MICROORGANISMS AT THE INTERSECTION OF HYDROLOGY AND CO_2 EFFLUX IN SUBALPINE SOILS

by

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ABSTRACT

Subalpine forests are responsible for a substantial fraction of carbon (C) cycling in the western United States, with over 70% of the C sink activity taking place at elevations exceeding 750 meters. Soil microbial communities are key drivers of C cycling in these ecosystems, yet, factors that influence the composition of these communities and their activities across these heterogeneous subalpine landscapes are not well understood. Ten geographically distinct coniferous forest watersheds across western Montana were subjected to characterization of soil properties, carbon dioxide (CO_2) efflux, and community composition to determine the influence of heterogeneity in these watersheds on these properties. Moist, alkaline riparian soils had a higher net CO_2 efflux than drier, more acidic upland soils; soil temperature had no detectable effect on CO_2 efflux. The composition of microbial communities was also significantly correlated to variations in soil moisture content and pH. Dominant bacterial phyla in riparian soils were Proteobacteria while those in upland soils were Acidobacteria, suggesting that these components of these respective soil communities are at least partially responsible for variations in CO_2 efflux. Together, these data suggest that patchiness in subalpine soil properties within a watershed drive variation in the composition of soil microbial communities and their C cycling activities.

CHAPTER ONE

INTRODUCTION

The goal of this thesis is to understand interactions between carbon dioxide (CO_2) fluxes from subalpine forest soils, landscape heterogeneity, and the composition of soil microbial communities in subalpine forest ecosystems across western Montana. Subalpine forest ecosystems in the region are overall carbon (C) sinks. Due to the fundamental role that subalpine forest ecosystems play in global C cycling, it is important to gain a better understanding of how microbial communities influence carbon flux across heterogeneous landscapes. Interactions between environmental characteristics, microbial community structure, and soil CO_2 efflux are understudied in subalpine forest ecosystems, specifically how these components influence landscape level carbon fluxes from soil.

There are roughly 3.2×10^{24} grams (g) of C on earth (Marty, 2012). This C is heterogeneously distributed throughout the atmosphere, hydrosphere, lithosphere, and biosphere. Of the global C pool, roughly 4×10^{19} g of C is active at the earth's surface. Soils contain 2.3×10^{18} g of this C, and constitute its largest terrestrial reservoir (Schleshenger, 2005). In an ecosystem context, soils are important sites of C exchange, with fluxes into and out of the soil controlling C flow through ecosystems, and variable C residence times in soils controlling overall rates of C movement (Janzen, 2004).

C fixation and assimilation by terrestrial vegetation serves as a primary vector for atmospheric C input into soils, with land vegetation estimated to fix $1.2x10^{17}$ g C yr^{-1} (Schlesinger, 2005). Globally, half of the C fixed via photosynthesis is respired by the plants themselves (Farrar, 1985), while excess carbon is assimilated and eventually transported into the soil, where it can be respired via heterotrophic activity (Schimel, 1995), leached out of the soil as dissolved organic/inorganic C (Freeman et al., 2001; Kindler et al., 2011), or stored long term as soil C (Post & Kwon, 2000). These processes can happen over different time scales, which can lead to different residence times for the C within the system. Much of total soil C is lost to the atmosphere via carbonaceous gas fluxes from soil (Raich & Potter, 1995).

C turnover in surface soils is faster than deeper soils, as organic C at depth is less responsive to environmental changes (Schimel et al., 1994). Soil C fractions with the fastest C turnover times have been shown to be comprised of the least decomposed soil organic matter, with more decomposed organic matter showing slower turnover times (Trumbore, 2000). The top down addition of soil organic matter in forest soils leads to more labile C in the upper most depths, and more recalcitrant C with increasing depth. This influences C turnover rates with depth in soils. Moreover, higher microbial biomass and metabolic rates in surface soils can drive faster C turnover rates (Blume et al., 2002). Soil CO_2 efflux is a major component of this carbon loss, with an estimated $6x10^{16}$ g of C per year lost to the atmosphere as CO_2 from soil microbial heterotrophic respiration (Shao et al., 2013).

Variations in soil nutrient availability have been shown to influence C cycling in soils. Increased nitrogen (N) concentrations can decrease labile C turnover times in soils, while less change is seen in turnover rates of the soil C pool as a whole (Neff et al., 2002). When N is limiting in soils, increased rates of organic matter degradation has been shown, as soil microorganisms use N from these organic pools, leading to an increase in heterotrophic respiration (Craine et al., 2007). C use efficiency, or the ratio of growth over C uptake, can be affected by nutrient availability and temperature, affecting rates of heterotrophic respiration (Manzoni et al., 2012). Increases in this ratio can increase C storage in soils, while decreases in the ratio can lead to higher soil CO_2 production, ultimately influencing soil CO_2 efflux.

Changes in the soil environment (i.e., soil temperature) can cause shifts in the microbial community leading to changes in activity (i.e., C decomposition rates) (Zogg et al., 1997), or changes in the activity and size of the community, with changes in microbial biomass influencing the magnitude of soil C flux (Rochette & Gregorich, 1998). Soil microbial communities have been shown to be heavily influenced by their environment. Environmental characteristics such as soil pH (Lauber et al., 2009), soil water content (SWC) (Brockett et al., 2012), substrate availability (Myers et al., 2001), and soil temperature (Zogg et al., 1997) have been shown to influence the composition of soil microbial communities.

Soils are heterogeneous environments, with micro-scale complexity driving variation in soil parameters (i.e., pH, SWC, etc.). This creates a diversity of niche space across small distances (Vos et al., 2013), that can influence the composition and activity

of microbial communities. SWC has been shown to drive changes in microbial communities at the phylum level within soils, with different survival strategies enabling microorganisms to occupy different ecological niches across SWC gradients (Lennon et al., 2012). Moreover, differences in environmental characteristics create niches for evolutionarily adapted organisms to inhabit, and these niches can vary spatially within soils and across landscapes and temporally, as soils are subject to season variation in precipitation and temperature (Scott-Denton et al., 2003). Elevated microbial diversity has been demonstrated at the micron scale (Grundmann & Normand, 2000), to within a soil profile (Eilers et al., 2012), to across entire continents (Lauber et al., 2009). Because of the variable spatial and temporal scales across which partitioning of niches can occur, it is imperative to understand the scale at which environmental variation affects soil microbial communities and community function.

Environmental characteristics have been shown to influence soil CO_2 efflux via changing CO_2 production rates across a variety of environments. For example, in semiarid regions, soil rewetting events, such as heavy rainfall, have been shown to drive pulses in soil heterotrophic respiration (Rodríguez-Iturbe & Porporato, 2007). Decreases in SWC have also been shown to reduce metabolic activity of microorganisms, including a decrease in heterotrophic respiration (Schimel et al., 2007). In heterogeneous landscapes, such as subalpine forests, soils with drastic differences in environmental characteristics (i.e., SWC) can be found in close proximity due to topographic layout and heterogeneous distribution of soil properties (Gessler et al., 2000). Coniferous forest soils contain $1.6x10^{17}$ of the $2.3x10^{18}$ g of C found in soils. Although this is not the largest C

reservoir by soil type, coniferous forest soils are important sites for C cycling due to the large amount of land they make up in the northern hemisphere, as well as the magnitude of C exchange between terrestrial and atmospheric pools mediated by these ecosystems.

Subalpine forests, a subset of global coniferous forests, are one ecosystem where landscape level topographic controls can drive differences in soil characteristics. Subalpine forests are important C sinks, making up over 70% of the C sink in the western US (Schimel et al., 2002). These ecosystems can be topographically heterogeneous, leading to differences in SWC, soil pH, and nutrient availability across the landscape (Griffiths et al., 2009). This heterogeneity creates gradients in soil pH, temperature, SWC, and nutrients, which in turn, can drive variation in microbial community composition across topographically diverse landscapes (Swallow et al., 2009). Since microbial communities are responsible for an estimated 45.8% of CO_2 production in soils (Hanson et al., 2000), it is important to understand how geochemical gradients influence the composition of soil microbial communities. This thesis aims to fill gaps in the field of carbon biogeochemistry, and to develop a better understanding of how microbial communities influence CO_2 efflux in subalpine forest soils.

Here, I present an analysis of CO_2 efflux, geochemistry (SWC, soil temperature, soil pH), and microbial community composition in soils sampled from 10 subalpine forest watersheds. We show that Proteobacteria dominate riparian soils and soil CO_2 efflux is positively correlated with proteobacterial relative abundance, while Acidobacteria dominate upland soils, and soil CO_2 efflux is negatively correlated with acidobacterial relative abundance. These observations add to an evolving body of work

focused on microbially mediated C cycling and help to close a major gap in understanding by presenting new data on subalpine ecosystems that were previously understudied. These results provide new insights into how patterns in biogeochemical C cycling are influenced by soil microbial communities across heterogeneous landscapes.

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CHAPTER TWO

MICROBIAL HETEROTROPHIC RESPIRATION IN SUBALPINE SOILS

Introduction

Soils serve as an important global carbon (C) reservoir, storing an estimated 1.5 x 10^{18} g of C (Schlesinger & Andrews, 2000). This global C reservoir contributes an estimated 7.7 x 10^{16} g C yr^{-1} to the atmosphere, largely in the form of carbon dioxide (CO_2) (Raich & Potter, 1995). The CO_2 released is primarily the result of heterotrophic respiration by soil biota, making this biological activity the largest contributor of CO_2 flux to the atmosphere. The magnitude of the flux of CO_2 from a given soil is determined by the relative CO_2 consuming and producing activities of autotrophic and heterotrophic soil community members and plants (Welles et al., 2001; Raich et al., 2002). An estimated 30 to 50% of respired soil CO_2 is derived from the heterotrophic activity of plant roots, with the remaining CO_2 being derived from heterotrophic activity of soil microorganisms (Bowden et al., 1993).

Over 70% of the C sink activity in the western United States takes place above 750 meters (Schimel et al., 2002). Subalpine forest ecosystems tend to be at elevations greater than 750 meters and therefore are likely to have a substantial impact on the C cycle in the western United States. While subalpine ecosystems tend to be overall C sinks, the magnitude of the soil CO_2 efflux is a determining factor in the size of this sink. Moreover, the extent of the soil CO_2 efflux is influenced by soil water content (SWC) (Sjögersten et al., 2006), pH (Xu & Qi, 2001), and soil temperature (Raich &Potter,

1995), which vary across time and the heterogeneous topography associated with these ecosystems. Despite the complexity of these environments, SWC has emerged as the predominant control on soil CO_2 flux across heterogeneous subalpine ecosystems that exhibit moisture differences. For example, riparian soils exhibit higher rates of soil CO_2 efflux when compared to more dry, upland soils within these ecosystems (Pacific et al., 2008). Similarly, a previous study conducted in a ponderosa pine forest in a subalpine environment showed that soil temperature and SWC explained temporal variation in soil CO_2 efflux, while root and microbial biomass explained the spatial variation in efflux (Xu & Qi, 2001). In contrast to subalpine forests that appear to be primarily influenced by SWC, a study in North American hardwood forests showed pH to be a predominant control, where more acidic soils exhibited higher levels of CO_2 efflux (Xu & Qi, 2001). While identifying relationships between environmental characteristics and soil CO_2 efflux can help elucidate landscape level patterns of soil mediated carbon cycling (Qi et al., 2002), application of molecular genetic approaches to characterize soil microbial community members provides insight into the organisms potentially responsible for the observed changes in soil CO_2 efflux.

SWC and pH have been shown to exert an influence on the CO_2 efflux activity of soil microbial communities in forests (Xu & Qi, 2001), while soil pH appears to be the predominant factor influencing the taxonomic composition of those soil bacterial communities (Lauber et al., 2009; Rousk et al., 2010; Du et al., 2015; Kaiser et al., 2016). In addition to pH, other environmental variables that have been shown to influence the composition of soil microbial communities include SWC, nutrient availability, soil

temperature, and C availability (Zogg et al., 1997; Drenovsky et al., 2004; Lazzaro et al., 2009; Du et al., 2015). The heterogeneous nature of soils in subalpine forest watersheds results in gradients in soil pH, SWC, and temperature, which would be expected to influence the composition and activity of soil communities. Here, we examined the relationship between gradients in subalpine forest soil pH, SWC, temperature, the composition and abundance of soil communities, and their activity at the level of CO_2 efflux. We hypothesized that the composition of soil microbial communities contributes to patterns in soil CO_2 efflux across the landscape and thus varies with SWC. We also hypothesized that SWC exerts a significant and positive relationship with soil CO_2 efflux across subalpine forest ecosystems. To test these hypotheses, we identified unique subalpine watersheds within western Montana and subjected their soils to analyses of SWC, pH, temperature, CO_2 efflux levels, and microbial community composition.

Methods

Site Description

Ten watersheds were selected from across western Montana, U.S.A., for detailed hydrological, geochemical, and biological analyses. Five of these watersheds are in the Tenderfoot Creek Experimental Forest (TCEF) in Meagher County (Lewis & Clark National Forest), two of these watersheds are in the Lubrecht Experimental Forest in Powell County (Bureau of Land Management), one watershed is in the Big Belt Mountains in Meagher County (Lewis & Clark National Forest), one watershed is in the Swan Range in Lake County (Flathead National Forest), and one watershed is in the

Gallatin Mountains in Gallatin County (Gallatin National Forest) (Fig. 1). The elevation of these watersheds ranges from 1,000 to 2,218 meters, with the watershed in the Swan Range representing the lowest elevation and the watersheds within TCEF comprising the highest elevations. GPS coordinates and elevations of specific sampling sites are provided in Supp. Table 1.

Bedrock geology for each watershed was determined using USGS bedrock geology maps (Raines & Johnson, 1995). The bedrock underlying three of the TCEF watersheds is classified as sedimentary clastic or carbonaceous, while the bedrock underlying the other two TCEF watersheds is classified as tertiary and coarse grained igneous. The Big Belt watershed was located on Newland Limestone whereas the Gallatin watershed is located on Pre-Belt metamorphic bedrock. The two watersheds located in the Lubrecht Experimental Forest are underlain by the igneous Boulder batholith. The watersheds in the Swan Range are underlain by alluvially dominated sediment and metamorphic Grinnell argillite.

Sample Collection

Soil samples were collected between July 8, 2015 and August 15, 2015. At each watershed, soil samples were collected from depths of 5 cm and 20 cm at 4 sites along a riparian transect and 4 sites along an upland transect (Fig. 1). Sites along each transect were located roughly 100 m apart, and riparian and upland transects were located roughly 100 m apart. Soils were sampled with a sterile soil core and spatula, then were placed in sterile 15 mL Falcon tubes, and were placed on ice during their transport to Montana

State University where they were immediately frozen at -80°C until processed for molecular analyses.

Corresponding SWC and soil temperature measurements were made at each sample site, and samples were collected for determining soil pH in the laboratory. Soil temperature was measured for each site using an Omega Engineering handheld digital thermometer Model HH508 with a 25 cm stainless steel soil probe equipped with an internal type E thermocouple. The probe was inserted to a depth of approximately 12 cm (single measurement made for each sample location). SWC was measured using a HydroSense Soil Water Measurement System (Campbell Scientific, Logan UT) over the upper 12 cm. SWC is reported as the volumetric mean of triplicate measurements from each site. The pH of the soils was measured using an Oakton pH 2700 pH meter. Prior to measurement, soil subsamples were mixed with deionized water to create a 1:2 (gram to gram) slurry of soil:water. Following vortex mixing, the pH probe was inserted into the slurry, allowed to stabilize, and the pH was recorded. pH values are reported as single measurements for each soil sampled from each depth from each site (Supp. Table 1).

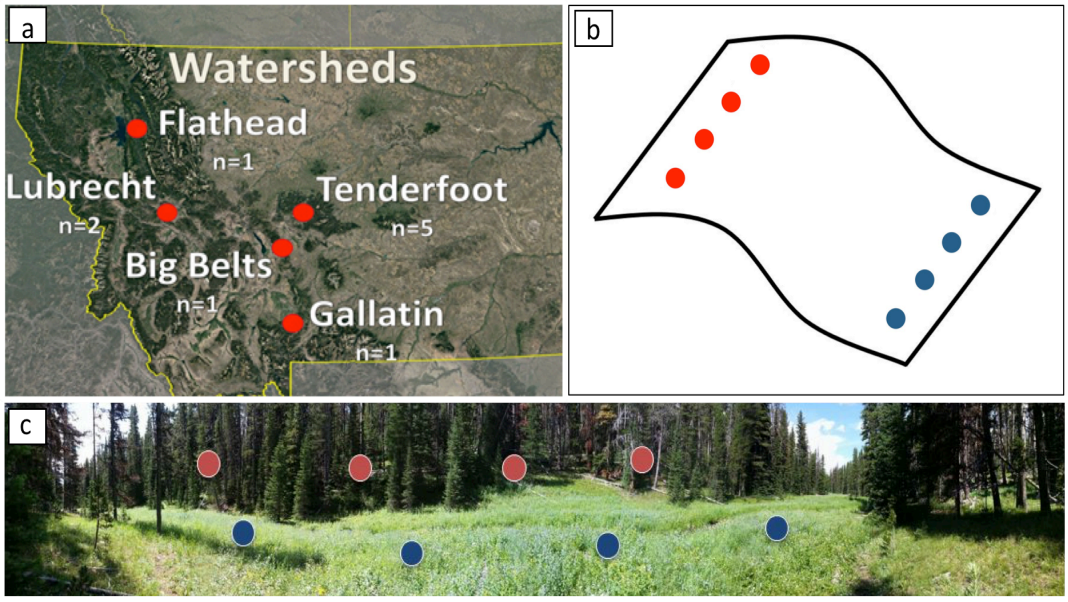

Figure 1. a) Location of watersheds across western Montana. The number of watersheds located within each sampling location is shown beneath each location. b) Schematic showing transect sampling design within an individual watershed, with riparian sampling locations (blue) in the bottom right, and upland sampling locations in the upper left (red). c) Stringer Creek Watershed, located in TCEF, with an example riparian transect (blue) and upland transect (red) (photo courtesy of Fabian Nippgen).

The efflux of CO_2 from soils was determined using an infrared gas analyzer (IRGA) with an attached soil respiration chamber. We used an EGM-4 portable IRGA coupled with an SRC-1 flux chamber (PP Systems, Amesbury, MA), which was allowed to normalize to atmospheric levels of CO_2 prior to placement of the chamber into the soil. Gas flux was measured based on the rate of increase of CO_2 in the chamber. No soil collar was used; instead, the sharp lower rim of the chamber was manually inserted about 1-2 cm into the soil to prevent ambient air leakage. The SRC-1 chamber has a 79 cm^2 footprint. The stated accuracy of the EGM-4 is ± 1%; however, it was calibrated with a certified 20,000 ppm CO_2 standard (Norlab, Boise, ID) with a stated accuracy of ±2%. CO_2 efflux values are reported as the mean of triplicate measurements for each site.

DNA Extraction/Sequencing

DNA was extracted from ~0.25 g subsamples of soil using the MoBio Powersoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer's instructions. Extracted DNA was quantified using a Qubit 2.0 Fluorimeter (Invitrogen, Carlsbad, CA, USA) and a Qubit dsDNA HS Assay kit (Molecular Probes, Eugene, OR, USA). Community 16S rRNA genes were PCR amplified from genomic DNA using the "universal" 515F-806R primers, while specifying the following cycling conditions: initial denaturation (4°C, 180 s) followed by 35 cycles of denaturation (94°C, 45 s), annealing (50°C, 60 s) and extension (72°C, 90 s), and a final extension at 72°C for 600 s. Amplicons of the 16S rRNA gene were sequenced using the Illumina MiSeq sequencing platform (2 x 151) at the Institute for Genomics & Systems Biology Next Generation Sequencing Core, Argonne National Laboratory.

Processing of Sequence Data

Roughly 17.2 million 151-base pair, paired end reads were generated for the 168 amplicon pools. DNA sequences were demultiplexed and merged using the 64-bit USEARCH version 7 (Edgar, Bioinformatics, 2010). Paired end reads were trimmed to a length of 253 base pairs (overlap of the 151 bp paired end reads). Using USEARCHv7, reads with an expected number of errors greater than 0.5 were discarded, singletons were discarded, and remaining sequences were assigned to operational taxonomic units (OTUs) using the UPARSE-OTU algorithm based on 97% sequence identity. A total of 6249 16S rRNA gene sequences were subsampled from each community. Sequences were classified using the Greengenes database (DeSantis et al., 2006), aligned using the PyNAST algorithm (Caporaso et al., 2009), filtered with the Greengenes lane mask file, and a phylogenetic tree was constructed using FastTree (Price et al., 2009). QIIME was used to generate a weighted Unifrac dissimilarity matrix from the phylogenetic tree (Caporaso et al., 2010).

Statistical Analysis

Statistical analysis of data was completed with the R statistical computing software (R Foundation for Statistical Computing, Vienna, Austria). Environmental statistics were completed using the entire environmental dataset, regardless of sites being excluded from biologic/genomic analysis. Pearson correlation was used to identify relationships between environmental variables and the relative abundance of taxa. Principle coordinates analysis (PCoA) was used to ordinate the 16S rRNA gene phylogenetic dissimilarity matrix and to examine this ordination for patterns with respect

to the taxa that contribute to patterns of clustering. The Inverse Simpson Diversity Metric (ISDM) was used to describe the alpha diversity of soil microbial communities. Communities with higher ISDM values indicate higher levels of diversity. A two-tailed t-test was used to identify statistical significance between soil environmental parameters measured among upland and riparian sites.

Results

Watersheds and Environmental Characteristics

The range in soil temperature for upland sites was 7.8°C to 21.4°C, while the range for riparian sites was 8.2°C to 17.2°C (Fig. 2). The mean (±sd) soil temperature in upland sites was (12.5°C±3.4) and in riparian sites was (11.6°C±2.1). Across the sampling sites, soil temperatures associated with riparian and upland sites were not significantly different ($P = 0.126$) (Fig. 2). However, within individual watersheds, the differences in soil temperature between riparian and upland sites were often significant, in particular in Bond Creek ($P < 0.001$), Lubrecht North ($P < 0.01$), and Lubrecht South ($P < 0.01$) sampling locations (Fig. 2).

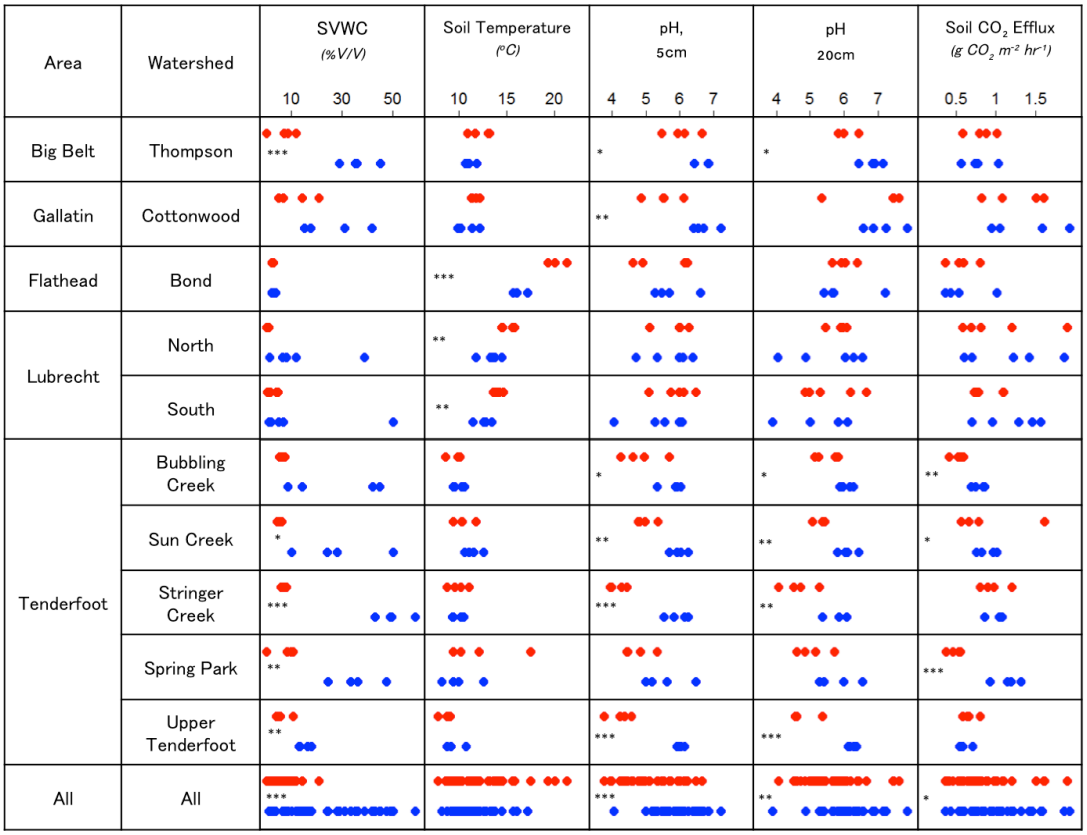

Figure 2. Plots of measured environmental variables within watersheds, colored by riparian (blue) or upland (red), as compiled for a total of 168 sampling locations across western Montana. Variables include SWC, soil temperature, soil CO_2 efflux, and soil pH at 5 and 20 cm. Asterisks within each figure panel represent significance, at *(P<0.05), **(P<0.01), and ***(P<0.001).

Volumetric SWC ranged from 1.4% to 58.9% at riparian sampling sites, and from 0.5% to 21.1% at upland sampling sites (Fig. 2). Riparian soils were wetter on average (SWC = 24.4% ±17.5) when compared to upland soils (SWC = 5.7% ±4.2). As such, SWC differences between upland and riparian sites were significant across the study (P<0.001). The differences in SWC between upland and riparian sites were not significant within Cottonwood (P = 0.089), Bond (P = 0.138), Lubrecht North (P = 0.090), Lubrecht South (P = 0.293), and Bubbling Creek (P = 0.067) watersheds (Fig. 2).

At riparian sites, soil pH ranged from 4.06 to 7.24 at 5 cm, and from 3.89 to 7.88 at 20 cm. In upland sites, the pH ranged from 3.77 to 6.67 at 5 cm, and 3.77 to 7.64 at 20 cm (Fig. 2). Soil pH differences between upland and riparian sites were significant across the study at both 5 (P<0.001) and 20 cm (P<0.01) depths. Soil pH was significantly (P<0.05) lower at 5 cm (mean=5.23) than at 20 cm (mean=5.60) in upland sites, while there was no significant difference (P>0.05) in pH with depth in riparian sites (Fig. 2).

Soil CO_2 efflux ranged from 0.37 g CO_2 m^{-2} hr^{-1} to 1.92 g CO_2 m^{-2} hr^{-1} in riparian sites, and from 0.37 g CO_2 m^{-2} hr^{-1} to 1.89 g CO_2 m^{-2} hr^{-1} in upland sites (Fig. 2). Soil CO_2 efflux differences between upland and riparian sites were significant (P <0.05) across the study. Within individual watersheds, the difference between soil CO_2 efflux in riparian and upland sites was significant within Bubbling Creek (P<0.01), Sun Creek (P<0.05), and Spring Park watersheds (P<0.001) (Fig. 2).

Relationships Between Environmental Characteristics

Pearson correlations of individual environmental variables (temperature, pH at 5 cm, pH at 20 cm, SWC, CO_2 efflux) were calculated using data collected from all sites (Table 1). All relationships between measured variables were significant, except between soil temperature and soil CO_2 efflux ($P = 0.47$), soil temperature and soil pH at 5 cm (P=0.19), and soil temperature and soil pH at 20 cm (P=0.25). SWC and soil CO_2 efflux were positively correlated across all sites (R=0.28, P=0.01). SWC and soil temperature were inversely correlated (R=-0.40, P <0.001). Soil pH at 5 cm was positively correlated with SWC (R=0.35, P <0.01), soil pH at 20 cm (R=0.72, P <0.001), and soil CO_2 efflux (R=0.26, P=0.02). Soil pH at 20 cm was positively correlated with soil CO_2 efflux (R=0.26, P=0.02) and SWC (R=0.32, $P < 0.01$).

Table 1. Correlations (Pearson *R*) between environmental measurements from all 168 sampling locations (8 sites per location, 10 for both Lubrecht watersheds) analyzed in this study. Bold values denote significance at a $P < 0.05$.

	Soil pH 5 cm	Soil pH 20 cm	Soil Temperature *(°C)*	Soil CO_2 Efflux *(g CO_2 m^{-2} hr^{-1})*
Soil pH, 20 cm	**0.72**			
Soil Temperature *(°C)*	0.14	0.13		
Soil CO_2 Efflux *(g CO_2 m^{-2} hr^{-1})*	**0.26**	**0.26**	-0.08	
Soil Water Content *(%V/V)*	**0.35**	**0.32**	**-0.40**	**0.28**

Microbial Diversity Metrics

A total of 6249 16S rRNA gene sequences were subsampled for each of the 168 soil communities analyzed in the present study. This subsampling excluded 34 sites due to a low number of reads from the sequencing run. The samples that were excluded due to subsampling are noted with an asterisk in Supp. Table 1.

16S rRNA gene sequences were subjected to analyses of their diversity, structure, and composition, as they relate to the characteristics of the soils from where they were collected. Significantly ($P<0.05$) more 16S rRNA gene OTUs were detected on average (1258 ±394) in riparian soils when compared to upland soils (923±320). Riparian soil communities were also found to be comprised of significantly ($P<0.05$) more diverse 16S rRNA gene assemblages (average ISDM = 122.0) when compared to those from upland soils (average ISDM = 76.6). Soil sampling depth was not significantly correlated with variation in the number of 16S rRNA gene OTUs in the soil communities analyzed. Significant positive correlations were found between SWC and the number of 16S rRNA gene OTUs ($R=0.39$, $P<0.05$) and the ISDM ($R=0.36$, $P<0.05$). Also, significant positive correlations were found between pH and the number of 16S rRNA gene OTUs ($R=0.42$, $P<0.05$) and ISDM ($R=0.47$, $P<0.05$).

Composition of Soil Microbial Communities

Principal coordinates analysis of the weighted Unifrac 16S rRNA gene dissimilarity matrix revealed that 52% of the total variance in the data can be explained by the first two axes, PCoA1 and PCoA2 (38% and 14% of the total variation, respectively) (Fig. 3). Assigning colors to communities based on site type (riparian versus

upland) showed that riparian soil communities (blue) were patterned based on soil microbial community composition. Over 80% of riparian sites had PCoA1 values less than 0, while over 60% of upland sites had PCoA1 values greater than 0 (Fig. 3). Over 63% of riparian sites had a PCoA2 values less than 0, while over 54% of upland sites had PCoA2 values greater than 0. Environmental variables and the relative abundance of the top 4 most abundant phyla (Proteobacteria, Acidobacteria, Verrucomicrobia, and Actinobacteria) are represented as vectors on the principal coordinates analysis.

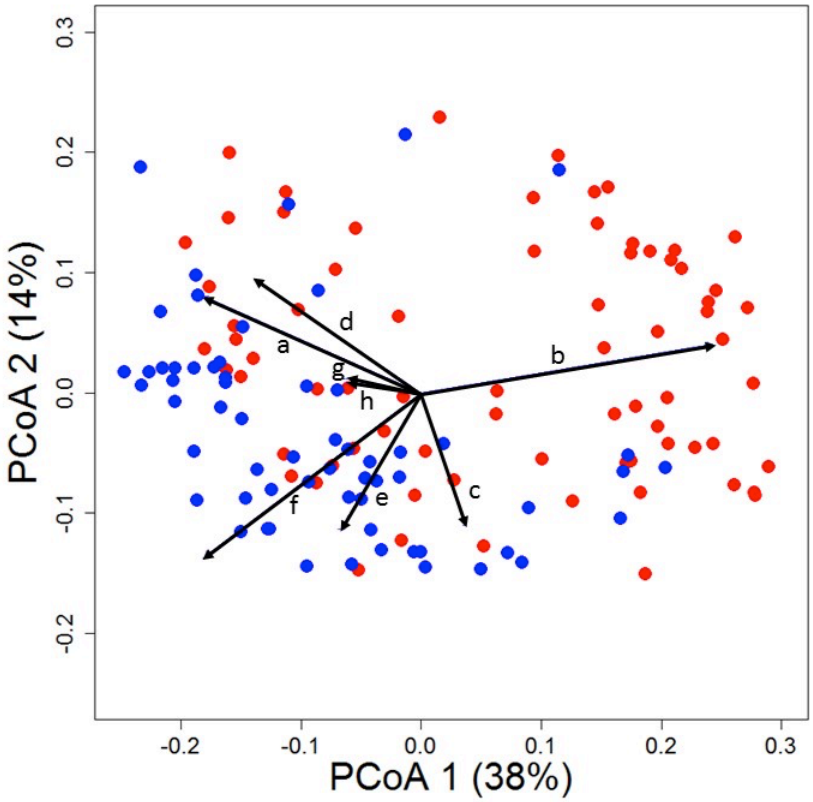

Figure 3. Principle coordinates analysis depicting the dissimilarity in Weighted Unifrac distances of community 16S rRNA gene sequences. Sampling sites were overlaid with landscape position, with blue dots depicting communities from riparian sampling locations and red dots depicting communities from upland sampling locations. Relative abundance of a) Proteobacteria, b) Acidobacteria, c) Verrucomicrobia, and d) Actinobacteria are overlaid on the ordination as vectors. Environmental variables e) SWC f) pH g) soil temperature and h) soil CO_2 efflux are also overlaid on the ordination.

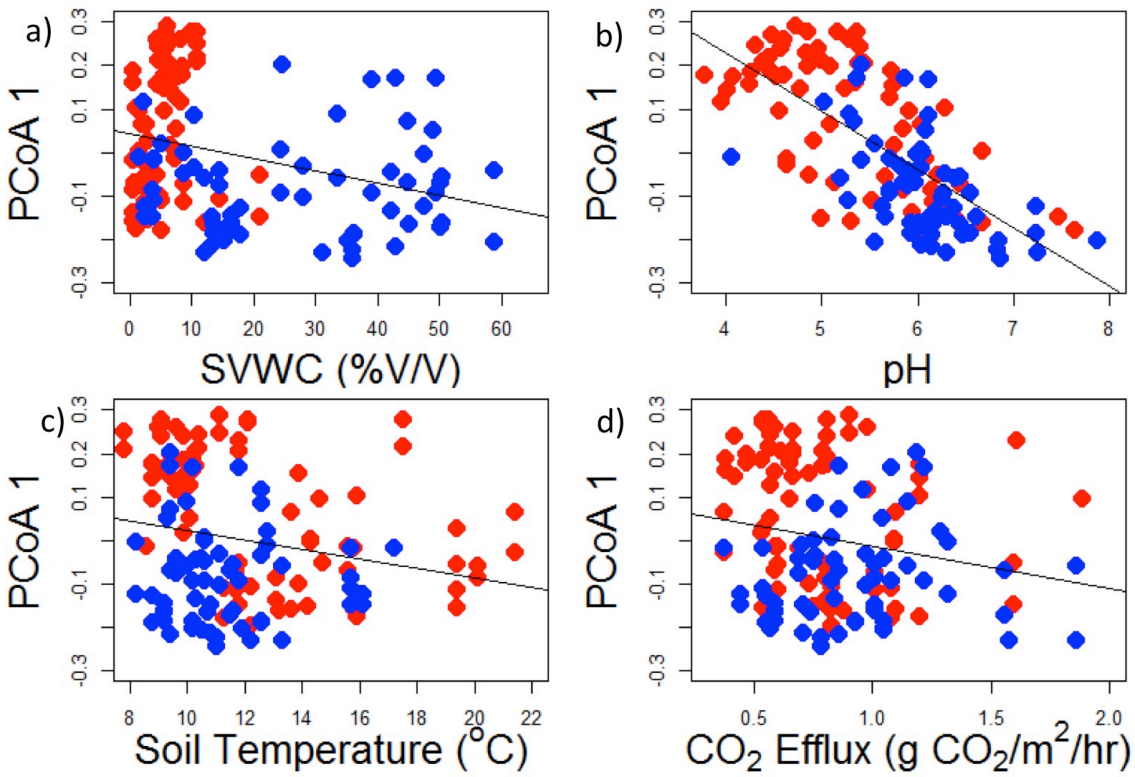

Figure 4. Plots of environmental variables against PCoA1. Pearson R values are listed by environmental variable. (a) SWC, R=-0.30, (b) pH, R=-0.70, (c) Soil temperature, R=-0.21, and (d) CO2 efflux, R=-0.20. All relationships are significant at P<0.05. Red points represent upland sites, blue points represent riparian sites

To identify which of the measured environmental factors were associated with this pattern of clustering, PCoA1 and PCoA2 axis values were regressed against measured environmental data. All environmental variables were significantly correlated with PCoA1, with soil pH exhibiting the strongest relationship (R=-0.70, P <0.001) (Fig. 4). Soil pH (R=-0.33, P <0.001) and SWC (R=-0.33, P <0.001) were significantly correlated to PCoA2 (data not shown).

A total of 64 bacterial phyla were identified across the sampling sites (Fig. 5), however, 4 dominant phyla accounted for between 52.4% and 94.6% of the total sequence reads. Sequences affiliated with Proteobacteria represented between 14.1% and 59.0% of the communities across sites whereas those affiliated with Acidobacteria ranged from 2.8% and 51.7% of the total communities across sites. Sequences affiliated with the Verrucomicrobia represented between 1.0% and 33.1% of the total communities across sites while those affiliated with Actinobacteria ranged from 0.6% to 34.3% of the total communities across sites.

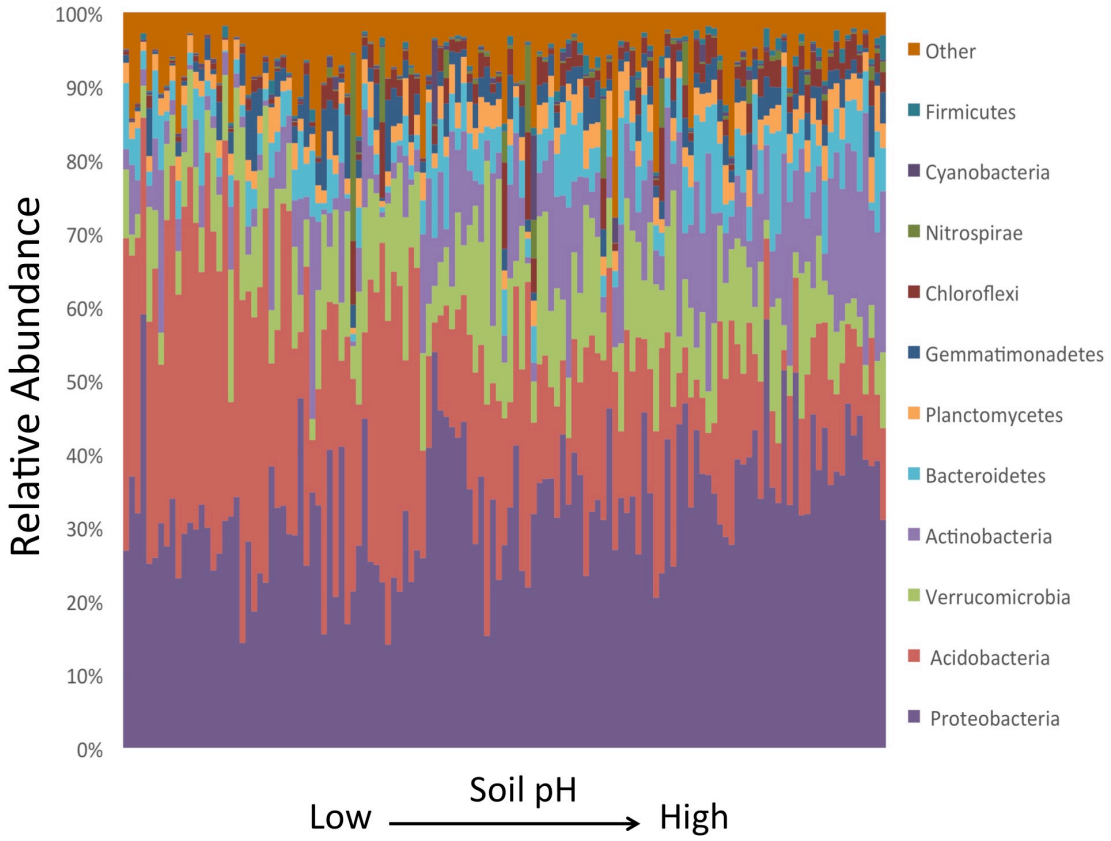

Figure 5. Composition of soil 16S rRNA gene assemblages as binned at the phylum level. The communities are arranged by the pH of the soils from which they were sampled, with those from the most acidic sites on the far left and those from the most alkaline sites at the far right. Soil pH ranges from 3.77 (left) to 7.88 (right).

To identify which of these phyla were contributing to the observed changes in community composition, we plotted vectors corresponding to their relative abundance on the PCoA plot (Fig. 3) to determine phylum-level compositional changes in ordination space. Along with the most abundant phyla, we plotted environmental variables and CO_2 efflux in ordination space to determine if trends in community composition were related to trends in environmental characteristics (Fig. 3). The vector representing acidobacterial relative abundance showed an increase in the direction of the upland site grouping in ordination space. Proteobacterial and actinobacterial vectors showed similar direction and magnitude when plotted in ordination space, towards lower PCoA1 values and higher PCoA2 values, populated by both upland and riparian communities. Verrucomicrobia show an increased relative abundance towards lower PCoA2 values. SWC and pH showed increasing values towards lower PCoA1 and lower PCoA2 values, populated by mostly riparian sites. Soil temperature and soil CO_2 efflux vectors are both low magnitude, and directed towards lower PCoA1 values.

To further characterize the influence of SWC, soil temperature, and pH on the taxonomic composition of subalpine soil communities, the relative abundances of the most prominent phyla were plotted against environmental variables (Fig. 4). The relative abundance of Proteobacteria was significantly related ($P<0.05$) to SWC ($R=0.30$), pH ($R=0.35$), and CO_2 efflux ($R=0.31$). Acidobacteria showed significant relationships ($P<0.05$) with soil temperature ($R=-0.26$), SWC ($R=-0.22$), pH ($R=-0.67$), and CO_2 efflux ($R=-0.28$). Verrucomicrobia showed significant relationships with SWC ($R=-0.26$) and

CO_2 efflux (R=-0.34). Actinobacteria showed significant relationships (P<0.05) with soil temperature (R=0.23), pH (R=0.49), and CO_2 efflux (R=0.25).

To identify if patterns observed at the phylum level were driven by trends from a lower taxonomic rank, we identified the most abundant classes within Acidobacteria. The most abundant classes within Acidobacteria included Acidobacteriia (0.0-27.9% relative abundance), DA052 (0.00-28.2% relative abundance), Acidobacteria-6 (0.00-10.8% relative abundance), Solibacteres (0.1-7.4% relative abundance), and Chloracidobacteria (0.0-14.4% relative abundance). The relative abundance of Acidobacteriia affiliated sequences was significantly correlated with soil pH (R=-0.75, P<0.01) and soil temperature (R=-0.33, P<0.01) while the relative abundance of DA052 affiliated sequences was significantly correlated with pH (R=-0.67, P<0.001), SWC (R=-0.31, P<0.001), and soil temperature (R=-0.21, P<0.05). The relative abundance of Acidobacteria-6 affiliated sequences was positively correlated with pH (R=0.76, P<0.001) and soil temperature (R=0.20, P<0.05). Solibacteres relative abundance was correlated with pH (R=-0.66, P<0.001) and SWC (R=-0.32, P<0.001). Chloracidobacteria relative abundance was significantly correlated with pH (R=0.37, P<0.001), SWC (R=0.18, P<0.05) and soil temperature (R=0.26, P<0.01). Soil CO_2 efflux showed significant relationships with the relative abundances of DA052 (R=-0.26, P<0.01) and Solibacteres (R=-0.39, P<0.001).

The most abundant classes within Proteobacteria included Alphaproteobacteria (4.33-39.51% relative abundance), Betaproteobacteria (0.34-19.59% relative abundance), Deltaproteobacteria (1.14-20.04% relative abundance), and Gammaproteobacteria (0.31-

37.30% relative abundance). The relative abundance of Alphaproteobacteria showed significant relationships with pH (R=0.25, P<0.01) and soil temperature (R=0.34, P<0.001), whereas the relative abundance of Betaproteobacteria showed significant relationships with pH (R=0.41, P<0.001) and SWC (R=0.45, P<0.001). Deltaproteobacteria relative abundance showed significant relationships with SWC (R=0.49, P<0.001) and soil temperature (R=-0.25, P<0.01).

A total of 253 known bacterial families were detected across the sampling sites. Unclassified families (from 13.4% to 56.3% relative abundance) made up the highest fraction of all samples. The most common classified families in the study included *Chthoniobacteraceae* (phylum Verrucomicrobia, ranged from <0.1% to 29.7% in relative abundance), *Koribacteraceae* (phylum Acidobacteria, ranged from 0% to 26.4% in relative abundance), and *Bradyrhizobiaceae* (phylum Proteobacteria, ranged from 0.5% to 17.8% relative abundance). Chthoniobacteraceae showed a significant, negative correlation with SWC (R=-0.31, P<0.001), while soil CO_2 efflux showed a negative correlation to the family's relative abundance (R=-0.30, P<0.001). Koribacteraceae showed a significant, negative correlation with soil temperature (R=-0.35, P<0.001) and pH (R=-0.57, P<0.001). Bradyrhizobiaceae showed a significant, negative correlation with SWC (R=-0.25, P<0.01) and a significant, positive relationship with soil temperature (R=0.34, P<0.001).

Discussion

Upland and riparian sites from geographically distinct subalpine watersheds across Montana differed in their hydrological and geochemical properties and thus offered a unique opportunity to examine how such differences influence microbial community composition and function. In this study, soil pH was shown to differentiate soils sampled from riparian and upland ecosystems, regardless of watershed location across Montana. This finding is consistent with a previous study conducted in a single watershed in Montana that also showed significant differences in soil pH in these landscape positions (Du et al., 2015). Likewise, SWC, albeit to a lesser extent, also differentiated these environment types. This observation is consistent with previous studies looking at landscape effects on these soil variables (Jencso et al., 2009). Other soil characteristics have been shown to differentiate across these landscape positions in subalpine forests, including organic N and various forms of C. Soil N concentrations in riparian soils at 20 cm within Stringer Creek were shown to be higher when compared to upland soils (Pacific et al., 2011). This study also showed a trend of decreasing C:N ratios moving from upland to riparian sites. The molar C:N ratios within riparian soils in *Pacific et al.* approached levels from previous literature that indicated enhanced microbial decomposition compared to the upland soils (Gholz et al., 2000).

We showed that the abundance of OTUs was significantly greater within riparian areas of watersheds when compared to upland areas. *Du et al.* also showed that within a subalpine watershed, riparian and midslope soils have higher levels of microbial diversity than upland soils (Du et al., 2015). Riparian zones have higher SWC when compared to

upland zones, and water limitation is a stressor on microbial life, which limits habitability for certain microorganisms and thereby limits overall diversity (Rothschild & Mancinelli, 2001). In contrast, increases in SWC have been shown to lead to increases in diversity. In forest soils, SWC has been shown to be positively correlated with the functional potential of soil microbial communities, which increases with diversity (Brockett et al., 2012).

Interestingly, the diversity of soil communities sampled in the present study was not influenced by soil depth, with samples collected from 5 and 20 cm depths showing similar levels of diversity. Soil organic matter and organic carbon decrease with depth (Jobbágy & Jackson, 2000), which would be expected to lead to a decreased potential for heterotrophic activity and thereby a decrease in microbial diversity, since soil microbial communities tend to be heterotroph dominated (Fierer et al., 2007). It is possible that we would have seen more significant differences with depth had we sampled deeper soils. Indeed, other studies have shown a reduction in soil microbial diversity of 20-40% in soils at a depth of 50 cm when compared to surface soils (Eilers et al., 2012).

While SWC and pH were shown to differentiate the environments into upland and riparian zones, soil pH appears to exert the strongest influence on the composition of the microbial communities. Previous studies have documented the prominent role of pH in shaping the composition and diversity of microbial communities in a variety of environments, including soil (Fierer & Jackson, 2006; Eilers et al., 2012), marine biofilms (Witt et al., 2011), and hot springs (Colman et al., 2016). It is not clear from the data collected in the present study why pH exerts such a strong control on microbial community composition in these soils that vary in pH from 3.77 to 7.88. We do, however,

see a very large range in pH values (4 orders of magnitude) across the study, while the range in other environmental variables was much smaller. This could account for the large explanatory power shown by pH. Considering that pH influences the bioavailability of metals and other nutrients (Violante et al., 2010), and influences the speciation of key nutrients such as ammonia, allowing it to potentially volatilize out of the system (Amend & Shock, 2001), it is possible that the strong influence of pH on these soil properties indirectly exerts a strong influence on the composition of communities. Along with influencing the availability of nutrients and metals, pH influences microbial cells at a physiological level. Many microbial cells maintain an intracellular pH close to neutral (Madigan et al., 1997), and more energy is required for cellular maintenance in suboptimal pH. In our study, we show a positive relationship between soil pH and observed OTUs, and previous studies have shown positive relationships between soil pH and microbial biomass (Bååth & Anderson, 2003). These pH based physiological constraints could partially explain the strong influence of pH on these communities.

The pH of soils was also associated with shifts in the predominant phyla observed in the soils, consistent with previous studies showing soil pH to be the primary variable associated with shifts in the taxonomic composition of communities (Lauber et al., 2009). The two most abundant phyla identified in this study were Acidobacteria and Proteobacteria, both of which are known to harbor members with broad metabolic and physiological diversity (Boone at el., 2005; Garrity, 2010). However, a large fraction of characterized members of both phyla are heterotrophs (Bergey, 2004; Ludwig et al., 2010), which dominate surface soils (Nannipieri et al., 2003) and can influence soil CO_2

production (Zhou et al., 2007). Proteobacteria tended to dominate riparian environments whereas Acidobacteria tended to dominate upland environments.

In understanding shifts in community composition across landscape position relative to these two dominant phyla, placing the observed trends in the context of the copiotroph-oligotrph spectrum described in *Fierer et al. 2007* helps us better understand these organisms roles in an ecological context. Copiotrophic microorganims are more competitive when there are high levels of available C and high levels of nutrient availability (low C:N, riparian soils), while oligotrophic microorganisms are more competitive in environments with low nutrient availability (high C:N, upland soils) due to higher substrate affinities of these microorganisms (Fierer et al., 2007). While we did not directly measure substrate availability in this study, previous studies in subalpine forests have helped us infer potential differences in substrate availability and nutrient ratios within our soils.

Proteobacteria show a positive relationship with both soil pH and SWC, as they tend to be found in higher abundances in riparian soils. These riparian soils, which past studies have shown tend to have lower C:N ratios (Pacific et al., 2011), have higher nutrient availability, which is associated with higher rates of microbial decomposition. Proteobacteria dominate these soils with nutrient availability and potentially high rates of microbial decomposition, indicating they fall on the copiotrophic end of this spectrum. Proteobacteria communities were dominated by Alphaproteobacteria in the study, which showed a positive relationship with pH. Betaproteobacteria, while less abundant overall, showed a positive relationship with both pH and SWC. In soils, Betaproteobacteria have

been shown to behave copiotrophically, responding to high levels of organic C and low C:N ratios (Fierer et al., 2007). Previous studies in soils have shown positive relationships between Betaproteobacteria and soil pH, while a study in Danish lakes showed the opposite trend with regard to pH (Ren et al., 2015). When we look at lower levels of taxonomy, however, the betaproteobacterial communities in the lake ecosystems were vastly different than those in soil environments. The lake betaproteobacterial communities were dominated by *Polynucleobacter* and *Ferrovum*, ande were shown to prefer lower pH (Ren et al., 2015). Within the soils from our study, the dominant members of the betaproteobacterial communities were *EB1003*, *Commonadaceae*, and *Bulkholdeiaceae*, which have been previously shown in soil environments. While we do see different responses of the betaproteobacterial communities to pH in these two studies, it is not surprising that the membership differs between lacustrine and soil environments.

Acidobacteria, on the other hand, show negative relationships with both soil pH and SWC, as they tend to be found in higher abundances in upland soils. These soils have been shown to have higher C:N ratios (Pacific et al., 2011), and are more nutrient limited. These conditions can limit rates of microbial decomposition. The dominance of Acidobacteria in these more nutrient limited upland soils pushes them onto the oligotrophic end of the spectrum (Fierer et al., 2007), where higher substrate affinities allow them to outcompete more copiotrophic microorganisms. Along with this, Acidobacteria have been shown to have higher tolerance for lower pH environments, like the upland soils within our study (Jones et al., 2009). The three classes of Acidobacteria (*Acidobacteriia, DA052, and Solibacteres*) that dominated the acidobacterial

communities in upland sites, showed negative relationships with soil pH. These classes, which dominate the acidobacterial communities in upland sites, fall on the oligotrophic end of the spectrum due to their prevalence in upland soils which likely have higher C:N ratios.

Observed differences in CO_2 efflux across our study indicated higher efflux levels in riparian soils and lower efflux levels in upland soils. This statistically significant difference across landscape position could be explained by a number of things. First, more optimal C:N ratios in riparian sites allows for higher rates of C mineralization, leading to higher CO_2 concentrations in soil pore spaces and potential for higher soil CO_2 efflux (Pacific et al., 2011). Along with more optimal C:N ratios in riparian areas, higher root biomass in riparian soils could account for increased soil CO_2 efflux via C mineralization by roots (Riveros-Iregui et al., 2009). Belowground root heterotrophic respiration influences CO_2 efflux, and although we were unable to separate microbial and root influences on CO_2 production and eventual efflux, we believe the changes in microbial community composition associated with changes in soil CO_2 efflux indicate these communities play a key role in this process within these soils. Considering dominant members of the microbial communities within our study in the context of the copiotroph-oligotroph spectrum can help us better understand this role. Proteobacterial (copiotroph) dominated communities in the riparian soils, with low C:N ratios, allow for higher C mineralization and hence, likely higher levels of soil CO_2 efflux. Acidobacterial (oligotroph) dominated communities in the upland soils with high C:N ratios can be limited by lower soil nutrient concentrations, leading to lower levels of soil CO_2 efflux.

While a number of variables and conditions can influence CO_2 efflux within soils, we propose the C mineralization rates of these microbial communities are influenced by soil nutrient availability and pH, limiting C mineralization rates in upland soils and increasing these rates within riparian soils.

This study illustrated relationships between soil physiochemical properties, microbial community membership, and soil CO_2 efflux. Using physiochemical properties to differentiate landscape position, we showed that pH strongly influences microbial communities, while community variation correlates with observed patterns of soil CO_2 efflux and can be influenced by soil nutrient concentrations. This information lends insight into microbial soil heterotrophic respiration in these environments, and will guide further investigations regarding the influence of these heterogeneous landscapes on relationships between soil properties, soil microbial communities, and soil CO_2 efflux.

Conclusion

SWC and pH showed significant, positive relationships with soil CO_2 efflux across the study. SWC and pH were also shown to drive differences in community composition across our watersheds, indicating that these variables influence community composition, which in turn influences CO_2 efflux via heterotrophic respiration within these watersheds. Some trends were seen at a phylum level, while it took a finer taxonomic lens to parse out other taxonomic patterns in relation environmental variables, community composition, and the ecosystem function of CO_2 efflux across subalpine forest ecosystems.

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CHAPTER THREE

CONCLUSION/FUTURE DIRECTIONS

This thesis aimed to define relationships between environmental variables, soil microbial communities, and ecosystem function at the level of CO_2 efflux. Much of the previous work on soil microbial community ecology has been largely limited to descriptive studies and has focused on describing the presence and abundance of organisms within or across ecosystems and physical and chemical gradients. While these studies revealed new insights into the factors that influence soil community composition, they largely overlooked how such shifts in community composition related to ecosystem function. This work aims to bridge this gap by studying the composition of communities along physiochemical gradients while at the same time measuring the function of communities via heterotrophic respiration at the level of CO_2 efflux.

In this work, I showed that environmental characteristics change across landscape position in subalpine forests and that this in turn influences community composition and soil CO_2 efflux. Major shifts in the phylum level taxonomic composition of soil communities were observed in soils sampled from upland versus riparian sites, which themselves are differentiated by changes in SWC and soil pH within the top 20cm. Specific class level groupings drive the changes in the two most abundant phyla, Proteobacteria and Acidobacteria. We saw that Proteobacteria dominate riparian soils, while Acidobacteria dominate upland soils. Within Proteobacteria, Alphaproteobacteria are the dominant taxa, but are also responsible for trends observed at the phylum level.

Within Acidobacteria, we see three classes (Acidobacteriia, DA052, and Solibacteres) that show inverse relationships with SWC and pH, while CO_2 efflux shows an inverse relationship with the relative abundance of these classes. The two acidobacterial classes that show opposite trends with these environmental variables (Acidobacteria-6 and Chloracidobacteria) are prominent members of the group responsible for the still notable presence of Acidobacteria within riparian sites.

With predicted increases in average temperature alongside predicted changes in precipitation quantity, frequency, and timing, it is important that we understand potential changes to global biogeochemical cycles. As previously stressed in Chapter 2, subalpine forest ecosystems are important in global carbon cycling, especially in the western United States. However, the patchy and heterogeneous characteristics of these watersheds make them difficult for study and it is easy for overgeneralizations to be made. Adding a community-scale microbial component to current literature will help with future studies and predictions to changes in CO_2 efflux in response to climate change.

We were unable to separate root and microbe derived CO_2 in this study. Previous studies have looked at root biomass across landscape position within subalpine forest soils, which could help us better understand the contribution of root driven C mineralization to the total. Going one step further, future work to separate these sources coupled with microbial community analysis could help us better understand which microbial taxa are responsible for C mineralization, and which taxa may be forming relationships with the roots that are responsible for this CO_2 production. Including a more

comprehensive survey of plant diversity across sites would also allow for a better understanding of CO_2 sources and plant-microbe interactions.

While our study allows us to gain greater insight into soil microbial communities present within subalpine forest soils and their relationships to environmental characteristics and soil CO_2 efflux, one major limitation is our inability to identify the taxa directly responsible for below ground CO_2 production. Observed changes in community composition across the landscape due to changes in environmental variables can help to lead us the direction of possible taxa responsible for CO_2 production, but further study and different techniques would be necessary to fully understand this relationship. Adapting this study using RNA rather than DNA to better understand which organisms are active and potentially responsible for changes in CO_2 production via heterotrophic respiration would be important complimentary work in this regard.

Another potential future approach would involve using stable isotopically enriched isotopes (^{13}C) to trace carbon through the system and identify which organisms are responsible for CO_2 production, with the assumption that community members that are mineralizing C are also assimilating a portion of that C into biomass. Using a microcosm approach, soils from distinct landscape positions, exhibiting different physiochemical variables (e.g., pH, SWC) would be chosen and microcosms would be installed as described in Chimner et al. (2003). ^{13}C labeled sucrose would be added to individual microcosms in order to observe which community members respond to sucrose additions (Padmanabhan et al., 2013). The amount of labeled C added to each microcosm would be standardized to the amount of available C present in each soil prior

to microcosm installation to account for shifts in established communities reacting to new available C inputs.

Using a time series approach, samples would be taken before substrate addition, at time 0, and throughout the experiment in order to observe temporal responses to the substrate additions. Soil property (pH, SWC, etc.) measurements would be made at each time point, headspace CO_2 would be sampled, and soil samples would be taken for DNA extraction. ^{13}C labeled bicarbonate additions would constitute controls to account for potential autotrophic assimilation of labeled CO_2 within microcosms. The DNA would be extracted and separated using buoyant density gradient centrifugation, as shown in Neufeld et al. (2007). This would allow the separation of DNA that has incorporated the ^{13}C from non-labeled nucleic acids. The V4 region of the 16S rRNA gene from the labeled DNA would be amplified and sequenced in order to understand the community members responsible for C mineralization. These sequences would be compared to communities from total soil 16S rRNA gene sequencing of the microcosms to better differentiate functional groups within these communities responsible for CO_2 production and C assimilation. With this additional component, we could identify which taxa are responding to changes in specific environmental variables (e.g., pH, SWC) at the level of CO_2 production. Pairing this with more in-depth characterization of soils, we could quantify diffusion rates and physical limitations between CO_2 production and ultimately soil CO_2 efflux.

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APPENDICES

APPENDIX A

ENVIRONMENTAL MEASUREMENTS

Supplementary Table 1. Name, location, elevation, environmental variables, and sampling date for each sampling site in the study. A soil sample from 5 cm and 20 cm was taken from each site for pH and molecular analysis. Samples excluded from molecular statistical analysis due to low number of reads after sequencing are denoted with an asterisk under the pH columns.

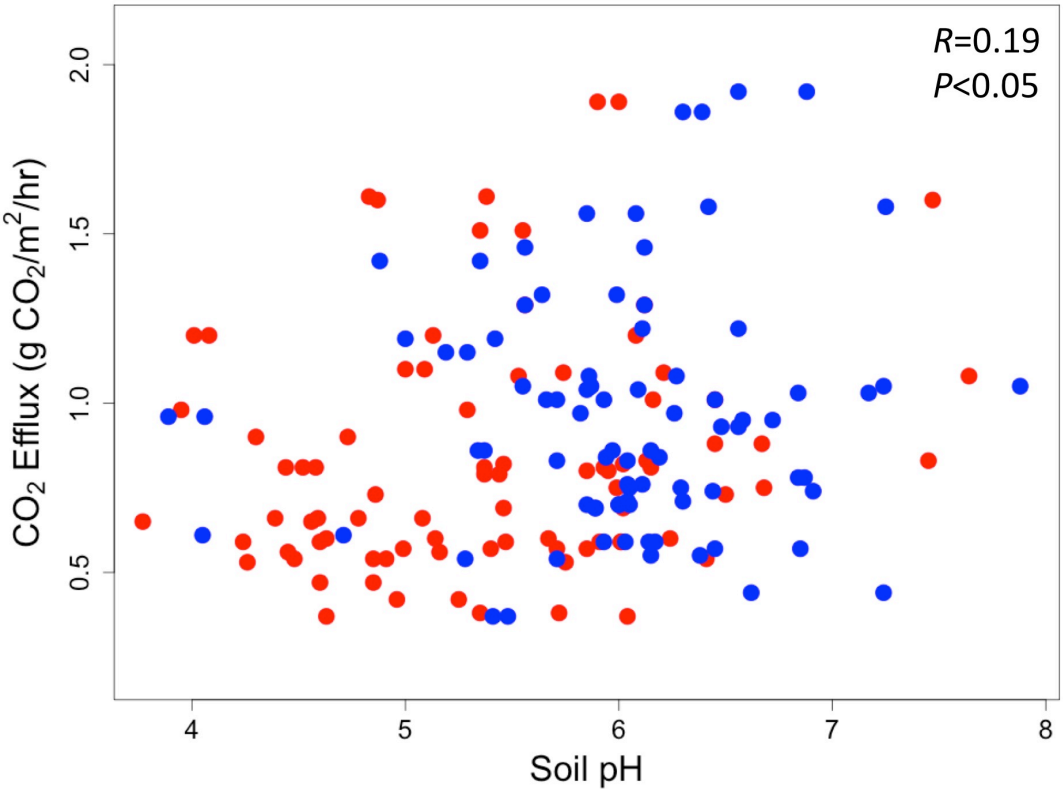
Watershed	Transect		Latitude Longitude	Elevation *(Meters)*	Soil CO_2 Efflux *(g CO_2 m^{-1} hr^{-1})*	SWC *(%V/V)*	Soil Temp. *(°C)*	pH *5 cm*	pH *20 cm*	Bedrock	Sample Date, Julian *(2015)*
Thompson Gulch	R	1	46.5157 -111.2153	1934	1.03	29.0	11.1	6.84*	7.17*	Newland Limestone	197
	R	2	46.5158 -111.2148	1932	0.57	35.3	11.9	6.85	6.45*	Newland Limestone	197
	R	3	46.5162 -111.2142	1927	0.78	35.9	11.0	6.87	6.84	Newland Limestone	197
	R	4	46.5164 -111.2129	1921	0.74	45.1	10.7	6.44	6.91*	Newland Limestone	197
	U	1	46.5162 -111.2155	1936	0.59	7.2	10.9	5.47*	6.01*	Newland Limestone	197
	U	2	46.5164 -111.2149	1931	1.01	8.7	11.7	6.16	6.45	Newland Limestone	197
	U	3	46.5167 -111.2142	1923	0.88	12.1	13.2	6.67	6.45*	Newland Limestone	197
	U	4	46.5169 -111.2134	1906	0.80	0.5	13.1	5.95	5.85	Newland Limestone	197
South Cottonwood Creek	R	1	45.5342 -111.0801	1768	1.58	31.1	12.2	6.42*	7.25	Pre-Belt Metamorphic	219
	R	2	45.5338 -111.0798	1770	1.92	17.7	11.4	6.56*	6.88*	Pre-Belt Metamorphic	219
	R	3	45.5332 -111.0791	1771	0.95	41.8	9.9	6.72*	6.58*	Pre-Belt Metamorphic	219
	R	4	45.5329 -111.0786	1771	1.05	15.3	10.2	7.24	7.88	Pre-Belt Metamorphic	219
	U	1	45.5337 -111.0806	1798	1.60	21.1	11.8	4.87	7.47	Pre-Belt Metamorphic	219
	U	2	45.5333 -111.0802	1808	1.51	6.9	11.4	5.55*	5.35*	Pre-Belt Metamorphic	219
	U	3	45.5329 -111.0794	1805	0.83	14.4	12.2	6.13	7.45	Pre-Belt Metamorphic	219
	U	4	45.5327	1793	1.08	5.2	11.3	5.53	7.64	Pre-Belt	219

			-111.0790							Metamorphic	
Bond Creek	R	1	47.9230 -113.8123	1000	0.44	2.4	16.1	6.62	7.24	Alluvium/ Grinnell argillite	182
	R	2	47.9230 -113.8125	1013	1.01	3.7	15.7	5.71	5.66	Alluvium/ Grinnell argillite	182
	R	3	47.9229 -113.8128	1018	0.54	3.9	15.7	5.28	5.71	Alluvium/ Grinnell argillite	182
	R	4	47.9230 -113.8128	1018	0.37	3.9	17.2	5.48*	5.41	Alluvium/ Grinnell argillite	182
	U	1	47.9238 -113.8126	1043	0.54	3.0	19.4	4.91	6.41	Alluvium/ Grinnell argillite	182
	U	2	47.9237 -113.8128	1043	0.60	2.5	19.4	6.24	5.67	Alluvium/ Grinnell argillite	182
	U	3	47.9237 -113.8130	1045	0.81	3.0	20.1	6.15	5.93	Alluvium/ Grinnell argillite	182
	U	4	47.9236 -113.8134	1046	0.37	2.8	21.4	4.63	6.04	Alluvium/ Grinnell argillite	182
North Lubrecht	R	1	46.8869 -113.2980	1721	0.61	8.3	14.5	4.71*	4.05*	Boulder batholith	227
	R	2	46.8866 -113.2978	1710	1.86	12.1	13.3	6.39	6.30	Boulder batholith	227
	R	3	46.8862 -113.2974	1732	1.42	6.8	13.8	5.35*	4.88*	Boulder batholith	227
	R	4	46.8860 -113.2970	1734	1.22	39.0	11.8	6.11	6.56	Boulder batholith	227
	R	5	46.8860 -113.2968	1736	0.70	1.7	13.6	6.00*	6.05*	Boulder batholith	227
	U	1	46.8868 -113.2970	1724	1.20	0.9	15.9	6.28	5.97	Boulder batholith	227
	U	2	46.8868 -113.2967	1747	0.59	0.7	15.6	5.13	6.08	Boulder batholith	227
	U	3	46.8866 -113.2964	1747	1.89	1.4	14.6	6.01*	5.91	Boulder batholith	227
	U	4	46.8866 -113.2963	1750	0.69	0.5	15.8	6.00	5.90*	Boulder batholith	227
	U	5	46.8865 -113.2961	1743	0.82	1.0	14.5	6.02	5.46*	Boulder batholith	227
South Lubrecht	R	1	46.8924 -113.3013	1761	0.96	2.3	12.6	5.27*	5.02	Boulder batholith	227

	R	2	46.8926 -113.3013	1749	0.70	1.4	12.8	4.06	3.89*	Boulder batholith	227
	R	3	46.8926 -113.3013	1739	1.56	50.2	11.5	6.00	5.85	Boulder batholith	227
	R	4	46.8928 -113.3013	1737	1.46	6.9	13.5	6.08*	5.85*	Boulder batholith	227
	R	5	46.8928 -113.3012	1739	1.29	5.1	12.8	5.56	6.12*	Boulder batholith	227
	U	1	46.8924 -113.3010	1761	1.10	2.0	13.6	5.09	5.30	Boulder batholith	227
	U	2	46.8924 -113.3010	1758	0.75	0.8	14.2	6.12*	5	Boulder batholith	227
	U	3	46.8926 -113.3009	1761	1.09	1.4	14.3	5.99	6.68	Boulder batholith	227
	U	4	46.8928 -113.3009	1755	0.73	4.8	13.9	5.74	6.21	Boulder batholith	227
	U	5	46.8929 -113.3009	1756	0.79	4.3	14.7	6.50*	4.86	Boulder batholith	227
Bubbling Creek	R	1	46.9202 -110.8955	2082	0.84	42.3	10.3	5.94	6.19	Sed. clastic carbonaceous	188
	R	2	46.9208 -110.8958	2073	0.69	14.4	9.6	5.89	5.89	Sed. clastic carbonaceous	188
	R	3	46.9214 -110.8962	2068	0.75	8.7	10.6	6.05	6.29	Sed. clastic carbonaceous	188
	R	4	46.9219 -110.8964	2059	0.86	44.9	9.4	5.34	5.97	Sed. clastic carbonaceous	188
	U	1	46.9203 -110.8964	2095	0.57	7.5	10.1	5.71	5.85	Sed. clastic carbonaceous	188
	U	2	46.9212 -110.8971	2087	0.53	6.5	9.9	4.26	5.75	Sed. clastic carbonaceous	188
	U	3	46.9222 -110.8970	2074	0.42	5.6	9.9	4.96	5.25	Sed. clastic carbonaceous	188
	U	4	46.9232 -110.8983	2062	0.60	7.2	8.6	4.63	5.14*	Sed. clastic carbonaceous	188
Lower Sun Creek	R	1	46.9177 -110.8787	2156	0.76	10.3	12.6	6.04	6.11	Sed. clastic carbonaceous	188
	R	2	46.9182 -110.8787	2146	0.83	24.4	10.6	5.71	6.04	Sed. clastic carbonaceous	188

	R	3	46.9188 -110.8787	2141	1.01	50.4	11.6	5.93	6.45	Sed. clastic carbonaceous	188
	R	4	46.9192 -110.8789	2136	0.97	28.1	11.1	6.26	5.82	Sed. clastic carbonaceous	188
	U	1	46.9181 -110.8796	2169	0.66	6.1	9.4	4.78	5.08	Sed. clastic carbonaceous	188
	U	2	46.9186 -110.8798	2167	0.57	4.6	10.4	4.99	5.40	Sed. clastic carbonaceous	188
	U	3	46.9192 -110.8798	2161	0.79	6.4	10.3	5.37	5.44	Sed. clastic carbonaceous	188
	U	4	46.9198 -110.8798	2158	0.61	5.4	11.8	4.83	5.38	Sed. clastic carbonaceous	188
Stringer Creek	R	1	46.9477 -110.8858	2190	0.86	43.0	9.4	6.15	5.37	Course grained igneous	189
	R	2	46.9471 -110.8862	2182	1.04	49.0	9.3	5.85*	6.09	Course grained igneous	189
	R	3	46.9465 -110.8864	2179	1.05	58.9	10.5	5.55	5.87	Course grained igneous	189
	R	4	46.9458 -110.8868	2182	1.08	49.6	10.2	6.27	5.86	Course grained igneous	189
	U	1	46.9480 -110.8863	2199	1.20	6.6	8.8	4.01	4.08	Course grained igneous	189
	U	2	46.9475 -110.8866	2191	0.98	8.3	9.6	3.95	5.29	Course grained igneous	189
	U	3	46.9468 -110.8870	2192	0.90	6.0	11.1	4.30	4.73	Course grained igneous	189
	U	4	46.9463 -110.8874	2189	0.81	7.8	10.2	4.44	4.52	Course grained igneous	189
Spring Park	R	1	46.9305 -110.8715	2181	0.93	36.2	12.6	6.48	6.56	Course grained igneous	188
	R	2	46.9300 -110.8722	2179	1.19	24.6	9.4	5.00*	5.42	Course grained igneous	188
	R	3	46.9297	2182	1.15	33.5	10.0	5.19	5.29	Course	188

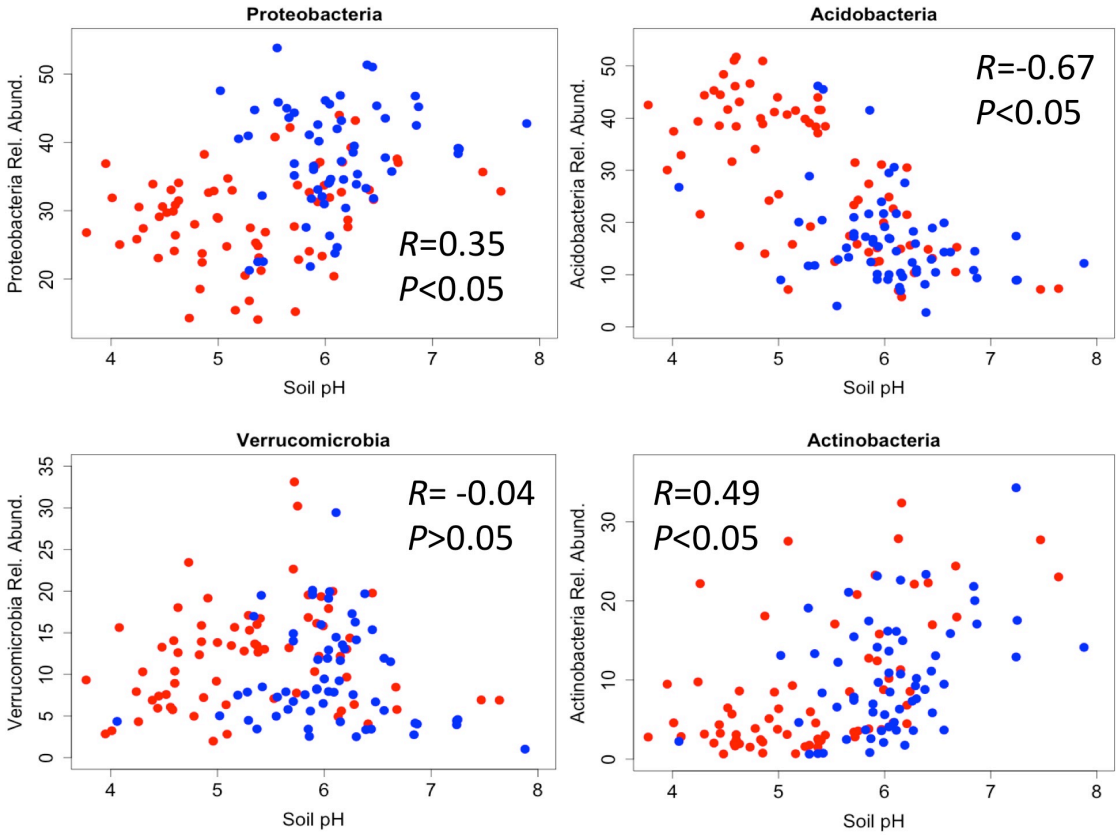
			-110.8728							grained igneous	
	R	4	46.9290 -110.8733	2179	1.32	47.6	8.2	5.64	5.99	Course grained igneous	188
	U	1	46.9302 -110.8710	2195	0.47	8.5	9.4	4.85	4.60	Course grained igneous	188
	U	2	46.9298 -110.8720	2191	0.56	11.0	17.5	4.45	5.16	Course grained igneous	188
	U	3	46.9293 -110.8725	2189	0.54	10.0	12.1	4.48	4.85	Course grained igneous	188
	U	4	46.9288 -110.8727	2189	0.38	0.5	10.2	5.35	5.72	Course grained igneous	188
Upper Tenderfoot Creek	R	1	46.9165 -110.8604	2217	0.55	18.0	8.8	6.15	6.15	Sed. clastic carbonaceous	189
	R	2	46.9169 -110.8612	2211	0.59	13.5	9.2	5.93	5.93	Sed. clastic carbonaceous	189
	R	3	46.9173 -110.8622	2206	0.71	13.4	10.8	6.04	6.04	Sed. clastic carbonaceous	189
	R	4	46.9175 -110.8631	2207	0.59	16.4	9.2	6.03	6.03	Sed. clastic carbonaceous	189
	U	1	46.9168 -110.8604	2218	0.65	5.5	8.8	3.77	3.77	Sed. clastic carbonaceous	189
	U	2	46.9172 -110.8613	2205	0.66	10.8	7.8	4.39	4.39	Sed. clastic carbonaceous	189
	U	3	46.9175 -110.8617	2204	0.59	4.3	9.0	4.24	4.24	Sed. clastic carbonaceous	189
	U	4	46.9180 -110.8626	2212	0.81	5.8	9.1	4.58	4.58	Sed. clastic carbonaceous	189



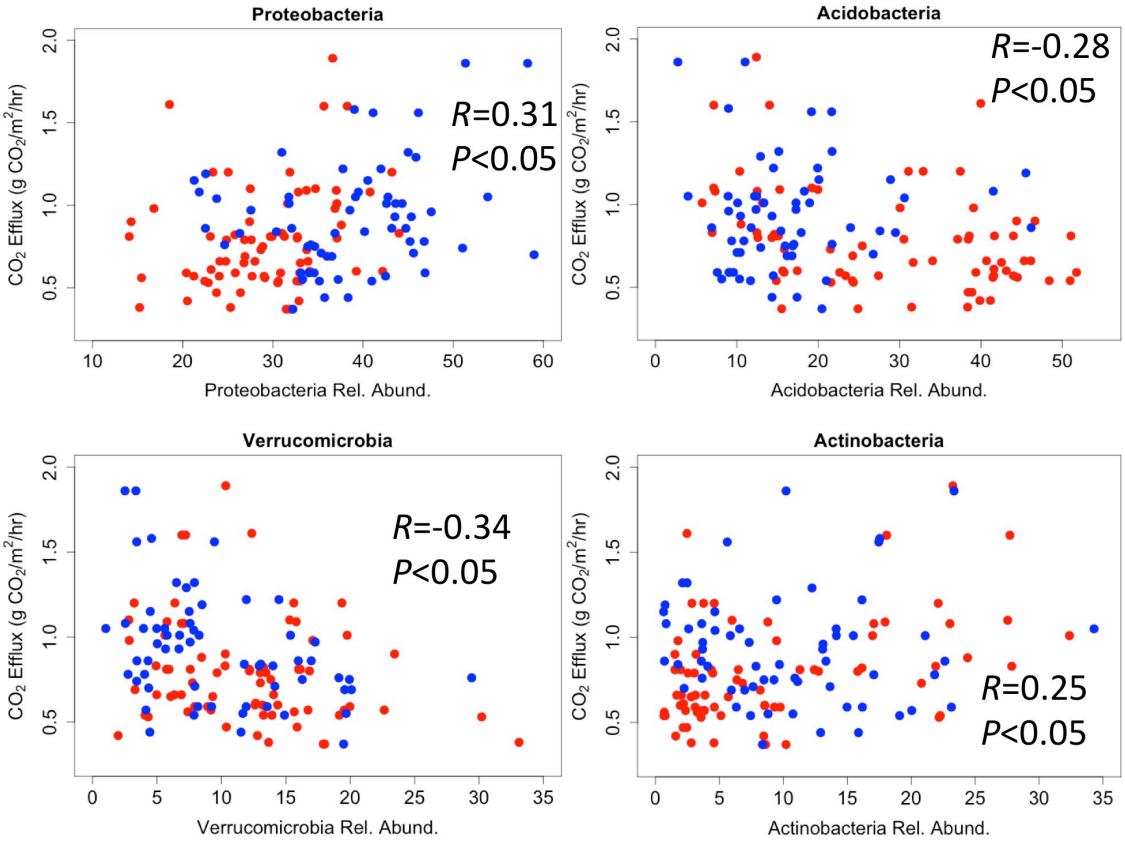
Supplementary Figure 1. Soil CO_2 efflux plotted against soil pH. Values for the Pearson *R* correlation and the corresponding *P*-value are shown for the relationship. Blue points represent riparian sites, while red points represent upland sites.

APPENDIX B

TAXONOMY & ENVIRONMENTAL MEASUREMENTS



Supplementary Figure 2. The relative abundance of the top 4 most abundant phyla from this study plotted against soil pH. Pearson *R* correlations and *P*-values are shown for each relationship. Blue points represent riparian sites, while red points represent upland sites.



Supplementary Figure 3. Soil CO_2 efflux values plotted against the relative abundance of the 4 most abundant phyla from the study. Pearson *R* correlations and *P*-values are shown for each relationship. Blue points represent riparian sites, while red points represent upland sites.