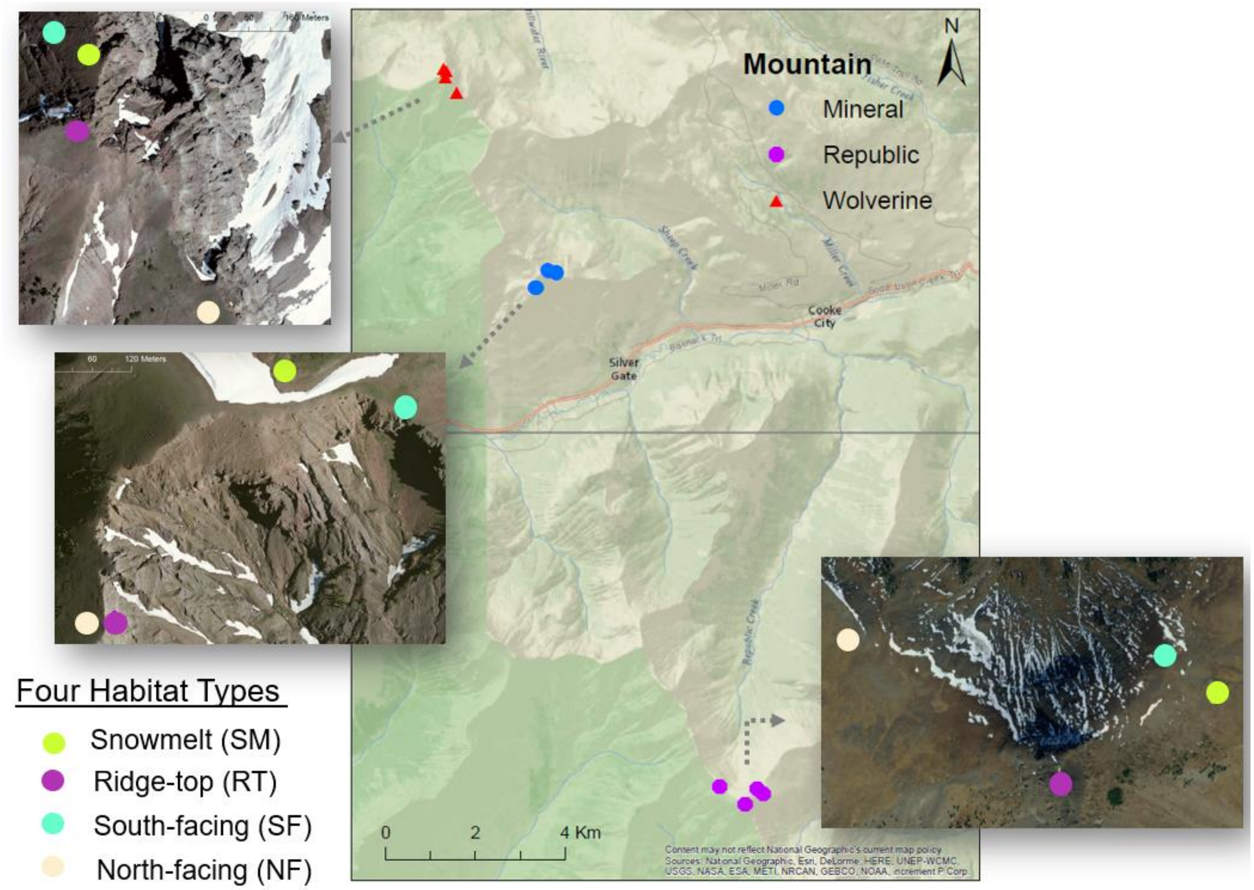

Figure 1. Map of the study area near Cooke City, MT. Insets and bottom key show habitat types.

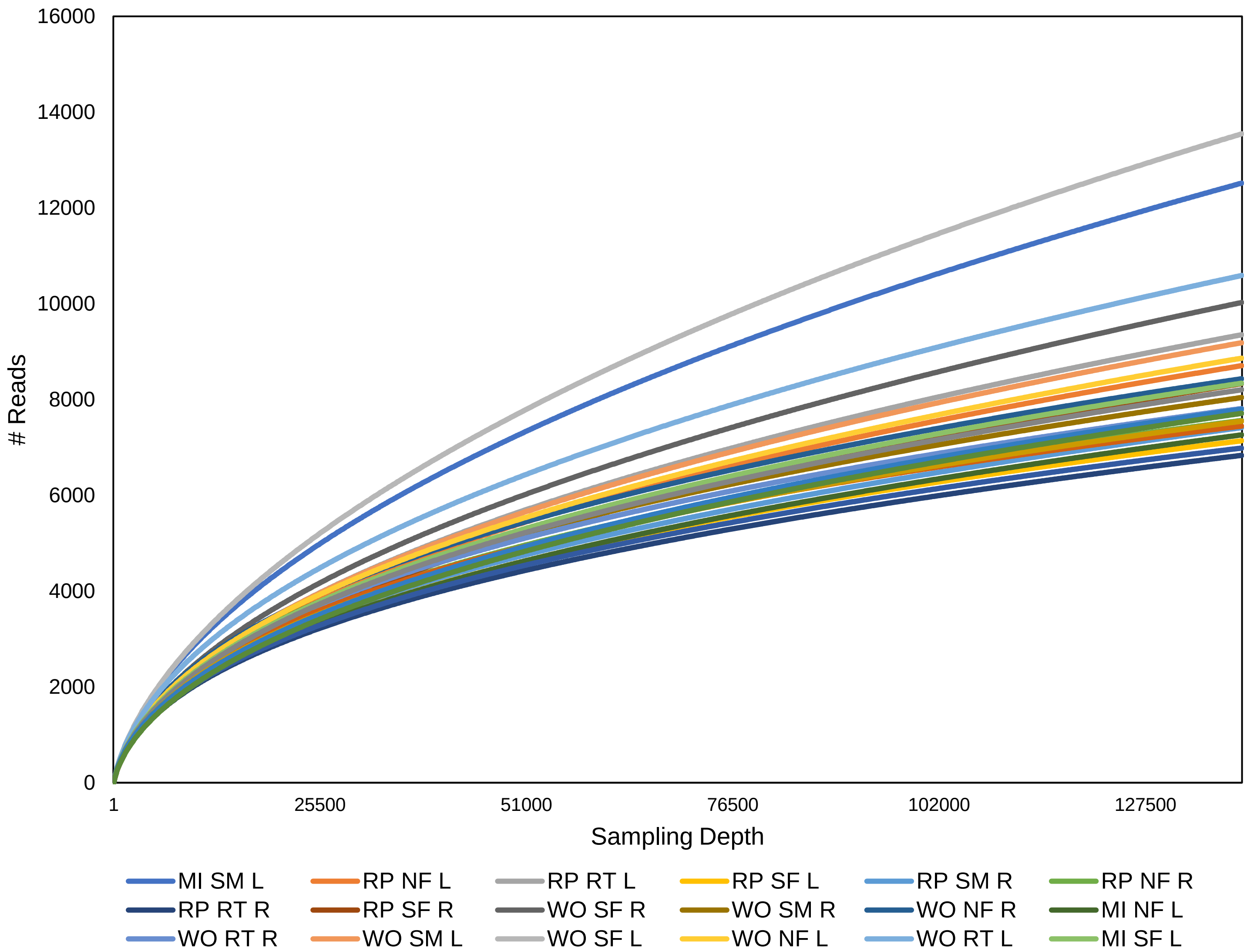

Figure 2. Rarefaction curves for sequenced samples of alpine soil bacteria. Total sampling depth was ~497,900. Sites associated with Mineral Peak have the lowest number of reads and most closely approach asymptote. MI, RP, and WO represent Mineral, Republic, and Wolverine Peak, respectively. Habitats are denoted by SM (late snowmelt), NF (north-facing), SF (south-facing), and RT (ridgetop). L indicates loose, or rhizosphere soil. R indicates root, or rhizoplane soil.

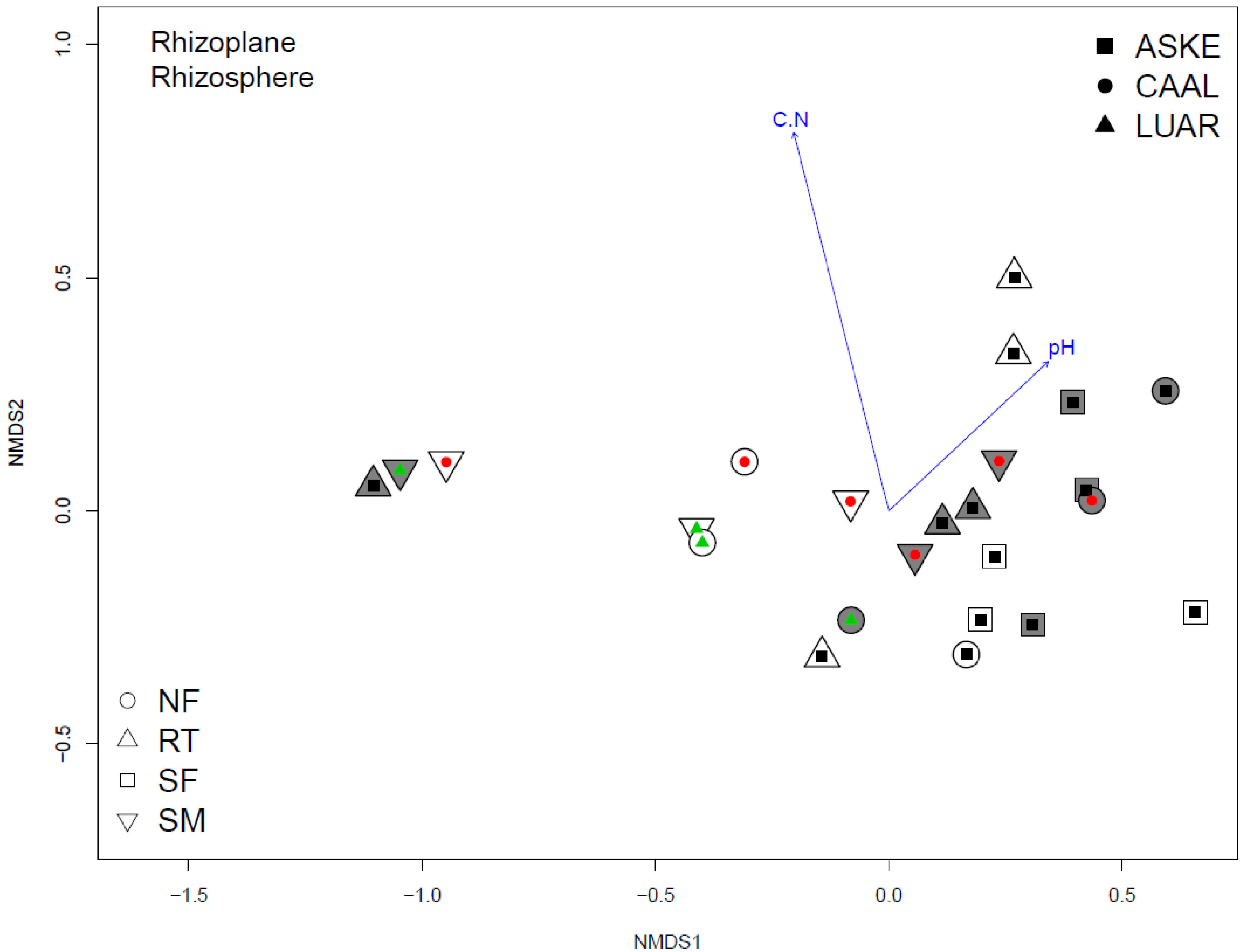

Figure 3. NMDS of soil bacteria composition of soil type, plant species (ASKE = *Astragalus kentrophyta*, CAAL = *Carex albonigra*, LUAR = *Lupinus argenteus*), habitat, and soil type. Blue arrows show significant soil chemistry values, C:N and pH. Symbols with a grey background represent rhizoplane samples. Habitats are denoted by NF (north-facing), RT (ridgetop), SF (south-facing), and SM (late snowmelt). Stress = 0.1, k =2.

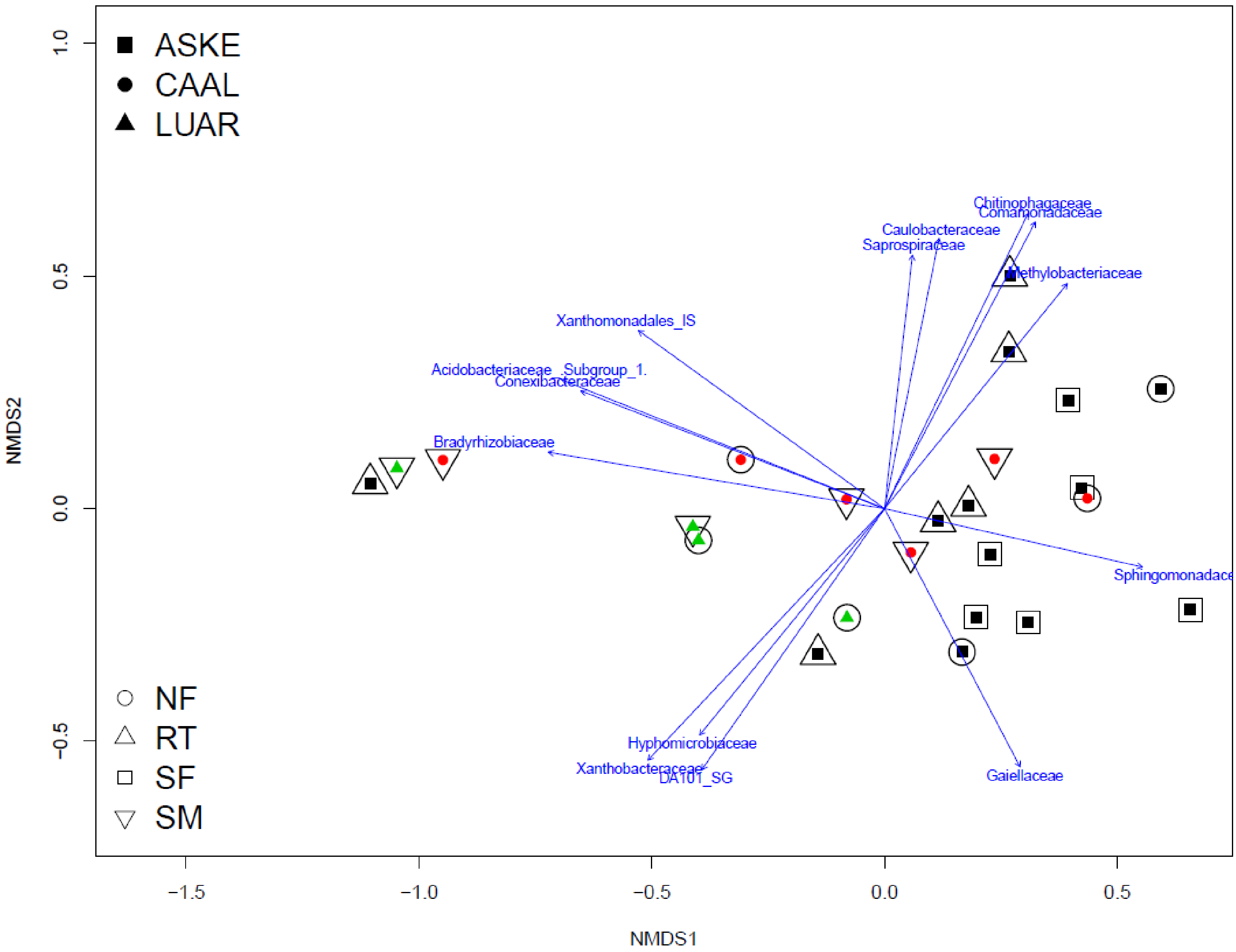

Figure 4. NMDS of soil bacteria composition of soil type, plant species (ASKE = *Astragalus kentrophyta*, CAAL = *Carex albonigra*, LUAR = *Lupinus argenteus*), and habitat. Blue arrows show C show dominant families of soil bacteria. Habitats are denoted by NF (north-facing), RT (ridgetop), SF (south-facing), and SM (late snowmelt). Stress = 0.1, k =2.

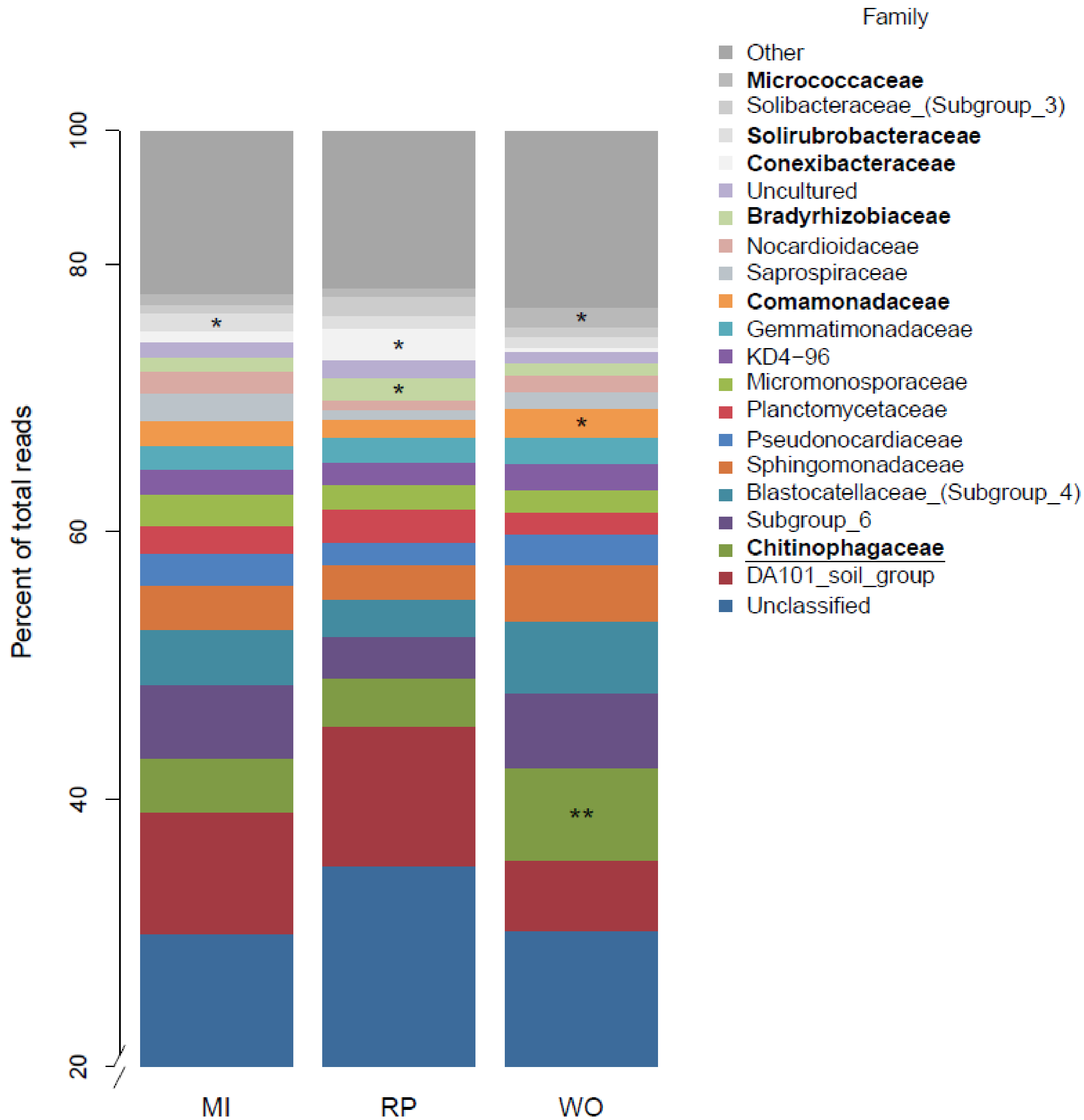

Figure 4. Stacked bar graph showing dominant bacterial (highest read) families by mountain. * = significance at alpha = 0.05, ** = significance at alpha = 0.05 after controlling for FDR. Families in the plot and key are arranged in order from top to bottom.

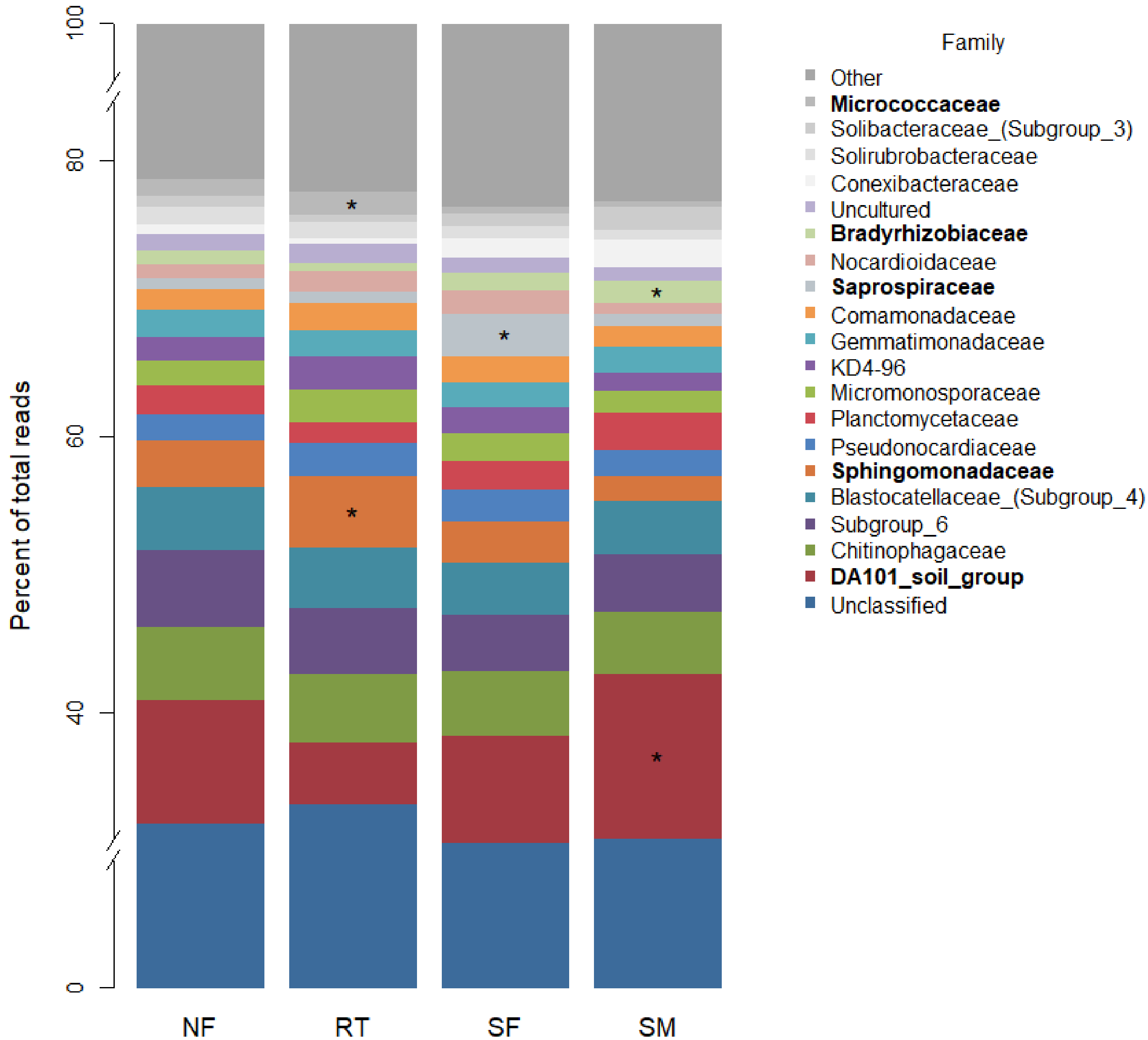

Figure 5. Stacked bar graph showing dominant (highest read) bacterial families by habitat.* = significance at alpha = 0.05. Families in the plot and key are arranged in order from top to bottom.

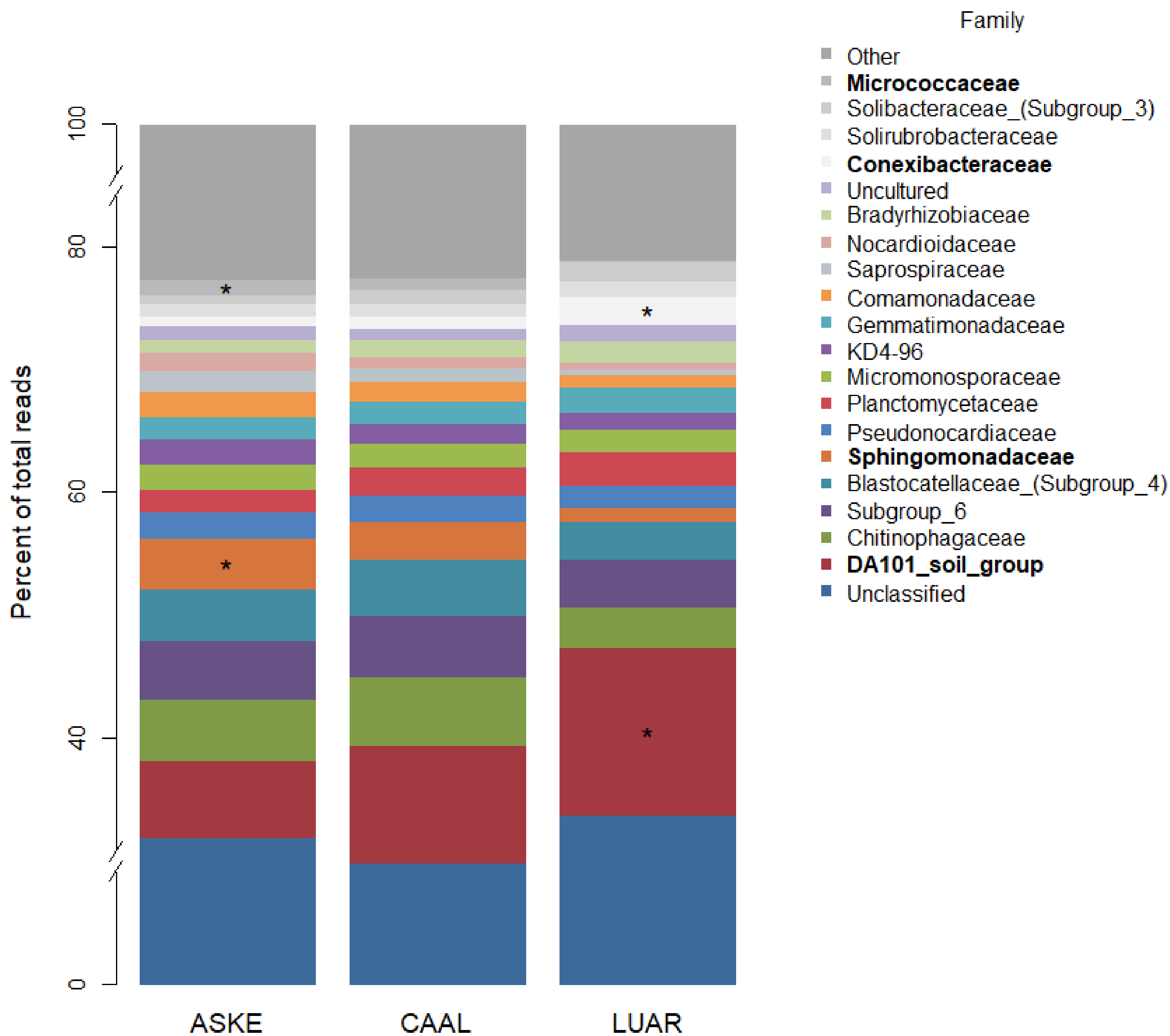

Figure 6. Stacked bar graph showing dominant bacterial (highest) families by focal plant species. ASKE= Astragalus kentrophyta, CAAL = Carex albonigra, LUAR = Lupinus argentus. Families in the plot and key are arranged in order from top to bottom.

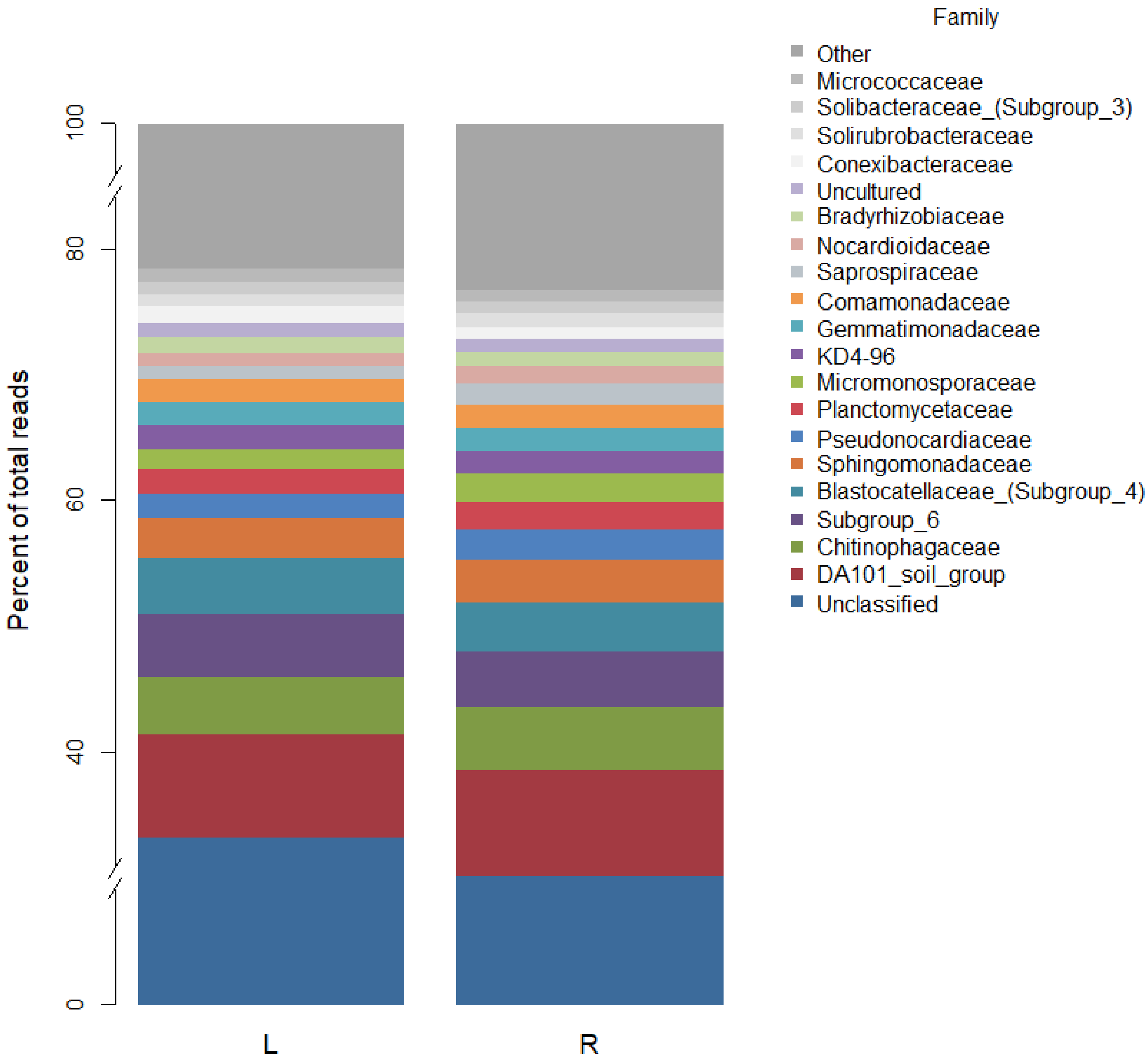

Figure 7. Stacked bar graph showing dominant bacterial families (highest read) by proximity of soil to plant. L= rhizoplane, R = rhizosphere. Families in the plot and key are arranged in order from top to bottom.

CHAPTER 3: Atmospheric bacteria assemblages across an alpine landscape

1. Introduction

A growing number of papers have reported spatiotemporal patterns in the distribution of aerosolized bacteria (Striluk *et al.,* 2016). Such patterns may be particularly pronounced in the proximal atmosphere of alpine environments, due to short, punctuated, growing seasons, the potential scouring action of strong winds, and high levels of condensation from adiabatic expansion (Bowers *et al.,* 2009; Smith *et al.*, 2013, Sattler *et al.*, 2001; Yamaguchi *et al.,* 2012; Weber, 2016).

Long-term research in alpine areas indicates that significant shifts have occurred in plant community composition due, in part, to direct and indirect anthropogenic effects such as the burning of fossil-fuels, land development, and large-scale agriculture (Dirnbock *et al.,* 2003; Harrison *et al.,* 2010; Gottfried *et al.,* 2012; Zorio *et al.,* 2016). While climate change is predicted to have largely indirect effects on microbial communities (Burrows *et al.*, 2009; Donhauser & Frey, 2018), higher annual temperatures, and increased incidence of drought and fire alter plant communities that result in changes to the activity of bacteria important for biogeochemical cycles (Lladó *et al.*, 2017; Teacon & Or, 2017).

Similarities between the bacteria of leaf-surfaces, plant/soil environments, and the atmosphere indicate that the atmosphere acts as a dispersal mechanism for many microbes (*e.g. Pseudomonas syringae*; Morris *et al.,* 2011; Braga *et al.,* 2016). The potential for global dispersal is of practical concern because many common airborne bacteria, like Pseudomonads, contribute to the spread of disease in agricultural and wild populations of plants and animals (Brown *et al.,* 2002; Smith *et al.,* 2013), whereas other airborne microorganisms provide positive important ecosystem services (Brown *et al.,* 2002; Smith *et al.,* 2013; Striluk *et al.* 2015).

This project utilized permanent high-altitude vegetation plots in Yellowstone National Park (YNP) and the surrounding Gallatin National Forest to investigate microbial communities in proximal atmospheric environments. Aho (2012) measured vegetation and abiotic attributes for 152 high altitude (> 3000 m) randomly selected plots, including 110 plots established in 2006 (Aho, 2006; Figure 1) within five topographic types (plateau, acute ridgetop, north-facing, south-facing, and late snow-melt) on eight mountains located in or adjacent to Northeastern YNP. I revisited a subset of baseline plots established by Aho (2006, 2012) to describe previously unconsidered microbial characteristics in the region.

Our primary goal was to consider the effects of biotic and environmental filtering on assemblages of near-terrestrial atmospheric bacteria at multiple, repeatedly measured alpine locations. Sequenced bacterial rRNA (16S) was collected from atmospheric samples at four alpine habitats (N. facing, S. facing, ridgetop, and late snowmelt), on three mountains, and three months during growing season (June, July and August). These data were used to consider three hypotheses:

H_1: Composition and abundance of atmospheric bacteria will shift throughout the growing season. Specifically, I predicted that July, the peak growing season for alpine plants in the region, will have the greatest abundance of bacteria, whereas June's patchwork of early and late snowmelt sites will drive a compositional mosaic of bacterial taxa with relatively high levels of β-diversity.

H_2: Nodal alpine habitats (Aho & Weaver, 2010) will be associated with patterns of atmospheric bacteria throughout the growing season. Compositional distinctions will be particularly pronounced between north-facing and south-facing sites due to aspect-related differences in in prevailing winds and incident radiation. Relatedly, I anticipated that polarized conditions for ridgetop (high wind exposure) and late snow-melt samples (lee sites) will drive the differences in airborne bacterial composition.

H_3: Microclimatic conditions specific to each mountain will have a will be associated with the composition of atmospheric bacteria throughout the growing season. In particular, I expect that bacterial composition of sparsely vegetated peaks will be distinct from peaks with relatively high levels of vegetation biomass.

2. Methods

2a. Study Area

Encompassing 89,030 km^2, the Greater Yellowstone Ecosystem (GYE) encompasses one of the largest relatively intact temperate-zone ecosystems on Earth. The Absaroka Mountain Range is a sub-range on the eastern side of the Northern Rocky Mountains that stretches across Montana and Wyoming for approximately 241 km. The three mountains considered in this study (Wolverine Peak, Mineral Peak, and Republic Peak; >3000 m) are located in the GYE, within or near the northeast boundary of Yellowstone National Park (YNP) and Cooke City—Silver Gate, MT (45.0160° N, 109.9166° W; elevation 2,319 m; Figure 1). The annual average maximum and minimum temperatures are 9.1°C and -5.9°C, respectively. Average yearly precipitation is 628 mm, and average annual snowfall is 513 cm (NOAA, 2017). The alpine regions here experience a shorter growing season (~ 3 month frost-free) than similar ecosystems in Colorado (Billings, 2000; Zorio *et al.*, 2016). This area has been largely spared from anthropogenic impacts, such as land development and road-building that can lead to habitat fragmentation and the introduction of invasive species.

2b. Field Methods

I visited 12 permanent vegetation transects on three mountains (Republic Peak, Wolverine Peak, and Mineral Peak) near the northeastern border of Yellowstone National Park during the summer of 2017 (Figure 1). Transect start and end points were located with Trimble JunoTM and Garmin eTrex® GPS units. The start point of a transect determined placement of the air sampler. To quantify seasonal effects on microbial communities three sampling periods occurred: 1) pre-growing season, *i.e.*, early June, 2) early growing season, *i.e.*, mid-July, and 3) senescence/late growing season, mid-late August. To deduce effects of local environmental conditions sites in four habitat types were sampled each month on each mountain. These habitats are ridge-top, late snow-melt, south-facing, and north-facing sites.

Methods used for atmospheric sampling largely follow Weber and Werth (2015). Air samples were collected at each habitat type on each mountain during June, July, and August with a SAS SUPER 180 (Bioscience International, Rockville, MD). The SAS SUPER 180 impacted air samples (180 L min-1) onto 100 mm agar plates overlaid with autoclaved 0.2 µm polycarbonate membranes (EMD Millipore, Darmstadt, Germany). A replicate plate was collected at each site. The membranes were removed from plates immediately after sampling (1,500 L per plate) and stored at -20 °C until DNA extraction was performed for bacterial 16S rRNA gene amplification and sequencing.

2c. Laboratory Methods

2c.1. DNA extractions from air samples

DNA was extracted from polycarbonate membranes by aseptically dividing each membrane into two pieces and placing each half one into a separate bead-beating tube from the ZR Fungal/Bacterial DNA MiniPrep extraction kit (Zymo Research, Irvine, CA). Eluted DNA (~20 µL) from duplicate extractions were combined and purified using steps 14 to 21 of the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). Included in the extractions were control groups consisting of an unused autoclaved filter and a blank (no DNA or filter) to detect sources of contamination, if any. Purified extracts were stored at -20°C until pyrosequencing.

2c.2. 16S SSU rRNA gene amplification, Illumina Mi-seq sequencing, and analysis

Bacterial 16S SSU rRNA gene fragments from air (36) samples were amplified in triplicate via polymerase chain reaction (PCR) with 515F and 806R barcoded primers (Caporaso *et al.*, 2011). Each 25 µL PCR sample included 3.0 µL of template DNA, 0.8 µL of uniquely barcoded reverse primer and 21.4 µL of master mix that contained 10X HotMaster Buffer and HotMaster TAQ 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 ISU Molecular Research Core Facility

(MRCF). The barcoded sequence libraries were trimmed, examined for quality, and analyzed with the software package "MOTHUR" version 1.39.5 (Schloss *et al.*, 2009). 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 (Huse *et al.*, 2010). Sequences were aligned against the SILVA (Pruesse *et al.*, 2007) bacterial 16S rRNA gene reference alignment. Operational taxonomic units (OTU's) were clustered at ≥ 97% similarity, using the average neighbor algorithm (Drechsel *et al.*, 1995). The resultant 24-libraries were normalized to contain the same number of sequences (n = 16,000) prior to all comparative analyses. OTU-based rarefaction curves for each sample were calculated using 1,000 sampling iterations in the MOTHUR software package. All sequences were taxonomically classified using the Ribosomal Database Project (RDP) Classifier (Wang *et al.*, 2007) within MOTHUR with the confidence thresholds set at ≥ 80%.

2d. Data Preparation and Statistical Methods

2.d.1. Data preparation: Atmospheric Bacteria Survey Data

The taxonomic classification output from sequencing was organized into two matrices for analysis. A matrix was created for all OTUs for each site. Another contained family-level OTUs comprising ≥ 1% of total abundance (all sites) for each site. Due to abundance, unclassified bacteria were included in the phyla and family barplots. Unnamed families with low abundance were removed. Metagenome data are currently being processed in the MG-RAST database (https://www.mg-rast.org/).

2.d.2. Tests of community diversity and species distributions

The Shannon-Weiner index was used to measure α-diversity within bacteria communities (Magurran, 2013). The Shannon-Weiner index assumes all species are represented and is relatively sensitive to the occurrence of rare species (Magurran, 2013). Beta diversity was measured using average Bray-Curtis dissimilarity (Bray & Curtis, 1957). Univariate ANOVA was applied to all diversity metrics.

Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) was used to test null hypotheses of identical composition of airborne bacteria with respect to month, habitat, and mountain. Bray-Curtis dissimilarity was used as the underlying PERMANOVA resemblance metric. Indicator Species Analysis (Dufrêne & Legendre, 1997) provided a permutational approach to test for the statistical significance of specific taxa associated with habitat, month, and mountain. The exception were ISA tests. In this case, I report both raw test results and results controlling for false discovery rate (see Benjamini and Hochburg 1995) across taxonomic families under consideration for an associated factor level. The value $\alpha = 0.05$ was used as the significance level for all significance tests.

2.d.3. Ordination

Non-Metric Multidimensional Scaling (NMDS) ordination was used for indirect gradient analysis (Jongman *et al.,* 1995; Legendre and Legendre, 1998). Bray-Curtis dissimilarity (Bray & Curtis, 1957) was used as the underlying resemblance metric in NMDS. Environmental data, including physical site characteristics and soil chemistry, were taken from raw data collected by Aho (2008, 2012). The correlation of each environmental variable to the orientation of communities within ordinations was tested with vector-fitting (*i.e.*, individual multiple regressions for each environmental variable with ordination dimensions as predictors; Oksanen *et al.*, 2013).

2e. Software

The statistical software package R was used for all analyses (R Core Team, 2015). I relied on multiple packages, especially vegan (Oksanen *et al.*, 2013) for ordination, PERMANOVA, and other community level analyses. I used the package plottrix (Lemon, 2006) for graphical applications and labdsv (Roberts, 2016) for indicator species analysis and bar plots.

3. Results

A total of 41,449 operational taxonomic units (OTUs), defined at the 97% nucleotide-sequence identity level, were identified among all sequences. DNA extraction concentrations were insufficient for sequencing 12 out of a total of 36 samples for sites from June-August. Mineral Peak, and more generally, north-facing sites comprised most of the unsuccessfully or poorly sequenced samples. Rarefaction analysis revealed that the number of unique reads in most of the samples was close to or reached saturation by sequencing (Figure 2). A reduced dataset containing taxa appearing at ≥ 1% relative abundance was used for ordinations and diversity metrics.

One-way ANOVA showed that α-diversity varied significantly ($F_{2,21}$ = 19.02, P = <0.001) across mountains, where Mineral Peak ranked lowest and Wolverine highest (Table 1). Neither habitat or month were appreciably different for α- or β-diversity. Republic Mountain had the highest β-diversity, although mountain was weakly significant ($F_{2,21}$ = 4.61, P = 0.022; Table 1).

NMDS ordinations ($k = 2$, stress = 0.1) of the relationship of sites to bacterial taxa formed two main clusters driven largely by Wolverine Peak's association with unique taxa (Figure 3). Additionally, sites in June and August are diffusely spread along the second and first dimensions, respectively (Figure 3). Clustering in the third dimension was not as well-described with dominant taxa. PERMANOVAs detected airborne bacterial OTU compositional differences with mountain ($F_{2,23} = 6.89$, $P = 0.001$) and month ($F_{2,23} = 3.37$, $P = 0.009$), but not habitat ($F_{2,23} = 1.57$, $P = 0.128$; Table 1). The correlation of each environmental variable to the spread of communities within ordinations was analyzed using vector fitting (Figure 3). The strength of the correlation for vectors is graphically represented by the length of the arrows in the ordination diagrams, whereas the direction of arrows indicates the direction of most rapid increase for a variable among samples (Figure 3). Slope ($R^2 = 0.32$, $P = 0.02$) and aspect ($R^2 = 0.28$, $P = 0.03$) were correlated with the scatter of microbial communities represented by points in the ordination (Figure 3).

Indicator species analysis (Dufrêne & Legendre, 1997) found a number of families highly associated with specific mountains, less so with month, and none with habitat (Figure 4-6). I report only family-level taxa here because of decreased resolution of some taxa at the genus level and the relatively low concentration of bacterial DNA extracted from the polycarbonate filters. Enterobacteriaceae was the sole significant indicator for Mineral Peak. Republic Peak was distinguished by Alteromonadaceae, Burkholderiaceae, Micrococcaceae, Bacillaceae, and Rhizobiaceae. Wolverine peak had the highest number of indicator taxa, including: Sphingomonadaceae, Chitinophagaceae, Solirubrobacteraceae, Chthoniobacteraceae, Pyrinomonadaceae, Xanthobacteraceae, and Caulobacteraceae (Figure 6). June was associated with families Moraxellaceae and Psuedomonadaceae. July had no indicators taxa and August was associated with Enterobacteriaceae (Figure 4).

4. Discussion

We found reasonable evidence to support two of three hypotheses from a unique dataset of atmospheric bacteria collected from three alpine mountains bordering YNP. Specifically, we detected temporal and spatial shifts in composition. No changes were detected, however, at the habitat scale (Figure 4-7). Ostensibly, habitats seemed to be more distinguished by a dearth of north-facing sites available for analysis. Additionally, all samples were dominated by taxa associated with soil. Our findings are similar to other studies in the western U.S. that report seasonal shifts in bacterial composition and high incidence of soil bacteria in the near-ground atmosphere of subalpine and alpine environments (Bowers *et al.*, 2010, 2012; Weber & Werth, 2015). I note that my results are confounded by artifacts that largely exist due to low concentrations of atmospheric bacteria collected here, a twin challenge for studies in the high-atmosphere (Burrows *et al.*, 2009; DeLeon-Rodriguez *et al.*, 2013). The near-terrestrial environment is a continual flux of bacteria upwelling from the surface and settling from the atmosphere. The study of it provides important insight into the movement of bacteria from alpine soil, and more generally, how communities of bacteria vary from the surface to the troposphere (DeLeon-Rodriguez *et al.*, 2013; Ciccazzo *et al.*, 2015). The topography of mountains provides unique routes for wind dispersal of bacteria. Part of long-term management of federal lands includes incorporating knowledge about human-driven inputs that defy borders, such as eroded topsoil or airborne plant and animal pathogens (Després *et al.*, 2007 Morris *et al.*, 2011).

Our first hypothesis that a detectable shift in the composition of atmospheric bacteria would occur over the growing season is supported by PERMANOVA and clustering within ordinations (Table 1, Figure 3). In indicator species analyses, differences seem primarily determined by relative abundance of the most common taxa (Figure 5). Similar studies of atmospheric bacteria in alpine environments have shown seasonal shifts across a year (Christner *et al.*, 2008; Bowers *et al.*, 2012). To our knowledge, our study is one of the first to consider atmospheric bacteria in alpine air specifically over the summer, which is a period of peak productivity for many organisms in alpine ecosystems (Pauli & Grabherr, 1996, Lipson *et al.*, 2002). July did appear to have the highest total abundance of bacteria, however its overrepresentation in analysis (n = 10) compared to June and August (both n = 7) underscores the importance of repeat sampling for firmer estimation. June and August harbored comparatively low levels of α- and β-diversity. Conversely, communities of atmospheric bacteria were most diverse during peak growing season (July), which was somewhat unexpected since active vegetation and warmer temperatures create conditions favorable to only mesophilic taxa that thrive on labile carbon sources (Tecon & Or, 2017). Alternatively, niche space created by increased resource availability could promote diversity during peak growing season. While latitude has not been found to heavily effect bacterial communities, the combination of elevation and latitude in the study area create temperature variations that may not allow some taxa to be competitive (Tecon & Or, 2017). Thus, contributing to the lack of diversity seen in June and August when snow cover is assured for the former, and not unusual for the latter. Further, these transition periods between seasons may act as ecotones for the dispersal of bacteria.

Unlike month, habitat was not a reliable predictor for atmospheric bacteria over the summer for PERMANOVA. Likewise, ISA did not detect identifying families among habitats. This is likely due to the scouring effect of strong and constant wind, although it is unclear if this pattern remains year-round. The most prominent families were the Enterobacteriaceae, Moraxellaceae, and Burkholderiaceae. These families contain taxa that that exert beneficial effects on plant growth (as well as phyto- and animal pathogens) and can survive in high-UV and low temperature environments (Ewing, 1986; Coenye, 2014; Teixeira & Merquior, 2014). Pseudomonadaceae, which contains plant pathogens, and the highest number of ice-nucleating taxa made up approximately 5% of families at each habitat. Chitinophagaceae, a family associated with the breakdown of fungal mycelia (Lladó *et al*., 2017), comprised approximately 15% of total reads in each habitat (Figure 6). As in forest soils, soil material inputs from plants include roots and seeds in addition to leaves. All of which have been shown to be an important source of soil organic matter, particularly in poorly-developed soils (Lladó *et al.*, 2017). Culture-dependent techniques often skew towards gram-positive bacteria, whereas the dominant taxa identified here from culture-independent techniques are gram-negative (Fierer *et al.*, 2008).

Among habitats, late snowmelt sites had the highest β-diversity (rare taxa), while south-facing sites had the highest α-diversity (richness and evenness, Table 1). Late snowmelt sites at the study area often harbor densely growing and diverse vegetation and are more likely to include coniferous species such as the low-growing *Juniperus communis* and seedlings of *Pinus albicaulis* (Aho, 2007). South-facing sites face the prevailing wind, promoting potential capture of a wider variety of bacteria in higher abundance through increased impaction of bacteria onto filter plates. North-facing sites, however, still experience appreciable wind, thereby potentially mitigating the effect of prevailing airflow. Increased sampling in these areas, especially beyond the growing season may reveal unique characteristics across habitats that I was not able to detect here.

The three mountains are superficially similar with respect to their geography, parent material, and vegetation. However, airborne bacterial composition of the mountains were distinct (Table 1). If species composition was affected by distant weather systems in a strong and consistent way I could expect to see similarities across mountains, especially as the prevailing wind comes from the south-west for all three (Figure 7 and 8). The significant variation at the mountain level suggests that microclimatic factors could be more important than prevailing wind at the near-terrestrial scale. However, I stress that the incredible spatio-temporal variability of bacteria may mask the previously described trends and likely drive the contrasting findings of previous studies on communities of atmospheric bacteria (Fierer *et al.*, 2008; Lladó *et al.*, 2017). Another potential driver of differences between mountains is proximity to areas burned by wildfire. Wolverine Peak has been essentially free from wildfire while both Republic and Mineral Peaks share borders with extensively burned areas from the Yellowstone fires of 1988 (Romme *et al.*, 2016) Current data indicates that soil microbial communities tend to return to pre-burn composition within five years post-fire event, although seasonal shifts and differences across soil horizons have been observed (Bissett & Parkinson, 1980; Dooley & Treseder, 2012; Romme *et al.*, 2016). I am limited in speculating across the year as this study focused on the late spring and summer.

Each mountain was associated with different groups of bacteria primarily due to proportion rather than lack of presence. Wolverine Peak had the highest number of associated taxa (8) via ISA and unclassified bacteria, followed by Republic Peak (5), and Mineral Peak (1). Each mountain was highly associated with taxa commonly found in soil and aqueous environment, including beta-proteobacteria such as the Enterobacteriacae and Burkholderiaceae and Chthoniobacter from the phylum Verrumicrobia. The family Rhizobiaceae, important symbiotic N-fixers, were significantly associated with Republic Peak which is distinguished by its relatively gentle saddle-like slopes covered with vegetation. Chitinophagaceae, a family of in the phylum Bacteroidetes, was most abundant on Wolverine Peak. Previous studies have linked mycophagous bacteria with peak fungal biomass in fresh plant litter (Lladó *et al.*, 2017), yet it is unclear whether there are significant differences in the density of fungal mycelia between mountains.

A major trend in all samples was the high incidence of soil bacteria, which include all the previously identified taxa. These results suggest dispersal along elevation due to strong and steady wind and are consistent with studies that do not show weak altitudinal effects on the composition of atmospheric bacteria (Lazzaro *et al.*, 2015). The proportion and activity of soil bacteria is altered by species turnover in plant communities. The alteration and wind-assisted spread of soil bacteria could potentially prime high-elevations for colonization as range expansion increases with climate change. Ultimately, however, functional redundancy may preserve ecosystem function in the face of climate change as many bacterial taxa have a high degree of metabolic flexibility and tolerance to changing environmental conditions (Allison & Matriny, 2008; Teacon & Or, 2017).

There was a sizable difference in the pattern of wind flow for 2016 and 2017. In June 2016, a dry year, wind originated from the south, moving up from Nevada and southern California. In June 2017, a wet year, wind originated from the southwest, traversing across from Washington. At the regional scale, overall microbial community composition is delimited by general precipitation patterns in tandem with temperature, elevation, vegetation, and soil characteristics (Teacon and Or, 2016). Previous microbial studies on rain events and high-atmosphere environments indicate that origin of wind packet or storm event can have far-reaching influence on bacterial composition (Morris *et al.*, 2011; DeLeon-Rodriguez et al., 2013). The same could happen here, although our study may have been too close to the ground to catch the effects of varied wind masses. Bacteria on the windward side of the mountains, or more generally above-tree line, are likely to be more easily lofted into the air. Bacteria, dust, and other aerosol particles from afar could be incorporated into these near terrestrial feedback systems; thereby, forming a pathway to potential alterations in microbial function in the soil (Allison & Matiny, 2008; Fierer *et al.*, 2011; Lladó *et al*., 2017).

5. Conclusions

In this dissertation chapter, we describe our initial attempts to characterize atmospheric bacteria in alpine areas within the north-western USA through analysis of sequenced amplicons of the small subunit ribosomal RNA gene (SSU rRNA). Our results provide important insights into the species composition and abundance of bacterial communities of the lower atmosphere. This research is worthwhile because even fresh snow has been found to harbor distinct microbial assemblages from the atmosphere (Bowers *et al.*, 2009), therefore ground samples may not be a reliable measure or estimation of atmospheric bacteria (Fierer *et al.*, 2011). This research would complement studies on the soil microbiome and alpine plant ecology. Specifically, if bacteria from plant surfaces (Fierer *et al.*, 2011) were collected in tandem with soil and atmospheric sampling we may observe the connection and cycling that occurs between atmosphere, plant, and soil. In addition, this study highlights the advantages of using pre-established sites. Long-term plant sampling locations offer information on major species. Among many other possibilities, sampling plant surfaces and soil in these areas could reveal established patterns of movement and dispersal of bacteria. Barring overuse, additional projects at established sites are an efficient and low-impact way of performing research in protected spaces.

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7. Tables

Table 1. Summary statistics for bacteria composition of air samples including mean abundance, Shannon-Weiner diversity, and Bray-Curtis similarity.

Mountain	Abundance	Richness		Shannon	Bray-Curtis
MI	**2,613,264**	**425.8**	**(± 25.2)**	**2.8 (± 0.36)**	**0.51 (± 0.03)**
RP	**1,426,379**	**456.7**	**(± 8.92)**	**3.71 (± 0.21)**	**0.41 (± 0.02)**
WO	**1,307,540**	**484.6**	**(± 8.99)**	**5.14 (± 0.17)**	**0.49 (± 0.03)**
Site					
NF	646,107	472.5	(± 8.39)	4.31 (± 0.37)	0.43 (± 0.04)
RT	1,370,025	453.1	(± 22.2)	3.83 (± 0.46)	0.46 (± 0.03)
SF	1,310,376	449.5	(± 13.9)	4.37 (± 0.22)	0.42 (± 0.02)
SM	1,129,270	458.9	(± 19.4)	3.18 (± 0.59)	0.53 (± 0.04)
Month					
June	**1,333,895**	473.1	(± 14.0)	3.97 (± 0.39)	0.45 (± 0.028)
July	**1,877,302**	450.2	(± 17.9)	4.14 (± 0.43)	0.49 (± 0.03)
August	**1,430,452**	451.0	(± 8.17)	3.5 (± 0.32)	0.43 (± 0.031)

8. Figures

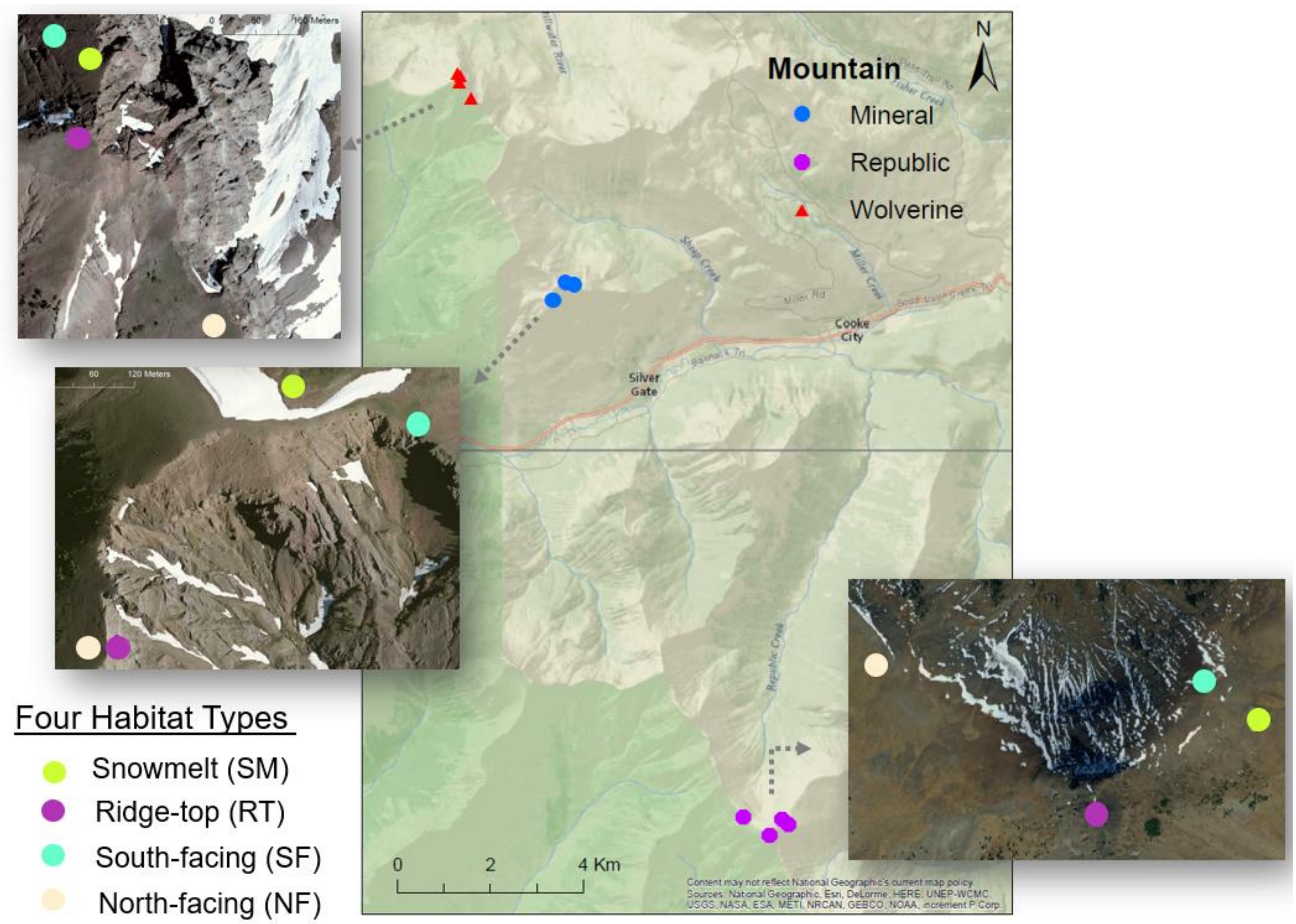


Figure 1. Map of study area near Cooke City, MT. Insets and bottom-left legend show habitat types.

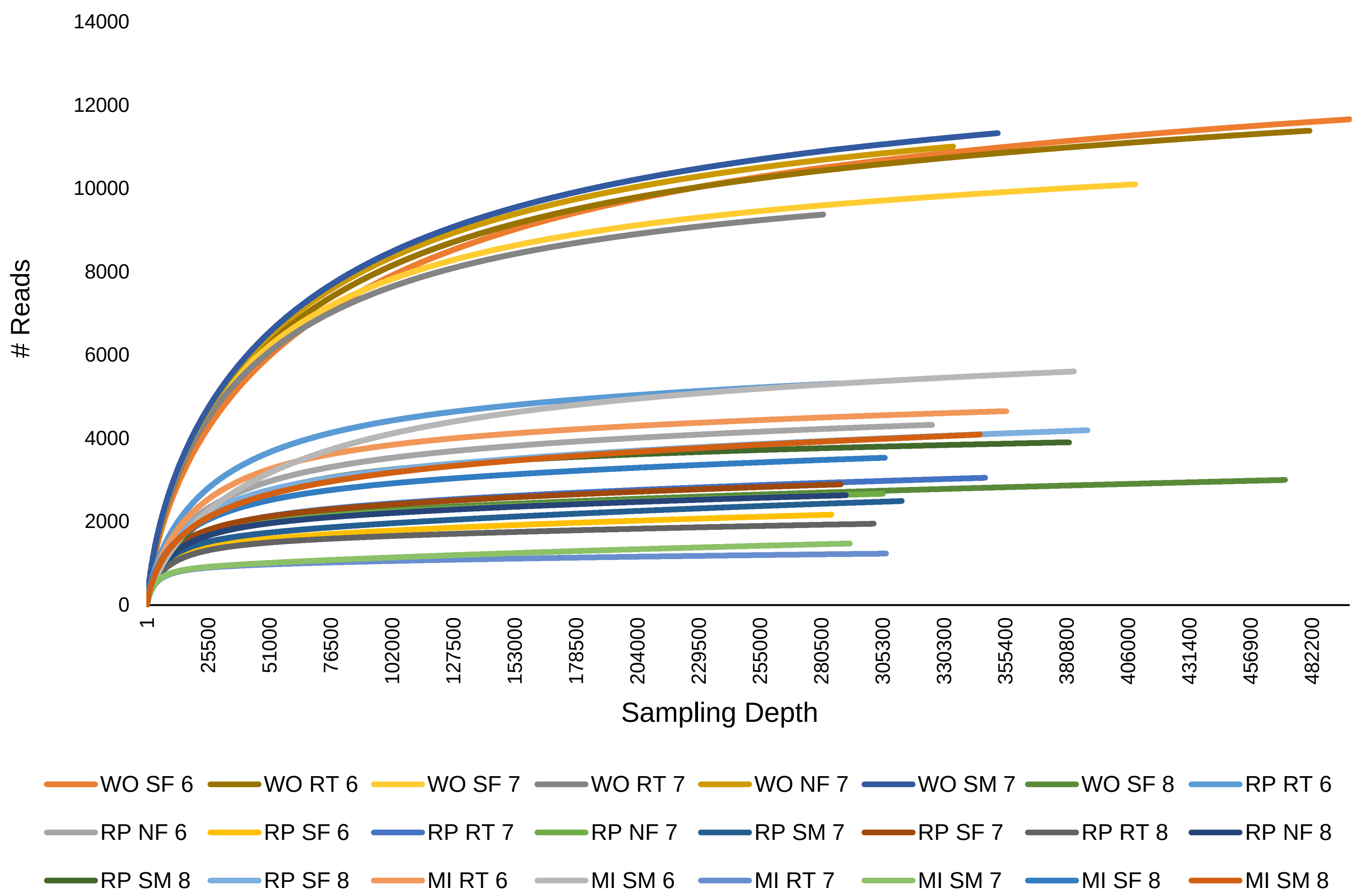


Figure 2. Rarefaction curve for sequenced samples of alpine atmospheric bacteria. Total sampling depth was ~127,500. All lines that are >6000 reads belong to sites on Wolverine Peak. MI, RP, and WO represent Mineral, Republic, and Wolverine Peak, respectively. Habitats are denoted by SM (snowmelt), NF (north-facing), SF (south-facing), and RT (ridgetop). Month is represented by: June (6), July (7), August (8).

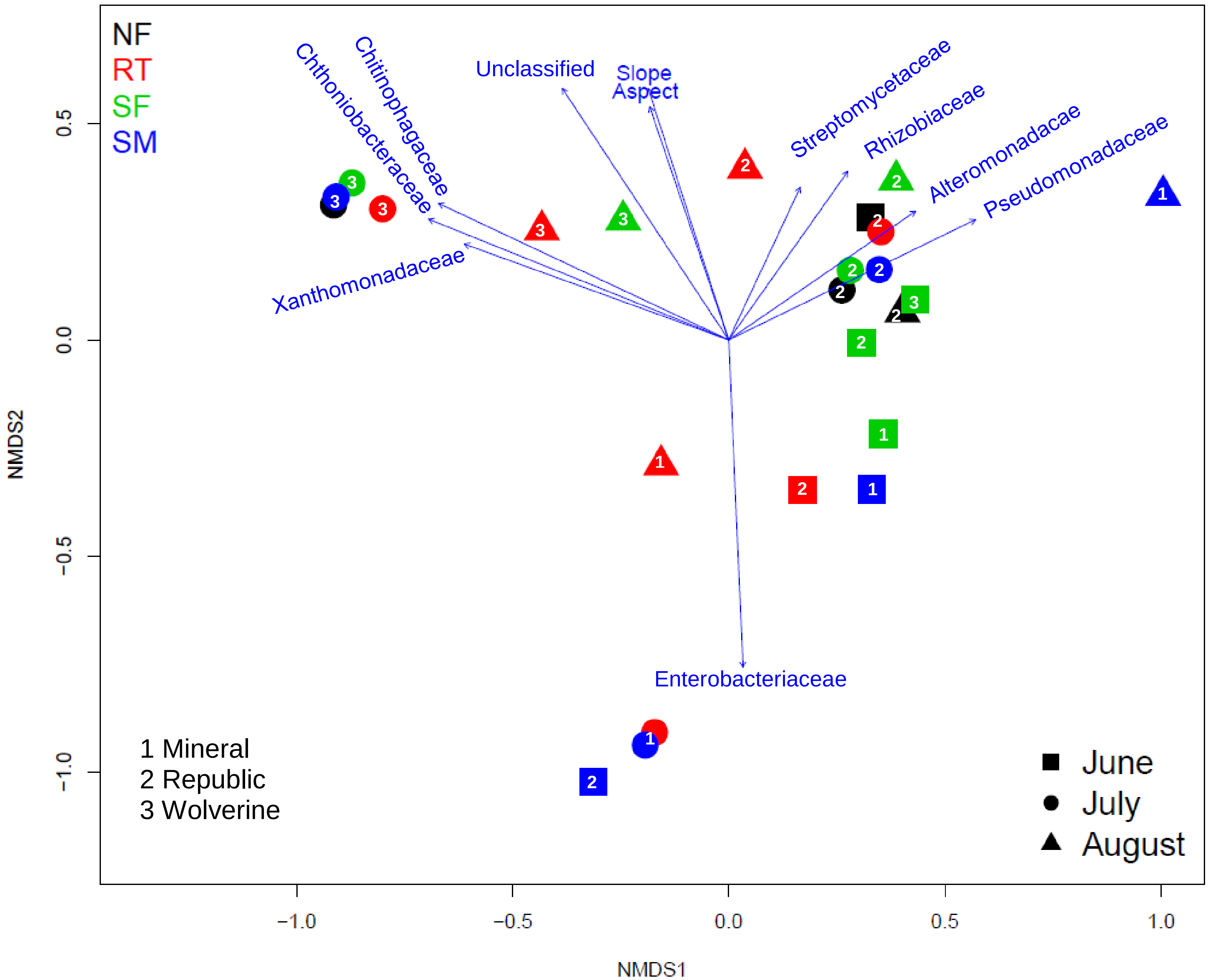


Figure 3. NMDS of sites in relation to dissimilarity values of atmospheric bacteria. Colors represent habitat types: north-facing (NF), ridgetop (RT), south-facing (SF), late snowmelt (SM). Numbers indicate mountain. Vectors show significant environmental variables and dominant families. Stress = 0.1, k = 2

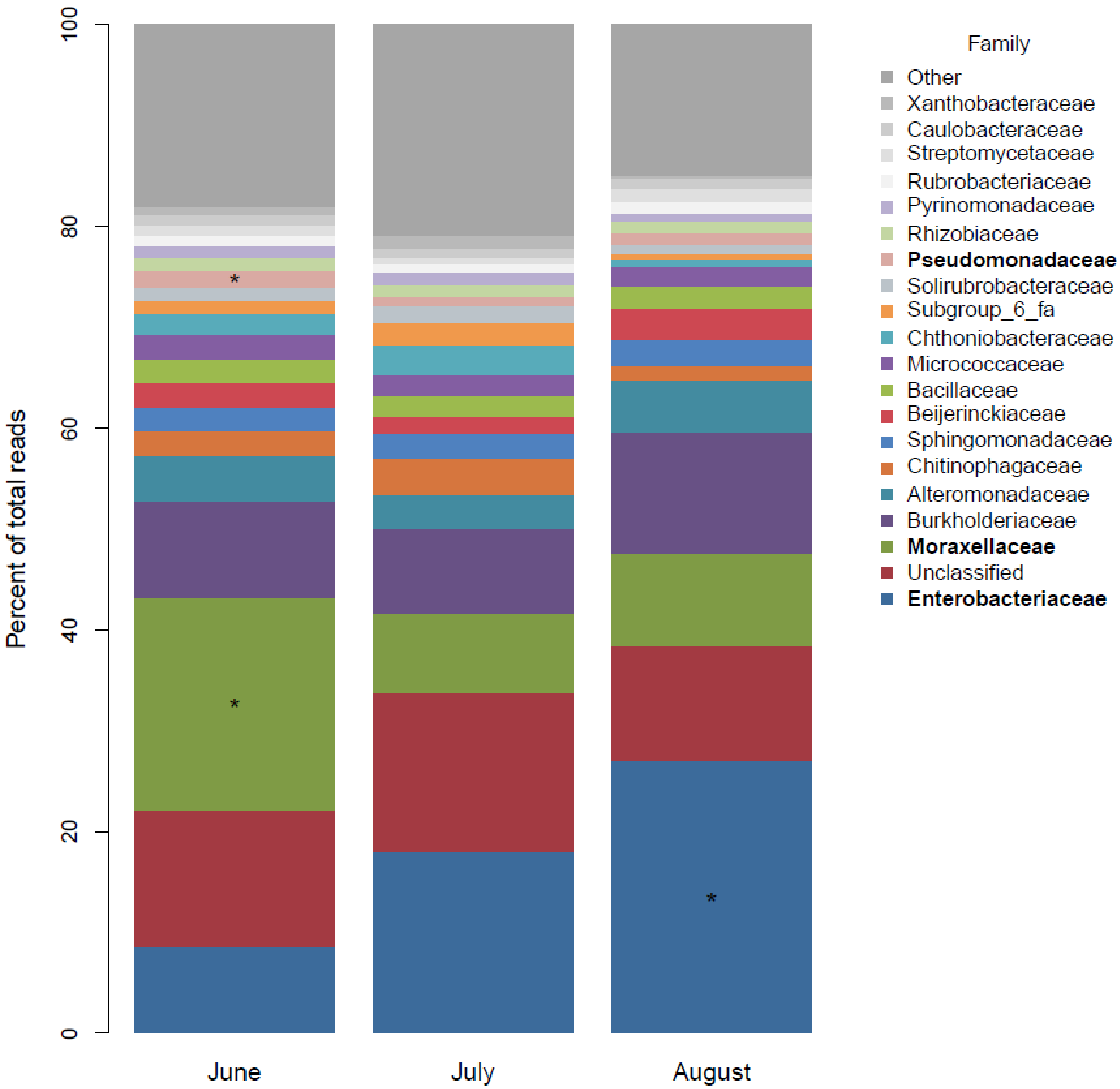


Figure 4. Stacked bar graph. Dominant bacterial families by month with significantly associated taxa via ISA. *: $P < 0.05$. ** indicates significant at alpha = 0.05 after controlling for FDR.

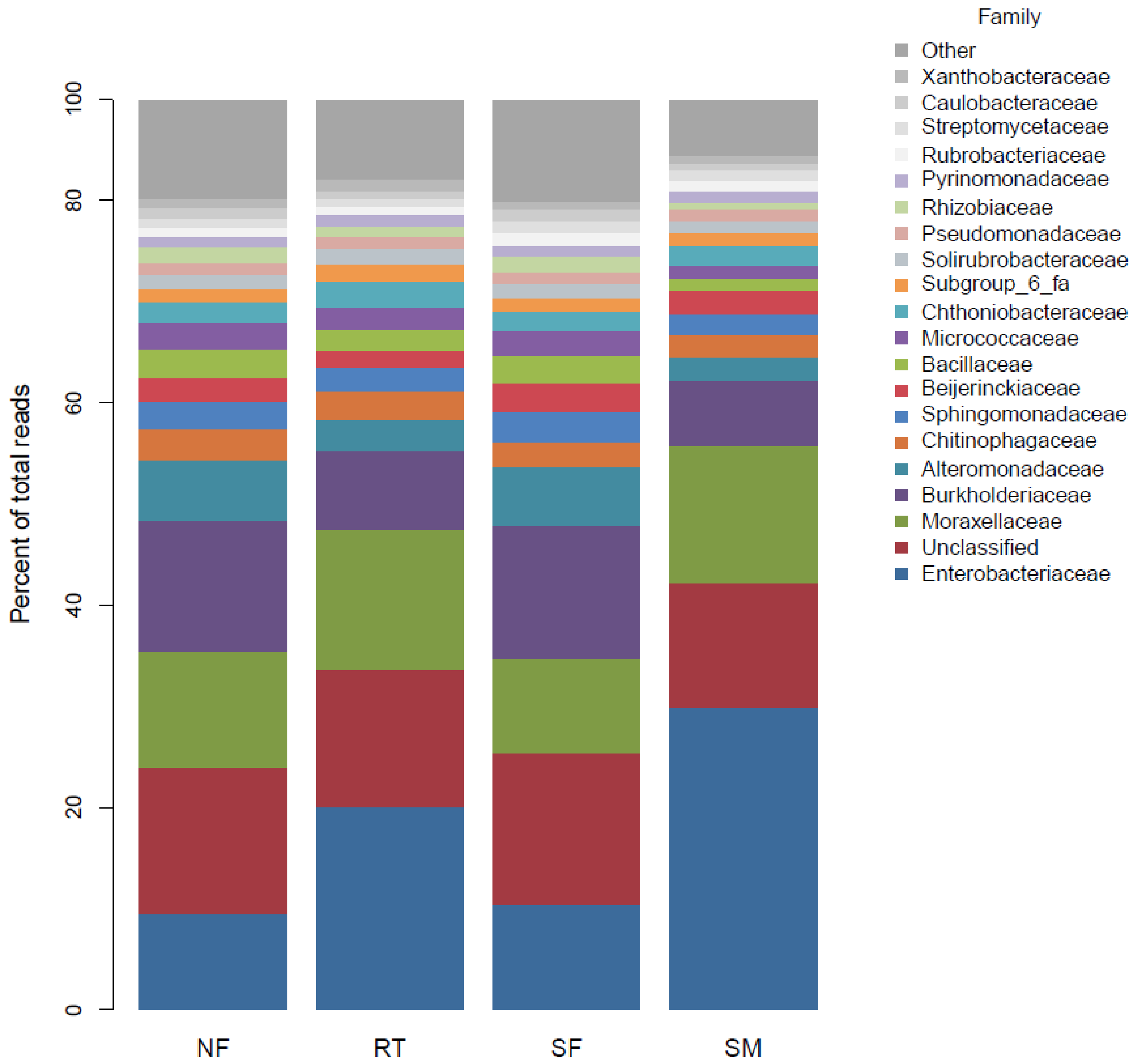


Figure 5. Stacked bar graph. Dominant bacterial families by habitat with significantly associated taxa via ISA. . *: $P < 0.05$. ** indicates significant at alpha = 0.05 after controlling for FDR.

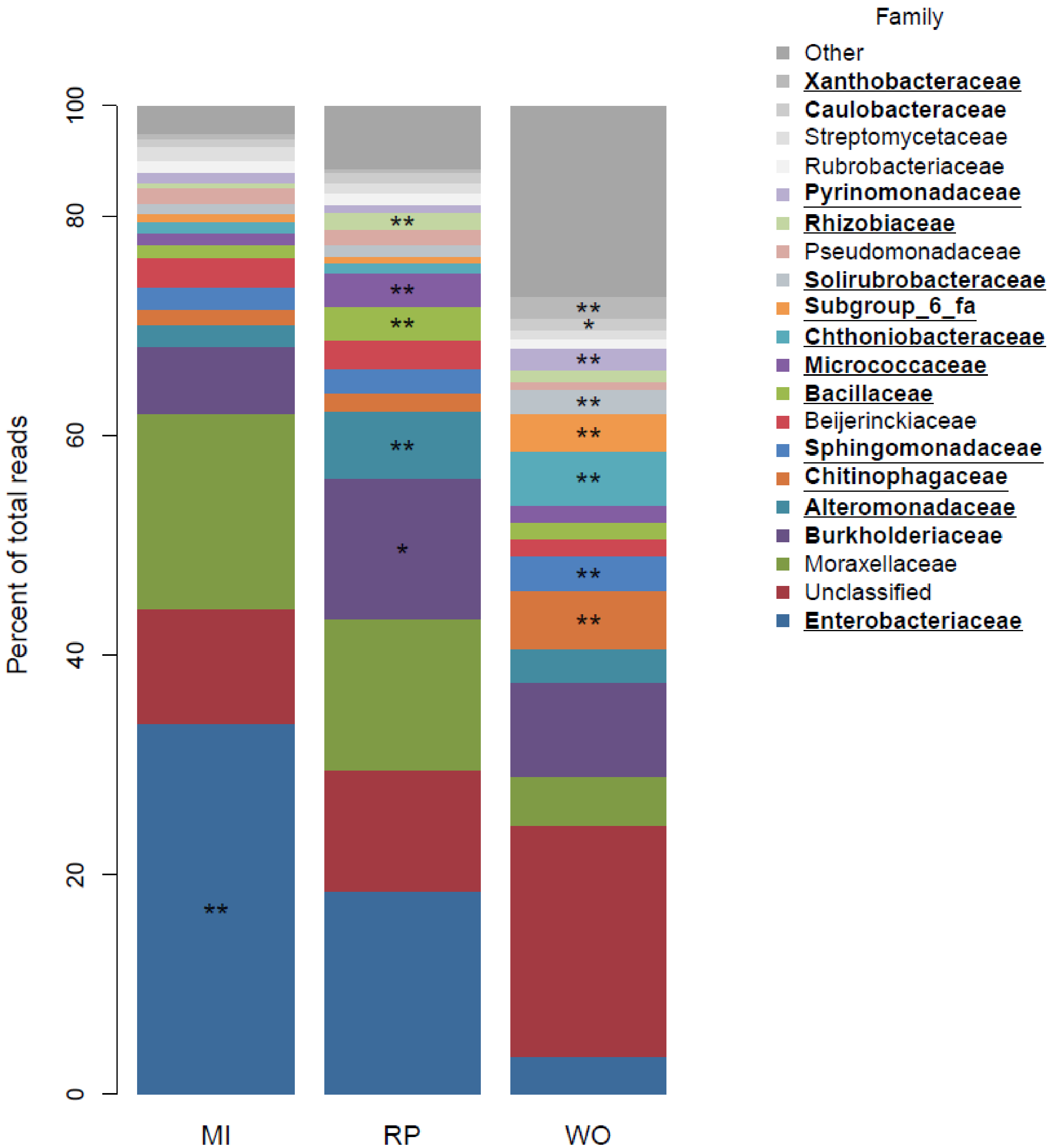


Figure 6. Stacked bar graph. Dominant bacterial families by mountain with significantly associated taxa via ISA. . *: $P < 0.05$. ** indicates significant at alpha = 0.05 after controlling for FDR.

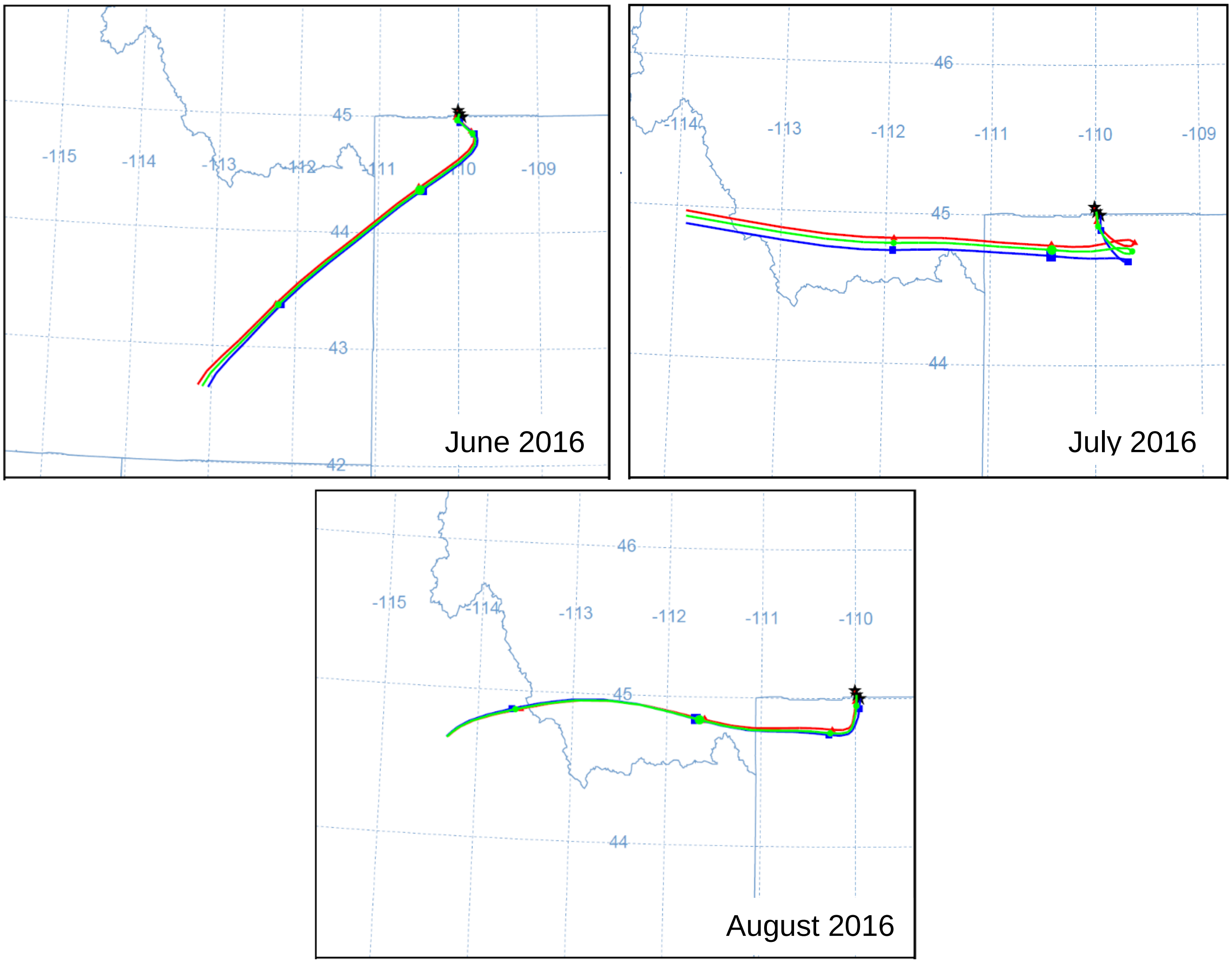


Figure 7. Backward wind trajectories (24 hrs.) for three mountains near Cooke City, MT calculated from meteorological data for 2016 accessed through the NOAA HYSPLIT modeling tool. Mountains are represented by stars. Red, green, and blue lines lead to, Wolverine, Mineral, and Republic Peaks, respectively. Air masses are measured at 500m AGL.

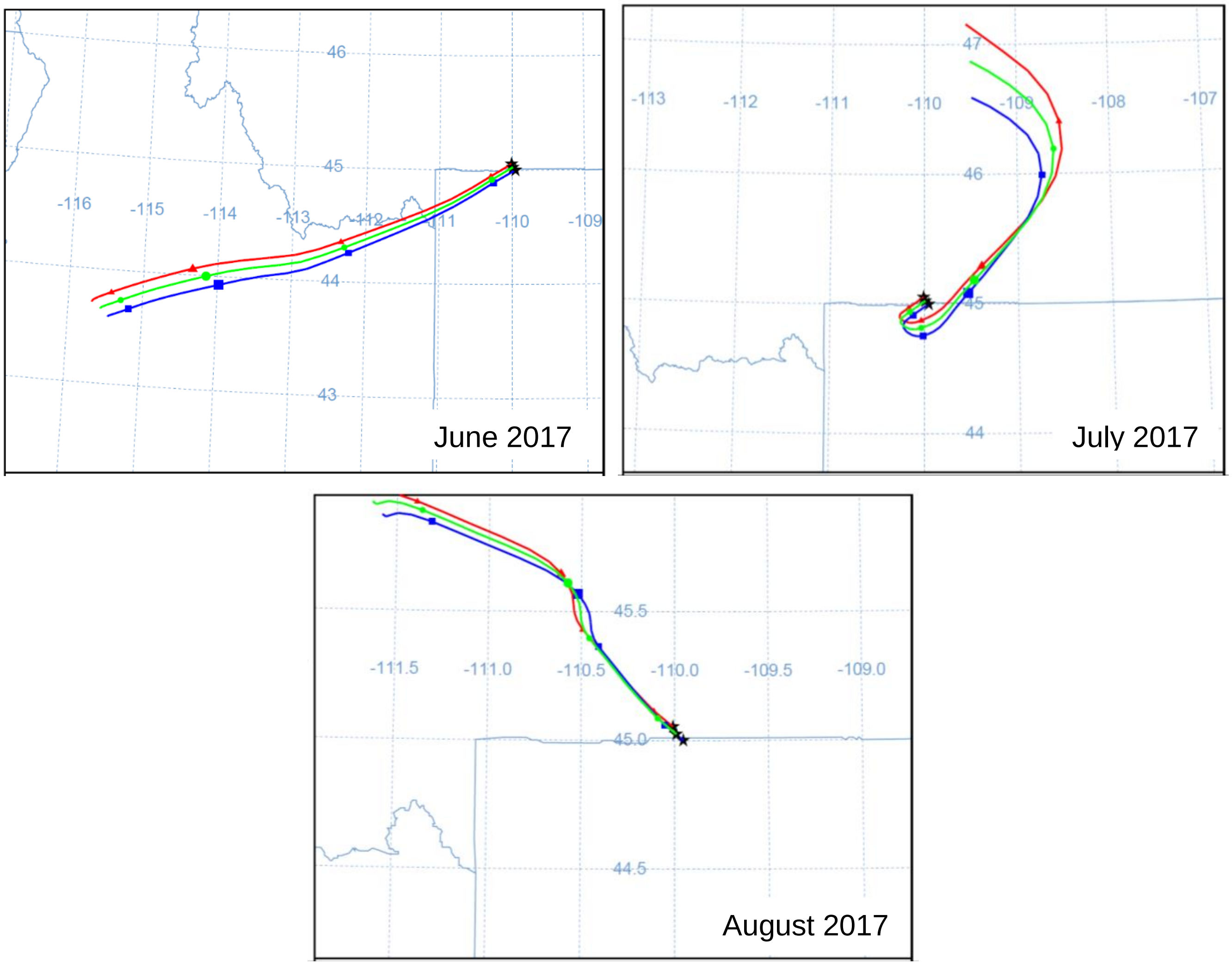


Figure 8. Backward wind trajectories (24 hrs.) for three mountains near Cooke City, MT calculated from meteorological data for 2017 accessed through the NOAA HYSPLIT modeling tool. Mountains are represented by stars. Red, green, and blue lines lead to, Wolverine, Mineral, and Republic Peaks, respectively. Air masses are measured at 500m AGL.

UNIT 2: PEDAGOGY

The primary goal of the Doctors of Arts Biological Sciences program is to prepare students for teaching at colleges and universities while honing their research abilities. Through seminars and Supervised Teaching Internships (SuTIs) I have gained content area expertise, theoretical background on learning, and curriculum design based on recommendations in the Vision and Change Report for undergraduate Biology education (AAAS, 2009). In this unit of my dissertation I will briefly describe my primary activities relating to biology education, followed by a final report from each of my three internships along with advisor and student reviews.

1. Teaching Philosophy

I aspire to be a well-rounded scientist whose skillset is applicable to a variety of careers in the public or private sector. One likely possibility is that I will pursue a faculty position with a focus on teaching and undergraduate research. Other job interests include science policy or working for an agency like the USGS or the National Forest Service as a research scientist. In all scenarios, I plan to emphasize effective communication, promote diversity in the STEM fields, and assist with citizen science. Writing a teaching philosophy requires deep self-reflection. The personal nature of a teaching philosophy presents a challenge to scientists accustomed to technical writing. Through this practice, I will identify a core set of values and a theoretical basis that form my motivations for teaching. A good teaching philosophy incorporates those values, general (or specific) pedological goals, teaching theory, and specific teaching techniques. Like the CV, a teaching philosophy can be viewed as a “living document” that changes, and frequently lengthens, over time as we gain perspective and experience.

For now, I've identified the major tenets of my teaching philosophy based on some phrases I find myself repeating to my students and dictate how I structure lessons: everything is connected, everything changes. These simple tenets are reflected in teaching goals outlined in McKeachie and Svinicki (2013). By encouraging students to develop their abilities and think critically I help students learn to think in depth about the subject matter by giving them practice in evaluating logic (McKeachie & Svinicki, 2013). For example, during my internship in Plant Ecology students had to identify and explain factors that could have skewed results of a global warming demonstration, then suggest and implement ways to improve the demonstration. During one field trip in my second internship, Fall Flora, I brought my students to my own backyard to demonstrate diversity and turnover in disturbed areas, the processes of natural selection and evolution, and the wild heritage of common agriculture species. In my last internship, a non-majors Biology course, we stepped away from reliance on textbooks and used case studies and current news to increase the depth and breadth of their scientific literacy. Activities like this help students articulate what they've learned in an inquiry-based environment with a high amount of social interaction (McKeachie & Svinicki, 2013; AAAS, 2015).

2. Internships and Projects

2a. Plant Ecology (BIOL 4408/5508), Spring 2017

I developed several components of a Plant Ecology course as a requirement of a teaching seminar in 2016. The research I did for that project combined my previous experience in teaching Ecology and Plant Ecology, assisted with the creation of a suite of inquiry-based laboratory activities (Appendix A). A major focus of mine is to connect applied research with the ecological concepts that students are introduced to in class (AAAS, 2015). These labs were designed to explore climate change while testing the validity of common methods of demonstrating related phenomena, such as global warming.

2b. Fall Flora (BIOL 2213), Fall 2017

This was my first experience having full course responsibilities. Although I do frequently review the material, my background in plant sciences allowed me more space to concentrate on my teaching style, learning aids, assessments, and time management. This internship aligned with my teaching goals in two major ways, 1) science-based courses that build practical knowledge are important for non-majors, and 2) skill in plant identification is crucial for conserving biodiversity. This class is historically notable for its broad appeal to undergraduates across majors. Therefore, this is an opportunity for sharing the process and utility of science to a wide audience.

2c. Senior Seminar Curriculum Survey

I adapted a curriculum assessment survey from the Vision & Change in Undergraduate Biology Education (AAAS, 2015) report for educators into a survey for graduating seniors. This is a relatively simple way to learn how and if our students are introduced to and utilize opportunities like working in a research laboratory, and more generally, perceive our efforts to align our curriculum to nationwide STEM education goals. The survey is one-page in length and consists of six questions.

During the Spring 2017 semester I distributed surveys to ~30 students in three Senior Seminar sections and received six completed surveys. In hindsight, I would have the students complete the surveys in class instead of setting a due date and collecting the surveys. I also found that, while not all faculty members agreed with the usefulness of surveys in general, they were supportive of the project. I plan to distribute the survey to at least one additional cohort of graduating seniors. Recently, the results from the pilot run of the survey were used by faculty members at a teaching conference.

2d. Biology for Non-majors (BIOL 1100), Fall 2018

As my final internship, I had the opportunity to not only test many of the teaching techniques that I had learned, but to design a course from the ground up. Further, with such a challenging time period to hold class (Tuesday evening, 7-10pm) traditional lecture methods seemed increasingly ineffective. Instead of requiring my students to purchase a textbook I designed the course around current events, student interests, book excerpts, and free online textbooks. This design meant that students were expected to participate in discussion and clearly explain their reasoning in the heavily short-answer question-based exams. One challenge was the large range of ability and interest within the cohort of students. However, by the end of the course students unanimously agreed that they not only have increased their scientific literacy, but also became excited about biology and science in general.

CHAPTER 1: Internship 1, Plant Ecology

I. General Information:

A. Student: Stephanie Zorio
B. Faculty supervisor: Dr. Ken Aho
C. Course description: BIOL 4408/5508 Plant Ecology, Spring 2017
BIOL 4408L/5508L Plant Ecology, Spring 2017
D. Extent of internship: One guest lecture, at least 1 co-generated multi-week laboratory exercise
E. # internship credits: 1 credit for proposal and final report, 1 additional credit for teaching activities

II. Rationale for teaching internship:

This internship provided a low-stakes introduction to SuTIs by concentrating on my research interests and strengths. A major focus of my teaching goals is to connect applied research with the ecological concepts that students are introduced to in class (AAAS, 2015). I accomplished this through the development and implementation of multiple connected laboratory activities. Dr. Aho and I usually decided on the specifics of each lab exercise a week to a few days before the lab section met. This was helpful in that it kept the assignments flexible. Each week we could customize the lab based on how the equipment and procedure worked and comprehension of concepts by the students. One disadvantage to this method is that sometimes we were scrambling to collect equipment and demo the lab ourselves before introducing it to the students. This was a small class with one lab section. In a larger class with multiple sections and TAs it would be more important to have clear goals and procedures lined out before each lab. For instance, the number of Plant Ecology students varies widely between semesters. In Spring 2017 class there were 9 registered students. In contrast, there were 25 students in the class when I took Plant Ecology during my Masters work at ISU. Overall, the labs that were developed could be easily scaled up or down to suit any number of students.

The goals for my first SuTI were to 1) develop a guest lecture to augment Dr. Aho's instruction during the community ecology portion of the Plant Ecology course, 2) to develop a rubric and grade weekly "Habitat of the Week" presentations, 3) help modify and develop new Plant Ecology labs that are more inquiry-based than demonstration-based (Nilson, 2012; Shields, 2006). The course syllabus had to be modified. As a result, I was not able to do a full guest lecture. I did, however, lead a discussion about using observer reports and satellite technology to measure spring-time phenology of plants using free information and visuals from the USA National Phenology Network (USA-NPN; https://www.usanpn.org/data/spring). The "Habitat of the Week" assignment was modified. I did develop a rubric, but it was not needed. Assisting with lab development was originally a minor goal, but I found that it became my focus.

III. Teaching strategies

Students used major concepts and principles from plant ecology to describe and explain plant-related phenomena. In-class discussions about phenology and the different ways we can gather and use ecological information about plants to determine trends over time was one teaching strategy I employed. The other major strategy was through the development and presentation of three inquiry-based lab activities. After every lab exercise students had to complete a reflection assignment that asked them to synthesize information, think critically about their results, and propose additional experiments or ways to improve the current experiment. For example, with the greenhouse effect lab, the students were able to test their improved experimental design the week after the initial lab. We discussed how greenhouse effect demonstrations can be problematic. Oftentimes, the students are not truly observing the greenhouse effect from increasing amounts of

CO_2, but rather ambient heat from a light source, or increasing heat caused by increasing pressure within a small container. I think these challenges were a great way to discuss the utility and caveats of using model systems.

IV. Assessment Strategies

Dr. Aho and I met weekly. The students filled out a short assessment of the lab activities as a summative assessment via Google Survey. Most students felt that the labs effectively meshed concepts and real-world situations while drawing upon skills they have developed in other courses. They were, however, a bit more ambivalent about open-ended labs than I expected and would have liked more detailed procedure and background information. If I were to teach this again I would spend more time on explaining isotope analysis since, understandably, that was where most students demonstrated confusion. The reflection exercises after every lab were useful formative assessments because I could immediately modify my instruction based off their misconceptions and interesting ideas for experiments. An example of a completed reflection assignment is attached. Dr. Aho provided summative feedback after the internship. Student and advisor assessments are attached.

Supervisor Evaluation: Plant Ecology Spring 2016

SuTI Plant Ecology, Spring 2017

Supervisor Survey for Lab Packet

1. *Success with connecting scientific concepts and skills with current events*
a. little to marginal success
(b.) somewhat to mostly successful, more difficult topics were not as clearly connected
c. very successful

2. *Adaptability of labs to teaching goals, time, and materials*
(a.) labs could be taught individually, more background may be useful
b. labs could be taught individually, but more concept review and background would be necessary
c. these labs are not appropriate for piece-meal use

3. *Lab activities and assignments had an appropriate amount of work required for the course level and number of lab credits (or no-credit lab).*
a. Strongly Disagree
b. Disagree
c. Neutral
(d.) Agree
e. Strongly Agree

5. *Lab organization allowed all students to fully participate*
a. Strongly Disagree
b. Disagree
c. Neutral
(d.) Agree
e. Strongly Agree

4. *Post-lab exercises consistently engaged students in higher-order thinking, questions were often used as a segue to the following week's lab activities.*
a. Strongly Disagree
b. Disagree
c. Neutral
d. Agree
(e.) Strongly Agree

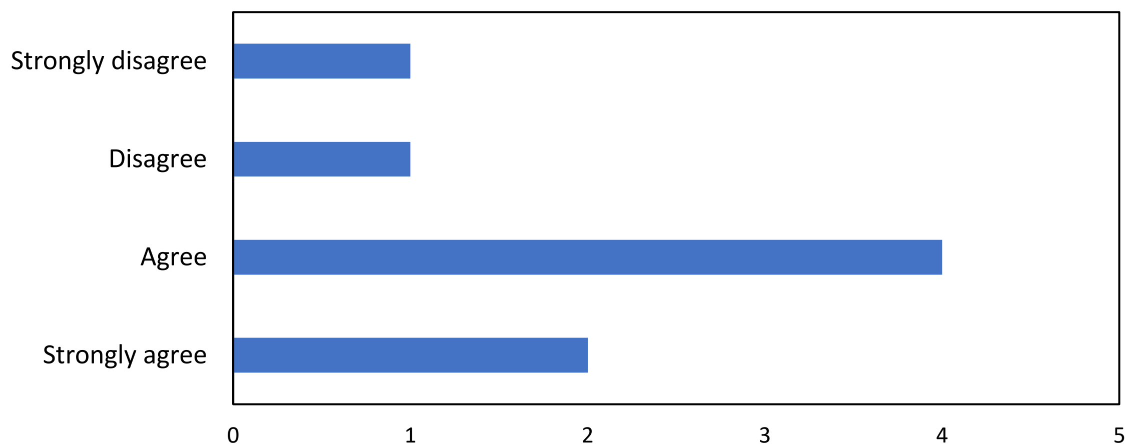
General comments and suggestions for improvement (more background information, figures/diagrams, etc.)

Imperfections of labs in 4408L/5508L ~~are~~ were due mainly to me (K. Aho). I personally found the labs to be exciting and interesting, although I sensed that some of the students wanted more of a rote exercise. Additionally, most of the undergraduate found the use of R to be daunting and/or frustrating.

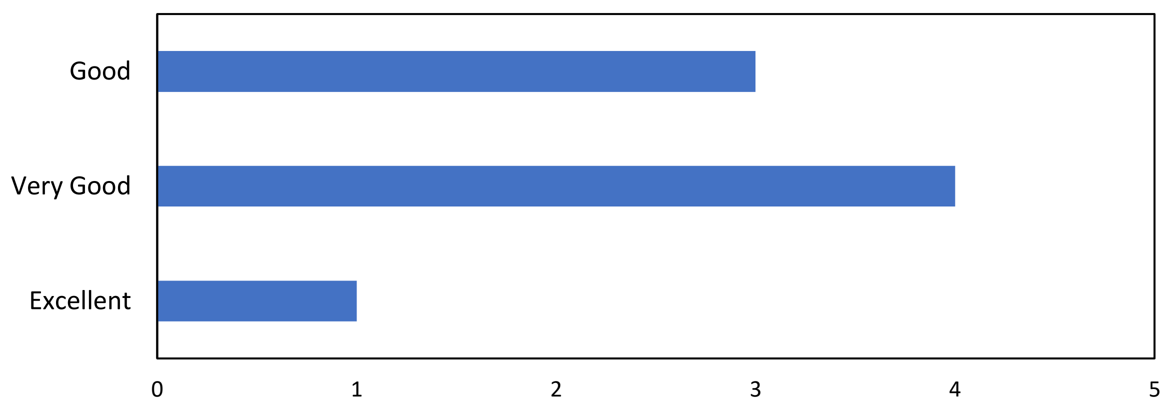
Student Lab Evaluations: Plant Ecology Spring 2016

*Choices with no responses are omitted from graphs.

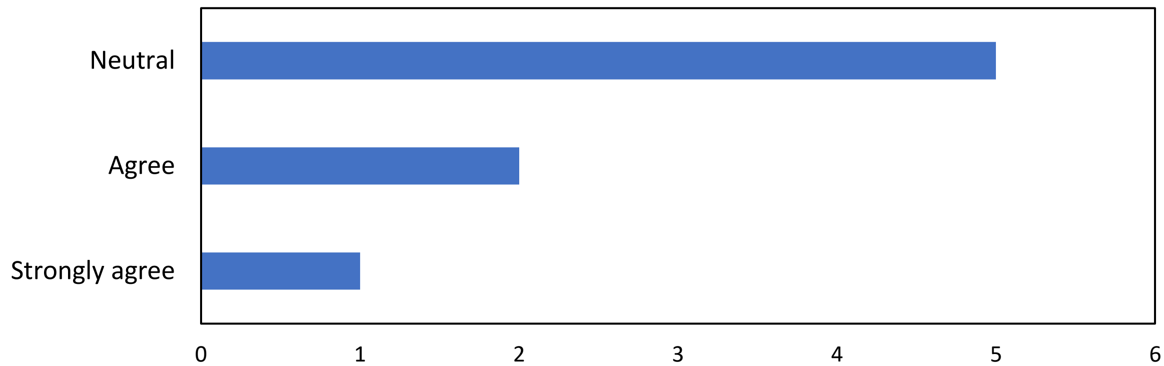
1. This suite of labs helped me connect scientific concepts with current events



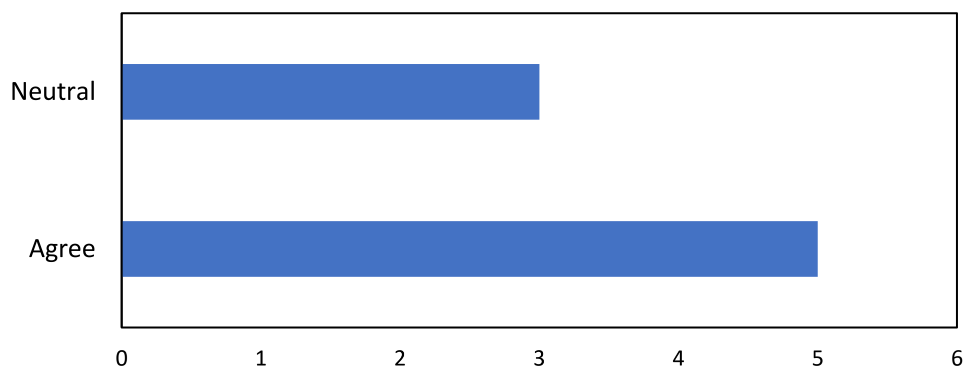
2. Opportunities to use concepts and skills previously learned (in other courses) were...



3. I enjoy open-ended labs.



4. I would have felt more comfortable with some written procedure and background about a lab before out weekly session.



5. One thing I would change about the greenhouse labs is...

Require reading more background info, and assign procedure ideas due ~1 week before the activity so that it could be more interactive

I felt that I wasn't really good at coming up with a experiment of my own.

6. One thing I enjoyed about the greenhouse labs was...

They build upon each other, and integrated multiple concepts in ecology

The hands on portions

The freedom to create our own scientific thought and procedure

7. One thing I would change about the isotope labs is...

Require reading more background info, and assign procedure ideas due ~1 week before the activity so that it could be more interactive

Isotopes are confusing, maybe some more time spent on them would be nice

Better explanation on how the isotope analysis works

8. One thing I enjoyed about the isotope labs was...
Really interesting data!
Going to the camis lab to see how it's all done.
Physically collecting samples

9. One thing I would change about the photosynthesis labs is...
Require reading more background info, and assign procedure ideas due ~1 week before the activity so that it could be more interactive

10. One thing I enjoyed about the photosynthesis labs was...
IRGA!
The introduction to new equipment

CHAPTER 2: Internship 2, Fall Flora

I. General Information:

A. Student: Stephanie Zorio
B. Faculty supervisor: Dr. Charles Williams
C. Course description: BIOL 2213 Fall Flora, Fall 2017

D. Extent of internship: Full course responsibilities: includes preparation for lectures, classroom activities, and logistics for field trips
E. # internship credits: 1 Credit for proposal and final report, 2 additional credits for teaching activities

II. Rationale and goals for teaching internship:

This internship allowed me to gain experience in course instruction while incorporating teaching strategies and goals outlined in the Vision and Change report (AAAS, 2015). In addition to field trips, my time in the classroom was a blend of lecture and lab activities and allowed me to practice combining multiple types of instruction each week. In addition to teaching techniques, I also became more well-acquainted with the administrative and preparation side of course management. This included diligent grade-keeping and organization, and student safety-based actions like repeatedly making requests to Facilities and Public Safety that the outside lights to the Plant Sciences building be set to turn on after dusk. Class prep activities included visiting field trip locations to ensure that a reasonable number and variety of species could be found, organizing the laminated plant sets so that students could easily follow along as I lectured, and collecting loose specimen examples that students could manipulate. These actions seem mundane and can take up a surprising amount of time, but are vital to keeping a course running smoothly. Overall, this internship experience increased my confidence as an instructor and I look forward to offering to develop a course like this in a campus job interview.

My goals for this SuTI were to: 1) Develop a syllabus, 2) update materials used in lecture, 3) develop a visual training exercise, 4) incorporate the use of free or low-cost digital keys and observation apps (*i.e.* iNaturalist), and 5) casually incorporate concepts from ethnobotany, ecology, and human society as students increase their knowledge of local flora (Nilson, 2012; Shields, 2006). Some specific actions towards these goals included revising the lectures to be more easily readable and replacing photos with higher quality versions. I created 10 quizzes on Moodle about taxonomy, plant anatomy, and species from each plant list. Five decks of plant family identification cards were purchased from the author of *Botany in a Day*. Additionally, a portion of the last field trip was spent in my backyard garden where we discussed the origin and wild relatives of many common garden plants. The backyard visit culminated in a scavenger hunt for a variety of leaf and fruit types.

III. Assessment of goals

My progress toward the goals previously mentioned was assessed several times over the semester. One of the most useful sources was from my SuTI advisor, Dr. Williams. He observed my teaching continuously over the 16 week semester. We would hold casual debrief session after class that led to more detailed discussions during our weekly meetings. Some helpful criticisms were that I sometimes meander away from my main point during lecture, I could be more serious, and that those traits are related to a fear of silence, as if I'll lose the attention of the students. He reminded me that I am already able to establish good rapport with my students and that they can tell that I am excited about the topics. The inclusion of my strengths empowered me to work on my weaknesses. While I am still progressing, I can say that I improved my lecture delivery by becoming more measured in my speech, using notes as a guide instead of feeling like I was cheating, and allowing myself time to collect my thoughts when needed.

I adapted examples of mid-term and final class assessments developed by Dr. Williams and Dr. Rosemary Smith for my students in Fall Flora. Students often forget to do online assessments and their structure can result in vague or unconstructive feedback. The in-class assessments that the students were given were formatted to easily elicit helpful information. The only drawback to hand-written assessments during class was that not all students were present on the days they were given. In the future I could set aside some time over two weeks to increase the likelihood that that a greater proportion of students are represented in the assessments.

The mid-semester student assessments were shorter than the final assessments, but still produced a significant amount of useful information. Students found the quizzes and lectures the most helpful. Many mentioned enjoying field trips although they were not asked about them specifically. None of the students chose the most negative descriptor (*e.g.* Strongly Disagree, Very unhelpful) except when they were asked about the plant family card game. Gamification is an increasingly popular pedogeological tactic for interactive learning (Becker *et al.*, 2017; Zurek, 2017). Studies on gamification have reported increased student engagement and cognition, but individual students generally vary in their preferred methods of instruction. In my small sample, some students enjoyed playing with the cards and suggested adding more families to the base deck. Conversely, some students found the cards to be infantizing or likened them to busy work. I think it's most beneficial to use multiple forms of instruction throughout a course although this may generate more work for the instructor, especially for the first semester of incorporating new activities. Student responses support this idea as most reported that they enjoy learning through a variety of methods. As reflected later in the final assessments, incorporation of the cards may have been more effective and accepted if there were regularly scheduled times to play with them every week and if more families that were covered in the course were represented in the card decks.

Student assessments at the end of the course were strictly short answer. In summary, students appreciated the lack of a required book. Lectures were well-received, although some felt that the amount of information was overwhelming. Worksheets were reported as somewhat helpful as a study guide, but also felt like a way to get easy points and were not taken seriously. Students liked the Moodle quizzes because they could access them remotely and retake them for practice. Since teaching this course I have found a way to resize the specimen photos. I was worried that the loss of resolution would make identification difficult, but the students did not share my fears. In addition, I could include a link to the original high-definition photo, thus promoting use of herbarium databases. Apart from Spring Flora, previous coursework in biological sciences was not a strong predictor of success in Fall Flora.

Field trips were nearly universally praised, as was the iNaturalist project. Most students would have liked to have more field trips spread throughout the semester, but the timing of the class makes that difficult. Some adaptations to future courses like this would be to focus on woody species and those that are conspicuous even when senesced. If possible, the class could be modified to two meetings a week where one meeting would be shorter and lecture-based followed by a longer, field-based meeting later in the week and with a meeting time early enough to not be affected by short days. In that case, trips may have to be limited to areas very close to campus. Students suggested that more field trips would have been useful for learning skills for identifying specimens in iNaturalist.

Exams were widely reported as difficult. I was surprised at the amount of negative feedback about the exams, especially since there were so many opportunities to gain extra credit points by knowing scientific names, including family names which could often be used repeatedly throughout an exam. One suggestion I may incorporate in the future is only requiring students to know one common name for each species. It would be interesting to see if removing that requirement would increase the frequency of correct answers concerning family and scientific names. Identifying what family an unknown species belongs to expedites the identification process and increases the likelihood of a correct ID. This is something I tried to emphasize in the class but was not very successful at. Many students did not seem to rely on family characteristics at all during exams, although they did access that information and were attentive to those characteristics during field trips.

Exam grades were a better indicator of progress than quiz grades, lab worksheets, or the iNaturalist projects because as students were obliged to access their recall memory without aids. The class average improved for each exam. Some reasons for this are because students became more comfortable with the exam format, and in later exams most of the questions about fruit, flower, and stem structures were directly related to important identifying characteristics of species they were being tested on.

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Final Course Evaluation

Fall Flora, Advisor Feedback

a. Suggested field guides and lack of required textbook: The use of detailed lecture PowerPoints and lab worksheets that emphasize important information about plant characteristics and identification aids somewhat negates the need for a text. Some students like to have a book, and a field guide and other ancillary texts are suggested. Since exams don't draw from information derived from textbooks – these are just supplemental for those students who want more information beyond the course material. Stephanie made good use of, and useful additions to the available PowerPoints and other visual aids during her lectures, and became more comfortable and confident integrating these resources to demonstrate the points in her lectures.

b. Lectures: Stephanie inherited a mature course with abundant materials to help illustrate and guide lectures and demos. While using and modifying existing materials when first teaching a course is standard procedure for most new faculty, it should only be a starting point for developing a course with one's own style and emphasis. Stephanie modified and updated many of the PowerPoint lectures with better graphics and better clarity of text. She also introduced some new materials and activities into the classroom. Her lecture style, mostly her familiarity with the material and hence confidence in her presentation, steadily improved throughout the semester. Improving her lecture style was probably the most frequently discussed aspect of her teaching that I gave feedback on during our weekly meetings. Content knowledge is the main factor that underlies effective teaching, and Stephanie worked diligently to learn the material before lectures so that she could confidently and effectively communicate it to the students. I think this was the biggest improvement I observed during the semester was her ability to prepare for and deliver an accurate, well-paced and informative lecture. She has a good speaking style – but sometimes can get sidetracked. During the course of the semester, I think she was able to better focus on the material, while maintaining good rapport with the students and deliver good lectures. Were she to teach this or a similar class again, I think she now has a very good foundation to develop a good course of her own.

c. Worksheets: These are an integral part of the laboratory for the class and are supposed to help the students identify the key concepts and information from lecture and specimen study. Stephanie was good at emphasizing the importance of the worksheets to aid student learning, and for the most part the students completed these assignments each week with due diligence. The worksheets are an opportunity for the students to work in groups and help each other with the material, and I think encouraging this kind of group study, both in filling out worksheets and in studying specimens to learn to identify them, is extremely important for success in the class. There are always a few students that don't willingly or comfortably participate in group work, and Stephanie for the most part was able to encourage more active group participation in lab.

d. Exams: Stephanie's ability to write and administer clear, effective and well-designed exams improved over the semester. This is a skill that requires practice, and I think that through writing, grading and discussing exams with me Stephanie's exams became much better over the semester. Writing unambiguous questions can be difficult, but by the end of the semester the exams were much better structured, covering the material more evenly, and the questions more clearly stated using the appropriate material (specimens) for each question.

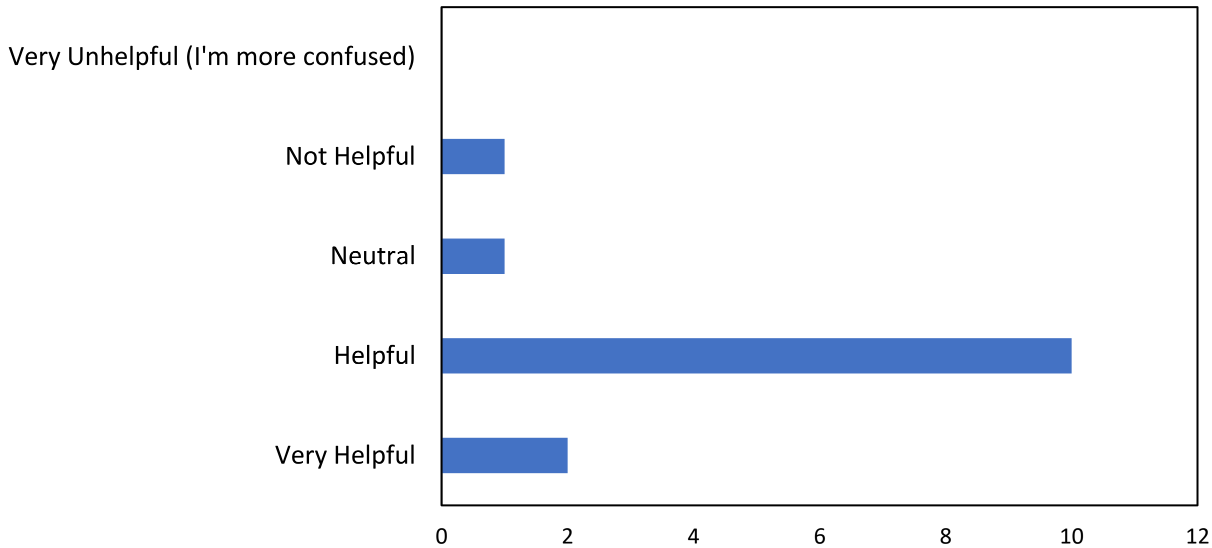
e. Moodle Quizzes: These were a good addition, and gave the student one additional mechanism to study for exams and learn plant IDs. There were some technical problems at first, but she was able to work these out and make the quizzes an important learning tool.

f. iNaturalist Project: Stephanie was very good a giving the students timely feedback on their plant observations for this project. iNaturalist can be most useful when there is an active dialog and identification hints from users and curators (Stephanie and I). I think the students appreciated the rapid feedback they got on their observations and it helped them correctly ID and learn their plants.

Overall, I thought that Stephanie did an excellent job of teaching the course and used our weekly meetings to get feedback and integrate that into her teaching techniques. This was probably the most successful DA internship I have mentored because of the frequent interaction and feedback, that was then incorporated into improvements in teaching. I think the experience also made it clear how much work goes into designing and delivering an effective course and Stephanie stepped up to the task and put in the work to make the course effective and rewarding for the students.

Mid-Term Evaluation
Fall Flora 2017

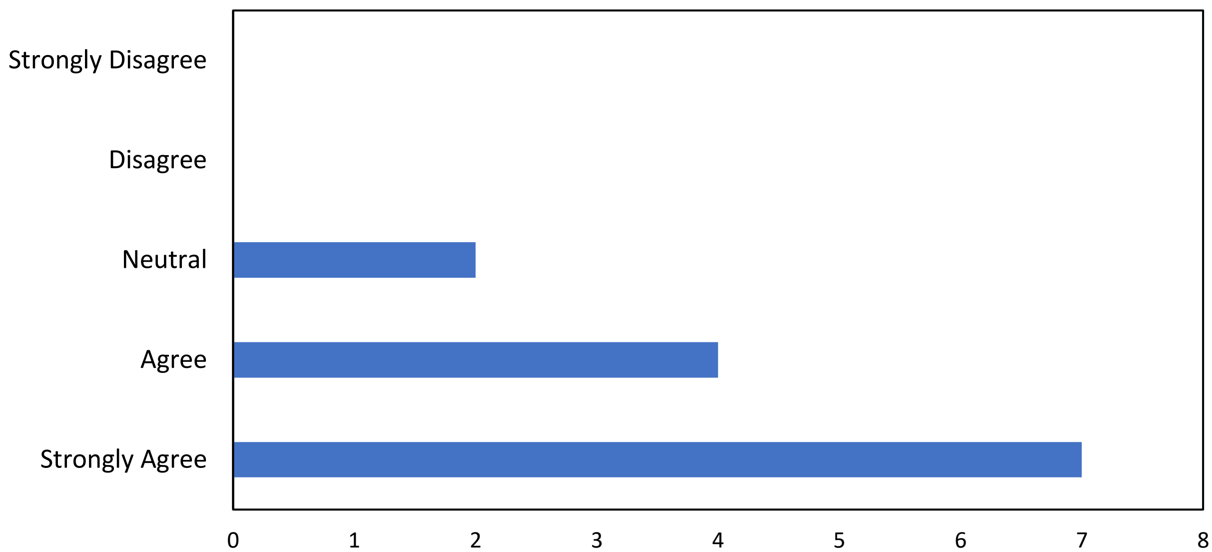
1. How helpful are the quizzes to you?



Please explain your answer:

- I haven't spent enough time on them yet so right now they are not helpful but that’s my fault.
- They give practice for the exam.
- They make you think about key concepts, which is nice.
- The quizzes are helpful but sometimes confusing. Especially the several answer multiple choice.
- Getting a review of the plants before class is helpful.
- They do not contain enough information to test knowledge and not enough to learn from.
- I do think they are helpful but not very challenging or extensive.
- It's a great study tool for after the lecture.
- I like the extra study help!
- The list quizzes are good. I understand why they are hi-res, but it makes it a giant pain to have to zoom in so far over and over.
- I like you can retake quiz many times, to get a feel for that exactly question is asking and help with knowledge.
- I like how it's another tool to help me learn.

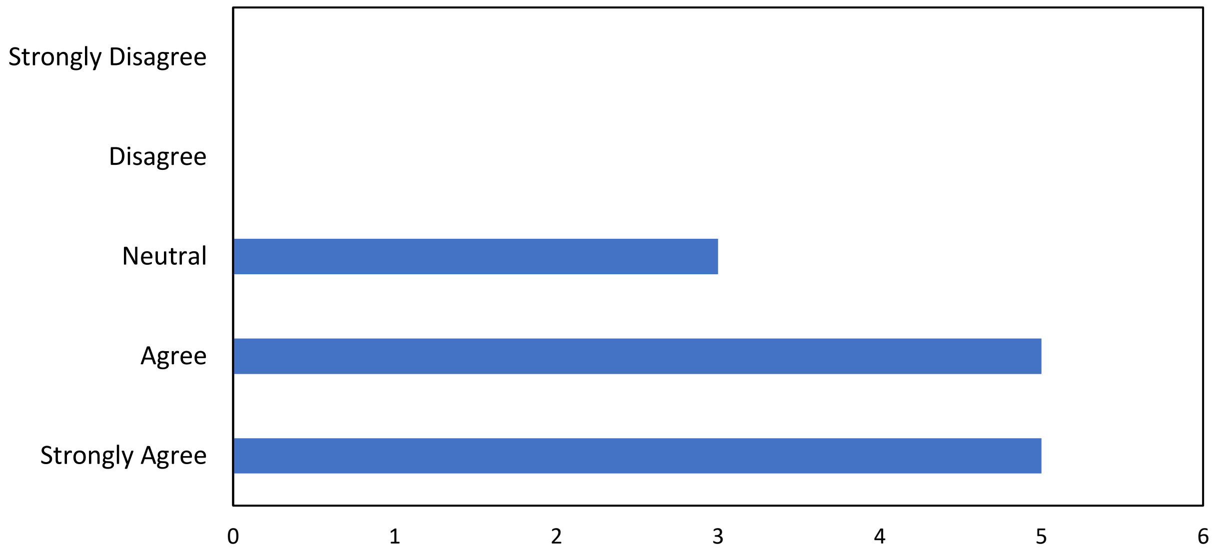
2. Lecture is easy to follow.



Please explain your answer:

- You [Stephanie Zorio] made it very easy to follow.
- Organized and to the point on what we need to know.
- Stephanie is a good lecturer. I appreciate her enthusiasm [smiley face].
- I wish this class was spaced out throughout the week. It's hard to care about plants for 3 hours straight.
- It goes by the lecture and its straightforward with some fun sidebars.
- Easy to follow along because it's organized. A little long though. This is a 2 credit class, but we are here for 3? What's up with that?
- Stephanie does a great job explaining the different plant and species.
- Lectures are to the point not a whole lot extra.
- Lots of visuals—everything is in depth which is interesting—BUT—hard to know what is a fun fact and what will be tested on.
- Ditto; pretty standard. Linear and concise, which is appreciated.
- Very easy to follow, and it is enjoyable so keeps your attention.
- I like how you stick to how Dr. Williams has done it, but you've added your own flair.

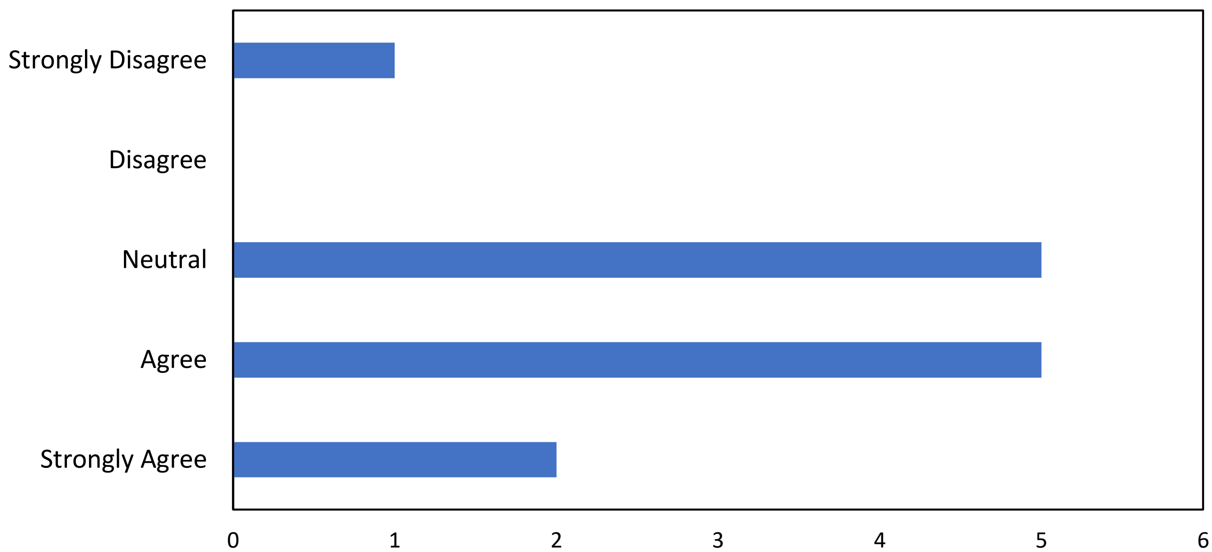
3. I enjoy learning through a variety of methods



Please explain your answer:

- I need multiple different ways to say the same thing for me to really get it.
- I like the lecture/lab situation.
- Don’t fall into a boring pattern. Keeps things fresh.
- Using PowerPoint, overhead slides, worksheets, and playing learning games are a good balance.
- I like being able to follow the PowerPoint slides while looking at the plants and then the lab directly after helps.
- Mix of lecture and hands-on is good!
- Audio-visual is the best w/ some written afterwards.
- I enjoy the hands on part, it helps w/my memory.
- I enjoy lecture for background knowledge and being hands-on with looking at different specimens.

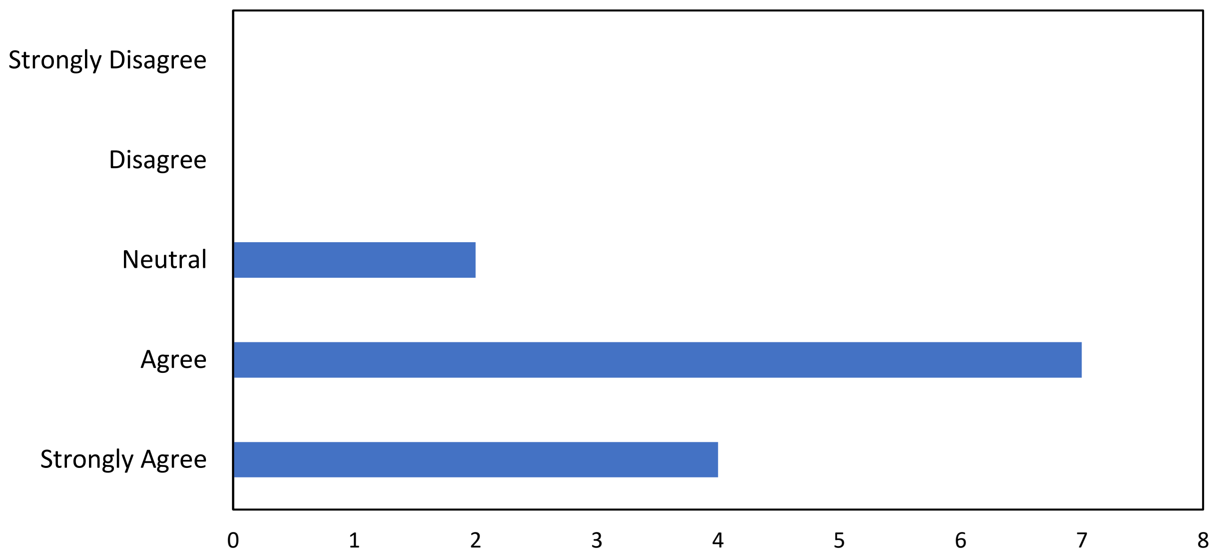
4. The plant family cards are a fun and useful way to learn about plants



Please explain your answer:

- I like the little games w/those cards, but we should use them more often to really be helpful.
- It was a great way to see the [ants before we dove into lectures.
- They're cool, I guess. Truthfully their [sic] is so many it kind of goes one ear and out the other.
- The more you practice the better you get. Some people don't enjoy them, but I think it's a good way to learn families.
- I'm not seven.
- We haven't played them enough! [Frown face]
- It's a great way to look at the plant characteristics quickly and the competitive helps make you want to learn.
- I wish there were more families! We should contact the makers and ask for Round 2.
- They don't really help me honestly. I'd rather just go through the lists and for family and common names.
- It helps you learn family/species and engage with others in class.
- It does help with familiarizing with plant names, but can be a time to pass the time by socializing.

5. My working knowledge of plants is much higher than at the start of the semester.



5. What would help you succeed in this course? (ex. More or different worksheets, dissections, lectures, field trips, game-like learning tools…)

- I really like the worksheets to fill out during lecture. Personally, it helps me follow along much better.
- Everything seems great, well-organized, and thought out.
- For someone who is not really interested in plants it is very hard to follow when you are going into such depth. Maybe build more of a base/ spend more time with the basic principles/vocabulary in the first few class sessions.
- Splitting the class and then it would be more exciting and entertaining.
- I seem to be learning well in this course, but field trips never hurt anyone.
- More focus on what we should study. I was unprepared for the first exam.
- Take home worksheets, more interactive games!
- Explaining simple meaning more such as monocots etc. Also letting us know what is more important to know or letting us know if it is extra information.
- I think we do a good job of going into depth and have enough repetition that it's all helpful. I only had to be better at studying the samples in my own time—at no fault to the teacher, that's totally on me.
- Field trips are invaluable, and I learn the most during those without question. Worksheets work well as study materials.
- Maybe just a bit slowly teaching on the parts of flora, kind of seemed we went through like. [sic]
- Maybe more depictive worksheets labeled better.

Final Course Evaluation

Please take a few minutes and fill out this evaluation so that we can continue to improve this course. Thank you for your feedback!

1. **How have each of the following affected your learning about plant-animal interactions? Briefly, suggest any changes to each that might improve your learning and comprehension.**

a. Suggested field guides and lack of required textbook

- I like no textbook.
- I like that there was no textbook, it's not really necessary!
- I downloaded the apps and those helped. I fail to see how a required textbook would help any.
- A field guide is more needed than a textbook. It works better. No bad comments.
- I never used a field guide and I don't believe a textbook would have been useful.
- Very helpful and affordable for students.
- I don't feel there should be a change.
- Field guides and textbook were really helpful to see what that plants look like while on field trips.
- I think you should make the best guide required cause it would help but I wasn't going to spend $ unless I have to.
- The field guides were awesome to have, they helped be able to identify all of the plants we found for INat.
- Not having a book was difficult.
- I did not purchase or use field guides. The less money I have to spend the better.
- Field guides provide a quick visual while being on field trips. I personally utilized mine, it was quick, convenient, and affordable. Even if I couldn't afford it Stephanie made sure to provide plenty copies and other resources.
- I really like the Plant of the Rocky Mts. guide.
- I never like to buy a textbook. The option available are good field guides.
- The PowerPoints were very informative. The field trips helped significantly for visual, hands-on information.

b. Lectures

- Ok.
- Love them, Stephanie did a great job keeping them interesting and upbeat.
- Good lecture, she has great enthusiasm about the topic.
- Thank you for not just word-for-word reading slides to us!
- Although long they are set up very well and informative.
- The lectures helped me to remember the plants and the important info about them.
- Good way of incorporating lecture (PowerPoint) and an activity like the worksheets.
- Lectures are great I don't think there should be a change! I love your positivity.
- Lectures were organized, Were gone through kinda fast but were okay to get the information from.

- Very helpful!
- Good to be able to sit and listen and look at specimens at the same time.
- Lectures were cool but again the specimens look different, so it was nice to hear about the plant but hard to retain.
- Powerpoints were useful and the lecture filled in the gaps. This helped the most.
- Lectures were well-guided and detailed. It was very nice that they were available on Moodle ahead of time, so I could print it and bring it to class.
- Ditto, I thought Stephanie did a great job all semester. Keep it up and become more comfortable as you go.
- I enjoyed her enthusiasm every time we had class.
- Lectures were enjoyable and very interesting, through at times there was a lot to memorize in just one day of class. The info on Moodle did help refresh and help memorize.

c. Worksheets

- Helpful and through, easy to answer and straight-forward.
- Very helpful!
- Definitely enhanced my comprehension of the subject matter. I like having them during lecture so I can fill it out as we go.
- Almost unnecessary, most of us would blast through it to get it done as quickly as possible.
- I found the worksheets semi-helpful in remembering info about the plants, but not with identifying them.
- Very helpful.
- Helped a ton and allow time to practice.
- Worksheets were helpful when studying for tests and trying to remember which characteristics each plant had.
- Not helpful.
- Good to use for studying.
- I think worksheets were cool but didn't really help because the specimens that are on the folder don't look like what's on the slideshow or worksheet.
- Sometimes just seemed tedious and like busy work. Good for study guides though.
- Worksheets were a good review. I think using the worksheet along with another review or practice resource would have been nice.
- Ditto (very helpful).
- Were extremely helpful especially for studying for exams.

d. Exams

- Not a fan of just memorizing names. I would rather write some specifics about a plant. I think this is a very difficult exam format for only two credits.
- I like the practical exams. Especially when there are fresh specimens.
- I wish exams were broken up into different parts. One where you just identify and then another section of just questions.

- Felt as if we were rushed because of the 1 minute at each station although there is not much that can be changed.
- The exams and especially studying for exams were helpful.
- First exam was very difficult and too in depth for a 2 credit class. However, the next exams were better.
- Personally, I feel like the characteristics of plants should be multiple choice and I wish there was a review session as a class.
- Exams were hard, but covered fairly well what we learned.
- Yikes the exams are ruthless. Didn't learn anything from them. I think learning two common names makes the exam a billion times harder. That's what killed my grade on them.
- Way to [sic] hard for a 2 credit class need to drop some of the material.
- Exams were literally harder then [sic] my A&P tests. So much information to memorize at least give a word bank or multiple choice.
- Were good for memorizing many different plant species. Sometimes seemed overwhelming.
- I really liked how the exams were given and organized. I knew what to expect each time. Maybe improve on the numbering system, sometimes I would get lost.
- Straight forward and easy enough to study for. It would maybe be useful to know how many specimens would be on the test prior, so I could have a way to know effectively how much of previous lists are going to be in it. This would help determine how much I feel I need to revisit older lists.
- For this fall semester Stephanie put more emphasis on habit of the plants and structure.
- At first were a bit overwhelming only because of the unfamiliar way of taking them. Once familiar with procedure, actually were quite fun.

e. Moodle Quizzes

- This is new, I love this! Very helpful. I like being able to retake for more points, helps comprehension.
- I LOVED these! They helped so much!
- Good way to get points. The species pics for the first few were too big/annoying but that got fixed.
- Very helpful! When I don't have specimens they would help.
- I didn't find the Moodle quizzes very helpful. I believe my learning would have been the same with or without them.
- Helpful and good for getting some extra points.
- Helped so much! I don't think anything should change about it. Maybe add some quizzes about characteristics.
- Moodle quizzes helped to cement the images and names.
- Helpful, but you should check them again. Yarrow was on all quizzes and replaced a plant that was supposed to be on.
- Really helpful to be able to test yourself before an exam.
- Those were awesome! Helpful!
- I thought they were useful as study guides. There needs to be a better way to display the species list portions though.
- Good! The sample pictures were clear and being able to take the quiz unlimited attempts was nice because I could use it later on to review for the exams.

- Somewhat helpful, but I find quizzing other students in the hallway with the pressed specimens to be more helpful here. There's more variation to see, which is why I find it more helpful. This is also why iNat us very helpful, the multiple pictures that are available.
- Moodle quizzes were very helpful and another good study aid.
- Extremely helpful to learn species, with the images available to you. And having them open to retake, really help with identification.

f. iNaturalist Project

- Very helpful and fun!
- Helped a good amount.
- I think iNat is so fun and rewarding. Plus, it's a great alternative to actually having to collect plants.
- Much easier I think then collecting/pressing also I use this app for bugs ALL THE TIME now.
- Hard to do at first week, due to not knowing much. Felt like a chicken with my head cut off.
- Seeing the specimens in nature is helpful in identifying them.
- Fun!
- Helps learn the plants. I did however enjoy the project at the end of the semester. However, I know we couldn't do that during this semester it might be nice to break up the field trips, not all at once.
- iNat was fun but I didn't really benefit much from doing it.
- It was my favorite part. I'll have an account and add to it for myself as a hobby.
- Good thing to help learn the plants we have around, also easy points, and good to get extra points.
- It's nice to actually get to see and feel real plants instead of flattened ones. My favorite part of the class.
- I thought it was fun.
- Super fun! I can definitely see myself using it when I go hiking.
- Very helpful, this has helped me learn the plants just as well, if not more than the pressed specimens. Being able to have this w/me all the time on my phone is great.
- I always enjoy field trips. They are useful to learn and be exposed to live plants.
- Interesting and eye-opening. Was nice to have others give suggestions on my observations.

2. Did your background from previous biology/ecology/evolution courses prepare you adequately for this course? Alternatively, if you haven't had many biology etc. courses, do you feel like it hindered you at all?

- I took spring flora, that helped.
- Upper level bio courses weren't helpful or deleterious.
- Bio 102 helped the most.
- Not really.
- No. I wish I took Spring Flora first.
- Some, but not a lot.

- I haven't, but I don't think they would have helped.
- No.
- I don't think it made a difference. This class to me just seems like a lot of memorizing plant common names, which I did not like.
- Yes, I feel like they have helped, and this course has helped me in my other classes as well.
- I don't feel that it helped or hurt me.
- Yes, other classes helped a lot with this one for general knowledge.
- Spring Flora was very helpful.
- I didn't remember a lot of plant stuff from my biology courses.
- Yes, Bio 102 and Fall [note: maybe they meant Spring?] Flora helped a ton!
- I don't feel a lack of Bio classes hindered me.

3.Overall, what were the most beneficial aspects of the course? Why?

- The knowledge of Stephanie and Rick, they are always able and willing to answer any questions.
- Being able to identify the plants around me.
- Learning the different species was helpful for summer and fall field work that I have done, as I now have a better understanding of the biodiversity around me and their interactions occurring.
- The field trips because I can actually identify live specimens.
- Hands-on portions and worksheets because I could physically feel the differences, instead of just looking at pictures of laminated samples.
- The field trips. I think it is important to engage students with interactive things.
- I loved the instructor's sense of humor.
- I think all aspects were useful to understand the plant lists and families.
- Finding new cool places on field trips. I wish we took more.
- Extra credit saved me.
- Field trips and worksheets were the most beneficial along with the specimens.
- Knowing what plants you can eat.
- Lectures, labs, and field trips because they were hands-on.
- I loved learning of flora. Stephanie was great and so patient and helpful.
- Learning how to identify and use the plants outside of class.
- Worksheets, Moodle quizzes, iNat. These were fun activities that really helped with learning about plants.
- The field trips and specimens. They really help with remembering the plants.
- The pressed specimens, because that's the only way to learn.

4. Overall, what were the least beneficial aspects of the course? Why?

- Stephanie at times could be a little more serious. This may help her stay on track.
- Moodle quizzes, limited variation in specimens.
- The quizzes. They just seemed like busy work, but were good for beefing up the grade.
- I think I learned so many plants in such a short time I never appreciated the plan and its purpose or beauty. I just learn the names.

- Some of the exam questions were absurd. It takes a long time to memorize 40 types of plants, their names, family names. Then you also want me to randomly remember how many of this is common to this family and what was it used for. I think those questions were ridiculous.
- iNat was the least beneficial. Didn't really seem to help when trying to remember/learn plants.
- The exams. I feel like they were about memorization rather than comprehension.
- The fact that it is a 6 at night [frown face].
- I cannot think of any.
- The worksheets. Just rushed through them to get home.
- The Moodle quizzes.
- Learning little details that don't help outside of class.

5. Overall, how would you recommend changing the course format to get the most out of it?

- I think the way the course is set up succeeds in teaching its students.
- I would recommend more live samples during the lecture portion.
- More field trips, no tests.
- Take it outside more. I wanted to learn plants not really a slide show.
- I think a couple of changes need to happen: 1. The class needs to be a 3 credit class, not a 2 credit. It is way to [sic] much information to be remembered for a 2 credit class. 2. If it remains a 2 credit class some of the material needs to be dropped because I was spending more time on this than my upper level biology classes.
- I retained almost nothing of the plant formula and characteristics of fruit and stuff. So maybe make that a focus in lab or lecture.
- The course overall was well done, maybe discussing what we need to know on exam would help.
- Offer different ways to learn the material/games.
- Change the exams. I have put more effort into these exams than I have into my upper division courses, and I still do better in those. Or keep the exams, but make it worth more credits. It is a lot for 2 credits.
- Don't change anything! Spring and Fall Flora have been some of my favorite classes!
- I don't think there is a way to do this in the fall but I wish I had a few lectures before the first field trip because I felt completely lost on that trip…
- Maybe just slow down a bit and if first time taking a flora class, maybe explain certain aspects of class first [smiley face].
- Bad to say but brake [sic] the class into 2 days. 3 hours is a lot and can take a toll on retaining information especially at night.
- I don't recommend changing anything. Stephanie was great! [smiley face]
- I know a lot of this is out of your control! However, more practice w/characteristics of plants and having a review session or an idea of what will be on test besides naming the plants. Overall though you are an excellent teacher!

CHAPTER 3: Internship 3, Concepts Biology Human Concerns

I. General Information:

A. Student: Stephanie Zorio
B. Faculty supervisor: Dr. Jeffrey Hill
C. Course description: BIOL 1100 Concepts Biology of Human Concerns, Fall 2018
D. Extent of internship: Full course responsibilities: includes preparation for lectures, classroom activities, and assessments
E. # internship credits: 1 Credit for proposal and final report, 3 additional credits for teaching activities

II. Rationale and goals for teaching internship:

With the completion of this internship I have satisfied an important requirement of the D.A. degree. Personally, it also served as a capstone project, as it was an opportunity to design a course from conception and manage a classroom much in the way faculty would. The steady progression of responsibility throughout my internships meant that I was prepared to reap significant benefits from not just teaching but creating and implementing a non-majors biology course for my final internship.

My goals for this SuTI were to: 1. Create a syllabus (O'Brien *et al.*, 2009), 2. develop a portfolio of resources (article, book chapters, activities, *etc.*) to supplant the traditional requirement of a textbook, 3. relate scientific concepts to current events and everyday life (Nilson, 2010; Shields, 2006), and 4. foster a vigorously student-centered and inclusive learning environment. Overall, I feel these goals were met. In the following paragraphs I will provide specific examples and describe the overall structure of the course.

The course schedule included in the syllabus at the beginning of the course only delineated important dates, *e.g.* exams. It was largely filled over time by topics chosen through group discussion. More specifically, I would assist the class with selecting a broad topic, then use that to guide my lesson plans. I used this strategy to encourage a sense of ownership of course content by students, and as a way to promote just-in-time teaching practices (Novak, 2011) for myself and prevent falling into the rote progression of small- to large-scale concepts (*e.g.* enzymes to ecosystems) common to introductory biology courses.

The objectives for BIO 1100 in the undergraduate course catalog served as my primary objectives because they were a concrete way for me to assess myself as I developed materials and moved through the semester. I used the Biocore Guide developed by the authors of *Vision and Change* (AAAS, 2015) to cover a set of important principles in biology rooted in real-world examples sourced from book excerpts, news articles, publicly-funded online learning tools, videos, and case studies (Hobson, 2000; Klymkowsky, 2007). My approach was influenced by my background and teaching philosophy in that there was a strong underlying theme of biological connectedness and dynamic change uniting each portion of the course.

Exam dates provided tidy bookends to three distinct modules based on topics decided by vote after slight curation by the instructor. The three modules began with an investigation of food systems (*e.g.* trophic levels, GMOs, sustainable vs. unsustainable agriculture), followed by a deeper dive into evolutionary relationships between humans and the rest of the biosphere (*e.g.* phylogeny, taxonomy, diversity and connections of all life on earth). For instance, students read and discussed the potato chapter in Pollen's *Botany of Desire* during the first module and watched a portion of *Your Inner Fish* by Dr. Neil Shubin after learning about taxonomy and phylogenies in the second module. The topics of these sources seem divergent at first; however, it was a natural progression of student curiosity and wonder *in situ*. To paraphrase one student: "I'm more closely related to powdery mildew than a potato?!" The third module was focused on current and historical ethical considerations in biology, another natural move because issues that concern humans often include challenging moral/ethical situations that cannot be addressed through science alone. For example, during the phylogeny module we discussed conservation tactics asking, “Is it better to focus on biological hotspots of diversity like the Amazon rainforest, or is maintenance of phylogenetic diversity more important?” Throughout the semester students were asked to critically examine and discuss the validity of information from multiple news sites, common memes, and viral videos—sources that they were at times asked to find and give a brief oral report on.

The first half of the course was light on lecture, relying heavily reading assignments and small groups to keep discussion moving. This also had the benefit of forcing many student interactions, a priority in the constructivist framework of pedagogy (Tanner, 2013). The last third of the class was more case-study and lecture-based. Case studies were sourced from the National Center for Case Study Teaching in Science (NCCSTS; sciencecases.lib.buffalo.edu/cs/). The NCCSTS is a subscription service ($25/yr) limited to collegiate faculty. However, after my correspondence with the administrators, Dr. Hill was permitted to vouch for me and I gained access after payment. I would promote this tactic as opposed to clandestine use of a faculty account, for obvious reasons and to endorse our unique D.A. program. Overall, I found case studies to be an effective way to discuss some delicate topics, although keeping the discussion focused and lively without doing the conversational heavy lifting was challenging. We discussed historical medical cases like Henrietta Lacks’ immortal cells and the Tuskegee syphilis experiment. Recent news about beach access led to a move towards natural resources as the final case study. The water issues in the Klamath Basin, spanning the OR-CA border, are the same that farmers, tribes, and recreators face here in SE Idaho. Namely, who gets the water and how much under the destabilizing effects of climate change.

After this experience, I am convinced that the worst way to think about non-majors biology is as a chore or a diluted Bio 101. This is not to blame entrenched instructors. Most departments benefit from great teachers, but don't often include course development as an important component in their promotion evaluations. Ultimately, this is a systemic change that must be made at the administrative level. Scientists and engineers account for a scant 5% of the labor force (Sargent, 2017). Non-majors represent the majority; they are the people who will influence important decisions about public policy, funding for science, and more broadly, our collective future as a species. Bio 100 may be the last life sciences course these students have in their college career and beyond. Therefore, non-major biology courses are an opportunity to teach and communicate with the broader population, which is both a goal and necessity (AAAS, 2015; Becker, *et al.*, 2017). This internship has affirmed my desire to teach to a broad audience. Even if I should leave academia, I will always act as liaison between science and society.

III. Teaching strategies

The flexible course schedule combined with the solicitation of regular feedback from students allowed me to respond dynamically to the course without an overwhelming amount of extra work. However, since the class was not highly structured, at times I found it hard to trust myself and spent many hours trying to predict and prepare for any direction a given class meeting could go. My control over course content meant that I could include culturally diverse examples to connect biology concepts. I was able to incorporate a variety of active-learning strategies to promote an open and friendly atmosphere and encourage participation and engagement from all my students (Tanner, 2013).

The time of the class was a boon as well as a challenge. I was able to more seamlessly incorporate long-discussions and case studies because of the three-hour time period. In general, my strategy to foster an active learning environment was to begin with something familiar to students, then use that angle to jump into broad biological principles. We would then follow those principles through spatial and temporal scale. For example, when discussing GMOs I mentioned that there are glowing fish for sale at a local pet store, McKee's. The fluorescence of the fishes is mediated by proteins coded by a jellyfish gene added to their DNA. That lead naturally to review of macromolecules, enzymes, and the cell cycle—common topics for the start of a science class. Information and discussion skills were then transferred to the next module, again situated in the reasonably familiar (Tanner, 2013). I tried to incorporate lab topics into our material and provide a quick review every week, but evaluations indicate that it was not very useful.

This course was more group work and discussion-based than any class I've previously had an instructional role in. Most evaluation respondents identified group work as a critical learning tool but varied in their preferred style. Shy people preferred small group work. Both talkative and quieter students self-reported as benefitting from large group discussions. Students would often be asked to brainstorm on their own before breaking into randomly assigned groups. I also frequently included board work. With an average weekly class size of 30, I could easily divide the white boards in the Plant Sciences lecture hall into five sections. The entire class would be at the front of the room, discussing a question in their groups while jotting notes, answers, and lists on their section of board. Students seemed to feel more comfortable sharing thoughts when we were in a concentrated group at the front. I could also more easily direct students to talk to their classmates instead of at me. Moreover, instructor-chosen reporters from each group provided low-stakes public-speaking practice.

In future classes I will surely incorporate two combination assessment and learning exercises adapted from Dr. Hill. These are weekly (or class period) class summaries and student-led study guides. The last 10 minutes of each class was reserved for class summaries. The summaries were unstructured outside of the requirement of at least three important pieces of information. I wasn't so concerned with what they were writing or how it was phrased. Rather, it gave students another in-class opportunity to recall, synthesize information, and ask questions. For me, it provided an easy way to take attendance and assess the progress of each class.

Students would use their returned class summaries, notes, and Moodle materials to construct study guides. Guides were due two weeks before each exam. I asked them to identify at least three major concepts, important terms, and suggest short answer questions. Students generally earned full points if the preceding requirements were met. After giving detailed feedback on the first study guide, I imposed a two-page limit, further challenging them to choose the most salient points and to synthesize and apply information. I would use their guides to construct a final study guide for each exam. In course evaluations students had a positive view of study guides and weekly summaries.

This experience also made me reflect on course elements beyond teaching. I think that 1.5 hrs. twice a week is an ideal format for most biology classes. Three hours is long outside of laboratory sections, although students will fill those lecture sections out of necessity. Half as much time provides room for discussion, lecture, and activities. Further, this echoes student feedback I've received from teaching late evening classes. Interestingly, I found it difficult to find studies that investigate possible correlations between class length and student achievement. The teaching space was also challenging to work around. The Plant Sciences lecture hall was more amenable to active-learning than the originally scheduled classroom (LC 10); however, both spaces have immovable tables and chairs. Fortunately, multi-purpose and fluid spaces that include easy ways for students to share group work with the class are becoming more common over time. Again, these are systemic issues, similar to the course development issue identified earlier.

IV. Course Assessment

In terms of self-assessment, I think I can improve on grading, managing extended class discussions, and making my lessons more concise. I found that I was consistently impressed by the care that most students took with writing assignments and felt that if an answer was well-supported it deserved full credit. I also gave many opportunities to gain points throughout the semester which could have inflated grades. Free-form teaching is much different than answering questions about a prepared lecture. I've gained valuable experience, but I need more practice to become a truly effective class manager in discussion heavy classes. Another unforeseen and certainly contemporary weakness was penmanship while writing on the board. Fortunately, this is a skill that can easily be improved with practice. I created a detailed outline for each class, although I often found that I had overprepared. I assumed that I would need more material to fill dead space, especially because of my infrequent use of PowerPoint presentations. I was surprised by how much my students wanted to talk, especially as they became more comfortable over the semester. In the end, my skill as a discussion leader was perhaps more important than my ability to quickly recall and report facts.

Graded assignments and exams were one way for me to assess the impact of course activities on student learning. In general, by the end of the semester written arguments became more fact-based. They frequently made connections to past discussions and tried to apply biological principles to challenging, or plain interesting, human concerns. Formal and informal assessments beyond graded assignments and exams were conducted throughout the semester. Formal assessments include the mid-term and final course evaluations (Appendix A & B).

Informal assessments included live class votes using PollEverywhere (www.polleverywhere.com/) on perceived difficulty of a recent exam or initial feelings about science classes. Many students preferred that assignments were due on the same day and time every week. Another common criticism was that they felt that lab was not well-synchronized with lecture. That is likely a result from the way the course was structured. Anecdotally, however, even traditional lecture-based classes suffer from this disjunct. Students were initially uneasy about the lack of textbook and structured course schedule, but most appreciated both at the end of the class. I did not receive expected push-back about the amount of writing in the course. Assignments requiring some level of critical thinking were frequent. Most importantly, the common sentiment was that after taking this course they have a better appreciation and understanding of biology and how it relates to their college majors and lives.

V. Assessment of Supervisor Involvement

I consider this internship a success in large part due to the mentorship of my supervisor, Dr. Jeffrey Hill, who taught a concurrent section. His willingness to meet several times over the summer was critical for constructing the skeleton of the course. During the semester we met weekly after my Tuesday evening section to debrief and discuss the next week's schedule. Also helpful were several weeks of observing Dr. Hill's morning section. Likewise, Dr. Hill observed my teaching throughout the semester. His feedback was particularly useful for helping me learn how to distill the major objectives of each class and stick to them while teaching.

All D.A. internships can be greatly enhanced by frequent interactions between interns and mentors, and consistent exposure to different styles of teaching. Teaching seminars and collaboration with my mentors has given me the confidence to incorporate innovative teaching techniques into courses of my own design. Most importantly, both parties benefit, as D. A.

students are sources for current pedological technique, theory, and novel teaching tools, any or all of which can be incorporated into current or future classes by the mentor.

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BIOL 7700 Supervised Teaching Internship
Mentor's Assessment of Stephanie Zorio

December 2018

Faculty supervisor:
Dr. Jeffrey P. Hill

Internship Course Assignment:
BIOL 1100 Concepts Biology Human Concerns (biology for non-major's)

Scope of Student Internship:
Full course responsibilities: syllabus, lectures, classroom activities, and student assessments

Internship Context:
This internship occurred near the end of Stephanie Zorio's doctoral program, after completion of advanced coursework in college teaching methods and after a prior internship for a different course with a different faculty supervisor. The non-major's biology course under review here was essentially a capstone teaching responsibility in her DA.

The assignment posed some unique challenges for several reasons. First, several broad learning objectives are prescribed by ISU as part of the general education criteria (Objective 5) that all undergraduate students at the university are expected to meet to be eligible for graduation. Instructors in courses for majors do not face these externalities for course design. Second, specific decisions about content were under-prescribed compared to a traditional major's biology course because the class embraces the dynamic relationship between modern biology and human concerns – and "human concerns" in a changing world are neither fixed nor strictly defined. This creates a large instructor responsibility to make critical choices that illustrate how life science literacy informs current issues in the lives of a diverse student population. There is no single best strategy to accomplish this goal, so Stephanie had to grapple with navigation into some uncharted conceptual seas. This was a significant difference in professional responsibilities from her first teaching internship, but likely to be a situation encountered in future career settings in education. Third, due to unanticipated departmental staffing changes, Stephanie coincidently accepted the internship at the same time her faculty mentor (Hill) was assigned to the nonmajor's biology course for the first time. This added to her professional responsibilities since she was not presented with a "here's what worked before" course packet. Fourth, although there were two concurrent lecture sections of the course, Stephanie's course was the evening session that met weekly for three hours. This imposes special teaching and learning constraints and opportunities that do not exist for other class formats. Finally, the non-major's course includes a weekly student laboratory. Stephanie had to develop her philosophy about how to adapt knowledge in pedagogy into practice, integrating lecture with a lab taught independently by other graduate teaching assistants.

Mentor-intern Interactions:
By far, the main interaction activity during the internship was one-on-one meetings. These ~1.5 – 2.5 hour discussions were initiated during the summer for pre-course planning, and continued on a near weekly basis throughout the fall term. During the course, meetings were generally scheduled the day following the evening session to get fresh recollections about how the class meeting went and to project forward to the expected lecture content at the next class meeting. Additionally, Hill made some in-class observations during the term to evaluate Stephanie's lecture style, both in a lecture hall setting and when

she moved her class to the computer lab for an on-line activity on phylogenetic trees. Stephanie also committed time early in the semester to observe numerous lectures by Hill in the daytime section of the same course to get insights into how someone else was leading the non-majors class. Stephanie was given access to Hill's course Moodle page to find sources potentially useful in her own lecture section. There was also a string of ongoing email exchanges during the term about potential content and sources useful to the course.

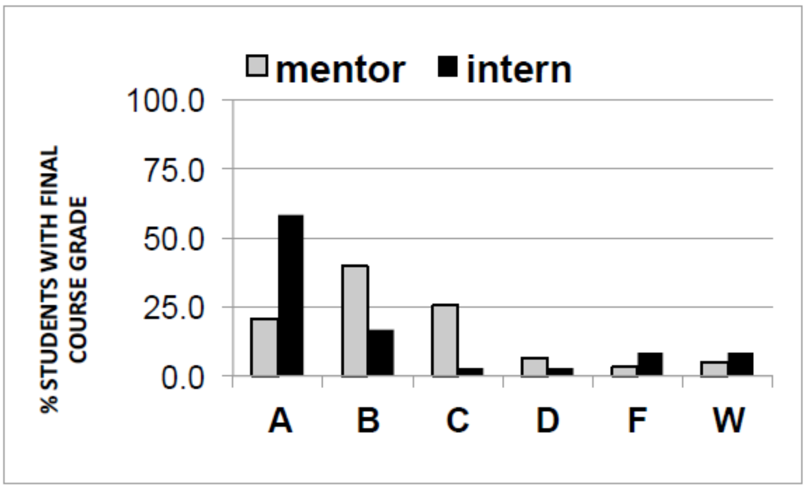
Mentor's Assessment of Internship:

Stephanie deserves genuine credit for accepting such a challenging teaching assignment that surely involved overcoming a palpable level of apprehension about embracing a relatively non-traditional open course structure. For example, to the keep the course responsive to topics deemed relevant to students' human concerns, the decision was made to switch from readings in an introductory text to open source materials, current news journalism, or selected readings from a variety of monographs and books. This decision (or leap of faith?) is a difficult one to make in a freshman course, even for a seasoned instructor, so her willingness to take the risk illustrates her progressive attitudes toward the use of experimental approaches to instruction. It is not easy to try to expand beyond your comfort zone, but taking such chances is part of the path to determine what works best for you as an individual instructor. In the science education literature, this sensibility also embodies the idea that teaching life science is itself an ongoing experiment and instructors should be proactively exploring approaches to discover data-driven solutions for what works. Stephanie didn't just automatically employ methods previously learned in teaching methods classes; she consciously and courageously pushed herself to experiment with her own teaching. In that regard, I submit that her internship represents a truly creative scholarly contribution to her doctoral degree. It reflects well on her expanding professional maturation.

Stephanie's overall approach and execution of university level instruction was as effective as any I have seen for someone at her career stage. As a lecturer, she had an excellent presence in the classroom and offered an engaging style that was both professional and personal. Based on our pre-class interactions, she put real effort into being (over!) prepared for each class meeting and she taught with a visceral level of commitment: she really cared about whether her efforts were successful. She was ready to be both self-critical and to accept constructive criticism as positive input for improvement. I think these are essential values for someone potentially considering a longer-term commitment to teaching the public. She also demonstrated on ongoing awareness of the constraints of an evening session scheduled for three hours. Tradition might dictate stringing three one-hour lectures together while standing at the podium. Stephanie instead designed a wide variety of activities to change up the learning format within each class meeting. Students did pre-class assignments (i.e., the flipped class method?), small group work, moved around to use the whiteboard, visited the computer lab, and participated in class discussions throughout the semester. In the end, the decision not to use a textbook seemed to fade from our conversations since we were involved in more basic deliberations about how to get our students to engage and learn certain ideas in ways they would remember. She identified and deployed numerous useful sources (e.g., a case study archive for instructors) that I was not familiar with. She took initiative. In short, she adapted well to her course setting at numerous levels, spanning the mechanical, pedagogical and intellectual.

Overall, Stephanie's commitment and prowess as an instructor was never really in question. As a mentor, I tried to use our interactions to provide additional professional guidance about creating a reasonable balance between her teaching and other important demands (in her case, completing the doctoral dissertation). In my experience, early career interns often spend extra time in class preparation compared to established faculty members. My impression from debrief sessions and a review of her course experience suggest this may have also been true at times for Stephanie, particularly early in the term. She was having difficulty predicting how much preparation would fit into the prescribed class meeting time. Early on, she lamented not getting through much of the material she had planned to cover with students. This is an acquired skill and I feel like she had already started to master it as the course progressed. She benefited from the weekly practice.

Another course management feature that I noticed in Stephanie's section was the number of student assessment activities she integrated into her class; she had quite a few more student opportunities for earning points than my lecture section did. Going forward, I encourage Stephanie to think through the value of these activities for learning (i.e., return on investment) because they can demand a lot of the instructor's professional time. It is all but impossible to implicate cause and effect, but students in her section had a higher GPA than mine did:



One potential explanation is that they had more chances to earn course points outside of the pointed stress of periodic midterm examinations. This is potentially a positive course attribute. However, decisions will always need to be made about how much assistance average university students should get to learn content and concepts without transferring too much responsibility for their performance to the instructor. Decisions need to be made about how many students in a class, on average, can be expected to demonstrate mastery of the subject at an "A" level while the instructor still commits a reasonable amount of time on their behalf. This is an observation to think about rather than a criticism.

As a final comment, it would useful for Stephanie to reflect on her efforts to function with an open format in a new course, particularly with respect to the responsibilities she had to drive the selection of content. In my own section, I ended up with three conceptual units that aligned with three midterm exam assessments, and those units could be unified into broader themes that provide satisfying integrated explanations of phenomena in the natural world. Those explanations also had important implications for addressing our collective human concerns. After a review of content on her course Moodle page at the end of the term, it was clear that she had emphasized some topics that I did not, so I was not entirely sure of what larger conceptual levels of organization and linkages she wanted to emphasize. These are emergent properties of the class that appear as subtopics are tied together into bigger themes. As an exercise, it might be useful to revisit on the first run through with this course to identify whether those larger themes exist, how well they were developed, and how the approach might be improved going forward if the course were taught again. I have no doubt she would be restless and she would strive for improvements.

In summary, Stephanie has successfully completed an ambitious teaching internship and she performed at a high level with admirable levels of independence and intelligence. Our conversations during her teaching assignment were very interactive and she has well informed and well developed points of view that reflect well on her professional development. It is reassuring to see this level of accomplishment and ability.

Mid-Term Evaluations
Bio 1100 2018

1. What do you like about this course?

I like how there is a free spirit thing going on. She doesn't put pressure on us at all. I feel that some people in my class aren't participating how they should, but she's really nice and I like her hippy attitude.

I like how open it is and the discussions. There isn't a strict guideline or calendar to get through and its a pace I like.

My favorite part is the interaction with class and teacher that promotes a casual environment. This makes it so much easier to participate and function in the social parts of this class.

I like how we connect what we are learning about scientifically to real life, and that we aren't lectured its interactive. We are encouraged to have our own ideas and question things. I have struggled with science but found myself learning so much from this class.

The instructor makes it interesting with good lectures and anecdotes. The group sessions help me understand the terms and concepts better.

I like that it's open and full of discussion. I also like all the ways in which she presents info—articles, videos, graphs, etc.

The professor is very enthusiastic and obviously loves what she does and it shows in our discussions. The topics are very interesting and really makes you think.

Our instructor is very knowledgeable and makes class fun. She deeply cares about our opinion and is more than willing to help.

The instructor is lively so it helps to stay focused. Also not textbook based which is good and bad.

I appreciate that the class is geared to our interests rather than strictly by a book.

I like her originality. I like that she is making us a part of deciding what to study.

I enjoy the freedom to come up with topics I am interested to learn about that potentially steer the class.

2. What would you like to change about this course?

I wouldn't change anything.

No

Some topics are pretty vague and its difficult to see exactly where we're headed with a topic sometimes.

I would prefer if it could be spread to shorter but multiple days throughout the week. The long time period has its benefits but also can cause zoning out due to its length.

Nothing! [smiley face]

She used to just give assignments through email. She starts putting them on Moodle. That way there is one place to look for assignments. I get too many emails and hers got lost a couple of times.

Cover some of the lab info to help us better understand or comprehend the lab work.

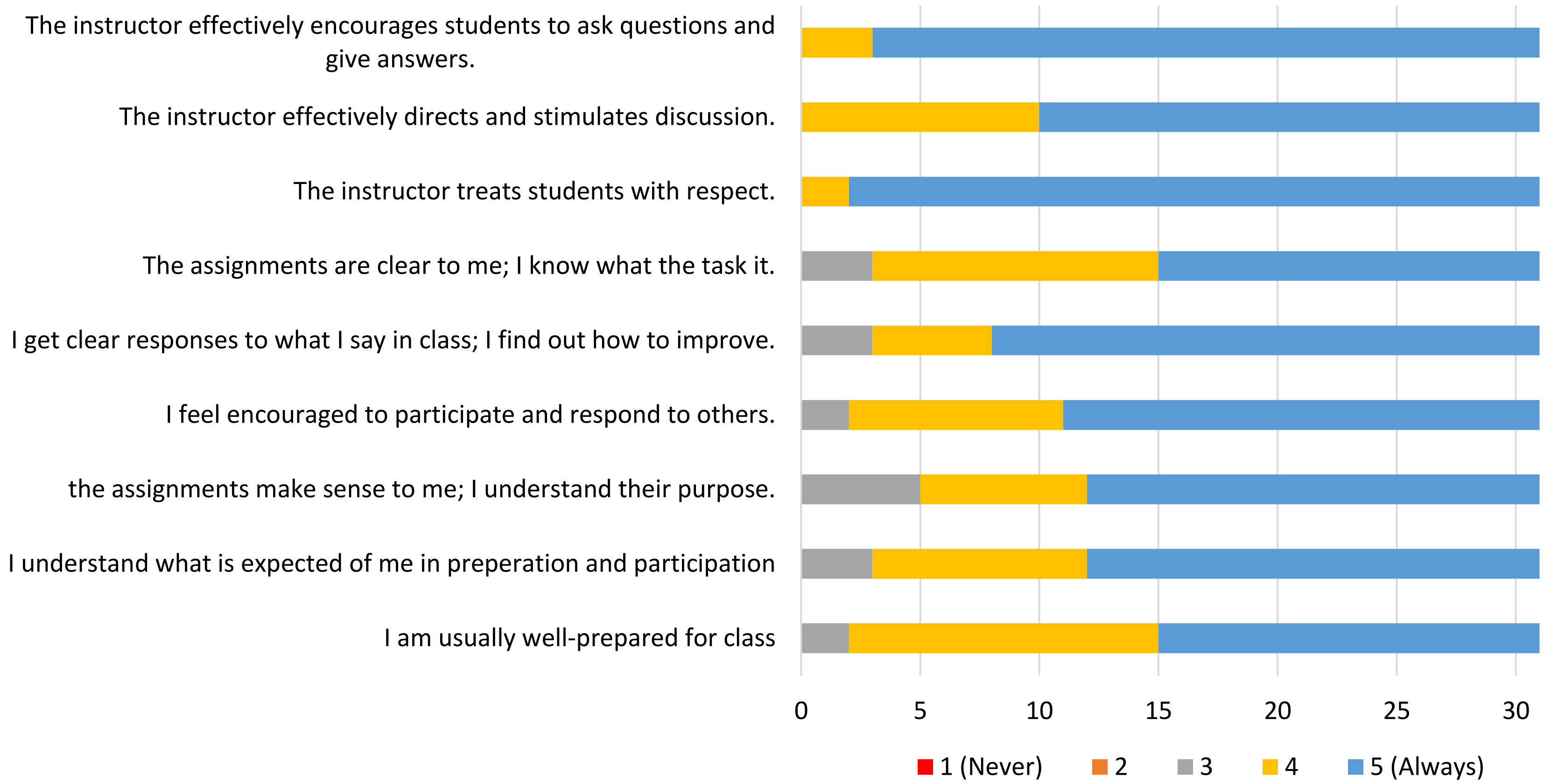
I would like more time spent in formal lecturing, possibly a slide presentation each week.

I feel it is a little too unstructured for my liking. I would like if there were some powerpoints.

I like not having a textbook, but maybe an online manual we can refer to for some topics

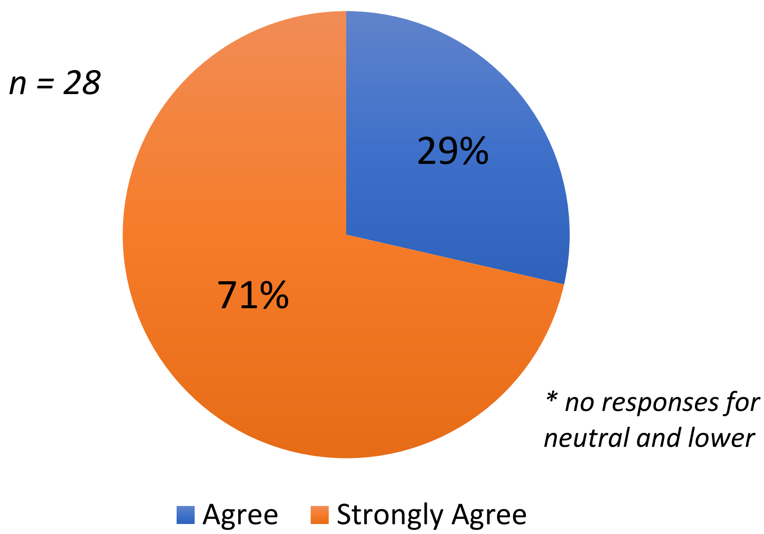
I wouldn't change anything, although I wish a class was offered in IF.

Not learning the same material between class and the lab makes the lab more difficult. It forces you to learn the lab material completely separate from the class, almost making the lab feel like a lecture, not being able to focus on the lab itself. It makes you need to work much harder to learn all the material. If this class didn't need a lab, it would rock!


The instructor effectively encourages students to ask questions and give answers.
The instructor effectively directs and stimulates discussion.
The instructor treats students with respect.
The assignments are clear to me; I know what the task it.
I get clear responses to what I say in class; I find out how to improve.
I feel encouraged to participate and respond to others.
the assignments make sense to me; I understand their purpose.
I understand what is expected of me in preperation and participation
I am usually well-prepared for class
0
5
10
15
20
25
30
1 (Never)
2
3
4
5 (Always)

Final Course Evaluations
Bio 1100 2018

1. I have a greater understanding of biological concepts and their relation to society than at the beginning of the course.



2. How have each of the following affected your learning? Briefly, suggest any changes to each that might improve your learning and comprehension.

a. Lack of required textbook, use of free sources, current *etc.*

- Helpful for the wallet for sure. The free sources are helpful in many ways. First, they tend to be more current and as such more relevant and relatable. Second, it helps to lean the credibility by forcing us to look at the aspect and possible issues.

- Stephanie Zorio was phenomenal at providing resources like articles, papers, books that helped us get quality knowledge about the subject we discussed in class. No changes.

- I loved having no textbook, it required me to be more active in class.

- I enjoyed not having a textbook and being able to use free sources. I think it made an overall better learning experience and the class enjoyable.

- I love that there wasn't a required textbook. It allowed for more of a diversity in subjects and room for us to talk freely. I wish more of my classes used some of the sources we used in this class. I would suggest maybe a weekly news site to scan before class to discuss in the first couple minutes of class.

- I liked this very much. Not being trapped in the curriculum of a book was liberating.

- Not using a textbook seemed weird at first but it turned out really good and Stephanie gave a lot of info to help us!

- It was actually even better without a textbook. Steph really was amazing!

- I feel as though I learned more from sources outside of textbooks as I had more motivation to read and the sources typically were more interesting than a text book.

- Love everything about all of these. Much better knowledge retention.

- I still learned a lot and now understand biology more than before.

- The sources were very helpful.

- I actually enjoyed not having a textbook. I though the class was a lot more interesting when we utilized different types of articles and even current event news. I think future classes would enjoy a similar format. Reading articles made the class entertaining and easy to digest versus just reading out of a textbook.

- The lack of a textbook and free learning style of the class was fun and made things interesting. It was difficult in the lab at times because we would be on two separate topics, so being prepared for the lab was harder. Besides that, it was awesome.

- No need for changes.

- I didn't feel a textbook was needed. I feel getting the reading material on Moodle was better. I am not upset I didn't have a heave textbook to lug around.

- The lack of a textbook was not a loss for me. I was still able to learn a lot and incorporate the free sources and current news very well. I actually prefer this method of learning. It felt more hands-on and an active way of learning.

- I appreciated it. It saved me money and the energy of carrying a book around.

- A lack of textbook did not hinder the learning process. A better use of Moodle would help with online sources.

- I liked that we had more freedom/ open-ended learning. I've never been on to lean well from a textbook. I would have liked, if possible, to have known that the book wasn't needed beforehand.

- At first it felt a bit awkward not to be following a textbook along with lab. However, the work and freedom were great and a lot of it fell in line with our lab work. I liked reading and learning new concept through articles and newspapers.

- No, it was better to not have a textbook, more affordable as well.

- It was nice not having a book, but it was hard to study for exams.

- It was refreshing to not have a textbook for this course and to be able to choose what content we wanted to cover.

- She provided great resources on Moodle and in class. It was current and even stuff you don't think about.

- I feel like the course structure and lack of textbook benefitted my learning because it allowed the learning to be more free-flowing and interest specific. I was able to take more interest and take more in.

- Current news kept me updated. Not having a textbook is cool, we don't have to worry about forgetting it at home.

- Keep this please! Free sources have been very helpful. As well as talking about current events. Perhaps using more real world/ current events.

b. Classroom exercises (including group work and lectures)

- I enjoyed the interactive set-up in this class. It's so easy to get lost in the background and in so doing get complacent about the information, as we regularly discuss the information it forces us to be somewhat knowledgeable beforehand.

- We were pushed to do group work and a lot of class discussion that really helped me understand more and dig deeper. I've never understood more in a science class.

- The classroom exercises helped tremendously with learning and I loved how interactive everything was.

- At the beginning we did a lot more hands-on and worked in groups. I know some students had mentioned that they preferred the traditional lecture, but I enjoyed the hands-on and group exercises. I felt like I retained the information much more by being in the middle of it. It also made the time go by fast and with this being a night class it made it nice.

- I didn't like the large group projects since it kind of disrupted the flow. But the lectures and small groups were beneficial and informational.

- Very well throughout creating a good learning environment.
- Great lectures! Never felt boring, very interactive and fun!
- Incredible, Steph was very informative and enthusiastic. I really enjoyed the class.
- I enjoyed more formal lectures to give clear direction, but also enjoyed discussion based lecture to keep engaged during the 3 hours.
- Fun. Better retention when it's fun. Also gives students ore of a vested interest in the class.
- Perfect. They really helped understand and get broader ideas of the subjects.
- Love them. She was amazing at making sense, you feel?
- I enjoyed the lecture in class and the way they were formatted, the group work was fine and helpful, but in some instances, I think it was unnecessary.
- I liked how the class was interactive and allowed for everyone to be involved. I learn better when actually doing something. Maybe we could've done things with our hands? Create models, built things…
- Got me more engaged, made me memorize concepts better.
- I feel they were extremely helpful and worked well. Especially once I got a hang of the process for the class.
- Lots of classroom exercises were helpful and very important to my learning so much in this class. She made classroom exercises fun and enjoyable and made sure we had enough information to work with.
- At first I didn't like all of the group exercises, but talking to others and bouncing ideas off of each other helped me understand the concepts.
- Greatly increased learning awareness. The groups could be better structured so more people participated.
- I like slide show presentations. Group work was okay. Didn't like standing and presenting, as my anxiety flared up, but that's just me [smiley face].
- Working in groups helped me the concepts in different ways and I could see how other people looked at things and learned a lot that way. The lectures were always interesting and easy to follow.

- Useful and helped with exams. Interesting topics.
- Class was never boring
- It was nice to be able to hear others' ideas in a group, but I would have liked less of that.
- She did a great job in lectures making sure we could add in our ideas.
- They were beneficial as they added to the conclusions and knowledge that was already formed from the assignments.
- It was interesting to learn and search new stuff. Group work helped me learn about others' view on the subject matter.
- Very helpful, group work has also been very helpful, me being I still group work is helpful.

c. Exams

- The exams were helpful, the information easy to use as we had in-depth conversation before taking them.
- The exams were NOT meant to trick us and Professor Zorio made that clear beforehand and in her actual exams. I was well-prepared and felt that the exams covered very fair material.
- They were not trickery and had questions about everything we had talked about.
- I liked the way the exams were set up. It challenged what we knew but also made us think hard about what we thought on certain subjects and why we thought that way.
- The exams tested me on all the material and there were no surprises. I would suggest more short answer questions instead of multiple choice or T/F questions.
- Very well written—they created though and efficiently measured concept understanding.
- Loved the exams, very straight forward. Exactly what we learned.
- She followed the study guide to a 'T'. she never gave a surprise questions, what was on the study guide was on the test!
- I feel the exams summarized learned content well.

- Perfect. I also like that the grading was not too critical. Other professors tend to put more importance on making exams difficult rather than use as a teaching aid.
- Perfect as well. Everything om the study guide was on the exams and it made it easy to understand and learn.
- Use nothing but some we learn in class which I loved.
- The exams were great it covered class lectures and were to the point. I never felt like I was answering trick questions.
- The exams were good. No part seemed an attempt to trick you like other exams I've taken.
- No effect on learning, but how much of the information I was able to retain. Leave the exams how they are.
- I felt challenged and I think they were good format and set up well.
- Exams were organized and well packed with the appropriate materials from what we learned.
- I really liked the fact that we wrote our own study guides, it prepared me for the test more than if I was handed one.
- The exams were very well thought out, no changes.
- I thought they were well-written, covered everything, and very much enjoyed how you could defend your answer, even for multiple choice questions.
- Good use of different layouts, multiple choice, T/F, and matching. Really liked the more in-depth questions, giving us a chance to express our knowledge.
- Similar to what we learned, no difficult. Review similar to exam.
- The exams were very straight forward
- Exams were good reviews of course content.
- Her exams came straight from lectures and didn't offer any bad surprises.
- They were easy to understand. I think the last was the hardest because it was the most open to interpretation and there was more room for misunderstanding.

- Great as is, as well with study guides and doing them together in class.

3. Overall, what were the most beneficial aspects of the course? Why?

- Getting engaged and having to do research. This gets your memory going.
- The way upbeat environment. Because it felt more inviting and exciting, made me want to learn more. As well as getting discussions started.
- The materials and topics chosen [sic] were excellent and very beneficial to my learning.
- Preparing study guides and learning from it.
- Everything! To be less vague, study guide, exercises, presentations, homework helped a lot.
- The fact that we were taught the subjects and topics we were interested in and not what the textbook said as it made it more engaging.
- For me in particular, I felt the hands on approach was extremely useful. I do so much better discussing what I am learning. It helps correct misconceptions right away while allowing me to present the information as I have interpreted it.
- Learning about current problems and showing how we can help.
- Stephanie Zorio! She most the beneficial aspect of this course! She was amazing and so fun to listen to.
- I learned a lot, a lot more than any other biology I ever took. I truly learned the meaning of biology.
- Guided group discussions. More participation means more knowledge retention.
- Learning a link of biology to human concerns. Biodiversity and environment.
- Liked that e got to make our own study guides for exams, but overall, I learned a lot in this class.
- There was no course work associated with a textbook. Very easy and interesting bio work.
- The way that we kind of bounced from subject to subject then in the end it was all related. I felt it was easier to grasp the concepts.

- Groupwork helped me learn the concepts better. I enjoyed going to the lab and working in the National Geographic app.

- Learning how to apply biology in my life/ in my career because I never considered it beforehand.

- The class lectures and the types of topics we discussed! It wasn't just the typical subjects I was expecting.

- No required textbook and in class discussion was very useful.

- Overall, I really enjoyed this class. Stephanie made it very interesting and fun. I looked forward to Tuesday night class.

- I liked the interactions and the exposure to different aspects of biology.

- The most beneficial aspect was the group discussion because it kept me engaged in the class.

- The group sessions were good because you got to interact and test ideas off classmates.

- The environment of free thought and acceptance of out of the box thinking.

- All of Stephanie's posts on Moodle really helped.

- I though the home work and articles were one of the most important because they have time to think and were interesting.

- Stephanie was the most beneficial she is an amazing educator who is truly innovating the way we should teach. She is helping us understood the way science connects to life instead of lectures. She truly cares about her students.

- The class discussion because it helped me think out loud and become comfortable

4. Overall, what were the least beneficial aspects of the course? Why?

- The class didn't match with the lab, made the lab more difficult.

- Personally, most of the things in this course were beneficial to me. But I'd have to say the one on one discussions with an individual. You learn more from bigger groups.

- I think this class could have benefitted from being spread out throughout the week. Kind of like brushing your teeth, best not squeeze it all in to one long session.
- Nothing was not beneficial. Everything I learned I know I'm going to use in my future. This class will help me with my major.
- The lab could have lined up better but I understand that is not any specific person's fault.
- Sometimes I wasn't really sure when she was asking questions what exactly she wanted or where she was wanting us to go with the topic.
- Maybe font be in too big of a hurry to think a student has a negative objective (so to speak).
- Some of the group work seemed unnecessary or repetitive.
- Steph Z. was amazing.
- I didn't like the way we did some of the discussions. In the large group it took more time and focus away from another subject we could be focusing on instead.
- The least beneficial aspect was the minor inconsistency in the way coursework was meant to be turned in.
- Nothing. Besides the fact that I spent over a hundred dollars on a book we didn't need. I was very upset about that.
- Some of the reading I thought weren't [sic] as helpful and TED talks as well as they didn't help me as much.
- None, they were all beneficial!
- No changes within the classroom! I LOVED Professor Zorio's class and have never been so successful in a bio class before.
- Nothing
- Not being able to submit assignment online all the time.
- I wish towards the end we could have done more hands on.
- There were a few parts of the group work that could have been a little more clear.

- As I said before, I didn’t love the various group presentations because I have anxiety, but that’s NOT to say they were a bad idea.
- I liked the way this course the way this course was structured already! It fit my way of learning! Thank you so much for making a late class so fun! [smiley face]
- I didn’t like how assignments were due at different times and days every week. I felt some class time was wasted on irrelevant info.
- How late the class was.
- Not having a book. Some of the stuff I didn’t really get.
- Very long class. Sometimes talked about irrelevant topics.
- The times, which can’t be helped. I feel people were tired and not as eager to participate.

5. How would you recommend changing the course format to get the most out of it?

- I wouldn’t change anything, in the first class we talked about interested us. We stuck to that and I think it worked well.
- I don’t think I’d change anything.
- The reading material was good, but I am a more visual person so maybe more videos or powerpoint presentations to so along with reading material. For example, the syphilis study maybe a youtube video about it if there is one.
- Better schedule times for the course.
- None! Stephanie Zorio is amazing!
- I would use 1/3 of class time for formal lecturing to provide notes.
- I actually really enjoyed the format of this course. If only it hadn’t been so late in the evening [smiley face].
- Nothing
- No changes.

- The only change I would recommend would be the time. It's kind of late. I realize it was my choice to take it at this hour. I am grateful I did though. A wonderful teacher and overall a great class.

- The class setup and material were great for me. I learned a lot of appreciated how knowledgeable and helpful the instructor was.

- Nothing, it was a great class.

- Maybe more get ideas about what we are learning about. It felt like we were learning but sometimes info seemed scattered! Great course overall!

- Nothing personally.

- Doing more hands on activated. Going somewhere to observe different aspects of biology in person.

- More current events, more interaction.

- Only discussing things related to class and class work. In all, very good class and interesting.

- I think it's good and does not require changes.

- Nothing! I really enjoyed this class! It was my favorite and it made me like science.

- I thought it was already beneficial, but 3 hours is a long time to be in one room. Nothing against the class though. 2-2.5 hours max.

- Have assignments due at the same time each week.

- Nothing, everything she taught was very formatted.

- I loved Stephanie's style for teaching (and her dress too, lol!). She was very enthusiastic and the only change I recommended is more of an outline for subjects to discuss and go off from there but those subjects are set for the year.

- I would go back to the hands-on and group atmosphere. I think the students get a Better idea of what other people think a different perspective. Stephanie—I really enjoyed your class! I think you are an amazing instructor! You ROCKED it! Good job!

- I truly think nothing should change I have never been so interested and educated in a science class as this one.