2015-05-01

Tree Islands of Fertility Structure Bacterial Community Assembly and Functional Genes Contributing to Ecosystem Processes

Tayte Paul Campbell
Brigham Young University - Provo

Tree Islands of Fertility Structure Bacterial Community Assembly and Functional Genes Contributing to Ecosystem Processes

Tayte Paul Campbell

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Zachary Aanderud, Chair
Alan Harker
Bruce Roundy

Department of Plant and Wildlife Sciences

Brigham Young University

May 2015

ABSTRACT

Tree Islands of Fertility Structure Bacterial Community Assembly
and Functional Genes Contributing to Ecosystem Processes

Tayte Paul Campbell
Department of Plant and Wildlife Sciences, BYU
Master of Science

In arid and semi-arid ecosystems, dominant tree species create dramatic mosaics of plant islands of fertility and relatively barren plant interspaces that exert immense pressure on ecosystem processes and offers an ideal opportunity to explore the impact of bacterial communities. We evaluated potential links between soil respiration and N mineralization, and community co-occurrence networks and predicted gene function across three tree island microsites (i.e., beneath tree canopies, at the canopy edge, and in interspaces) in a replicated field experiment in thirty-eight woodlands sites in the Great Basin Desert in UT, USA. Additionally, we potentially intensified the effects of tree islands by creating a treatment where whole trees were shredded and the resulting fine woody debris (FWD) was deposited onto the soil surface and measured a suite of characteristics relating to the metabolic functional state of communities (i.e., microbial efficiency as the microbial quotient, C substrate quality, biomass, and dissolved organic C) to improve our interpretation of potential links between function and structure. We found that tree islands were the predominant driver, creating highly complex and connected assemblies of bacterial populations and easily discernable differences in abundance and composition of predicted functional genes. Specifically, communities directly beneath *Juniperus* and *Pinus* canopies were comprised of at least 5.2-times more connections between bacterial taxa than present in networks from interspace and edge. Using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to predict the gene expression, differences in the functional potential mirrored shifts in network complexity. Tree island communities expressed 236 genes with many related to the degradation of polyaromatic or polycyclic compounds, while interspace communities expressed only 66 genes associated with the decomposition of more labile C substrates. We observed a robust tree island microsite effect on all ecosystem processes, with soil respiration rates increasing 12% and N mineralization decreasing 29% in canopy than interspace soils demonstrating that a more recalcitrant substrate from a sole C source provided high amounts of low quality of DOC and lead to a decrease in metabolic efficiency, but ultimately selected for a specific community assembly. Alternatively, communities at the edge of canopies, experiencing both tree island and interspace soil conditions generated similar levels of soil respiration as canopy soils regardless of not selecting for a highly connected community and/or specific genes suggesting that a diverse composition of labile and recalcitrant C substrates from multiple sources (e.g., trees, perennial grasses, annual grasses, and forbs) potentially elevates function by promoting the activity of a wide range of taxa. Our results identify that tree islands exert enough pressure to create distinct interactions between bacteria and alter gene expression resulting in changes in ecosystem function, but the link between structure and function is mediated through the diversity and quality of C substrates.

Keywords: microbial ecology, target metagenomics, fine woody debris, PICRUSt

ACKNOWLEDGEMENTS

I would like to thank my committee members, Zachary Aanderud, Bruce Roundy, and Alan Harker for their support in completing this project. I would also like to thank David Robinson, Erin Jones, and Tylan Magnusson for their help with lab techniques and data analysis. Finally, I express my deep appreciation to my wife, Tenery, for her support and encouragement through this entire process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

One of the overarching goals of microbial ecology is to link the immense bacterial diversity within communities to ecosystem functioning. This seemingly straight-forward task is complicated by the fact that: phylogenetically diverse groups of bacteria often exhibited high levels of functional redundancy by perform similar processes (i.e., they are functionally redundant); a large fraction of the bacterial species may effectively be metabolically inactive or dormant awaiting to resuscitate and contribute to pulses of ecosystem activity (Prosser et al., 2007; Lennon et al., 2011); and rare bacteria may contribute disproportionately to certain biogeochemical processes from ubiquitous relations associated with trace gas flux (Aanderud et al., 2015) to specific functions like methane oxidation (Bodelier et al., 2013) and sulfate reduction (Pester et al., 2010). For more universal processes, early attempts to identify links assumed that species contribute according to their relative abundance and in doing so assumes biomass or its surrogate, relative recovery for bacteria, dictates function. However, there are multiple novel techniques emerging to push beyond biomass and potentially investigate bacterial contributions within communities and to processes in a different light. One such is the construction of network co-occurrence models that quantify bacterial population interactions (negative and positive, i.e., competition and facilitation). For example, network models have linked nutrient characteristics such as nitrate, P, organic matter, and aboveground biomass with annual grassland plants (Elmendorf and Moore, 2008). Further, predictive metagenomics uses phylogenetic marker genes to infer the composite metagenome and has been used to determine bacterial functional relationships. For example, predictive metagenomics was used to infer the enzymes that may be utilized to degrade recalcitrant litter (Jimenez et al., 2014). Both of these

approaches offer a more detailed characterization of community interactions and metabolic function that may open new opportunities to link function and structure.

Plant-islands of fertility in desert ecosystems robustly influence ecosystem processes and create unique bacterial communities providing an ideal opportunity to investigate links between function and more detailed understanding of community interactions and gene expression. Across arid and semi-arid ecosystem shrubs and tree species create mosaics of plant islands and relatively barren plant interspaces as soils beneath shrubs become enriched in organic C substrates and other essential elements (e.g., N, P, and Ca) due to litter and root inputs, root translocation of elements from interspace soils, and shrub canopies' trapping of aeolian material (Schlesinger et al., 1996; Schlesinger and Pilmanis, 1998; Van Breemen and Finzi, 1998). Plant islands enhance soil respiration, (Liao and Boutton, 2008), elevate N mineralization, nitrification (Schade and Hobbie, 2005; Kieft et al., 1998), and denitrification (Wainwright et al., 2002), stimulate biomass (Aanderud et al., 2010), and also structure bacterial communities (Gallardo and Schlesinger, 1992; Vollmer et al., 1977). However, it remains unclear the extent that plant island functional responses relate to differences in bacterial community structure (Ludwig and Tongway, 1997; Puigdefabregas et al., 1999; Schlesinger et al., 1996). In these communities, canopy soils show an increase in gram-positive bacteria and fungi, but all of these differences are based on coarse determinations (e.g., plate counts and phospholipid fatty acid (PLFA) analysis) (Aanderud et al., 2008). Plant islands or a single dominant plant species may exert enough pressure to create distinct interactions between bacterial species and promote gene expression.

Many shrub and tree species in deserts deposit leaf and woody material on soil surfaces that contain polymers to enhance burning (Zedler, 1995; Behm et al., 2004), secondary defense compounds against insect herbivores (Mumm and Hilker, 2006; Wainhouse et al., 2008), and

polycyclic aromatic hydrocarbons (e.g., lignin and tannins; Horner et al., 1988; Kraus et al., 2003) that may need a consortium of bacterial species to breakdown. In particular, the additions of fine woody materials (FWD, e.g., small logs and brances) may drastically influence bacterial function and structure. For example, in forest and desert ecosystems woody debris stimulates soil respiration (Perez et al., 2004) and increases N mineralization (Hart, 1999) and P availability (Harmon et al., 1986). Across thousands of hectares of piñon-juniper woodlands tree islands are being mechanically shredded into fine woody debris (FWD) and deposited on the soil surface to create firebreaks. This immense field manipulation may further enhance the effects of tree islands on bacteria offering an unprecedented opportunity to explore links between processes and a more intense evaluation of bacterial communities.

In this study, we identified potential links between ecosystem processes and bacterial species interactions and functional genes within tree islands of fertility communities exposed to FWD additions in piñon-juniper woodlands. In a replicated field experiment, we measured soil respiration, N mineralization, and P availability in three microsites (i.e., beneath tree canopies, at the canopy edge, and in interspaces) representing a gradient of soils influenced by tree islands where FWD was added to soils across thirty-eight woodlands sites in the Great Basin Desert in Utah, USA and related microsite and/or FWD-induced changes to shifts in the structure of bacterial communities. Specifically, we evaluated bacterial interactions by constructing network co-occurrence models and predicted functional differences of metagenomes using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PiCRUST). Further, we measured a suite of characteristics relating to the metabolic functional state of communities (i.e., microbial efficiency as the microbial quotient, C substrate quality, biomass, and dissolved organic C) to improve our interpretation of potential links between function and structure.

2. MATERIALS AND METHODS

2.1 Site selection and sample collection

We conducted our study across 38 *Juniperus osteosperma* (Torr.) and *Pinus edulis* (Engelm.) woodland sites where these trees are expanding (i.e., mid-level encroachment) into sagebrush steppe/semi-desert ecosystems (Miller and Rose, 1999; Miller et al., 2000; Bates et al. 2011). Sites were selected using images from the National Agricultural Imagery Program (NAIP) at 1 m spatial resolution (USDA-FSA-APFO Aerial Photography Office, Salt Lake City, Utah, USA). We used ENVI Zoom version 4.5 (Exelis Visual Information Solutions, Boulder, Colorado, USA) to find active areas of tree encroachment (15-45% absolute tree cover). Dominant shrub species included *Artemisia tridentata* Nutt. (ssp. *wyomingensis* Beetle and Young, *tridentata*, and *vaseyana* [Rydb.] Beetle), *Atriplex canescens* (Pursch) Nutt., *Chrysothamnus viscidiflorus* (Hook.) Nutt., *Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom and Baird, *Purshia stansburiana* (Torr.) Henrickson, and *Purshia tridentata* (Pursh) DC. Across the sites, elevations ranged from 1637 – 2512 m.a.s.l., average annual precipitation from 272 – 483 mm, and average annual temperature from 4.5 – 13 °C (PRISM Climate Group, 2004; www.prism.oregonstate.edu; Supplemental Table 1).

2.2 Microsites, fine woody debris treatment, and soil sampling

We sampled microsites to determine the contribution of tree islands and FWD to microbial community structure and function. The three microsites included: soils directly beneath the tree at one-third the distance from the trunk to the edge of the canopy (CANOPY); soils at the tree canopy edge at the intersection between litter and duff deposition and barren soil

surfaces (EDGE); and tree interspace soils consisting of both barren surfaces and/or surfaces covered with grasses and/or forbs (INTERSPACE). We located three subplots (33 m × 30 m) for each microsite and treatment within each site and beneath *Juniperus* and *Pinus* when present. Both subplot and microsite locations were randomly located with microsites surrounding trees with a canopy radius of at least 3 m. The FWD additions were created by shredding live whole trees to mulch and depositing the residue on the soil surface using a Bull Hog® (tractor mulcher equipped with rotating blades). Shredding is commonly used to mitigate catastrophic wildfires in *Juniperus* and *Pinus* woodlands and creates FWD with diameters ranging from 2 – 7.6 cm (1- and 10- hour fuels; Young et al., 2014). Our range of woody debris diameters meets the requirements of 1-10 cm in diameter outlined for FWD in deciduous and coniferous forest studies (Norden et al., 2004; Yan et al., 2006). Time since shredding ranged from 1-8 years with an average of 3.9 years (2003-2010).

We collected soils over a 2-year period during the summer months (April-August, 2011-2012). Soils were removed from the three subplots with a soil corer (5 cm diameter and 10 cm in length), composited by microsite by treatment combinations within a site, and kept cold in the field and lab at 5 °C. Soils were sieved to 2 mm and biogeochemical analyses were started within 5 days after collection while soils for molecular analysis were frozen at -20 °C until further analysis.

2.3 Bacterial communities in tree island microsites following shredding

To investigate the effects of tree islands of fertility and FWD additions on microbial communities, we used target metagenomics of the 16S rRNA gene. We extracted genomic DNA from a subset of microsites in untreated and FWD addition soils (3 microsites × 2 treatments:

control and FWD additions × 2 tree species: *Juniperus* and *Pinus* species when both occurred at the site × 5 replicates = 40 samples). We extracted genomic DNA from 0.5g of soil using a PowerSoil DNA Isolation Kit (MoBio, Carlsbad, California, USA). The V4-V5 regions of 16S rRNA were amplified with polymerase chain reaction (PCR) with Q5 High-Fidelity PCR Kit (New England Biolabs, Ipswich, Massachusetts, USA) using bacteria specific primer set 515F and 806R primers with unique 12-nt error correcting Golay barcodes. Due to difficulty in amplifying the16S rRNA gene from CANOPY and EDGE samples, possibly from increased chemical inhibitors from FWD and duff influencing soils, we decreased number of replicates from five to four or three in both of these microsites for both tree species. The forward primers were designed with a Lib-L A Adapter, a unique barcode, and a GT-linker on the 5' end; and the reverse primer was designed with a Lib-L B Adapter and a GG-linker on the 5' end (Lauber et al., 2013). The thermocycler settings were: an initial denaturation step of 98 °C for 30 seconds, followed by 35 cycles of denaturation at 98 °C for 10 seconds, annealing at 57.5 °C for 30 sec, and extension at 72 °C for 30 seconds. Samples were purified using an Agencourt AMPure XP PCR Purification system (Beckman Coulter Inc., Brea, California, USA) and were quantified using a Quant-iT^{TM} PicoGreen dsDNA Kit (Invitrogen Corporation, Carlsbad, California, USA) to create equimolar concentrations prior to sequencing. The samples were then sequenced on a 454 Life Sciences Genome Sequence FLX instrument (Roche, Branford, Connecticut, USA) sequencer at the Brigham Young University DNA Sequencing Center (http://dnasc.byu.edu/). All sequences were analyzed using Mothur (Schloss et al., 2009). After removing barcodes and primers, we removed sequences that were <300 base pairs in length to assure the quality and accuracy of pyrosequencing. The sequences were denoised using AmpliconNoise (Quince et al., 2011) before removing chimeras using UCHIME (Edgar et al., 2011) and eliminating

chloroplast, mitochondria, archaea, and eukarya gene sequences using reference sequences from the Ribosomal Database Project (Cole et al., 2009). Operational taxonomic units (OTUs) were identified using Megablast at a minimum coverage of 99%, and minimum pairwise identity of 97%. Phylogenetic identities were aligned against the Greengenes database (DeSantis et al., 2006; http://greengenes.lbl.gov).

To assess differences in community composition due to tree island microsites and FWD, we first performed a principle coordinate analysis (PCoA) using a Bray-Curtis abundance-based distance matrix from the relative recovery of OTUs using the *vegdist* function in the vegan package (Oksanen et al., 2014) in R (R Development Core Team, 2013). Second, we tested for the main effects and interaction between the microsites and FWD treatment with site as a random variable using PERMANOVA (Anderson, 2001), which was performed with the *adonis* function in the vegan package. We were not able to add time since shredding as a factor or covariate in our model since time only applied to the FWD and not control treatment. Third, we quantified richness as the total number of OTUs, alpha diversity as the inverse Shannon index, and OTU evenness based on 1000 iterations of 900 random resampled sequences from each replicate. The effects of microsite and FWD additions on richness, diversity, and evenness were evaluated using two-way ANOVA with Tukey's HSD tests. Last, phylogenetic trends of dominant taxa (mean recovery $\geq$ 1.0%) from 10 phyla and 4 subclasses, as well as 23 families were shown in a heat map with hierarchal clustering using the *heatmap* function in the 'gplot' package in R (Oksanen et al 2013).

2.4 Co-occurrence networks within communities

We created network co-occurrence models using maximal information coefficient (MIC) analysis to evaluate bacterial relationships within microsites (Reshef et al., 2011). MIC was calculated using the *minerva* package in R (Filosi et al., 2014) to calculate linear and non-linear relationships between bacterial OTUs. The nodes in the network models represent OTUs at 97% identity and edges represent significant co-occurrence connections that occur in at least 75% of samples and have an MIC that is both > 0.7 and statistically significant (*P* value < 0.01; Junker and Schreiber, 2008). We calculated a series of topology parameters to describe the network and OTU interactions including: average node connectivity, cumulative degree distribution, average path length, modularity, and clustering coefficient (Barberan et al, 2011; Newman, 2003). We created network graphs using the *igraph* package in R (Reshef et al., 2011) and visualized the interactions with the open graph visualization platform Gephi (v. 0.8.2-beta; Bastian et al., 2009) to explore community interaction complexity.

2.5 Functional gene predictions

To begin to identify whether microsite location or FWD additions alter the functional potential of communities, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt (version 1); Langille et al., 2013) to predict genes from the target metagenome. PICRUSt predicts the metagenomic functional recovery from the 16S rRNA gene target metagenomes using a two-step process. First, gene content for each OTU is computed in a reference phylogenetic tree, and, second, these gene content predictions are combined with the relative abundance of 16S rRNA genes from collected samples, corrected to the expected 16S rRNA gene copy numbers. We used the Galaxy open, web-based platform

(Goecks et al., 2010; Blankenberg et al., 2010; Giardine et al., 2005) to run PICRUSt against the database of Clusters of Orthologous Groups of proteins (COG; Tatusov et al., 2000). To visualize changes in gene enrichment, we created a ternary diagram using the *ggtern* library (Hamilton, 2014), an extension of ggplot (Wickham, 2009) in R. We considered genes to have a higher abundance in a given microsite if the mean relative gene recovery, or the number of times that a gene is present in a sample relative to the total number of genes in that sample, for that microsite constituted ≥50% of the total recovery for the given gene.

2.6 Ecosystem processes

We identified potential links between bacterial communities and ecosystem processes by measuring shifts in soil respiration, N mineralization and P availability along with communities across microsites and the FWD addition. To calculate C mineralization, soil respiration rates (μg CO_2 g $soil^{-1}$ day^{-1}) were measured in a laboratory incubation using soil microcosm created by weighing 30 grams of soil into a specimen cup (155 mL), bringing the soil to a constant gravimetric water content (0.3 g H_2O g dry $soil^{-1}$), placing the microcosm in a quart-sized mason jar (935 mL) with a gas-tight septum, incubating the microcosm at 30° C; and measuring CO_2 concentrations at five time intervals (0, 1, 3, 6, and 10 days). CO_2 concentrations were measured by extracting 10 ml of air with a gas-tight syringe and measuring the CO_2 concentration using an EMG-4 (PP Systems, Amesbury, Massachusetts). The lid was opened after each measurement and allowed to equilibrate with ambient CO_2 concentrations. CO_2 concentrations were converted from ppm to μg using the Ideal Gas Law. All jars holding the microcosms contained 10 mL of water in an inner cup to keep samples moist during incubation. We measured N mineralization rates (μg N-NH_4^+ g $soil^{-1}$ day^{-1}) based on the change in NH_4^+ and NO_3^- concentrations over the

same 10-day experimental incubation used for C mineralization (Billings et al., 2002; Fierer and Schimel, 2002; Schaeffer et al., 2003; Schaeffer at al., 2007). Soils in the microcosms at the beginning and end of the incubation were extracted with 0.5 M K_2SO_4 (1:2 w/v) and NH_4^+ and NO_3^- concentrations were determined colorimetrically using a SpectraMax Plus 384 (Molecular Devices Corporation, Sunnyside, California, USA) following Forster (1995) for NH_4^+ and Miranda et al. (2001) for NO_3^-. For P (μg P g $soil^{-1}$), soils were extracted with a 0.5 M $NaHCO_3$ (1:20 w/v) using the Olsen Sodium Bicarbonate method (Olsen et al., 1954; Watanabe and Olsen, 1965; Thomas and Peaslee, 1973). P concentrations were evaluated colorimetrically using a SpectraMax Plus 384 (Molecular Devices Corporation, Sunnyside, California, USA).

2.7 Bacterial community metabolic function

To investigate the effects of microsites and FWD additions on the metabolic function of the bacterial community we measured microbial efficiency and C substrate quality as the microbial quotient and amount of microbial biomass supported per unit of dissolved organic C respectively. The microbial quotient (μg C-CO_2 g C_{mic} $hour^{-1}$) represents the amount of C from C substrates that was incorporated into biomass compared to how much was respired as waste, more CO_2 respired per unit biomass indicates a less efficient community (Liao and Boutton, 2008). To calculate the quotient, we used the CO_2 concentrations generated from the microcosms divided by the measured microbial biomass. We measured microbial biomass (SMB, μg C g $soil^{-1}$) using a substrate-induced respiration (SIR) technique (Anderson and Domsch, 1978). Briefly, 10 g of soil was weighed into a specimen cup with a gas-tight septum in the lid (155 mL) and 1.121 mL of a 4 M sucrose solution was added to the soil. Respiration rates were measured at 24 and 24.5 hours by capping the cups for 30 minutes and measuring the

change in headspace CO_2 concentration using the method described above for C mineralization. Microbial biomass was calculated using the equation (Bailey et al., 2002):

$$SMB\ (\mu g\ C\ g\ soil^{-1}) = (40.04\ X\ \Delta CO_2) + 0.37$$

where ΔCO_2 is the change in respiration rates caused by the input of sucrose solution in units of $\mu L\ CO_2\ g^{-1}\ h^{-1}$. We calculated C substrate quality ($C_{mic}\ C_{DOC}^{-1}$ %) by dividing SMB by dissolved organic C concentration (DOC). We specifically used DOC instead of total soil C to determine quality since DOC is the more labile and available C for bacteria metabolism. To measure DOC ($\mu g\ C\ g\ soil^{-1}$), soils were extracted with a distilled water extraction (1:2 w/v), passed through a 0.45 μm nylon filter, and measured on a TOC-N analyzer (Shimadzu, Columbia, Maryland, USA).

3. RESULTS

3.1 Bacterial communities in tree island microsites after shredding

Our results suggest that tree island of fertility microsites were the main driver of bacterial variation and that FWD additions only enhanced these variations. This inference was based on the recovery of 315,080 quality sequences and 25,411 unique OTUs. Communities from the three microsites clustered along the x-axis, which explained 19.85% of the variability among communities (Fig 1). FWD treatment caused a slight shift within microsites along the x- and y-axis. The PERMANOVA supported these results with microsite (df=2, P<0.001, R^2=0.17) and FWD additions to a lesser extent (df=1, P=0.04, R^2=0.03) altering community composition. Tree species had no apparent influence on communities. Despite large shifts in composition, microsite

difference did not affect richness, evenness, or diversity; however, FWD additions increased richness by 4% (one-way ANOVA, *df*=1, F=5.1, *P*=0.03), diversity by 2% (one-way ANOVA, *df*=1, F=8.7, *P*=0.006), and evenness by 1% (one-way ANOVA, *df*=1, F=8.9, *P*=0.005, Supplemental Fig 1).

The CANOPY and INTERSPACE microsites beneath both tree species selected for specific bacterial families regardless of FWD additions. Based on relative recovery and hierarchical clustering, CANOPY soil characteristics selected for 12 families, one phyla and four Proteobacteria subclasses, and INTERSPACE soils favored 8 families and four phyla (Fig. 2, Supplemental Fig 2). For example, in CANOPY soils Chitinophagaceae, Flavobacteriaceae, and Cytophagaceae were at least 66% higher in CANOPY than INTERSPACE and contributed to Bacteroidetes constituting upwards of 12.9% in soils beneath tree canopies. Many of the shifts in recovery caused by CANOPY characteristics occurred within the Proteobacteria with Hyphomicrobiaceae and Sphingomonadaceae (Alphaproteobacteria); Oxalobacteraceae and Comamonadaceae (Betaproteobacteria); Sinobacteraceae and Xanthomonadaceae (Gammaproteobacteria) each contributing up to 4% in these soils. Deltaproteobacteria was also more prominent in CANOPY soils but there was no specific family that demonstrated this relationship. Alternatively, families from Actinobacteria clearly distinguished INTERSPACE soil communities. Shifts in Actinobacteria families included Rubrobacteraceae, Geodermatophilaceae, Gaiellaceae, and Micromonosporaceae each contributing up to 3% in interspace soils. Similar to CANOPY soils, families from Alphaproteobacteria clearly distinguished the INTERSPACE microbial community, consisting of Beijerinckiaceae, Acetobacteraceae, and Bradyrhizobiaceae. Additionally, Phormidiaceae (Cyanobacteria) was more prominent in INTERSPACE with no recover in CANOPY. INTERSPACE also selected for

Chloroflexi and Gemmatimonadetes but this increase is not reflected in any family differences. Edge showed minor increases in Gemmataceae (Planctomycetes), Nocardioidaceae (Actinobacteria), and [Chthoniobacteraceae] (Verrucomicrobia) while selecting for Acidobacteria, Planctomycetes, and Verrucomicrobia at the phyla level.

3.2 Co-occurrence networks within communities

Of the three tree island microsites, soil communities beneath *Juniperus* and *Pinus* canopies were the most complex and highly connected. Although treatment slightly influenced community composition, based on co-occurrence network models there were no differences in topological parameters between FWD additions and control treatments, thus we collapsed FWD additions and controls to increase the replication for models investigating apparent differences in microsite and tree species (Fig 3). In addition, we originally collapsed network models by tree species based on the coarser PERMANOVA results; however, when communities beneath tree species were combined the complexity and connectivity in the models drastically declined, suggesting that tree species influenced both positive (i.e., facilitation) and negative (i.e., competition) interactions even when the composition of communities may be relatively similar. Overall, network complexity was greatest in CANOPY, intermediate in INTERSPACE, and lowest in EDGE soils (Fig 3). For example, *Juniperus* and *Pinus* networks in CANOPY soils had at least five-times the number of edges or significant correlations between nodes or OTUs, a 5-6 times higher mean degree or the average number of edges connected to a node, at least 4-times the density, a 2-fold reduction in the average shortest path length or the average number of steps between each node and every other node than any other microsite; and a 10-25% decline in modularity or the ratio of edges within sub-clusters to the number of edges between sub-clusters. Alternatively, the number of significant nodes were only substantially higher in *Juniperus*

CANOPY microsites. The increased complexity and connectivity in INTERSPACE relative to EDGE microsites was highlighted in a slightly higher number of nodes, edges, and lower mean path length.

Highly connected (hub) nodes represented families that were selected for in each microsite. Within each network model the hub OTUs were based on the top ten highest connected nodes (Table 2). Hub nodes in CANOPY were made up of Hyphomicrobiaceae (Alphaproteobacteria), Sinobacteraceae (Gammaproteobacteria), and Chitinophagaceae (Bacteroidetes). INTERSPACE had hub nodes of Geodermatophilaceae and Rubrobacteraceae (Actinobacteria), as well as Beijerinckiaceae (Alphaproteobacteria). Last, EDGE had hub nodes of [Chthoniobacteraceae] (Verrucomicrobia), but also included hub nodes that were prominent in INTERSPACE and CANOPY, including Acetobacteraceae (Alphaproteobacteria) and Micromonosporaceae and Geodermatophilaceae (Actinobacteria) from INTERSPACE and Solirubrobacteraceae (Actinobacteria) and Oxalobacteraceae (Betaproteobacteria) from CANOPY.

3.3 Functional gene predictions

Differences in the functional potential of the tree island communities mirrored the shifts in community composition, taxonomical groups, and network complexity. Specifically, the abundance and composition of predicted functional genes were easily discernible between soil beneath tree canopies and interspace soils. Based on PICRUSt, a metagenomics prediction tool, the 16S rRNA gene profiles of CANOPY soils resulted in the predicted expression of over 236 genes where recovery in CANOPY alone made up $\geq 50\%$ of the total recovery of any given gene (Fig 4). Of particular importance in CANOPY communities were multiple genes, beta-

galactosidase, beta subunit; beta-N-acetylglucosaminidase; exo-beta-1,3-glucanase; and formylmethanofuran dehydrogenase subunits A,B, and C, involved in the degradation of polyaromatic or polycyclic compounds. Interspace represented ≥ 50% of the expression of 66 genes, including: alanine-alpha-ketoisovalerate aminotransferase and ATP adenylyltransferase. These genes are involved in the degradation of more labile products. The edge community composition did not predict the recovery of any functional gene over 50% abundance.

3.4 Ecosystem processes and P availability

We observed a robust tree island microsite effect on all ecosystem processes, which reflected major differences among bacterial communities among tree island microsites. For example, soil respiration was at least 12% higher in soils influenced by tree canopies (i.e., CANOPY and EDGES) than interspace soils (three-way ANOVA, F=24.4, $P<0.0001$, $df=2$; Fig 5A). However, P availability was only 33% higher in soils directly beneath tree canopies than EDGE and INTERSPACE microsites (three-way ANOVA, F=14.31, $P<0.001$, $df=2$; Fig 5C). In contrast, N mineralization rates were highest in EDGE, intermediate in INTERSPACE, and lowest in CANOPY soils (three-way ANOVA, F=19.76, $P<0.001$, $df=2$; Fig 5B). FWD additions and tree species did not influence any of the processes.

3.5 Bacterial community metabolic function

Canopy provided high amounts of low quality DOC, which led to decreased microbial efficiency under tree canopies, especially for *Juniperus*. In general, the microbial quotient (qCO_2), or degree of CO_2 respired per unit microbial biomass, increased by at least 20% in CANOPY compared to EDGE and INTERSPACE microsites, regardless of tree species, thus,

indicating a decline in efficiency (one-way ANOVA, F=6.2, *P*=0.0023, *df*=2; Fig 6A). A potential cause for this decline in efficiency may stem from a reduction in DOC quality caused by changes in microsite, tree, and FWD treatment. Although DOC concentrations were at least 84% higher in CANOPY soils (one-way ANOVA, F=68.03, *P*<0.0001, *df*=2; Fig 6C) and the amount of DOC in microsites increased in *Juniperus* (one-way ANOVA, F=10.36, *P*=0.001, *df*=1), the DOC supported less microbial biomass. The quality of DOC, measured as the amount of microbial biomass per gram of DOC decreased at least 53% in CANOPY (three-way ANOVA, F=35.25, *P*<0.0001, *df*=2) and 30% under *Juniperus* compared to *Pinus* (three-way ANOVA, F=6.69, *P*=0.01, *df*=1). FWD treatment enhanced these effects in *Juniperus*, increasing DOC 46% (three-way ANOVA, F=18.44, *P*<0.0001, *df*=1), but had no effect in *Pinus* (three-way ANOVA, treatment and tree interaction, F=5.29, *P*=0.02, *df*=1; Supplemental Fig 3). FWD treatment decreased the quality of DOC 18% across all microsites and trees (ANOVA, F=4.24, *P*=0.04, *df*=1). Last, there were no differences in the microbial biomass among microsites or treatments with values ranging from 51.4 to 56.8 μg C g soil^{-1} (data not shown).

4. DISCUSSION

4.1 Islands of fertility drive community and function

The patchy mosaic created by tree islands exerts immense force on the plant community; however, it is unclear the extent that this dominant landscape feature influence the structure and function of bacterial communities (Ludwig and Tongway, 1997; Puigdefabregas et al., 1999; Schlesinger et al., 1996). Tree islands of fertility: generate nutrient-rich islands supplying limited essential nutrients enhancing the diversity of understory plant species; increase the water holding

capacity and water content available for establishing plant species (Bonanomi, 2008); and ultimately enhance plant and microbial biomass (Aanderud et al., 2010; Schade and Hobbie, 2005). We found that tree islands were the predominant driver by creating highly complex and connected assemblies of bacterial populations and easily discernable differences in abundance and composition of predicted functional genes. Specifically, networks directly beneath *Juniperus* and *Pinus* canopies were comprised of at least 5.2-times more connections between taxa than those describing interspace and edge communities. Many of these connected taxa influencing the facilitation and/or competition were both rare and abundant, suggesting that connected taxa are not constrained or dependent on biomass. Differences in the functional potential mirrored shifts in network complexity with the tree island communities expressing 236 genes, many related to the degradation of polyaromatic or polycyclic compounds, while interspace communities expressed only 66 genes including genes relate to the decomposition of more labile C substrates. We observed a robust tree island microsite effect on all ecosystem processes, with soil respiration rates increasing 12% and N mineralization decreasing 29% in canopy than interspace soils. Alternatively, soil communities at the edge of canopies, experiencing both tree island and interspace soil conditions generated similar levels of soil respiration as canopy soils regardless of not selecting for a highly connected community and/or specific genes. An explanation for this discrepancy may stem from differences in the quality and quantity of C substrates available to bacteria in the different microsites. The higher recalcitrant substrates from a sole C source provided high amounts of low quality DOC, which led to a decrease in metabolic efficiency, but selected for specific consortiums of populations. Alternatively, a diverse composition of labile and recalcitrant C substrates from multiple sources (e.g., trees, perennial grasses, annual grasses, and forbs) potentially elevated function by promoting the activity of a wide range of taxa. The

addition of FWD debris enhanced microsite overall community differences and bacterial diversity, richness, and evenness, but did not alter network complexity, gene expression, or ecosystem processes. Our results identify that tree islands exert enough pressure to create distinct interactions between bacteria and alter gene expression resulting in changes in ecosystem function, but these links are not necessarily concomitant and potentially mediated through the diversity and quality of C substrates.

4.2 Community assembly and interactions

We found that tree islands were the predominant driver creating highly complex and connected assemblies of bacterial populations. Deciphering these intricate relationships between specific taxa are especially complicated due to the high species richness in terrestrial soils (Berry and Widder, 2014). Additionally, it is estimated that less than 1% of soil microbes can be cultivated (Pham and Kim, 2012), making it impossible to construct laboratory assemblages of bacteria that can be used to infer interactions (Trosvik et al., 2010). Network co-occurrence models provide an important tool for measuring connections between thousands of bacteria to tease apart potential interactions between populations of bacteria from target-metagenomic data independent of cultivation (Ruan et al., 2006; Shokralla et al., 2012). Specifically, communities directly beneath *Juniperus* and *Pinus* canopies were comprised of at least 5.2-times more connections between bacterial taxa than present in networks from interspace and canopy, which resulted in the lowest average shortest path length. This highly connected assembly of bacterial taxa signifying a possible increase in responsiveness to environmental characteristics associated with tree islands and may confer a higher level of resilience following potential disturbances (Zhou et al., 2010). Canopy also had the highest average degree, average clustering coefficient,

and density detailing a more robust community that is resistant to random node failure (i.e., removal or death of a random taxa) (Gutteridge et al., 2007; Zhao et al., 2006). However, modularity decreased in canopy soils with a lower number of within-cluster edges than between-cluster edges, indicating a lower number of niches (Chaffron et al., 2010; Freilich et al., 2010). This potential decline in niches may result from a relatively homogenous set of environmental characteristics and C substrates generated by tree islands compared to the more heterogeneous conditions and C source inputs in edge and interspace soils.

Of all the bacteria in the networks there were both rare and abundant taxa that were highly connected (i.e., hub nodes, nodes with the most connections in networks) and potentially structured many of the interactions in the community. For example, in tree island soils relative recovery of the ten most highly connected nodes ranged from 0.01% to 1.06%. In our evaluation we defined rare OTUs as taxa with a relative recovery $\leq 0.1\%$ while abundant OTUs had $> 0.1\%$ relative recovery (Pedros-Alio, 2012). Highly connected nodes in canopy consisted mostly of rare taxa while interspace hub nodes were mostly abundant. These taxa spanned 5 phyla and 3 sub-phyla and 8 classified families with 35% of these bacteria in theAlphaproteobacteria phylum. Within the interspaces 4 phyla and 1 sub-phyla and 4 classified families were represented with 40% of these bacteria within Actinobacteria. Highly connected taxa in canopy and interspace mirrored specific shifts in relative recovery with families containing hub OTUs in the network. Conversely, edge contained hub nodes that reflected families in both canopy and interspace, with the edge microsite enhancing the recovery of no family. Thus, the duplication of hub nodes in edge soils may indicate an area of transition between the two other distinct communities. Typically, networks are robust to the removal of nodes with few links, but sensitive to the removal of hub nodes, linking hub nodes to the concept of keystone species

(Jeong et al., 2001; Steele et al., 2011; Paine, 1969). Many of the hub nodes within the canopy and edge microsites were several OTUs of Chitinophagaceae. These potential keystone OTUs may positively influence other bacteria by degrading recalcitrant litter into more labile substrates (Chung et al., 2012; Leschine, 1995). In interspace, Beijerinckiaceae is known to release plant growth regulators, amino acids, and pigments that may stimulate other microbes in the community (Daniela et al., 2003).

4.3 Functional differences across microsites

We found easily discernable differences in abundance and composition of predicted functional genes. The composition of canopy communities predicted 236 specific genes associated with the degradation of complex aromatic hydrocarbons. For example, exo-beta-1,3-glucanase degrades insoluble plant polysaccharides, such as callose, a glucose polysaccharide found in the plant cell wall. While interspace communities predicted 66 genes, including alanine-alpha-ketoisovalerate aminotransferase and ATP adenylyltransferase, which degrade simple amino acids and ATP, respectively. We focused our inquiry on genes associated with the degradation of C substrates. There were also many other genes associated with a wide diversity of genetic function from nitrogen cycling to cell reproduction.

4.4 Ecosystem processes linked to bacterial communities

Our results identified a robust tree island microsite effect on all ecosystem processes and potential links between the two more unique microsites and soil respiration and N mineralization. Along our tree island gradient, bacterial assemblies in tree island and interspace soils were distinct and associated with different gene expression that may in turn influence processes. For

example, soil respiration rates increasing 12% and N mineralization decreasing 29% in canopy than interspace soils. Similarly, Salles et al. found that increases in bacterial richness generally increased CO_2 emission but depended on interactions between specific bacteria and C substrates (Salles et al., 2009) Alternatively, soil communities at the edge of canopies, experienced both tree island and interspace soil conditions and generated similar levels of soil respiration as canopy soils regardless of not selecting for a highly connected community and/or specific genes. An explanation for this discrepancy may stem from differences in the quality and quantity of C substrates available to bacteria in the different microsites. The recalcitrant C substrates from a single predominant source in tree island canopies provided high amounts of low quality of DOC, which led to a decrease in metabolic efficiency, selecting for a specific population assembly and gene expression. Alternatively, a diverse composition of labile and recalcitrant C substrates from multiple sources (e.g., trees, perennial grasses, annual grasses, and forbs) potentially elevated function by promoting the activity of a wide range of taxa without the need for increased recovery of any gene(Miegrot et al., 2000). This increased soil respiration in tree islands agrees with the findings of Liao and Boutton that respiration per microbial biomass is higher in soils beneath trees than in interspaces despite a marked increase in soil organic C and microbial C (Liao and Boutton, 2008). Increased soil respiration in tree islands has been attributed to the decreased quality of pine needle litter. Low quality litter typically has high levels of polycyclic, aromatic C compounds with decreased levels of N.

P availability and N mineralization levels were also dependent on microsite. P availability was highest in the canopy microsite, which may be due to P not being a limiting nutrient in this ecosystem. As microorganisms degrade litter they release P into the soil. In edge and interspace microsites, grasses may be taking up P in the surface soils that we tested (0-2 cm)

while the tree roots may be too deep to deplete P levels. Additionally, N mineralization is not associated with any specific bacteria or process and is generally a result of degradation. However, specific microbial taxa may need higher amounts of N. The high rate of microbial respiration in edge indicates an increase in the rate of degradation, when coupled with an increased quality of litter may explain why N mineralization is so high in edge. Interspace has a better quality of DOC typically signifying increased N content. However, the decreased rate of microbial respiration may correlate with lower rates of N mineralization.

Although specific bacterial species are associated with certain ecosystem functions the majority of bacteria are hypothesized to contribute to general ecosystem processes. However, even among the high diversity of bacterial species we are beginning to identify ecological significance among taxa. For example, canopy microsites selected for Proteobacteria, specifically Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria, which are generally categorized as important sub-phyla for degradation, contributing to the increased soil respiration in tree islands (Tanahashi et al., 2005). Specifically, Hyphomicrobiaceae is intimately involved with C and N cycling and has a tendency to occur in areas with recalcitrant C substrates (Anderson et al., 2011). Actinobacteria are typically associated with plant roots and may be selected for in tree island interspaces due to a higher amount of root biomass in surface soils (Palaniyandi et al., 2013). Additionally, Beijerinckiaceae is known to degrade labile substrates such as methane and methanol (Lau et al., 2007).

4.5 Implications of fine woody debris treatment

Shredding had a minimal impact on the bacterial community as evidenced by the PCoA and PERMANOVA findings indicating that the tree island of fertility effect for bacteria remains

intact to some degree several years after the removal of the tree, which may be correlated to the maintained increases in organic matter and mineralizable N after tree removal (Walker et al., 2001). However, we did find that the FWD treatment increased microbial diversity, evenness, and richness which may be due to an increased amount of released C and/or water. Additionally, *Pinus* canopy control showed an interesting response in the heatmap analysis, possibly due to a relatively more labile carbon substrate compared to *Juniperus*. However, when large amounts of this relatively labile carbon substrate are added to the soil surface through shredding, the microbial community becomes more similar to *Juniperus* canopy communities

5. CONCLUSIONS

CANOPY and INTERSPACE microsites selected for a specific bacterial community, with a specific functional potential. The bacterial community in CANOPY is robust and highly connected, with the number of connections decreasing in INTERSPACE and to a greater degree in EDGE. The EDGE microsite showed little selection for bacterial communities and serves as a buffer between the distinct CANOPY and INTERSPACE communities. CANOPY soils showed high rates of soil respiration, and microbial quotient, most likely due to the decreased quality of DOC. However, CANOPY had the highest amount of available P, which may be due to the increased quantity of DOC. Conversely, bacteria in INTERSPACE received the highest quality litter and had the lowest rates of soil respiration and microbial quotient, despite the lowest quantity of available P and DOC. Last, EDGE had the highest N mineralization rates, followed by INTERSPACE and then CANOPY.

Table 1. Network co-occurrence metrics for each microsite and tree species.

	Canopy		Edge		Interspace
	Juniperus	*Pinus*	*Juniperus*	*Pinus*	
Nodes	411	305	297	291	331
Edges	9,498	6,421	1,118	629	1238
Mean Path Length	2.71	2.54	4.51	5.36	4.30
Mean Degree	46.2	42.1	7.53	4.32	7.48
Mean Clustering Coefficient	0.70	0.67	0.58	0.52	0.57
Density	0.11	0.14	0.03	0.02	0.02
Modularity	0.66	0.55	0.73	0.79	0.74

Table 2. Ten highest connected nodes for each microsite and tree species. Nodes are OTUs that occur in in at least 75% of the samples for a given microsite. Degree is the number of edges (significant correlations with other nodes) connected to the one node.

		Phyla	Family	Degree	Relative Recovery
Canopy	*Juniperus*	Alphaproteobacteria	Caulobacteraceae	66	0.03
		Alphaproteobacteria	Rhodospirillaceae	66	0.07
		Acidobacteria	Unclassified	65	0.05
		Actinobacteria	Unclassified	65	0.04
		Actinobacteria	Nocardioidaceae	65	0.05
		Actinobacteria	Unclassified	65	0.06
		Alphaproteobacteria	Hyphomicrobiaceae	65	1.06
		Alphaproteobacteria	Hyphomicrobiaceae	64	0.55
		Alphaproteobacteria	Hyphomicrobiaceae	64	0.38
		Bacteroidetes	Chitinophagaceae	64	0.05
	Pinus	Actinobacteria	Unclassified	105	0.08
		Acidobacteria	Unclassified	99	0.02
		Alphaproteobacteria	Rhizobiaceae	99	0.04
		Bacteroidetes	Chitinophagaceae	99	0.02
		Betaproteobacteria	Unclassified	99	0.15
		Betaproteobacteria	Unclassified	99	0.09
		Gammaproteobacteria	Sinobacteraceae	99	0.02
		Verrucomicrobia	[Chthoniobacteraceae]	99	0.01
		Alphaproteobacteria	Hyphomicrobiaceae	74	0.10
		Bacteroidetes	Chitinophagaceae	74	0.83
Edge	*Juniperus*	Actinobacteria	Micromonosporaceae	24	0.04
		Acidobacteria	Unclassified	22	0.37
		Verrucomicrobia	[Chthoniobacteraceae]	22	0.16
		Actinobacteria	Geodermatophilaceae	21	0.47
		Actinobacteria	Pseudonocardiaceae	21	0.96
		Bacteroidetes	Chitinophagaceae	21	0.03

		Verrucomicrobia	[Chthoniobacteraceae]	21	1.82
		Actinobacteria	Geodermatophilaceae	20	0.22
		Alphaproteobacteria	Acetobacteraceae	20	0.37
		Verrucomicrobia	[Chthoniobacteraceae]	20	0.19
	Pinus	Alphaproteobacteria	Unclassified	20	0.02
		Planctomycetes	Unclassified	19	0.07
		Alphaproteobacteria	Sphingomonadaceae	15	0.04
		Actinobacteria	Unclassified	13	0.59
		Betaproteobacteria	Oxalobacteraceae	13	0.26
		Alphaproteobacteria	Acetobacteraceae	12	0.19
		Bacteroidetes	Sphingobacteriaceae	12	0.02
		Chloroflexi	Caldilineaceae	12	0.20
		Actinobacteria	Solirubrobacteraceae	11	0.06
		Bacteroidetes	Chitinophagaceae	11	0.35
Interspace		Alphaproteobacteria	Beijerinckiaceae	24	0.11
		Actinobacteria	Geodermatophilaceae	22	0.07
		Actinobacteria	Unclassified	21	0.15
		Actinobacteria	Rubrobacteraceae	21	1.38
		Alphaproteobacteria	Unclassified	21	0.02
		Chloroflexi	[Kouleothrixaceae]	21	0.32
		Chloroflexi	Unclassified	21	0.08
		Actinobacteria	Unclassified	20	0.29
		Alphaproteobacteria	Beijerinckiaceae	20	0.13
		Planctomycetes	Unclassified	20	0.44

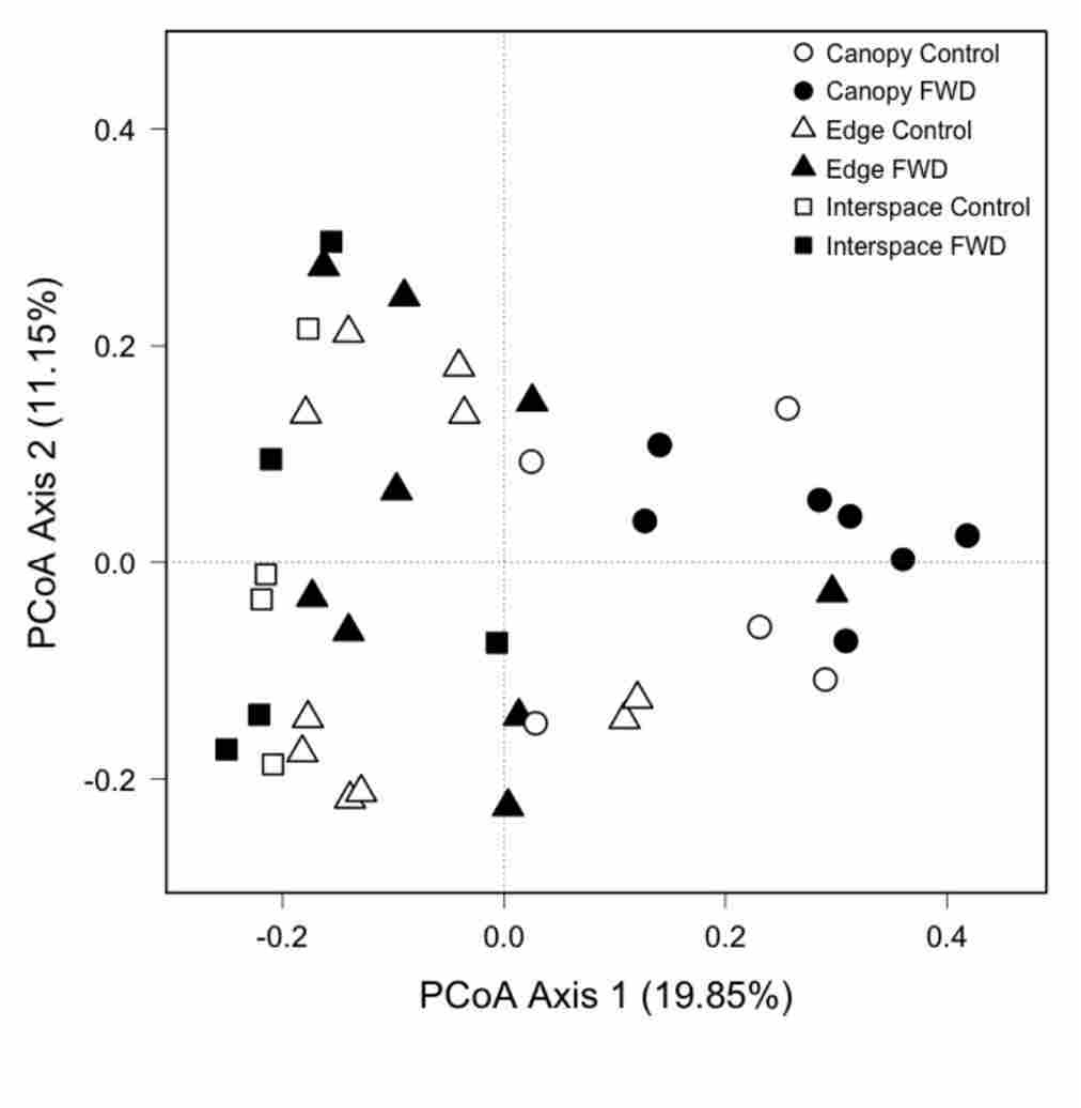


Figure 1. Tree island-of-fertility microsites structured bacterial communities and CWD additions enhanced these differences. The three microsites included: soils beneath tree canopies (CANOPY); soils at the tree canopy edge where litter and/or duff and barren soil surfaces intersect (EDGE); and tree interspace soils consisting of barren surfaces and/or surfaces covered with annual or perennial grasses and/or forbs (INTERSPACE). Microsites Three microsites, including, tree canopy, tree canopy edge, and interspace, as well as a coarse woody debris (CWD) treatment and control. The multivariate ordination was generated using principle coordinate analysis (PCoA) and a Bray-Curtis abundance-based distance matrix of samples × OTUs from 16S rRNA community libraries (97% similarity cut-off). The percent of variation among communities explained by each axis is noted in parentheses.

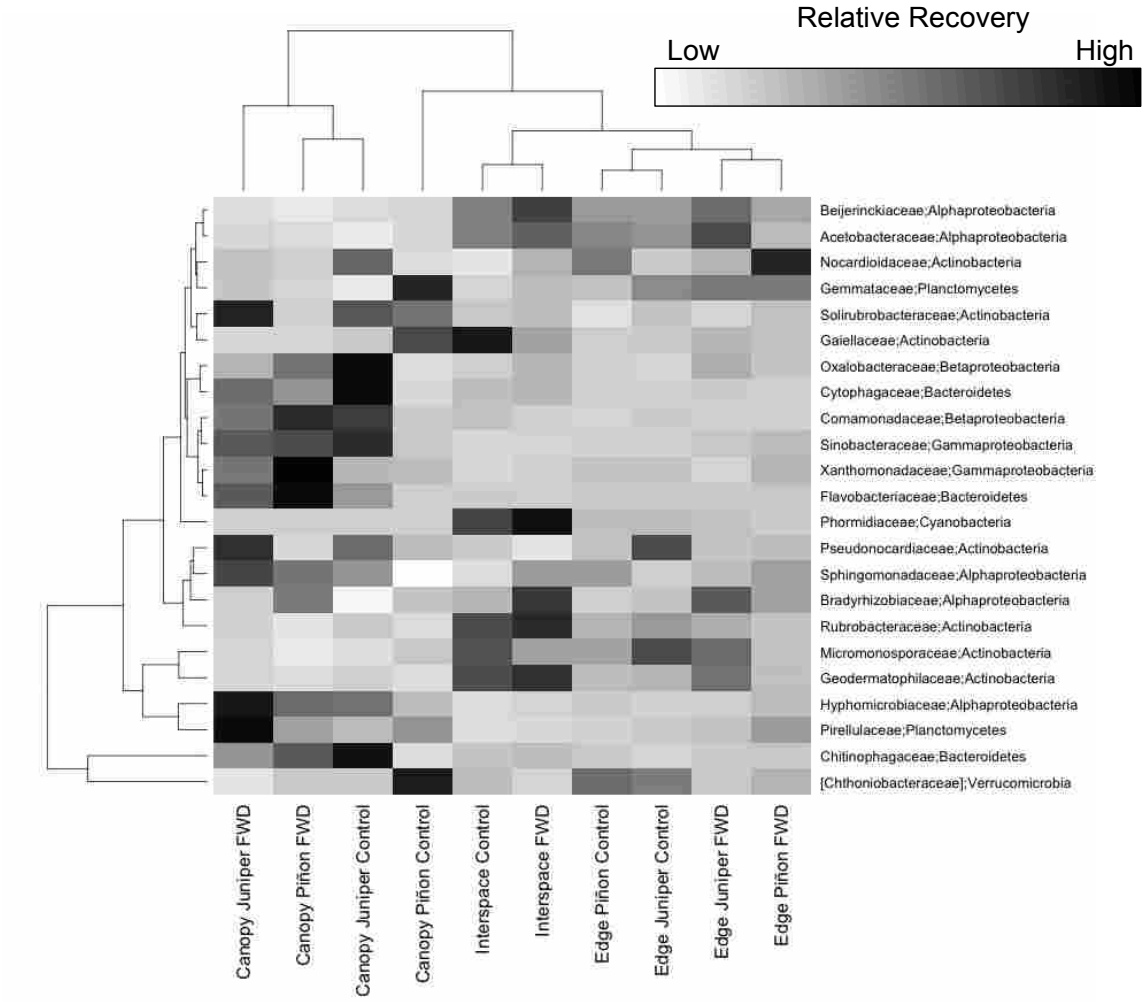


Figure 2. Canopy and interspace microsites selected for specific bacterial families based on a heat map and hierarchical clustering of the relative recovery of twenty-three bacterial families in the three microsites in soils beneath *Juniperus* and *Pinus* species. Values are based on means with hierarchal clustering of soil microsites for each tree species (bottom) and families (left). Only families that contributed ≥ 1.0% to the total recovery of communities are presented with recovery based on 16S rRNA libraries.

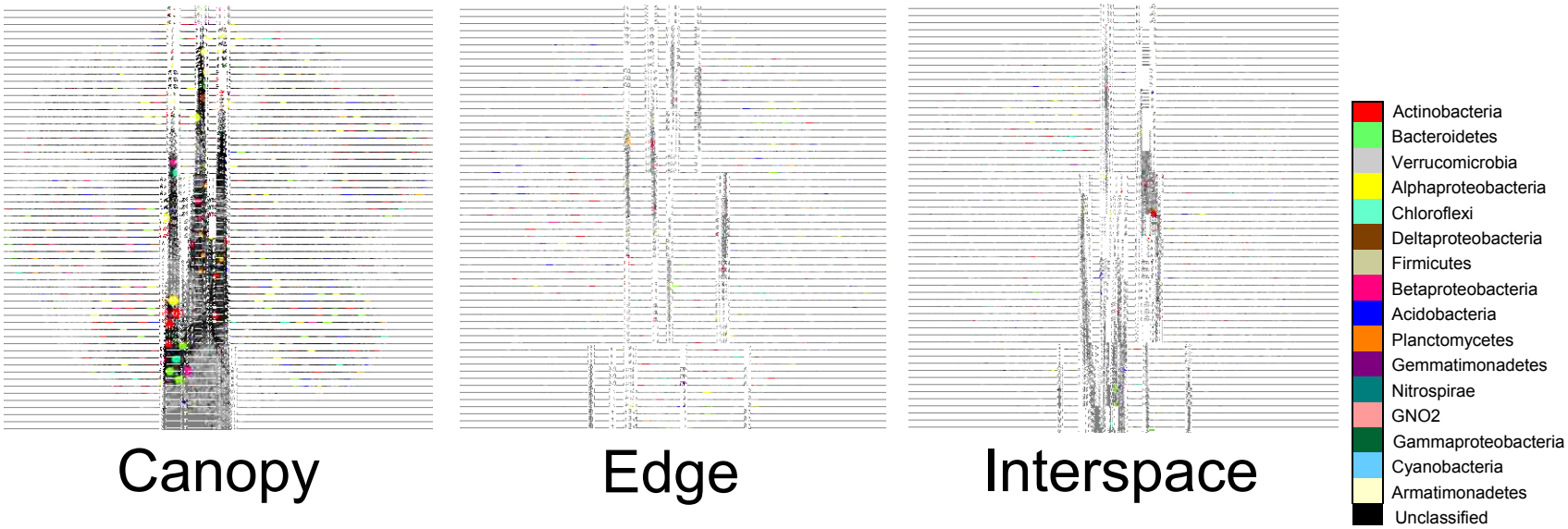


Figure 3. Network co-occurrence models using maximal information coefficient (MIC) analysis across *Juniperus* tree island microsites. Nodes (circles) in the network models represent OTUs from 16S rDNA community libraries (97% similarity cut-off) with edges (connecting lines between nodes) representing significant co-occurrence connections that occur in at least 75% of the samples for a given microsite and have an MIC that is both >0.7 and statistically significant (P<0.01). Colors represent different phyla as shown in the figure key.

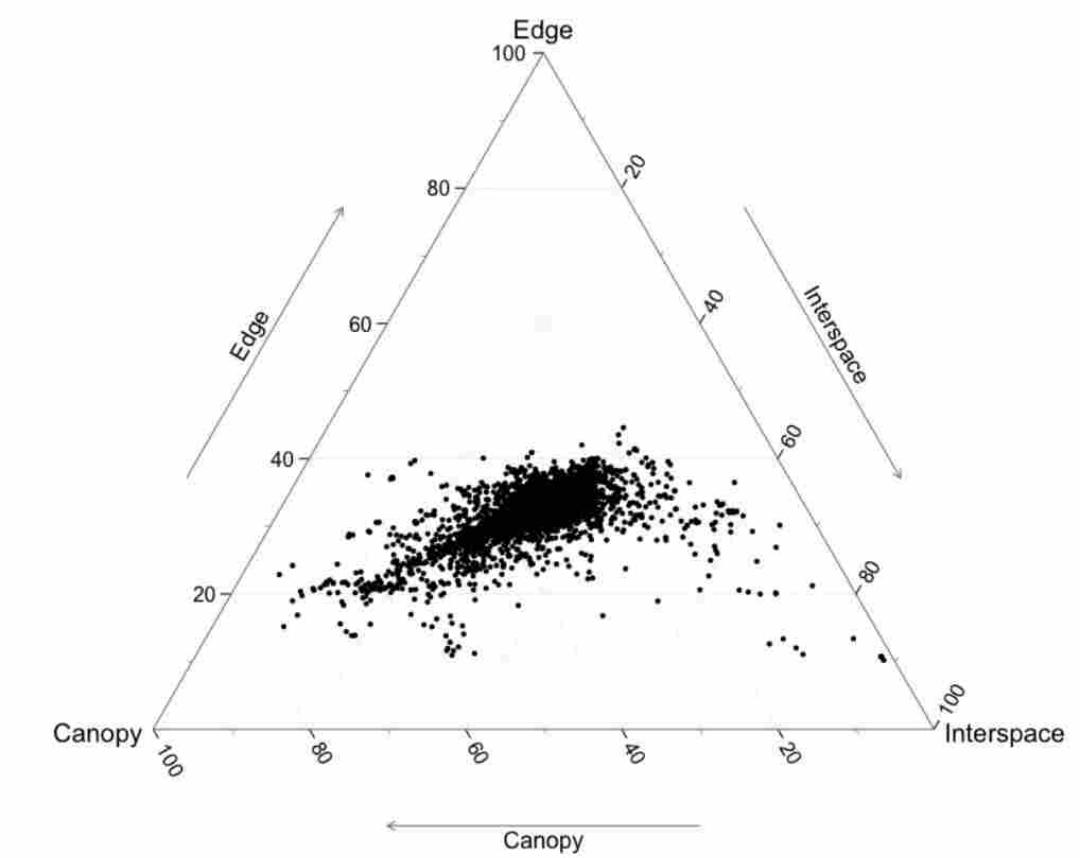


Figure 4. Functional gene differences between microsites predicted using phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) to predict genes based on the clusters of orthologous groups (COG) database. Relative gene recovery was calculated for each site and percent recovery for a gene was calculated for each microsite by dividing the gene recovery for a microsite by the total recovery for the given gene.

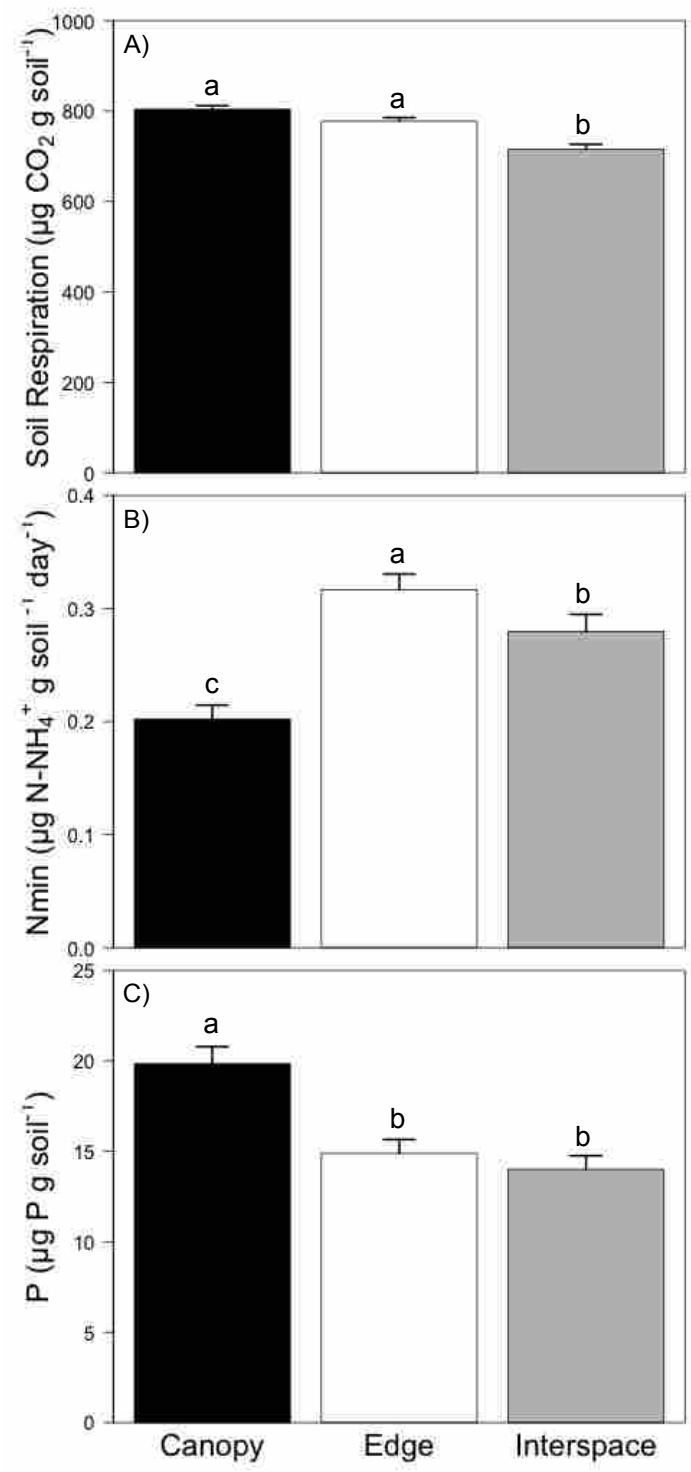


Figure 5. Soil respiration (A), N mineralization (B), and P availability in the three tree island microsites. Soil respiration (µg CO_2 g $soil^{-1}$ day^{-1}), N mineralization (µg $N\text{-}NH_4^+$ g $soil^{-1}$ day^{-1}), and P availability (µg P g $soil^{-1}$) values are means (n=340) ± SEM. Different letters indicate significant differences between treatments (P<0.05) based on one-way ANOVAs and Tukey's HSD tests.

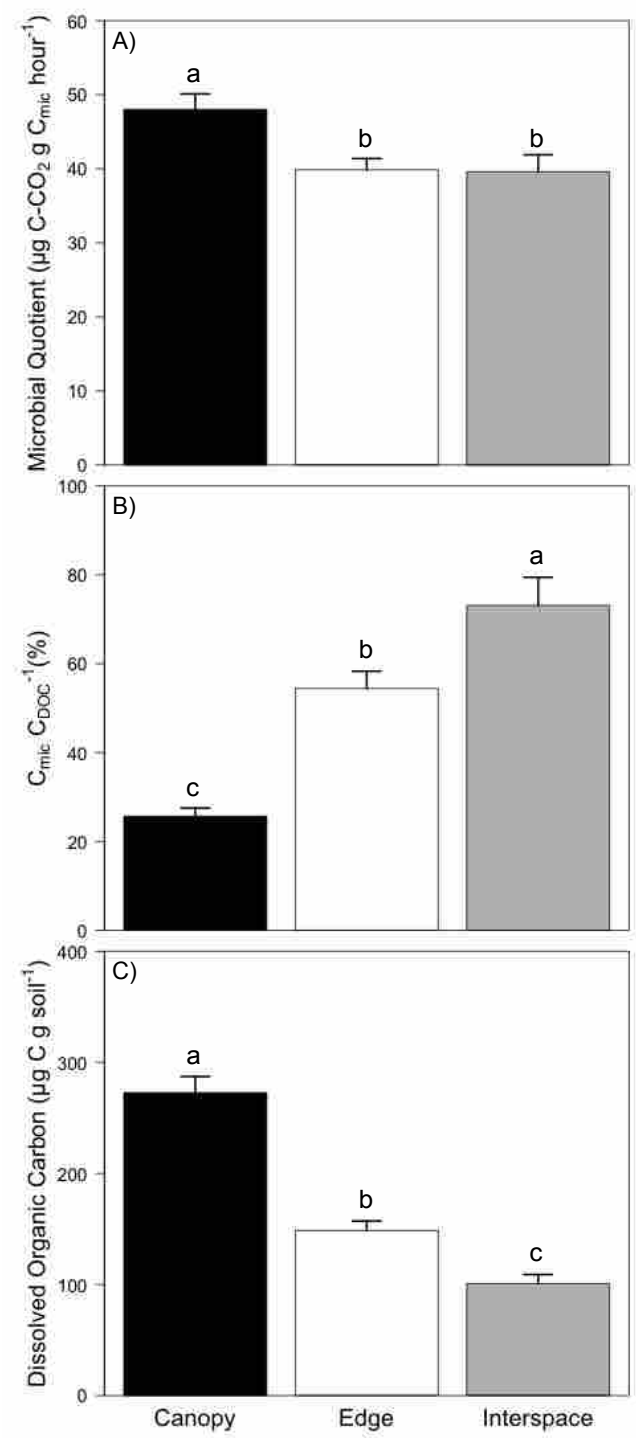


Figure 6. Differences in microbial metabolic function in soil microsites. Microbial quotient (A) (µg C-CO_2 g C_{mic} $hour^{-1}$) estimating microbial efficiency and C substrate quality (B) (C_{mic} C_{DOC}^{-1} %) identifying the amount C as biomass (C_{mic}) supported by dissolved organic C (C_{DOC}) values are means (n=338 and n=331) ± SEM. Last, to help interpret C substrate quality, DOC concentrations (C) (µg C g $soil^{-1}$) are presented (means ± SEM, n=337). Significant differences between treatments (P<0.05) are based on one-way ANOVAs and Tukey's HSD test.

REFERENCES:

Aanderud, Z.T., Jones, S.E., Fierer, N., Lennon, J., 2015. Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. Frontiers in Microbiology 6, 24.

Aanderud, Z.T., Lennon, J.T., 2011. Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. Applied and Environmental Microbiology 77(13), 4589-4596.

Aanderud, Z.T., Shuldman, M.I., Drenovsky, R.E., Richards, J.H., 2008. Shrub-interspace dynamics alter relationships between microbial community composition and belowground ecosystem characteristics. Soil Biology and Biochemistry 40(9), 2206-2216.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46.

Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R., 2011. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen, and phosphorous. Pedobiologia 54(5-6), 309-320.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10, 215-221.

Bailey, V.L., Peacock, A.D., Smith, J.L., Bolton, Jr., H., 2002. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biology and Biochemistry 34, 1385-1389.

Bastian, M., Heymann, S., Jacomy, M., (2009). Gephi: an open source software for exploring and manipulating networks. International AAAI Conference on Weblogs and Social Media.

Bates, J.D., Davies, K.W., Sharp, R.N., 2011. Shrub-steppe early succession following juniper cutting and prescribed fire. Environmental Management 47, 468-481.

Behm, A.L., Duryea, M.L., Long, A.J., Zipperer, W.C., 2004. Flammability of native understory species in pine flatwood and hardwood hammock ecosystems and implications for the wildland-urban interface. International Journal of Wildland Fire 13(3), 355-365.

Berry, D., Widder, S., 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Frontiers in Microbiology 5, 219.

Billings, S.A., Schaeffer, S.M., Evans, R.D., 2002. Trace N gas losses and N mineralization in Mojave Desert soils exposed to elevated CO_2. Soil Biology and Biochemistry 34, 1777-1784.

Blankenberg, D., Von Kuster, G., Coraor, N., Ananda, G., Lazarus, R., Mangan, M., Nekrutenko, A., Taylor, J. Galaxy: a web-based genome analysis tool for experimentalists. Current Protocols in Molecular Biology Chapter 19, Unit 19.10.1-21.

Bodelier, P.L.E., Meima-Franke, M., Hordijk, C.A., Steenbergh, A.K., Hefting, M.M., Bodrossy, L., von Bergen, M., Seifert, J., 2013. Microbial minorities modulate methane consumption through niche partitioning. ISME Journal 7(11), 2214-2228.

Chaffron, S., Rehrauer, H., Pernthaler, J., Von Mering, C., 2010. A global network of coexisting microbes from environmental and whole-genome sequence data. Genome Res. 20, 947-959.

Chao, A., 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11(4), 265-270.

Charley, J.L., West, N.E., 1975. Plant-induced soil chemical patterns in some shrub-dominated semi-desert ecosystems of Utah. Journal of Ecology 63, 945-963.

Christensen, O., 1977. Estimation of standing crop and turnover of dead wood in a Danish oak forest. Oikos 28, 177-186.

Chung, E.J., Park, T.S., Jeon, C.O., Chung, Y.R., 2012. *Chitinophaga oryziterrae* sp. nov., isolated from the rhizosphere soil of rice (*Oryza sativa* L.). IJSEM 62, 3030-3035.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Research 37: D141-145.

Coleman, D.C., Whitman, W.B., 2005. Linking species richness, biodiversity and ecosystem function in soil systems. Pedobiologia 49(6), 479-497.

Daniel, R., 2004. The soil metagenome – a rich resource for the discovery of novel natural products. Current Opinion in Biotechnology 15, 199-204.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72: 5069-5072.

Elmendorf, S.C., Moore, K.A., 2008. Use of community-composition data to predict the fecundity and abundance of species. Conservation Biology 22(6), 1523-1532.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16): 2194-2200.

Fierer, N., Schimel, J.P., 2002. Effects of drying-rewetting frequency on soil C and N transformations. Soil Biology & Biochemistry 34, 777-787.

Forster, J.C., 1995. Soil nitrogen. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, San Diego, pp 79-87.

Freilich, S., et al., 2010. The large-scale organization of the bacterial network of ecological co-occurrence interactions. Nucleic Acids Res. 38, 3857-3868.

Gallardo, A., Schlesinger, W.H., 1992. Carbon and nitrogen limitations of soil microbial biomass in desert ecosystems. Biogeochemistry 18, 1-17.

Gamfeldt, L., Hillebrand, H., Jonsson, P.R., 2008. Multiple functions increase the importance of biodiversity for overall ecosystem functioning. Ecology 89(5), 1223-1231.

Giardine, B., Riemer, C., Hardison, R.C., Burhans, R., Elnitski, L., Shah, P., Zhang, Y., Blankenberg, D., Albert, I., Taylor, J., Miller, W., Kent, W.J., Nekrutenko, A., 2005. Galaxy: a platform for interactive large-scale genome analysis. Genome Research 15(10), 1451-1455.

Goecks, J., Nekrutenko, A., Taylor, J., The Galaxy Team, 2010. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 11(8), R86.

Gutteridge, A., Kanehisa, M., Goto, S., 2007. Regulation of metabolic networks by small molecule metabolites. BMC Bioinformatics 8, 88.

Hamilton, N., 2014. ggtern: An extension to ggplot2, for the creation of ternary diagrams. R package version 1.0.3.2. http://CRAN.R-project.org/package=ggtern

Harmon, M.E., Franklin, J.F., Swanson, F.J., Sollins, P., Gregory, S.V., Lattin, J.D., Anderson, N.H., Cline, S.P., Aumen, N.G., Sedell, J.R., Lienkaemper, G.W., Cromack, K., Cummins, K.W., 1986. Ecology of coarse woody debris in temperate ecosystems. Adv. Ecol. Res. 15, 133-302.

Hart, S.C., 1999. Nitrogen transformations in fallen tree boles and mineral soil of old-growth forests. Ecology 80, 1385-1394.

Horner, J.D., Gosz, J., Cates, R.G., 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. American Naturalist 132, 869-883.

Hunting, E.R., Vijver, M.G., van der Geest, H.G., Mulder, C., Kraak, M.H.S., Breure, A.M., Admiraal, W., 2015. Resource niche overlap promotes stability of bacterial community metabolism in experimental microcosms. Frontiers in Microbiology 6, 105.

Jeong, H., Mason, S.P., Barabási, A.-L., Oltvai, Z.N., 2001. Lethality and centrality in protein networks. Nature 411, 41-42.

Jimenez, D.J., Dini-Andreote, F., van Elsas, J.D., 2014. Metataxonomic profiling and prediction of functional behavior of wheat straw degrading microbial consortia. Biotechnology for Biofuels 7.

Junker, B.H., Schreiber, 2008. Analysis of biological networks. John Wiley & Sons, Inc. New Jersey.

Kaye, J.P., Hart, S.C., 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology and Evolution 12, 139-143.

Kieft, T.L., White, C.S., Loftin, S.R., Aguilar, R., Craig, J.A., Skaar, D.A., 1998. Temporal dynamics in soil carbon and nitrogen resources at a grassland-shrubland ecotone. Ecology 79, 671-683.

Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems-a review. Plant and Soil 256, 41-66.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., a Reyes, J., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 8, 1-10.

Lau, E., Ahmad, A., Steudler, P.A., Cavanaugh, C.M., 2007. Molecular characterization of methanotrophic communities in forest soils that consume atmospheric methane. FEMS Microbiology Ecology 60(3), 490-500.

Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in soil microbial communities across land-use types. The ISME Journal 7, 1641-1650.

Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9, 119-130.

Leschine, S.B., 1995. Cellulose degradation in anaerobic environments. Annu. Rev. Microbiol. 49, 399-426.

Liao, J.D., Boutton, T.W., 2008. Soil microbial biomass response to woody plant invasion of grassland. Soil Biology & Biochemistry 40, 1207-1216.

Ludwig, J.A., Tongway, D.J., 1997. A landscape approach to rangeland ecology. In: Ludwig, J., Tongway, D., Freudenberger, D., Noble, J., Hodgkinson, K. (Eds), Landscape Ecology: Function and Management. CSIRO Publishing, Collingwood, Victoria (Australia).

Miegroet, H.V., Hysell, M.T., Johnson, A.D., 2000. Soil microclimate and chemistry of spruce-fir tree islands in northern Utah. Soil Science Society of America Journal 64(4), 1515-1525.

Miller, R.F., Rose, J.A., 1999. Fire history and western juniper encroachment in sagebrush steppe. Journal of Range Management 52, 550-559.

Miller, R.F., Svejcar, T.J., Rose, J.A., 2000. Impacts of western juniper on plant community composition and structure. Journal of Range Management 53, 574-585.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide-Biology and Chemistry 5, 62-71.

Montoya, J.M., Pimm, S.L., Solé, R.V., 2006. Ecological networks and their fragility. Nature 442, 259-264.

Mumm, R., Hilker, M., 2006. Direct and indirect chemical defence of pine against folivorous insects. Trends in Plant Science 11(7), 351-358.

Norden, B., Ryberg, M., Gotmark, F., Olausson, B., 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in termperate broadleaf forests. Biological Conservation 117(1), 1-10.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2014. Vegan: community ecology package. R package version 2.2-0. http://CRAN.R-project.org/package=vegan

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorous in soils by extraction with sodium bicarbonate. USDA Cir. No. 939.

Paine, R.T., 1969. A note on trophic complexity and community stability. Am. Nat. 103, 91-93.

Palaniyandi, S.A., Yang, S.H., Damodharan, K., Suh, J.W., 2013. Genetic and functional characterization of culturable plant-beneficial actinobacteria associated with yam rhizosphere. Journal of Basic Microbiology 53(12), 985-995.

Perez, C.A., Carmona, M.R., Aravena, J.C., Armesto, J.J., 2004. Successional changes in soil nitrogen availability, non-symbiotic nitrogen fixation and carbon/nitrogen ratios in southern Chilean forest ecosystems. Oecologia 140, 617.

Pester, M., Bittner, N., Deevong, P., Wagner, M., Loy, A., 2010. A 'rare biosphere' microorganism contributes to sulfate reduction in peatland. ISME Journal 4(12), 1591-1602.

Pham, V.H.T., Kim, J., 2012. Cultivation of unculturable soil bacteria. Trends in Biotechnology 30(9), 475-484.

Pregitzer, K.S., Euskirchen, E.S., 2004. Carbon cycling and storage in world forests: biome patterns related to forest age. Global Change Biology 10, 2052-2077.

PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, accessed 4 February 2013.

Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green, J.L., Green, L.E., Killham, K., Lennon, J.J., Osborn, A.M., Solan, M., van der Gast, C.J., Young, J.P.W., 2007. The role of ecological theory in microbial ecology. Nature Reviews Microbiology 5, 384-392.

Puigdefabregas, J., Sole, A., Gutierrez, L., del Barrio, G., Boer, M., 1999. Scales and processes of water and sediment redistribution in drylands: results from the Rambla Honda field site in Southeast Spain. Earth-Sci. Rev. 48, 39-70.

Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing noise from pyrosequenced amplicons. BMC Bioinformatics 12.

Ruan, Q., Dutta, D., Schwalbach, M.S., Steele, J.A., Fuhrman, J.A., Sun, F., 2006. Local similarity analysis reveals unique associations among marine bacterioplankton species and environmental factors. Bioinformatics 22, 2532-2538.

Reshef, D.N., Reshef, Y.A., Finucane, H.K., Grossman, S.R., McVean, G., Turnbaugh, P.J., Lander, E.S., Mitzenmacher, M., Sabeti, P.C., 2011. Detecting novel associations in large data sets. Science 334, 1518-1524.

R Development Core Team (2013). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.ISBN 3-900051-07-0, URL http://www.R-project.org.

Salles, J.F., Poly, F., Schmid, B., Le Roux, X., 2009. Community niche predicts the functioning of denitrifying bacterial assemblages. Ecology 90(12), 3324-3332.

Schade, J.D., Collins, S., Sponseller, R., Stiles, A., 2003. The influence of Mesquite on understory vegetation: effects of landscape position. J. Vegetation Sci. 14, 743-750.

Schade, J.D., Hobbie, S.E., 2005. Spatial and temporal variation in islands of fertility in the Sonoran Desert. Biogeochemistry 73, 541-553.

Schaeffer, S.M., Billings, S.A., Evans, R.D., 2003. Responses of soil nitrogen dynamics in a Mojave Desert ecosystem to manipulations in soil carbon and nitrogen availability. Oecologia 134.

Schaeffer, S.M., Billings, S.A., Evans, R.D., 2007. Laboratory incubations reveal potential responses of soil nitrogen cycling to changes in soil C and N availability in Mojave Desert soils exposed to elevated atmospheric CO_2. Global Change Biology 13.

Schlesinger, W.H., Pilmanis, A.M., 1998. Plant-soil interactions in deserts. Biogeochemistry 42, 169-187.

Schlesinger, W.H., Raikes, J.A., Hartley, A.E., Cross, A.E., 1996. On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77, 364-374.

Schloss, P.D., *et al.,* 2009. Introducing Mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75(23), 7537-7541.

Shokralla, S., Spall, J.L., Gibson, J.F., Hajibabaei, M., 2012. Next-generation sequencing technologies for environmental DNA research. Mol. Ecol. 21, 1794-1805.

Steele, J.A., et al., 2011. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. ISME Journal 5, 1414-1425.

Tanahashi, T., Murase, J., Matsuya, K., Hayashi, M., Kimura, M., Asakawa, S., 2005. Bacterial communities responsible for the decomposition of rice straw compost in a Japanese rice paddy field estimated by DGGE analysis of 16S rDNA and 16S rRNA fragments. Soil Science and Plant Nutrition 51(3), 351-360.

Tatusov, R.L., Galperin, M.Y., Natale, D.A., Koonin, E.V., 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Research 28, 33-36.

Thomas, G.W., Peaslee, D.E., 1973. Testing soils for Phosphorous. In: Walsh, L.M., Beaton, J.D. (Eds.), Soil Testing and Plant Analysis. Soil Sci. Soc. Am., Madison, WI. pp. 115-132.

Thuler, D.S., Floh, E.L., Handro, W., Barbosa, H.R., 2003. *Beijerinckia derxii* releases plant growth regulators and amino acids in synthetic media independent of nitrogenase activity. Journal of Applied Microbiology 95(4), 799-806.

Trosvik, P., Rudi, K., Straetkvern, K.O., Jakobsen, K.S., Naes, T., Stenseth, N.C., 2010. Web of ecological interactions in an experimental gut microbiota. Environ. Microbiol 12(10), 2677-2687.

Van Breemen, N., Finzi, A.C., 1998. Plant-soil interactions: ecological aspects and evolutionary implications. Biogeochemistry 42, 1-19.

Vollmer, A.T., Au, F., Bamberg, S.A., 1977. Observations on the distribution of microorganisms in desert soils. Great Basin Naturalist 37, 81-86.

Wainhouse, D., Staley, J.T., Jinks, R., Morgan, G., 2009. Growth and defence in young pine and spruce and the expression of resistance to a stem-feeding weevil. Oecologia 158(4), 641-650.

Wainwright, J., Parsons, A.J., Schlesinger, W.H., Abrahams, A.D., 2002. Hydrology-vegetation interactions in areas of discontinuous flow on a semi-arid bajada, Southern New Mexico. J. Arid Environ. 51, 319-338.

Walker, L.R., Thompson, D.B., Landau, F.H., 2001. Experimental manipulations of fertile islands and nurse plant effects in the Mojave Desert, USA. Western North American Naturalist 61.

Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67, 321-358.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorous in water and $NaHCO_3$ extractants for soil. Soil Sci. Soc. Amer. Proc. 29, 677-678.

Wickham, H., 2009. ggplot2: Elegant graphics for data analysis. Springer New York.

Yan, E., Wang, X., Huang, J., 2006. Concept and classification of coarse woody debris in forest ecosystems. Frontiers of Biology in China 1(1), 76-84.

Young, K.R., Roundy, B.A., Eggett, D.L., 2014. Mechanical mastication of Utah Juniper encroaching sagebrush steppe increases inorganic soils N. Applied and Environmental Soil Science, dx.doi.org/10.1155/2014/632757.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., et al., 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology 75, 2333-2347.

Zedler, P.H., 1995. Are some plants born to burn? Trends in Ecology and Evolution 10, 393-395.

Zhao, J., Yu, H., Luo, J.-H., Cao, Z.-W. Li, Y.-X., 2006. Hierarchical modularity of nested bow-ties in metabolic networks. BMC Bioinformatics 7, 386.

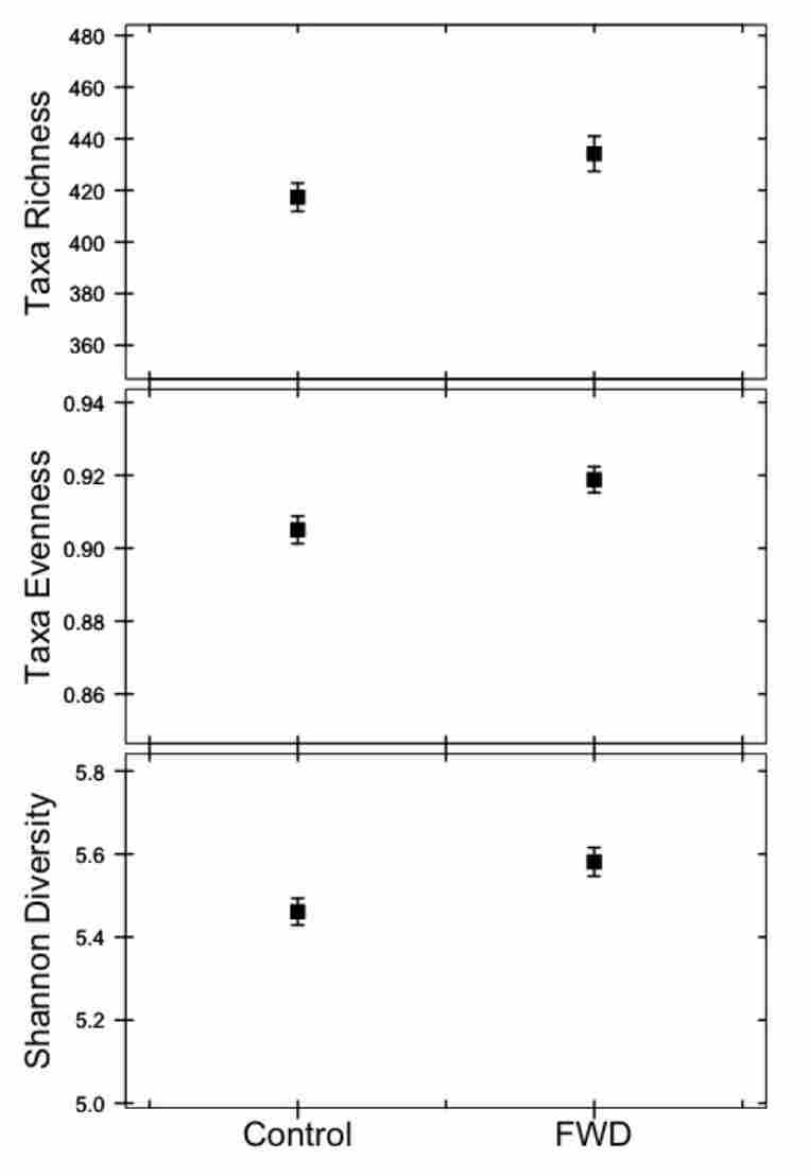
Zhou, J., et al., 2010. Functional molecular ecological networks. mBio 1, e00169-e00110.

Supplemental Table 1. General soil characteristics, elevation, mean annual temperature (MAT), and mean annual precipitation (MAP) of thirty-eight piñon-juniper woodland sites used to evaluate the effects of tree islands of fertility and coarse woody debris additions on soil bacteria. Soil characteristics values are means (n = 3) ± one standard error from surface and subsurface soils (0-2 cm and 15-17 cm depth). MAT and MAP data are from the Oregon State PRISM website (PRISM Climate Group, 2004). Mean temperature (temp) and precipitation (precip) are based on a thirty-year period (1982-2012).

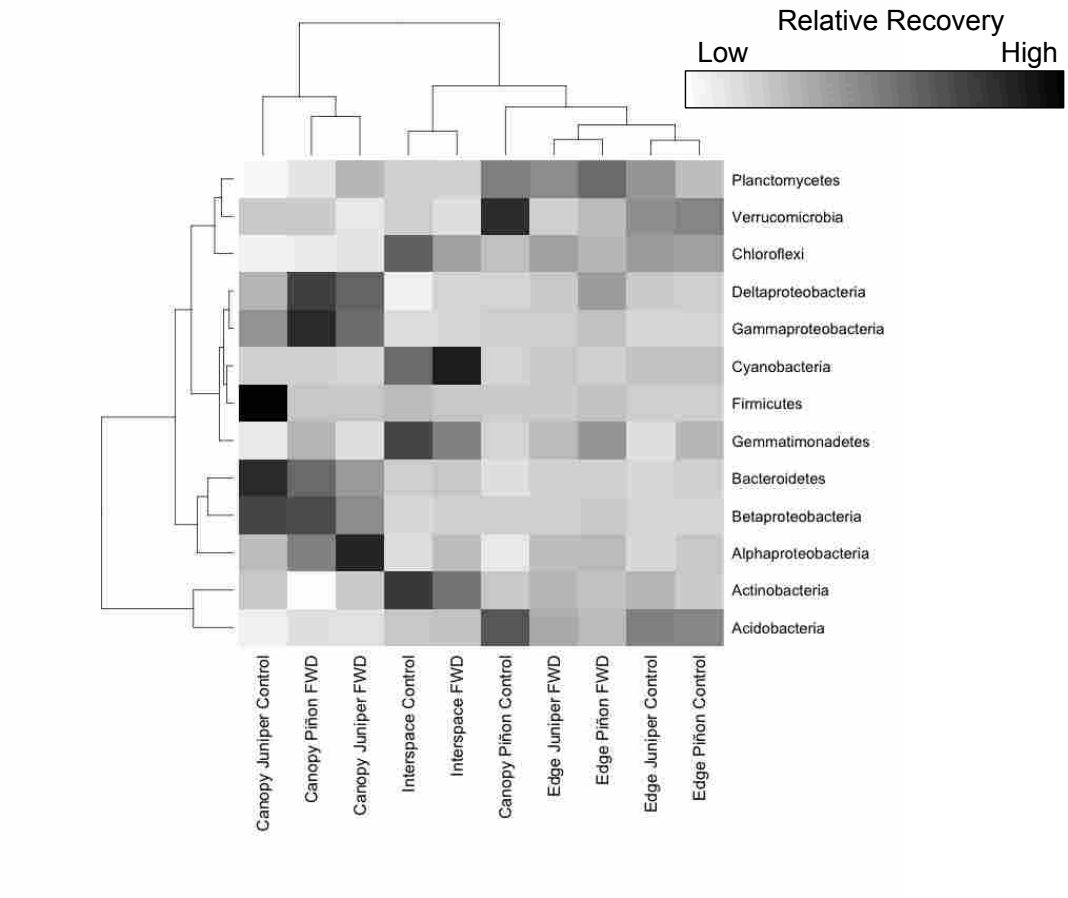
Site	pH	EC ds/m	% N	% C	% Sand	% Silt	% Clay	Elev (M)	MAT (ºC)	MAP (mm)
Anderson Mountain	6.3 ± 0.13	0.29 ± 0.06	0.10 ± 0.01	1.3 ± 0.28	51 ± 5.8	31 ± 3.0	19 ± 2.5	1914	9.7	348
Augusi	7.1 ± 0.06	1.0 ± 0.30	0.25 ± 0.04	8.3 ± 0.76	45 ± 3.9	28 ± 3.1	28 ± 6.5	2298	7.3	445
Black Dragon	7.5 ± 0.07	0.61 ± 0.02	0.27 ± 0.02	11 ± 0.65	37 ± 4.0	37 ± 4.0	26 ± 1.7	2512	4.5	483
Blue Valley	6.7 ± 0.24	0.72 ± 0.12	0.17 ± 0.01	2.9 ± 0.46	42 ± 2.2	31 ± 0.52	27 ± 1.7	1967	9.5	340
Chokecherry	6.6 ± 0.12	0.48 ± 0.04	0.15 ± 0.01	1.8 ± 0.33	46 ± 5.9	31 ± 3.4	24 ± 2.9	1835	8.2	300
Columbia	7.1 ± 0.03	0.54 ± 0.13	0.15 ± 0.01	7.2 ± 0.25	48 ± 1.1	29 ± 1.4	24 ± 2.5	1860	9.5	297
Cook Canyon	6.8 ± 0.14	0.46 ± 0.06	0.22 ± 0.04	3.9 ± 1.25	41 ± 2.3	39 ± 2.6	21 ± 0.42	1799	7.1	322
Goslin	6.1 ± 0.45	0.52 ± 0.12	0.14 ± 0.01	2.0 ± 0.58	55 ± 5.2	28 ± 4.5	18 ± 0.65	2034	6.8	333
Government Creek	7.2 ± 0.02	0.57 ± 0.02	0.25 ± 0.05	5.9 ± 1.2	34 ± 2.9	42 ± 4.3	24 ± 2.9	1768	10	406
Grantsville	7.3 ± 0.05	0.47 ± 0.10	0.23 ± 0.01	3.9 ± 0.42	39 ± 1.3	33 ± 3.5	28 ± 2.7	1746	11	470
Greenville	7.2 ± 0.30	0.52 ± 0.14	0.17 ± 0.04	3.8 ± 1.9	45 ± 1.3	31 ± 3.2	24 ± 3.3	1786	10	338
Hiawatha	7.4 ± 0.07	1.3 ± 0.35	0.20 ± 0.07	5.1 ± 3.3	62 ± 1.1	19 ± 6.7	19 ± 7.8	1896	7.8	282
Hwy 56 black sagebrush	7.4 ± 0.13	0.49 ± 0.08	0.16 ± 0.03	5.3 ± 1.9	49 ± 1.1	27 ± 0.39	24 ± 1.5	1682	12	351
Hyatt Springs	6.2 ± 0.28	0.23 ± 0.04	0.13 ± 0.01	2.4 ± 0.58	58 ± 1.2	24 ± 3.8	18 ± 2.0	1822	12	411

Indian Springs	6.5 ± 0.40	0.79 ± 0.26	0.26 ± 0.05	8.1 ± 1.9	22 ± 3.8	47 ± 2.4	30 ± 5.7	2320	6.5	462
James Ranch	7.1 ± 0.06	0.45 ± 0.02	0.15 ± 0.00	5.1 ± 0.26	29 ± 4.4	40 ± 4.7	31 ± 1.6	1653	10	351
Keg Springs	7.2 ± 0.03	0.51 ± 0.06	0.22 ± 0.01	6.3 ± 1.3	34 ± 2.3	37 ± 2.2	28 ± 1.1	1774	7.7	305
Muddy Creek	6.3 ± 0.22	0.21 ± 0.04	0.08 ± 0.00	0.63 ± 0.05	75 ± 6.4	13 ± 5.0	12 ± 2.2	1766	11	386
Natural Bridges	7.4 ± 0.05	0.49 ± 0.07	0.17 ± 0.03	4.3 ± 2.7	50 ± 3.1	28 ± 3.4	22 ± 1.6	1856	13	292
Onaqui	7.5 ± 0.06	0.81 ± 0.35	0.26 ± 0.09	6.6 ± 2.8	39 ± 1.5	37 ± 3.2	24 ± 3.3	1709	10	348
Ray Mesa	6.7 ± 0.20	0.46 ± 0.10	0.21 ± 0.04	4.2 ± 0.94	45 ± 3.6	42 ± 3.2	15 ± 0.82	2271	10	442
Sand Hollow	7.0 ± 0.03	1.1 ± 0.33	0.19 ± 0.03	4.8 ± 1.2	53 ± 8.0	25 ± 7.0	22 ± 4.5	1915	9.8	335
Scipio	7.3 ± 0.02	0.41 ± 0.07	0.17 ± 0.02	9.4 ± 1.5	46 ± 0.96	32 ± 1.9	22 ± 1.9	1728	11	381
Sharpes Valley	7.1 ± 0.04	0.58 ± 0.03	0.21 ± 0.03	6.9 ± 1.9	35 ± 2.5	39 ± 3.2	27 ± 1.5	1879	9.0	404
South Beaver	6.6 ± 0.17	0.70 ± 0.15	0.15 ± 0.02	2.3 ± 0.39	45 ± 6.5	32 ± 2.4	24 ± 5.2	2058	8.4	354
South Creek	6.0 ± 0.10	0.27 ± 0.06	0.13 ± 0.01	2.4 ± 0.32	57 ± 4.4	22 ± 2.2	22 ± 5.7	2247	4.9	368
South Hills	6.2 ± 0.26	1.4 ± 0.20	0.16 ± 0.03	3.2 ± 1.2	57 ± 4.4	28 ± 2.7	15 ± 1.9	2016	9.1	438
Stansbury	6.9 ± 0.18	0.54 ± 0.05	0.19 ± 0.02	3.0 ± 0.63	37 ± 1.9	37 ± 0.49	26 ± 1.8	1748	11	437
Steinaker	6.7 ± 0.71	0.65 ± 0.13	0.09 ± 0.02	1.2 ± 0.35	74 ± 3.3	15 ± 3.6	11 ± 0.47	1867	7.7	272
Terra East	7.4 ± 0.05	0.61 ± 0.06	0.19 ± 0.02	3.9 ± 0.89	35 ± 1.2	36 ± 2.2	29 ± 2.2	1637	11	376
Unit B	6.7 ± 0.29	0.83 ± 0.03	0.15 ± 0.02	3.1 ± 0.48	56 ± 3.6	27 ± 2.6	17 ± 1.5	1991	9.5	338
West Carbon	7.1 ± 0.03	0.54 ± 0.04	0.22 ± 0.04	7.8 ± 1.6	57 ± 4.1	28 ± 4.5	15 ± 0.50	2004	8.8	353
West Oakbrush	7.1 ± 0.06	0.51 ± 0.05	0.26 ± 0.03	4.6 ± 0.88	36 ± 0.97	40 ± 1.4	25 ± 1.7	1748	9.9	406

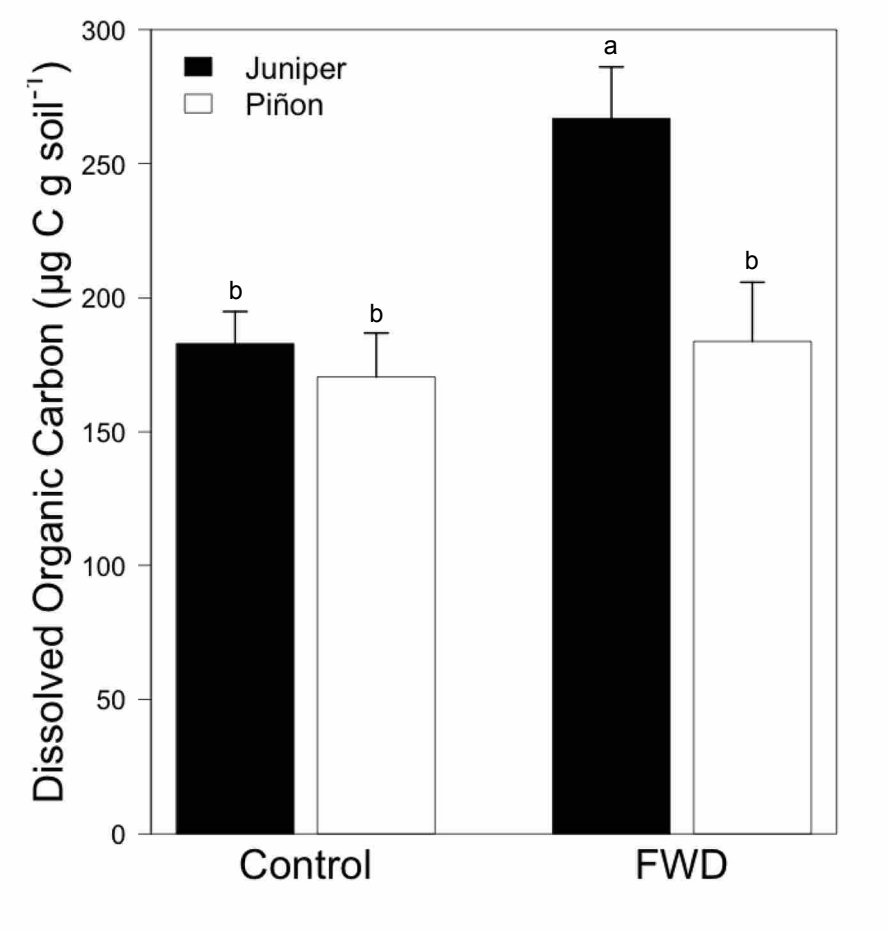
West Onaqui sagebrush	7.3 ± 0.06	0.55 ± 0.05	0.20 ± 0.02	8.2 ± 1.1	32 ± 4.2	38 ± 4.6	31 ± 2.4	1719	10	368
West Onaqui Mid	7.2 ± 0.02	0.60 ± 0.10	0.26 ± 0.05	9.4 ± 2.2	29 ± 2.1	46 ± 2.7	24 ± 2.3	1848	10	419
West Onaqui Upper	7.3 ± 0.07	0.61 ± 0.16	0.22 ± 0.04	7.5 ± 0.77	21 ± 1.4	43 ± 3.8	35 ± 2.9	1848	10	419
Winter Springs Low	7.3 ± 0.12	0.43 ± 0.09	0.16 ± 0.00	2.5 ± 0.36	44 ± 1.3	31 ± 2.4	26 ± 3.3	1653	11	330
Winter Springs High	6.9 ± 0.07	0.38 ± 0.05	0.25 ± 0.03	5.3 ± 1.4	29 ± 8.5	42 ± 7.2	29 ± 1.8	1850	10	417



Supplemental Figure 1. FWD treatment increased taxa richness, taxa evenness, and Shannon diversity. Taxa richness was based on the total number of OTUs, alpha diversity as the inverse Shannon index, and OTU evenness was based on 1000 iterations of 900 random resampled sequences from each replicate (*n*=41).



Supplemental Figure 2. Heat map and hierarchical clustering of the relative recovery of fifteen bacterial phyla in the three microsites in soils beneath *Juniperus* and *Pinus* species. Values are based on means with hierarchal clustering of soil microsites for each tree species (bottom) and families (left). Only phyla that contributed ≥ 1.0% to the total recovery of communities are presented with recovery based on 16S rDNA libraries.



Supplemental Figure 3. Fine woody debris (FWD) treatment increased dissolved organic carbon (DOC) but only in *Juniperus* tree islands. Significant differences between treatments ($P<0.05$) are based on three-way ANOVAs and Tukey's HSD test.