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### Authors

Collins, Courtney G  
Carey, Chelsea J  
Aronson, Emma L  
[et al.](#)

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## DIGGING DEEPER – HOW SOIL BIOTA DRIVE AND RESPOND TO PLANT INVASIONS

# Direct and indirect effects of native range expansion on soil microbial community structure and function

Courtney G. Collins<sup>1,2\*</sup>, Chelsea J. Carey<sup>3</sup>, Emma L. Aronson<sup>3</sup>, Christopher W. Kopp<sup>4</sup> and Jeffrey M. Diez<sup>1,2</sup>

<sup>1</sup>Department of Botany and Plant Sciences, University of California Riverside, Riverside, CA 92521, USA; <sup>2</sup>Institute for the Study of Ecological and Evolutionary Climate Impacts (ISEECI), University of California and UC Natural Reserve System, Riverside, CA 92521, USA; <sup>3</sup>Department of Plant Pathology and Microbiology, University of California Riverside, Riverside, CA 92521, USA; and <sup>4</sup>The Biodiversity Research Centre, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada

### Summary

1. Analogous to the spread of non-native species, shifts in native species' ranges resulting from climate and land use change are also creating new combinations of species in many ecosystems. These native range shifts may be facilitated by similar mechanisms that provide advantages for non-native species and may also have comparable impacts on the ecosystems they invade.

2. Soil biota, in particular bacteria and fungi, are important regulators of plant community composition and below-ground ecosystem function. Compared to non-native plant invasions, there have been relatively few studies examining how soil biota influence – or are influenced by – native species range shifts.

3. Here, we examined how a native range-expanding sagebrush species (*Artemisia rothrockii*) affects below-ground abiotic conditions and microbial community structure and function using next-generation sequencing coupled with other biotic and abiotic soil analyses. We utilized a range-expansion gradient, together with a shrub removal experiment and structural equation models, to determine the direct and indirect drivers of these interconnected processes.

4. Sagebrush colonization increased bacterial and archaeal richness and diversity and altered community composition across the expansion gradient. Soil organic C and N and soil moisture increased with sagebrush presence; however, results varied across the expansion gradient. We found no relationship between sagebrush and soil pH; however, pH strongly influenced microbial richness and diversity. Microbial (substrate-induced) respiration was influenced by soil organic N, as well as microbial diversity and functional group relative abundances, highlighting direct and indirect effects of sagebrush on microbial community structure and function. Microbial community composition of soils after 4 years of sagebrush removal was more similar to communities in shrub interspaces than underneath shrubs, suggesting microbial community resilience.

5. *Synthesis.* Our results suggest that native range expansions can have important impacts on soil biological communities, soil chemistry and hydrology which can further impact below-ground ecosystem processes such as nutrient cycling and litter decomposition. The combination of high-throughput sequencing and structural equation modelling used here offers an exciting yet underutilized approach to understanding how both native and non-native species' range expansions may affect the structure and function of soil ecosystems.

**Key-words:** alpine, bacteria, elevation gradient, global change, invasion, plant–soil (below-ground) interactions, resilience, sagebrush, structural equation model, woody encroachment

### Introduction

Changes in climate and land use, as well as anthropogenic introductions of non-native species, are creating novel communities of organisms around the globe (Tylianakis *et al.* 2008).

\*Correspondence author: E-mail: courtney.collins@email.ucr.edu

This is due largely to changes in species' ranges, which include range expansion, contraction and shifts (Sexton *et al.* 2009; Chen *et al.* 2011). In particular, range expansions occur with the introduction of non-native species to new areas, but also with native species expanding their ranges in latitude and/or elevation as they 'track' changes in climate (Parmesan *et al.* 2003; Valéry *et al.* 2008). Although similar processes govern distributions of native and non-native species, including dispersal ability and competitive interactions, native range expansions may differ from non-native invasions for several reasons (Morrien *et al.* 2010; Van der Putten, Macel & Visser 2010). First, native range expansions tend to be driven by changes in climate or land use, as opposed to intentional or unintentional human introductions, as the human introduction process may select for certain species' traits that affect subsequent spread and impacts (Buckley & Catford 2016). Secondly, native range-expanders are more likely to share evolutionary history with the communities into which they invade, potentially affecting processes such as enemy release or novel weapons that are important in non-native species' establishment in new areas (Callaway *et al.* 2008; Hill, Griffiths & Thomas 2011). Thirdly, natives have closer source populations that may continue to be connected via gene flow to the expanding range edge (Leger & Espeland 2010; Van der Putten, Macel & Visser 2010; Caplat *et al.* 2013), which may allow for improved adaptability to environmental conditions in the new range. Together, these differences may affect the mechanisms of range expansion and resulting ecosystem impacts of native and non-native species; however, species origin alone is likely not sufficient to accurately describe these complex ecological interactions (Buckley & Catford 2016).

Both native and non-native range expansions can create significant impacts on below-ground ecosystems by altering nutrient uptake, litter inputs, soil microclimate and disturbance regimes, which may have important effects on soil microbial communities (Wolfe & Klironomos 2005; Ehrenfeld 2010; Chapin *et al.* 2011). Altering the quality and quantity of litter inputs can shift the dominance of particular microbial groups within the soil (De Deyn, Cornelissen & Bardgett 2008). For example, plant litter with high carbon (C)-to-nitrogen (N) ratios often promotes fungal dominance in soils, due to differences in organismal C/N ratios and C-use efficiencies between bacteria and fungi (De Deyn, Cornelissen & Bardgett 2008; Bardgett 2011), and may also shift bacterial community dominance to oligotrophic groups with slower growth and turnover and higher nutrient use efficiency (Fierer, Bradford & Jackson 2007). Range-expanding plants may also alter below-ground competition for water and limiting nutrients with soil biota by shifting rooting depth, below-ground biomass and phenology during invasion (Schenk 2006; Gioria & Osborne 2014). These novel interactions may have strong influences on microbial community structure (composition and diversity) (Batten *et al.* 2006; Piper *et al.* 2015). For example, non-native grasses in California were shown to support higher abundances and diversity of ammonia-oxidizing bacteria than natives (Hawkes *et al.* 2005), and to reduce mycorrhizal fungal diversity of co-occurring native grass roots

(Hawkes *et al.* 2006). Expansion of native shrubs into grassland ecosystems can also increase soil bacterial and fungal diversity and select for distinct fungal community composition (Hollister *et al.* 2010; Yannarell, Menning & Beck 2014). Plant range expansions may also alter microbial activity and ecosystem functioning, such as when non-native invasive plants increase rates of decomposition and N cycling (Liao *et al.* 2008; Ehrenfeld 2010) or select for microbial species which preferentially degrade their own litter (Bardgett & Wardle 2010; Austin *et al.* 2014). However, below-ground responses to plant range expansions are highly variable, and can depend on time since establishment, plant trait variation and the microbial associations of the resident plant community (Liao *et al.* 2008; Castro-Diez *et al.* 2014).

Among the native range expansions occurring globally, the encroachment of woody plants into historically herbaceous dominated plant communities is particularly prevalent. Woody plants, in particular shrubs, are observed to be moving upslope in montane and alpine ecosystems and increasing in cover and abundance across diverse landscapes including arid grasslands, savanna, and arctic and alpine ecosystems (Wilson & Nilsson 2009; Myers-Smith *et al.* 2011; Naito & Cairns 2011; Saintilan & Rogers 2015). Similar to non-native invasions, increased density and cover of native woody vegetation can greatly affect the cycling of C, nutrients and water via changes in litter quantity and quality, rooting depth and woody biomass production (Huxman *et al.* 2005; Eldridge *et al.* 2011; Myers-Smith *et al.* 2011). These plant-induced changes can cascade through the soil ecosystem, altering microbial community structure and function. For example, global studies suggest that shrub litter has higher C/N than graminoid and forb litter and decomposes more slowly, thereby slowing microbial respiration and soil CO<sub>2</sub> flux into the atmosphere (Cornelissen *et al.* 2007; but see Wolkovich *et al.* 2010). Previous work has shown that shrub encroachment into grasslands can increase microbial biomass C, bacterial and fungal community diversity and fungi:bacteria ratios in soils (Liao & Boutton 2008; Hollister *et al.* 2010; Yannarell, Menning & Beck 2014). Mycorrhizal relationships in plants growing under shrub canopies (e.g. grasses and forbs) can also be affected by shrub-induced changes in soil nutrient availability (Shi *et al.* 2006) or secondary chemicals of shrub litter (Nilsson *et al.* 1993; Wardle *et al.* 1998). Shifts in soil microbial community structure and function as described above may persist even after the shrubs are removed or retreat (Kulmatiski & Beard 2011), and have the potential to create negative plant-soil feedbacks (PSFs) for other species by altering microbial decomposer communities and shifting pathogen to mutualist ratios (Bever 2003; Bardgett & Wardle 2010).

Despite the growing interest in the role of soil biota in plant range expansions (Suding *et al.* 2013), many of the relationships between abiotic impacts of woody plant encroachment and changes in soil microbial community structure and function remain speculative, as direct tests linking these below-ground processes are rare (Myers-Smith *et al.* 2011). In addition, general patterns for the effects of native range expansions on soil biota have not been well defined. Recent

reviews have examined mechanisms whereby native and non-native range-expanding species may be similar or dissimilar in their relationship with soil communities (Morrien *et al.* 2010; Van der Putten, Macel & Visser 2010; Van der Putten 2012). While conceptual frameworks such as these and the substantial literature base for PSFs of non-natives can help guide our predictions for (climate and land use driven) native range expansions, empirical tests are critical in order to fully fill this knowledge gap.

In this study, we examined how a native subalpine sagebrush species with a documented pattern of range expansion over the last 50 years (Kopp & Cleland 2014) is affecting abiotic soil properties and microbial community structure and function. In particular, we combined biotic and abiotic soil analyses with next-generation sequencing techniques to determine how soil microbial biomass C and N, bacterial and archaeal community structure, function (substrate-induced respiration [SIR]), and soil characteristics including C and N availability, pH and volumetric water content (VWC) were affected by the presence of sagebrush. The study was conducted across an altitudinal gradient of sagebrush expansion, where higher elevation sites were more recently colonized, allowing a chronosequence analysis of how sagebrush affects soil biotic and abiotic properties. We used structural equation modelling (SEM) to test multiple hypotheses for how the impacts of sagebrush expansion fit into a larger conceptual framework of soil biological communities, soil chemistry, hydrology and below-ground ecosystem processes such as nutrient cycling and decomposition (Fig. 1a,b). In addition, to estimate the possible consequences for resident herbaceous species, we quantified how colonization of arbuscular mycorrhizal fungi (AMF) of other native alpine plant (non-shrub) species was impacted by the presence of sagebrush. Finally,

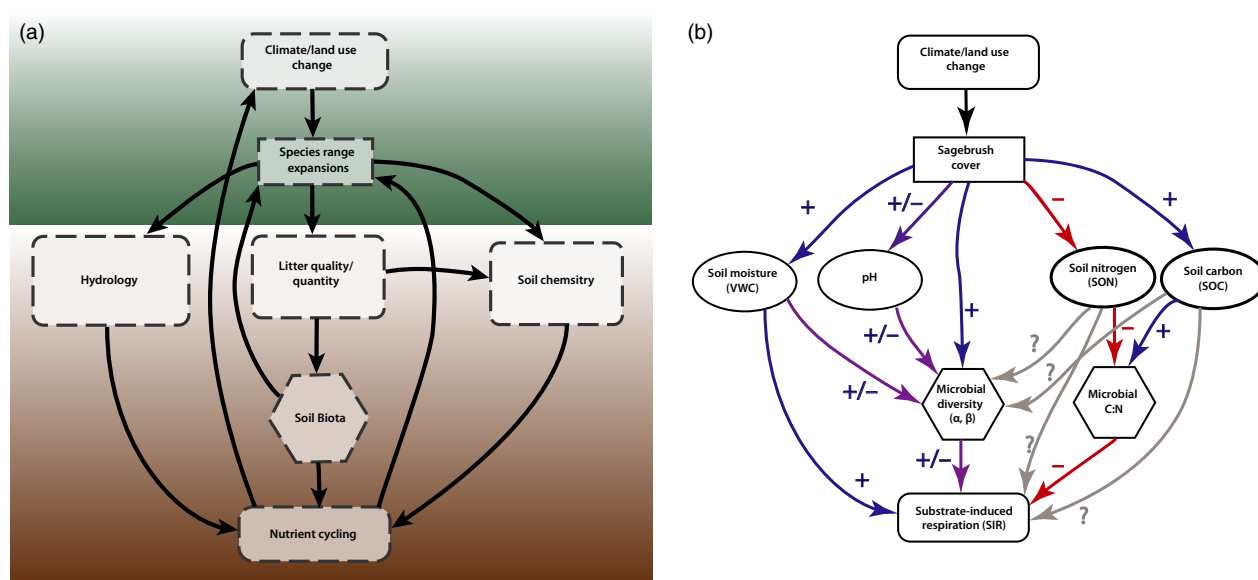
we used a 4-year sagebrush removal study to assess the resilience of soil bacterial and archaeal communities and test for a causal link between sagebrush presence and microbial community shifts.

## Materials and methods

### SITE DESCRIPTION

Research was conducted in the White Mountains of California near Crooked Creek (3094 m; 37°29'56"N, 118°10'19"W) and Barcroft (3800 m; 37°34'59"N, 118°14'14"W) research stations. This mountain range lies on the western edge of the Great Basin Floristic Province and in the rain shadow of the Sierra Nevada range. The climate is cold and dry, receiving between 150 and 450 mm of precipitation annually. Temperature declines with increasing elevation, with a mean annual temperature of 0.9 °C at Crooked Creek Station to -1.7 °C at Barcroft Station, while precipitation increases from 327 to 456 mm year<sup>-1</sup>, respectively (Hall 1991). These mountains contain a steep elevation gradient, ranging from 1220 m at its base in the Owens Valley to 4344 m at the summit of White Mountain Peak.

Due to dramatic changes in elevation, temperature and precipitation, this range contains five distinct plant communities: cold desert (1220–1980 m), montane (1980–2900 m), subalpine (2900–3500 m), alpine (3500–4000 m) and high alpine (4000–4344 m) (Rundel, Gibson & Sharifi 2008). Our research took place between subalpine and alpine communities, within the transition from sagebrush steppe to true alpine plant communities dominated by prostrate cushion plants and perennial bunchgrasses. Recent research has shown that *Artemisia rothrockii* A. Gray (sagebrush) is expanding upwards in elevation at a rate of 30 m per decade over the past 50 years (Kopp & Cleland 2014) and establishing patches up to 10 m wide in alpine zones. This range expansion is likely promoted by changes in climate such as increased temperatures and drought and changes in land use including cessation of grazing in the area (Kopp & Cleland 2014). Experimental warming



**Fig. 1.** Conceptual diagrams of plant range expansion impacts on soil and ecosystem processes. (a) Broad structural equation meta-model outlining general relationships among above- and below-ground processes affected by species introductions and range expansions; (b) specific parameters measured in our research ecosystem in California, with hypothesized relationships (grey = unknown) tested in structural equation model. Elliptical nodes represent abiotic variables and square and polygon nodes represent biotic variables in the system.

**Table 1.** Site-level soil characteristics for each elevation. Values shown are mean of all samples with standard deviation in parentheses. Middle-elevation sites did not include shrub removal soils

Elevation (m)	TOC (mg L <sup>-1</sup> )	TON (mg L <sup>-1</sup> )	WVC (%)	pH	Microbial biomass N (mg N kg soil <sup>-1</sup> )	Microbial biomass C (mg C kg soil <sup>-1</sup> )	CO <sub>2</sub> flux (μmol kg s <sup>-1</sup> )
3100 (N = 15)	1.7 (0.69)	0.18 (0.061)	1.9 (0.83)	6.2 (0.36)	11 (7.5)	170 (90)	0.097 (0.072)
3500 (N = 10)	2.5 (0.42)	0.29 (0.041)	5.3 (0.93)	5.9 (0.33)	25 (10)	320 (90)	0.2 (0.1)
3800 (N = 15)	2.6 (0.7)	0.34 (0.091)	8.1 (2.2)	6.1 (0.54)	36 (20)	480 (190)	0.22 (0.14)

and contracted snow pack periods have been shown to increase intrinsic growth rates of closely related *Artemisia* species in the intermountain west, thus providing a plausible hypothesis for range expansion in this system (Perfors, Harte & Alter 2003). In areas of sagebrush encroachment, there has been a decline in abundance and cover of native grasses and cushion plants (Kopp & Cleland 2014).

#### EXPERIMENTAL DESIGN AND SAMPLE COLLECTION

In order to determine impacts of *A. rothrockii* expansion on soil communities, we sampled soils under and outside sagebrush canopies at three sites located along the altitudinal transect at 3100, 3500 and 3800 m elevation. We also sampled soils in nearby (< 500 m distance) plots where sagebrush was manually removed 4 years before ('sagebrush removal') at 3100 and 3750 m elevations (described below). All sites span the observed gradient of sagebrush expansion from subalpine (< 3500 m elevation) to alpine (> 3500 m elevation) over the last 50 years (Kopp & Cleland 2014). In 1961, *A. rothrockii* was not present at the 3800-m site and was found in moderate-to-low densities at the 3500-m site and in high densities at the 3100-m site (Mooney, Andre & Wright 1962; Kopp & Cleland 2014). Therefore, this gradient is useful in assessing impacts of shrub expansion using the low-elevation sites as a historic reference and the high-elevation sites as representative of the leading edge of the expansion gradient where *A. rothrockii* transitions from an almost continuous population to isolated patches.

All sampling locations had granitic soils (Colluvium derived from granite) and east-/south-east-facing slopes to control for edaphic and aspect variation. In addition to consistency in parent material across sites, the middle- and high-elevation sites are in the same soil series (Pergelic Cryoborolls–Soakpak family association), while the low-elevation site is in the Hartig–Packham family association (Soil Survey Staff 2007; Appendix S1, Supporting information). Other site selection criteria included choosing areas that were located outside designated wilderness area and Ancient Bristlecone Pine Forest so that sagebrush removal and soil coring could be conducted.

We collected soils in August 2015 near the peak of growing season. Five sagebrush individuals (< 100 m apart) were sampled from each elevation site, with two replicate soil cores (1.3 cm diameter × 10 cm deep) taken from under (shrub) and outside (shrub interspace) of each individual. Shrub interspace cores were taken between 1 and 5 m from the edge of the canopy of each individual (based on sagebrush density of the site) and less than the distance to any other shrub canopy. In July 2011, 1-m<sup>2</sup> sagebrush removal plots were established at 3100 and 3750 m elevation by cutting sagebrush at the base of the stem and trimming any resprouting back yearly. Two replicate soil cores were taken from five sagebrush removal plots (within ~200 m radius) at the low- and high-elevation sites. The corer was sterilized between each sample with a 10% bleach solution to prevent cross-contamination, and cores were kept separate to assess within-individual variation in soil communities. For molecular

analyses, soil was stored in sterile specimen cups and placed on dry ice in the field and then stored in a –80 °C freezer prior to analysis. For all other analyses, one soil sample was taken from under and outside of the same sagebrush individuals at the same time using a garden trowel marked at 10 cm depth and then stored at 4 °C. These soil samples were sieved field-moist (0.5 mm) to remove roots and stones prior to analysis for soil abiotic characteristics, substrate-induced respiration (SIR) and microbial biomass C/N.

Additionally, roots of species co-occurring with *A. rothrockii* were sampled for mycorrhizal analyses in September 2014 and August 2015 in order to determine the effect of sagebrush expansion on mycorrhizal communities of alpine plants. Ten individual plants (including roots and intact rhizosphere soil) of a dominant alpine bunchgrass (*Koeleria macrantha*) and cushion plant (*Eriogonum ovalifolium*) were sampled at each of the three elevation sites, five from individuals growing directly below *A. rothrockii* canopies and five growing in adjacent shrub interspace. This provided a total of 30 individuals for each species (five individuals × three elevations × two locations). Soil samples for total (bulk) soil C and N were taken at the same time and location as individual plants sampled in September 2014 for each elevation site (five under and five outside sagebrush).

#### MOLECULAR ANALYSES

We extracted microbial DNA from 0.25 g of soil (± 0.025 g) using a MO BIO Powerlyzer PowerSoil DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) and quantified the extracted DNA using a NanoDrop 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). We then ran polymerase chain reaction (PCR) using primers targeting the V3-V4 region of 16S rRNA gene (S-D-Bact-0341-b-S-17 127 and S-D-Bact-0785-a-A-21; Klindworth *et al.* 2013). Specifically, samples were amplified in duplicate by combining 2.5 μL of DNA template, 5 μL each of 1 μM forward and reverse primers and 12.5 μL KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Inc., Wilmington, MA, USA). The thermocycler conditions were as follows: 95 °C for 3 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and finally an extension step for 5 min at 72 °C. We then conducted post-PCR clean-up using Agencourt AMPure XP Beads (Beckman Coulter Genomics, Danvers, MA, USA), followed by a second round of PCR to attach dual indices to each sample using the Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA). For this second round of PCR, we combined 5 μL DNA, 5 μL each of 1 μM forward and reverse index primers, 25 μL KAPA HiFi HotStart ReadyMix and 10 μL PCR-grade water. The thermocycler conditions were as follows: 95 °C for 3 min, followed by eight cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and finally an extension step for 5 min at 72 °C. We then conducted a second round of post-PCR clean-up (same as described above) on the indexed amplicons and quantified them with the Quant-iT PicoGreen<sup>®</sup> dsDNA assay kit (Life Technologies Inc., Grand Island, NY, USA). As a final step, the samples were pooled in equimolar concentrations

and then sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the University of California Riverside (UCR) Genomics Core Facility.

## SEQUENCE ANALYSES

We received the sequences already demultiplexed, and initially processed them using Quantitative Insights into Microbial Ecology (QIIME; Caporaso *et al.* 2010). We joined the forward and reverse reads (allowing for maximum 20% divergence in the overlap region) and used default QIIME parameters for quality control. Specifically, sequences were discarded if there were < 75% consecutive high-quality base calls, if there were more than three low-quality base calls in a row or if there were any ambiguous calls. Sequences were additionally removed if the Phred score was less than three and if the total length was < 75 bases (Bokulich *et al.* 2013). After quality filtering, we used UCLUST (Edgar 2010) to pick operational taxonomic units (OTUs) at 97% similarity and the 13.8 version of the Greengenes data base to assign taxonomy (McDonald *et al.* 2012). We used an open-reference OTU picking approach, where reads that had no sequence matches in the data base were clustered *de novo*. Two samples were removed due to low read counts, leaving 75 samples and resulting in 7 477 268 total reads. After removing unassigned sequences, we rarefied each sample to an even depth of 41 010.

To provide a robust analysis of alpha diversity for each sample (Haegeman *et al.* 2013), we calculated both the number of observed OTUs (richness) and Shannon diversity (richness and evenness). In addition, we compared the combined relative abundance of taxa that are generally considered to be copiotrophic (higher N demands, utilize labile C pools) and oligotrophic (lower N demands, utilize more recalcitrant C pools) across treatment and elevation in a similar way. Specifically, Actinobacteria, Betaproteobacteria and Firmicutes comprised the copiotrophic group, and Acidobacteria and Verrucomicrobia comprised the oligotrophic group (Fierer, Bradford & Jackson 2007; Ramirez, Craine & Fierer 2012). We tested the effects of treatment on microbial diversity and the relative abundances of taxa by performing analysis of variance (ANOVA) on data collected from the 3100-m and 3800-m elevation sites, followed by a Tukey post hoc analysis if the overall model was significant. Here, both treatment (shrubs, shrub removal, interspace) and elevation were included as factors in the model. When there was a significant treatment-by-elevation interaction, data were separated by elevation and individual ANOVAs were run. The 3500-m elevation site was analysed separately as it did not contain a shrub removal treatment. To account for multiple comparisons in the relative abundances of taxa, we performed a sequential Bonferroni correction; given this approach can be quite conservative (Moran 2003), we present both uncorrected and corrected values in the results and discuss the uncorrected values in the discussion.

Beta diversity was visualized using non-metric multidimensional scaling (NMDS) of the unweighted (presence-absence) and weighted (relative abundance) UniFrac distance. This metric uses overlap in branch lengths to estimate phylogenetic distance between pairs of samples (Lozupone & Knight 2005). The NMDS was graphed in R (R version 3.2.1; R Core Team 2015) using GGLOT2 (Wickham 2009) and the 'stat\_ellipse' function with 95% confidence intervals. We tested for differences in overall microbial community composition across treatments by performing a permutational multivariate ANOVA (PERMANOVA) implemented using the VEGAN function 'ADONIS' in R (999 permutations; Oksanen *et al.* 2016). Treatment (shrubs, shrub interspace and shrub removal) was used as the predictor variable, and

the elevation was included as 'strata' (a blocking variable), which restricted permutations to within sites.

## SUBSTRATE-INDUCED RESPIRATION

Substrate-induced respiration was measured using an LI-8100A (LI-COR Biosciences, Lincoln, NE, USA) automated soil gas flux system and a modified SIR method (West & Sparling 1986). By measuring CO<sub>2</sub> production over time, SIR estimates potential microbial respiration of those microorganisms that are active and glucose-responsive (Anderson & Domsch 1978), and therefore provides a coarse approximation of a key microbial function. Briefly, 15 g of field-moist soil was weighed out into 250-mL jars and 30 mL of a glucose solution (30 g glucose L<sup>-1</sup> H<sub>2</sub>O) was added to each. The jars were sealed with a lid modified with rubber tubing running from inside of the jar into the LI-8100A and shaken at 180 rpm for 30 min while continuous flux measurements were recorded. After 30 min, CO<sub>2</sub> flux rates (μmol kg<sup>-1</sup> s<sup>-1</sup>) were calculated using LI-COR software (Table 1) (LICOR Biosciences).

## MICROBIAL BIOMASS C/N

Microbial biomass C and N were measured using a chloroform fumigation-extraction (CFE) technique (Brookes *et al.* 1985). Briefly, 5 g of field-moist soil from each soil sample was separated into paired unfumigated and fumigated samples. Unfumigated samples were extracted immediately with 25 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> by shaking for 1 h on an orbital shaker and then filtering through a 1.2-μm glass fibre filter (Thomas C5500; Thomas Scientific, Swedesboro, NJ, USA). Filtered samples were frozen at -20 °C for subsequent analysis of dissolved organic C and N. Fumigated samples were weighed out into 250-mL Erlenmeyer flasks, exposed to 2 mL of ethanol-free CHCl<sub>3</sub>, capped and incubated for 24 h at room temperature. Following incubation, caps were removed and flasks were vented for 30 min under a fume hood, and the soil was extracted with 25 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> as described above. Unfumigated and fumigated extracts were shipped overnight on dry ice to the EcoCore Analytical Facility at Colorado State University, Fort Collins, CO, for analysis of total organic carbon (TOC) and nitrogen (TON) on a Shimadzu TOC-L autoanalyzer (Shimadzu Scientific Instruments, Inc., Carlsbad, CA, USA). Total organic C and N were calculated as mg L<sup>-1</sup> for each sample using the unfumigated values. We calculated microbial biomass C and N by subtracting organic carbon, nitrogen from the unfumigated samples by their paired fumigated samples, and by dividing the subsequent C- and N-flush by kEC and kEN coefficients of 0.45 and 0.69, respectively (Wu *et al.* 1990; Joergensen & Mueller 1996). Results are expressed as mg C, N \*kg soil<sup>-1</sup> (Table 1), where average site/treatment values of gravimetric water content (GWC) were used to calculate soil dry weight because GWC was measured on only three replicates per treatment (i.e. under and outside shrub) at each elevation.

## SOIL ABIOTIC PROPERTIES

Soil abiotic properties including VWC and pH (Table 1) were measured at the same time and sampling location of each soil core. VWC (%) was measured to 12 cm depth using a Campbell Scientific HS2 Hydrosense II probe (Campbell Scientific, Logan, UT, USA), and pH was measured by mixing soil from 10 cm depth in a sterilized cup with DI water and using an Extech PH100 ExStik pH meter (Extech Instruments, Nashua, NH, USA).

Total (bulk) soil C and N were determined by combustion using a Flash EA1112 combustion soil analyzer (Thermo Fischer Scientific,

Waltham, MA, USA) in the Environmental Sciences Research Laboratory at the University of California Riverside. Total C and total N values correlated strongly with the unfumigated values of TOC and TON by treatment (under and outside shrubs), and therefore, we used only TOC and TON values for this study [Pearson's  $r = 0.834$  (TOC and TC) and  $0.908$  (TON and TN)].

#### MYCORRHIZAL ANALYSES

Roots of the dominant alpine bunchgrass (*Koeleria macrantha*) and cushion plant (*Eriogonum ovalifolium*) were separated from intact soil, and then, 0- to 2-mm diameter roots were rinsed with distilled water and cleaned with fine brushes to remove soil particles. These roots were then cleared in a 10% KOH solution for 10 min at 60 °C and stained in a 5% ink–vinegar solution following the methods of Vierheilig *et al.* (1998). Colonization of arbuscular mycorrhizal fungi (AMF) was quantified at 40 $\times$  magnification using the magnified intersections method (McGonigle *et al.* 1990). We used a Bayesian multiple regression model with a varying intercept (Gelman & Hill 2007) to determine the effects of sagebrush cover (target plant growing under or outside shrub) and elevation (3100, 3500 or 3800-m site) on AMF colonization. The response variable was average per cent mycorrhizal colonization in the roots of each species. The regression intercepts varied by treatment (outside or under sagebrush) and elevation, and bulk soil C and N were also included as covariates. Non-informative priors were used for each parameter, and significance of each variable was determined by calculating the probabilities that posterior parameter distributions did not overlap zero (see below for additional detail on this approach).

#### STRUCTURAL EQUATION MODELLING

We used structural equation models to test and quantify the hypothesized connections between sagebrush expansion and soil biotic and abiotic parameters and sequence analyses (Fig. 1a,b). Structural equation modelling is a useful approach for disentangling complex sets of direct and indirect interactions (Grace *et al.* 2010), but remains relatively underutilized in soil ecology (Eisenhauer *et al.* 2015). We developed models based on *a priori* understanding about the functional relationships between soil variables in this and other ecosystems, and used the broad metamodel (Fig. 1a) to structure our specific hypotheses (Fig. 1b).

In the light of the large quantity and complexity of microbial sequencing data, we developed four separate models in order to best capture different hypotheses about how microbial community structure was affected by sagebrush. These models quantified the microbial community as follows: (i) diversity (Shannon diversity index), (ii) richness (number of OTUs), (iii) composition (first axis of weighted NMDS) and (iv) ratio of oligotrophic abundance to copiotrophic abundance (for description, see Sequence Analyses). Models were fit in a Bayesian framework using the R2JAGS package (Su *et al.* 2015) in R version 3.2.2 (R Core Team 2015). Non-informative priors were used on all parameters, and models were checked for convergence using visual assessment and the Gelman–Rubin diagnostic on three independent chains with sufficient burn-in periods discarded. Model structure was similar to that in Grace *et al.* (2014) except mixed models were used to allow for different effects of shrubs across elevations and random effects were used to account for the replicate soil cores in which microbial communities were characterized at each location. Model code is available upon request. Statistical significance and strength of relationships within the models were assessed using the

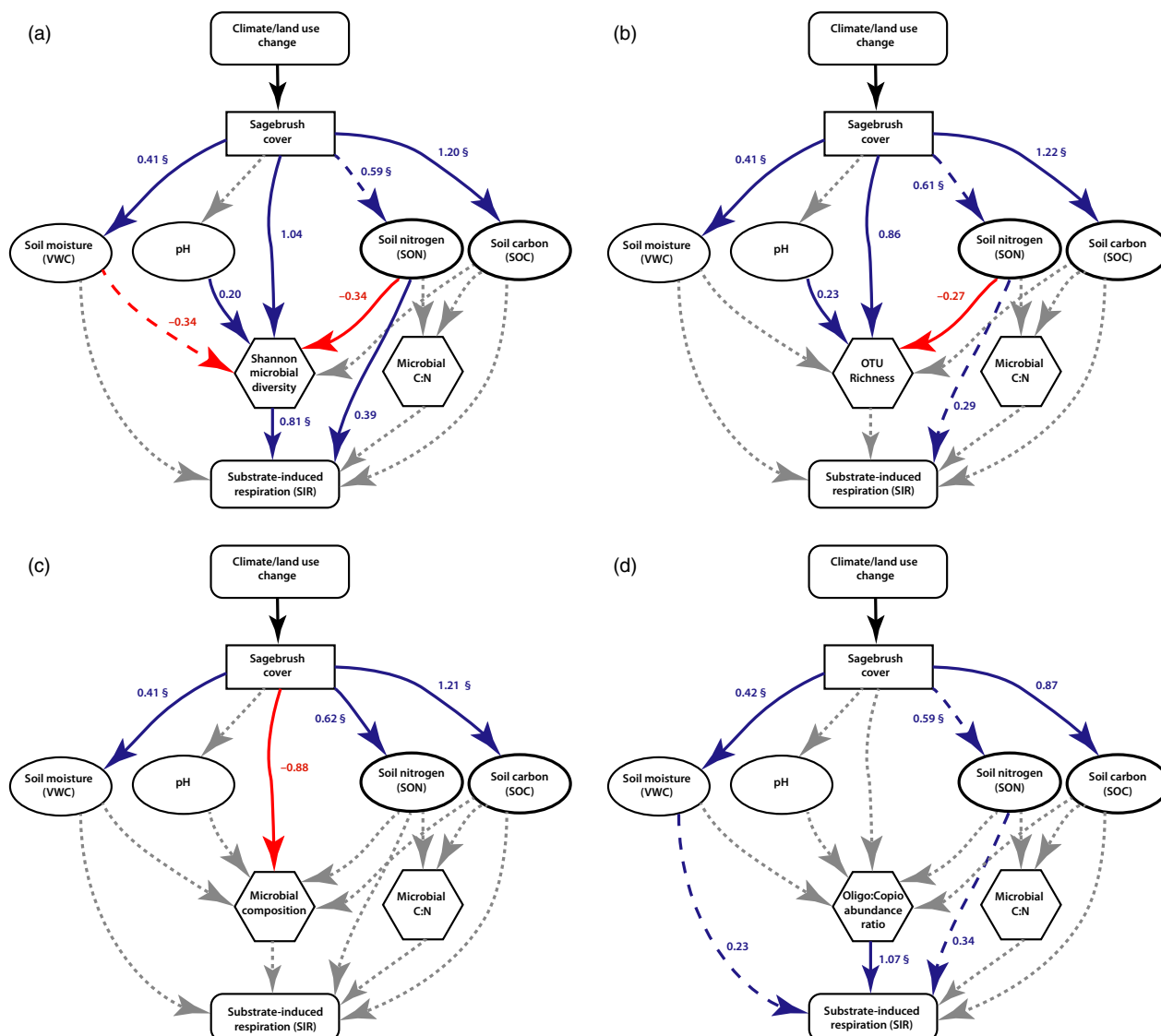
posterior parameter distributions of intercepts and slopes of mixed models describing each connection in Fig. 2a–d. Each variable was standardized before analysis in order to facilitate comparison of estimated path coefficients. We used the posterior distributions of each parameter to calculate the probabilities that it was different from zero, and three probability levels are reported (85, 90 and 95% probabilities (Fig. S1), respectively, that the relationship is different from zero). Because the effect of shrub cover in this study was a categorical test (samples were taken from underneath shrubs, shrub interspace and in shrub removal plots), these effects were calculated in the model as the difference between intercept terms that were allowed to vary by treatment. Therefore, the assessment of strength and statistical significance of shrub effects was based on the posterior distribution of the difference between shrub and shrub interspace intercepts calculated within the model.

## Results

#### MOLECULAR ANALYSES

At an even sequencing depth of 41 010 reads, our efforts yielded an average of 7462 OTUs per sample (standard deviation  $\pm 714$  OTUs). Overall, these OTUs belonged to 46 phyla, 161 classes, 311 orders, 495 families and 822 genera. Actinobacteria ( $20.6 \pm 5.1\%$ ), Verrucomicrobia ( $17.1 \pm 3.9\%$ ), Proteobacteria ( $15.8 \pm 2.5\%$ ), Acidobacteria ( $13.4 \pm 1.6\%$ ) and Planctomycetes ( $10.0 \pm 1.5\%$ ) were the dominant bacterial phyla, together accounting for more than 75% of sequences across all samples ( $76.9 \pm 14.5\%$ ; Fig. 3). Within the Actinobacteria, the Actinomycetales order was most prevalent (comprised  $40 \pm 2\%$  of the actinobacterial sequences), followed by the Gaiellales ( $21 \pm 2\%$ ) and Solirubrobacterales ( $19 \pm 1\%$ ). The Verrucomicrobia genus, *DA101*, was the most abundant genus recovered with an overall relative abundance of  $14.0 \pm 4\%$ .

Marked differences in microbial community structure were observed among shrub, shrub interspace and shrub removal treatments. Across all elevations, microbial communities underneath shrubs were more diverse (greater number of observed OTUs and higher Shannon diversity) than those in shrub interspace ( $P < 0.05$ ; Fig. 4a,b). At the lowest elevation site, shrub-associated microbial communities were also more diverse than in areas where shrubs had been removed ( $P < 0.05$ ; treatment  $\times$  elevation interaction  $P < 0.05$ ; Fig. 4a,b). In addition, microbial community composition was significantly affected by treatment (unweighted UniFrac  $R^2 = 0.07$ ,  $P < 0.001$ , Fig. 4c; weighted UniFrac  $R^2 = 0.15$ ,  $P < 0.001$ , Fig. 4d). Shrubs and shrub interspace plots harboured distinct microbial communities (unweighted UniFrac  $R^2 = 0.04$ ,  $P < 0.001$ , Fig. 4c; weighted UniFrac  $R^2 = 0.14$ ,  $P < 0.001$ , Fig. 4d), as did shrub and shrub removal plots (unweighted UniFrac  $R^2 = 0.05$ ,  $P < 0.001$ , Fig. 4c; weighted UniFrac  $R^2 = 0.12$ ,  $P < 0.001$ , Fig. 4d). Although the unweighted UniFrac metric also revealed significant differences in microbial composition between shrub interspace and shrub removal plots ( $R^2 = 0.04$ ,  $P = 0.02$ ), when the relative abundances of taxa were taken into account interspace-associated microbial communities were similar in composition to



**Fig. 2.** Structural equation modeling results for (a) microbial diversity, (b) microbial richness, (c) microbial composition, and (d) oligotrophic:copiotrophic ratios. Black lines show relationships that were significantly different from zero, whereas grey lines are used for relationships not significantly different from zero. Solid lines represent  $> 95\%$  probability that a parameter estimate was different from zero, while dashed lines had  $> 90\%$  probability of being non-zero, and dotted lines (in grey) had  $< 90\%$  probability of being non-zero. Results which were significant across all elevations show the average parameter estimate value for the three sites and elevation specific results are noted by § with the strongest elevation result displayed (further details in results and Fig. S1). Elliptical nodes represent abiotic variables and square and polygon nodes represent biotic variables in the system. Note that the top relationship between climate/land use change and shrub cover was not explicitly tested in this study.

those microbial communities from shrub removal plots (weighted UniFrac,  $R^2 = 0.04$ ,  $P = 0.26$ ; Fig. 4c,d).

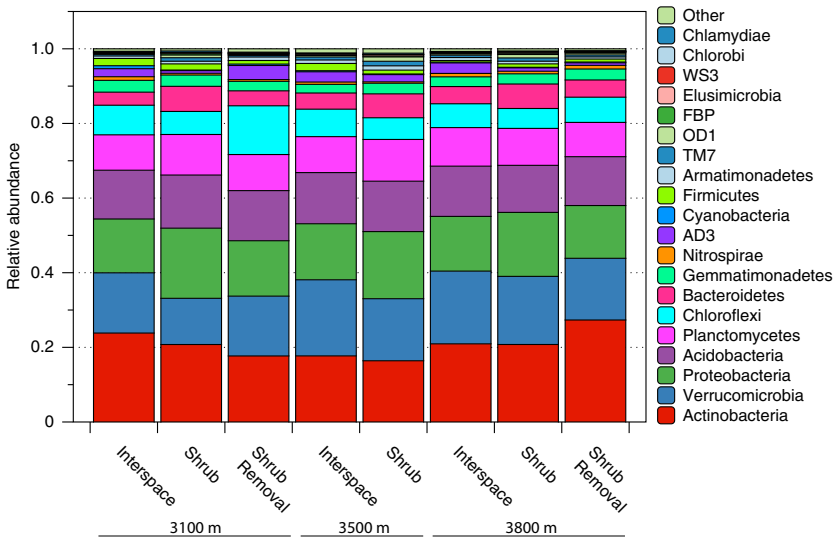
Treatment-induced shifts in community composition were accompanied by changes in the relative abundance of some dominant taxa. For example, Betaproteobacteria consistently increased in shrub plots compared to both shrub interspace and shrub removal plots ( $P < 0.01$ ). However, changes in the relative abundances of particular phyla did not always occur in the same direction at each elevation. Actinobacteria decreased with shrub removal compared to shrub interspace plots at 3100 m elevation ( $P < 0.01$ ; Fig. 3), whereas this phylum increased with shrub removal compared to interspace and shrub plots at 3800 m elevation ( $P < 0.05$ ; treatment  $\times$  elevation interaction  $P < 0.001$ ). Similarly,

Verrucomicrobia was unaffected by treatment at 3100 m elevation ( $P > 0.05$ ) but decreased with shrub removal compared to shrub interspace plots at 3800 m ( $P < 0.05$ ; treatment  $\times$  elevation interaction  $P = 0.05$ ; Fig. 3). However, after taking into account multiple comparisons, many of these significant trends disappeared; only the effect of treatments on Betaproteobacteria remained statistically significant.

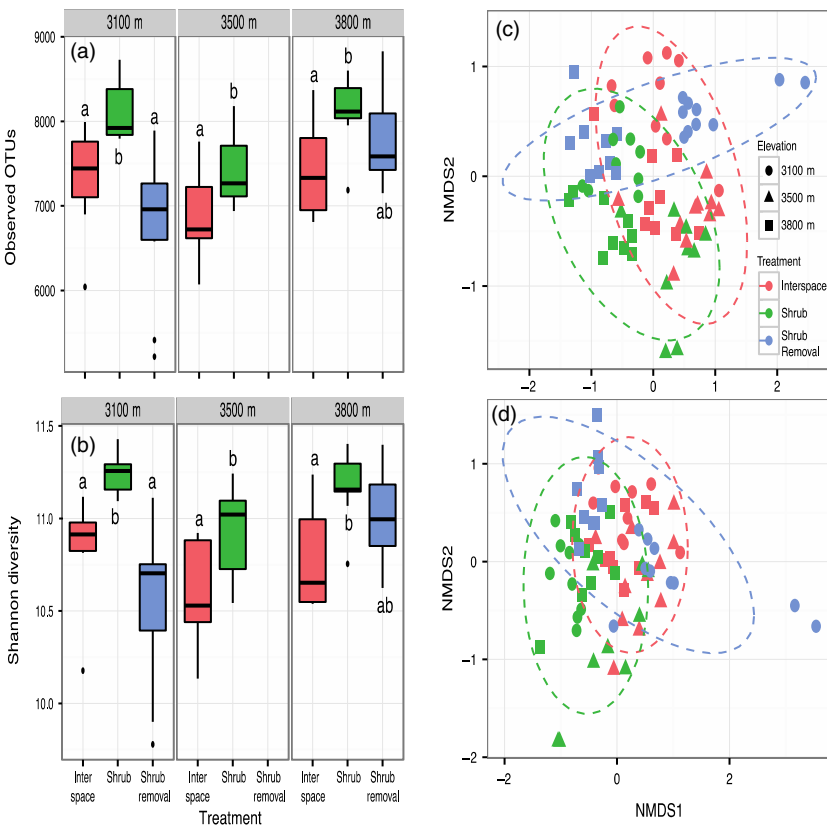
#### MYCORRHIZAL ANALYSES

Arbuscular mycorrhizal fungal per cent root colonization of the two species, *Koeleria macrantha* and *Eriogonum ovalifolium*, was highly variable within sites. The only significant effect of shrub cover was a tendency for greater AMF





**Fig. 3.** Relative abundances of bacterial and archaea phyla averaged across replicates ( $N = 9-10$  per treatment) from under (shrub) and outside (interspace) sagebrush canopies, and from shrub removal plots at 3100 m, 3500 m (no shrub removal), and 3800 m elevation sites. Both graph and legend share the same order, sequential from bottom to top. 'Other' indicates the combined relative sequence abundance of the lesser abundant phyla.



**Fig. 4.** Influence of treatment (interspace, shrub, shrub removal) on alpha diversity and overall community composition across all three elevations. (a) Number of observed operational taxonomic units, (b) Shannon diversity index, (c) non-metric multi-dimensional scaling (NMDS) of unweighted UniFrac metric, (d) NMDS of weighted UniFrac metric. In (a) and (b), different letters denote significant differences ( $P < 0.05$ ) among treatments within an elevation. In (c) and (d), each symbol corresponds to a sample collected from a particular elevation, and each color corresponds to a treatment. Points that are close together represent samples with similar bacterial and archaeal community composition, and the dashed ovals represent 95% confidence intervals of sample ordination grouped by treatment. The stress value for the unweighted (c) and weighted (d) metrics were 0.08 and 0.09, respectively. Shrub removal treatments were not sampled at 3500 m elevation.

colonization in plants growing underneath shrub canopies compared to outside for *E. ovalifolium* at the high-elevation site (0.943 probability that AMF colonization was greater underneath shrubs).

#### STRUCTURAL EQUATION MODELLING

Parameter estimates from each of the structural equation models are most easily visualized in Figs 2 and S1 and Table S1. Here, we summarize the primary results related to the hypothesized relationships in Fig. 1b. Sagebrush cover was strongly

associated with increased microbial diversity and richness (Fig. 2a,b), and with microbial community composition (Fig. 2c) at all elevations (Fig. S1). Sagebrush cover significantly increased TOC and TON (hereby referred to as *soil* organic C [SOC] and *soil* organic N [SON]) at the low-elevation site and VWC at the high-elevation site (Figs 2 and S1). SOC was also increased under sagebrush canopies at the high-elevation site in one version of our model (Fig. 2d). SON was positively related to SIR, and pH was positively associated with microbial diversity and richness. Volumetric water content and SON had inverse relationships with

microbial diversity, and SON alone was inversely related to microbial richness. Volumetric water content also had a positive relationship with SIR in one version of our model (Fig. 2d). When considering links between microbial community structure and function, microbial diversity and oligotrophic:copiotrophic ratios were positively related to SIR at the high-elevation sites only.

Although not directly included in the SEM, microbial biomass C and N were also higher under sagebrush canopies than outside ( $P < 0.001$ ).

## Discussion

In this study, we examined the below-ground impacts of a native species expanding its range over the last 50 years in the White Mountains of California (Kopp & Cleland 2014). Our approach utilized a structural equation modelling framework, in which *a priori* hypotheses (Fig. 1b) of relevant direct and indirect relationships between sagebrush cover and soil abiotic and biotic variables were tested. Although most measurements displayed variability among sites, several trends were strong across the entire sagebrush expansion chronosequence reflecting consistent impacts on soil microbial community structure and function. In addition, a sagebrush removal experiment showed that microbial community structure can return to pre-shrub composition relatively quickly ( $< 5$  years), demonstrating compositional resilience of the microbial community.

### CHANGES IN SOIL MICROBIAL COMMUNITIES WITH SHRUB EXPANSION

We observed a strong influence of sagebrush establishment on soil bacterial and archaeal community diversity (Shannon diversity index), OTU richness and overall community composition. Specifically, microbial communities were consistently more diverse and had higher richness under sagebrush canopies than outside, a finding that is congruent with prior research on woody shrub encroachment (Wallenstein, McMahon & Schimel 2007; Hollister *et al.* 2010; Yannarell, Menning & Beck 2014). This trend held true across all elevations. We hypothesized that microbial community diversity, richness and composition would be altered by sagebrush, potentially due to a higher diversity and altered abundance of litter sources (shrub, grass, cushion) entering the soil environment (Hooper *et al.* 2000). Studies suggest that litter sources can shift microbial biomass, community composition and structure by increasing substrate variability and diversity of chemical compounds and that this can vary through stages of decomposition (Meier & Bowman 2008; Chapman & Newman 2010; Chapman *et al.* 2013). An analogous explanation could be posed regarding the diversity of below-ground inputs, in regard to both root exudates and senesced root litter (De Deyn, Cornelissen & Bardgett 2008). Microbial biomass C and N were also higher under sagebrush canopies than outside, suggesting that sagebrush establishment promotes higher total microbial biomass in soils ( $P < 0.001$ ) in addition to altering composition.

In addition to microbial diversity and overall biomass, the relative abundance of particular microbial functional groups provides an ecologically relevant way to organize and draw inferences on complex molecular data (Fierer, Bradford & Jackson 2007). While sagebrush cover was not directly related to oligotrophic:copiotrophic ratios in the structural equation models, abundances of particular phyla did vary with shrub cover including an increase in Betaproteobacteria in shrub soils and shifts in Actinobacteria and Verrucomicrobia with shrub removal. The increase in Proteobacteria in shrub soils is consistent with Wallenstein, McMahon & Schimel (2007) who found increased Proteobacteria in arctic shrub soils, suggesting that these bacteria thrive in C and nutrient-rich soils under shrub canopies and exhibit copiotrophic attributes (Fierer, Bradford & Jackson 2007; Wallenstein, McMahon & Schimel 2007). In addition, there was a strong negative relationship between oligotrophic:copiotrophic ratios and SIR at the high-elevation site, consistent with glucose-induced respiration rates reported for copiotrophic microorganisms in other studies (Blagodatskaya *et al.* 2007; Hopkins *et al.* 2014). Therefore, these findings suggest that shifts in functional groups may significantly alter microbial activity which has important implications for the cycling of C in areas of recent sagebrush expansion (Metcalf, Fisher & Wardle 2011).

It is also important to note that the impacts of sagebrush expansion on soil fungal community composition were not directly tested in this study, although fungi are represented in several measured components of our system including microbial biomass C/N, mycorrhizal colonization and substrate-induced respiration. These measurements showed varying levels of response to sagebrush presence. In particular, mycorrhizal fungal colonization was higher for cushion plants (*E. ovalifolium*) growing under sagebrush canopies than outside at the high-elevation site only. We did not see a similar trend for the grass species (*K. macrantha*), or at lower elevation sites, suggesting high species- and site-level specificity in mycorrhizal responses to shrub encroachment. Soil fungi are known to be affected by, and have significant feedbacks on plant community composition and performance, including positive and negative PSFs of fungal symbionts and pathogens (Kulmatiski *et al.* 2008; Maron *et al.* 2011; Hilbig & Allen 2015). Therefore, determining changes in the composition of both free-living and symbiotic fungal communities will be important for a complete understanding of the impacts of native range expansion on soil microbial community structure and function.

### MECHANISMS BY WHICH ENCROACHMENT ALTERS THE SOIL COMMUNITY

We expected one way that sagebrush would influence microbial diversity is by modifying soil pH. Indeed, woody shrubs can alter pH (Buyer *et al.* 2016), which is one of the most important factors affecting soil bacterial community structure as many microorganisms have narrow pH niches for growth (Fierer, Bradford & Jackson 2007; Lauber *et al.* 2009; Rousk

*et al.* 2010). As predicted, soil pH strongly influenced microbial diversity and richness; however, sagebrush cover had no significant relationship with pH at any elevation. Soil pH also showed no clear trend across the elevation gradient suggesting that there is high within-site variability in pH in this ecosystem. Although soil pH had strong effects on the microbial community, and sagebrush had no detectable impact on soil pH, sagebrush cover had approximately two to five times stronger effects on microbial community structure than pH. This suggests that abiotic factors such as pH cannot fully explain the differences we observed in microbial communities in areas of sagebrush expansion.

We found evidence of altered soil nutrient levels underneath sagebrush with important cascading effects on microbial communities. In our study, soil organic C and N content were significantly higher under sagebrush canopies than in shrub interspace at low-elevation sites, and SOC was slightly higher at the high-elevation site in one version of the model. This local enrichment under shrubs is known as the 'island of fertility' effect and can be caused by many factors including accumulation of litter, trapping of airborne nutrients and reduced run-off under shrub canopies (Schlesinger *et al.* 1996; Ridolfi, Laio & D'Odorico 2008). This phenomenon is particularly important in dryland ecosystems, which is likely why we saw this effect most strongly at the low-elevation sites, where annual precipitation is significantly lower (Schlesinger *et al.* 1996). Furthermore, SON was positively related to substrate-induced respiration rates, suggesting increased potential for microbial decomposition of soil organic matter under sagebrush. This contradicted our predictions that sagebrush would have a dampening effect on SIR due to microbial acclimation to lower litter quality (Cornelissen *et al.* 2007). Finally, unlike the effects of sagebrush on microbial diversity, SON showed a consistently negative relationship with microbial diversity and richness, proposing that sagebrush may have indirect effects on soil microbial community composition via changes in soil nutrients.

By altering water use and shading, shrubs can influence the amount of water that is available for nutrient diffusion and microbial use in the soil (Gómez-Aparicio *et al.* 2005; Huxman *et al.* 2005). In our models, soil moisture (VWC) was associated with decreased microbial diversity, suggesting that in dry soil conditions, increased heterogeneity of microsites and spatial isolation of soil pores may promote microbial diversity and species coexistence (Frey 2007). However, the impact of sagebrush on soil moisture varied by elevation, with significantly higher soil moisture under sagebrush canopies at high elevation sites. These patterns are consistent with shrubs physically trapping snow under their canopies at high elevations, thereby increasing snowpack depth and delaying snowmelt (Leffler & Welker 2013). Research from arctic and alpine systems has suggested that snow trapped under shrubs may insulate soils and further stimulate winter microbial activity and nutrient breakdown (Weintraub & Schimel 2005; Leffler & Welker 2013). We predicted this would result in a positive impact of VWC on SIR, however we only observed this in one version of the model. VWC is known to be highly spatially and temporally

variable, and the impacts of woody plants on hydrologic cycles can be strongly influenced by climate (Bradford *et al.* 2014); therefore continuous measurements over time would be important in order to fully tease apart the net effects of sagebrush presence on soil water status. As climate continues to warm, the impacts of shrub expansion on soil moisture will likely become even more important at high-elevation sites as snowpack levels diminish and hydrologic cycles become increasingly altered (Callaghan *et al.* 2011).

Although not measured in this study, sagebrush is known to produce litter volatiles including terpenes, jasmonic acids and several other categories of secondary metabolic chemicals which may have direct or indirect influences on soil microbial communities (Weaver & Klarich 1977). Sagebrush has also been shown to have allelopathic effects on seed germination of heterospecific plants (Karban 2007), which may alter the soil microbial community associated with particular plant species. In general, plant volatiles such as those present in sagebrush litter can deter or attract different soil fauna to litter food sources (Austin *et al.* 2014), increase or decrease microbial respiration (Weaver & Klarich 1977; Asensio *et al.* 2012), alter microbial growth and nitrogen mineralization (Asensio *et al.* 2012) and disrupt plant-mycorrhizal associations (Nilsson *et al.* 1993; Wardle *et al.* 1998); however, the net impacts of these of plant volatiles on soil microbial communities are still poorly understood.

#### SHRUB REMOVAL, MICROBIAL RESILIENCE AND LEGACY EFFECTS

In addition to the observed changes in soil microbial communities due to sagebrush encroachment, we also found evidence that these changes are reversed with the subsequent removal of shrubs. Shannon diversity, OTU richness and overall microbial composition (based on weighted UniFrac metric) at 3100 m elevation differed significantly between shrub and shrub removal plots but were not different between interspace and shrub removal plots. This suggests a potentially high level of microbial resilience or ability to return to a 'pre-disturbance' condition over short time-scales (< 5 years) (Allison & Martiny 2008; Shade *et al.* 2012). Our findings also suggest that, in contrast to other studies, long-term microbial legacy effects of shrub expansion may not persist (Throop & Archer 2007). Although few studies have targeted microbial resilience after shrub removal, Shade *et al.* (2012) concluded that only 13–15% of studies testing resilience after a disturbance reported a reversal or return to pre-disturbance composition of soil microbial communities. One recent study focused on shrub thinning in a Namibian savanna found results similar to ours (Buyer *et al.* 2016), indicating that microbial communities may respond quickly to, but also recover quickly from, woody shrub encroachment. This type of compositional resilience, which could be leveraged for climate mitigation and habitat restoration, is likely promoted by rapid growth rates of bacteria and archaea, opportunistic species, flexibility in substrate use, and/or dispersal from neighbouring sites (Allison & Martiny 2008; Shade *et al.* 2012).

Identifying which of these mechanisms contributes to microbial resiliency after shrub removal, and measuring the rates at which microbial communities return to their previous composition, will allow for improved predictions of how microbially mediated ecosystem processes will respond to global change (Allison & Martiny 2008; Shade *et al.* 2012).

## CONCLUSIONS

Overall, we observed stronger effects of sagebrush on soil communities at high- and low-elevation sites versus middle-elevation sites, which is consistent with research proposing that changes in vegetation dynamics are likely to be strongest at the leading and trailing edges of range expansions (Svenning & Sandel 2013). However, while we aimed to control for variation in site-level environmental conditions – including parent material, aspect and plant community composition – we cannot fully isolate the chronosequence of sagebrush expansion from climatic changes along the altitudinal gradient. Specifically, precipitation increases with elevation and temperature decreases, as is common for alpine ecosystems. Nonetheless, the influence of sagebrush on microbial communities was strong across all elevations, and microbial community composition responded similarly post-sagebrush removal at the two opposing ends of our elevation gradient, suggesting a causal link between these factors.

While it is known that plant community composition is a major driver of soil microbial community composition (Wardle *et al.* 2004), specific mechanisms for how climate and land use driven changes in plant community composition will affect soil microbial communities have remained elusive (Classen 2015). We believe that coupling modern next-generation sequencing with soil biotic and abiotic measurements in a structural equation modelling framework offers exciting opportunities for disentangling the complex network of plant–soil interactions. Through this framework, we have uncovered interesting connections between plant range-expanders, abiotic soil parameters and the structure and function of soil microbial communities. In particular, our results show that sagebrush can have strong direct effects on soil microbial community structure including increased diversity and richness, and altered community composition, and important indirect effects on microbial communities by creating changes to soil nutrients and moisture. Teasing apart direct and indirect pathways of plant impacts on below-ground ecosystem function is a critical, yet unresolved area of ecological research. With additional studies, this approach could provide a more complete and predictive understanding of the impacts of native range expansions on terrestrial ecosystems.

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## Data accessibility

Raw data for all non-molecular analyses including soil nutrient, moisture, pH, microbial biomass, mycorrhizal colonization and substrate-induced respiration have been deposited in the Dryad repository <http://dx.doi.org/10.5061/dryad.b1m1h> (Collins *et al.* 2016). Raw sequences have been deposited to the NCBI Short Read Archive (SRA) BioProject ID PRJNA320310.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** SEM parameter estimates.

**Table S1.** Parameter estimates for structural equation models, with 95% credible intervals.

**Appendix S1.** Results of web soil survey query for three elevation sites.