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Soil fungal composition changes with shrub encroachment in the northern Chihuahuan Desert

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ABSTRACT

Woody species encroachment of grasslands globally causes many socioecological impacts, including loss of grazing pastures and decreased biodiversity. Soil microbial communities may partially regulate the pace of shrub encroachment, as plant-microbial interactions can strongly influence plant success. We measured fungal composition and activity under dominant plant species across a grassland to shrubland transition to determine if shrubs cultivate soil microbial communities as they invade. Specifically, soil microbial communities, abiotic soil properties, and extracellular enzyme activities were quantified for soils under four common Chihuahuan Desert plant species (three grasses, one shrub) in central New Mexico, U.S.A. Extracellular enzyme activity levels were fairly consistent under different plant species across the grassland to shrubland transition. Activity levels of two enzymes (alkaline phosphatase and beta-N-acetyl-glucosaminidase) were lower in the ecotone, presumably because soil organic matter content was also lower in ecotone soils. Community composition of soil fungi mirrored patterns in the plant community, with distinct plant and fungal communities in the shrubland and grassland, while grassland-shrubland ecotone soils hosted a mix of taxa from both habitats. We show that shrubs cultivate a distinct microbial community on the leading edge of the invasion, which may be necessary for shrub colonization, establishment, and persistence.

1. Introduction

In the Anthropocene, species distributions are shifting in response to global changes (Kelly and Goulden 2008; Chen et al., 2011; Virkkala and Lehikoinen 2014; Ash et al., 2017). Understanding the causes of range shifts will help to predict the distribution of species and communities into the future. Many factors interact to shape the magnitude and rate at which species ranges expand and/or contract (van der Putten et al., 2010; Engelkes et al., 2018), but the numerous regulating processes involved are poorly understood. Soil microbial communities and plant-soil feedbacks are important factors that could regulate the speed and success of plant migrations (Bever et al., 1997; Levine et al., 2006; Reinhart and Callaway 2006; Bardgett and van der Putten 2014; Dawson and Schrama 2016), yet these feedbacks have received limited attention within the context of woody plant encroachment.

Plant-associated microbes are essential to nearly all plant communities, regulating nutrient cycling (Collins et al., 2008; Bardgett and van der Putten 2014), influencing pollinators (Vannette and Fukami 2016), interacting with other trophic levels (Finkes et al., 2006), and shaping plant productivity and diversity (van der Heijden et al., 2008). In particular, the critical role of microbes in decomposition and nutrient cycling has long been of interest (Lindeman 1942). Within the soil, microbes exude enzymes to breakdown complex molecules prior to absorption, and many of the products of extracellular enzyme activity are also available for uptake by plants. Quantifying extracellular enzyme activity levels provides a measurement of both microbial activity and resource availability within the soil (Sinsabaugh 1994).

With the advent of more accessible and advanced sequencing techniques, we can also now characterize the diversity and composition of microbial communities to better understand their dynamics and complex associations with plants. As with plant communities, microbial community composition and function vary within a landscape (Johnson

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et al., 2018), through time (Williams et al., 2013; Kyaschonko et al., 2017), as well as across climatic and ecological gradients (Taylor et al., 2014; Mueller et al., 2016; Mureva and Ward 2017; Tripathi et al., 2017). This spatial and temporal variation in microbial composition will likely continue to influence future plant persistence.

Given the importance of plant-soil feedbacks in plant productivity, persistence, and diversity (Bardgett and van der Putten 2014; Bauer et al., 2017), it is likely that soil microbial communities may influence both plant distributions and the rate of plant invasions (Perkins and Nowak 2013; Bardgett and van der Putten 2014; Dickie et al., 2017; Policelli et al., 2019). While plants are dependent on microbes, plant-microbe interactions fall along a spectrum from beneficial to antagonistic. As antagonistic relationships between microbes and resident plants develop through time, soils can become more favorable to new plant species than to established species. These more favorable soil conditions can aid in expanding plant ranges, particularly if other necessary soil microbes are also present at the new location (van Grunsven et al., 2010; van der Putten et al., 2016, but see Suding et al., 2013). Alternatively, necessary microbes might not be present at new locations and plant-associated microbial communities may need to migrate with the plants, thus potentially limiting the rate at which plant expansions occur. In the latter case, it is unclear if the plants or microbes are the first to move, or if they move together as abiotic conditions change (Zorbel and Öpik 2014).

Shrub expansion into neighboring grasslands and open areas is a global phenomenon with widespread ecological and societal consequences (Tape et al., 2006; Briggs et al., 2007; Van Auken 2009; Eldridge et al., 2011; Ratajczak et al., 2012; Quero et al., 2013; Archer et al., 2017) including reduced forage production, altered biodiversity, increased soil erosion, and increased carbon sequestration (Archer et al., 2017). Oftentimes the encroaching shrub is a native or naturalized species that has recently expanded into adjacent habitats (Van Auken 2000; Heisler et al., 2003; Briggs et al., 2005; Fredrickson et al., 2006), and local soil microbial communities may influence the rate of woody plant expansion. In desert shrublands, microbial biomass is higher under shrubs relative to open areas between shrubs (Bacher et al., 2012; Creamer et al., 2016; Li et al., 2017; Mureva and Ward 2017), and in general soil microbial communities change as shrubs invade (Xiang et al., 2018). Encroaching shrubs can directly alter soil microbial communities (Collins et al., 2016) or indirectly shape soil microbes through changes to soil properties and chemistry (Creamer et al., 2016). Additional studies regarding soil microbial composition associated with shrub encroachment will allow us to look for generalities in the influence of microbes on the process of shrub encroachment.

We surveyed soil characteristics, microbial communities, and microbial activities under dominant plant species in grassland, shrubland, and the grassland-shrubland ecotone in a region where range expansion of a dominant shrub is actively occurring to ask: (1) To what degree is the soil environment (e.g. fungal composition and activity) under shrubs distinct from that of the grasslands they encroach? If the soil biotic environments are similar, then plant-soil feedbacks may not influence the rate of shrub encroachment. (2) If soil biotic environments differ between grasslands and shrublands, do invading shrubs in the ecotone alter soil microbial communities to reflect the composition and function of shrubland soils, or instead do invading shrubs adapt to the grassland soil microbial community? To assess microbial community function and composition associated with shrub encroachment, we measured microbial activity levels via extracellular enzyme assays and fungal species composition via DNA amplicon sequencing for soils under dominant plants across the grassland to shrubland transition.

2. Materials and methods

2.1. Study species and research site

This research was conducted at the Sevilleta National Wildlife Refuge (SNWR), also the site of the Sevilleta Long Term Ecological Research (LTER) program, located in central New Mexico, U.S.A. (34.33° N, 106.83° W). Average daily maximum temperatures range from 33.4 °C in June to 1.6 °C in January (Muldavin et al., 2008), and the site receives on average of 240 mm of rainfall annually, with 60% falling in summer monsoon events from June to September (Gosz et al., 1995; Pennington and Collins 2007; Petrie et al., 2014). Soils are a Typic Haplargid, with a 3.0–7.5 cm deep sandy surface soil composed of Aeolian material atop several argillic horizons (Buxbaum and Vanderbilt 2007; Bryan-Ricketts 2012). The SNWR includes both Chihuahuan Desert grasslands that are dominated by several grasses (e.g., *Bouteloua* spp., *Pleuraphis* spp., *Aristida* spp.) and Chihuahuan Desert shrublands dominated by *Larrea tridentata*.

Larrea tridentata (creosote bush) is a drought tolerant C_3 evergreen shrub native to the warm deserts of North America (Laport et al., 2012; Báez et al., 2013). Here in the northern Chihuahuan Desert, individuals are not clonal and reproduce only by seed (Duran et al., 2005). Although L. *tridentata* has very specific requirements for germination and establishment success (Ackerman 1979; Reynolds et al., 1999; Moreno-de la Heras et al., 2016), once established *L. tridentata* is extremely hardy and persistent (Bowers et al., 1995; Gibson et al., 2004; Ladwig et al., 2019), with some individuals estimated to be > 10,000 years old (Vasek 1980). Regionally, notable population expansions into neighboring grasslands have occurred since the 1850s (McCraw 1985; Gibbens et al., 2005), changing the community composition and function of these desert ecosystems (Pockman and Small 2010; Turnbull et al., 2011). Our site is located at the northern range extent of *L. tridentata* where its distribution is thought to be limited by cold temperatures (Pockman and Sperry 1997). Therefore, warmer and dryer conditions associated with climate change in this region (Rudgers et al., 2018) may aid in the continued expansion of *L. tridentata*.

The three grass species included in this study, *Bouteloua eriopoda, Bouteloua gracilis*, and *Pleuraphis jamesii*, are long-lived (>30 years), perennial C_4 grasses that are common throughout the region. All three grasses spread asexually. *Bouteloua gracilis* is caespitose and forms slowly expanding rings in our region (Ravi et al., 2008; Hoffman et al., 2020), whereas *B. eriopoda* and *P. jamesii* expand more rapidly by stolons and rhizomes, respectively (Peters and Yao 2012; Hoover et al., 2017). Collectively they contribute >80% of total grass biomass within grasslands at the SNWR (Rudgers et al., 2019). Currently, the Chihuahuan Desert grass *B. eriopoda* is increasing in areas dominated by the Great Plains species, *B. gracilis*, likely as a consequence of long-term regional increases in aridity (Rudgers et al., 2018; Maurer et al., 2020; Collins et al., 2020).

2.2. Field and lab procedures

To examine if microbial communities change across the grassland to shrubland transition, we measured fungal community composition, enzyme activity levels, and abiotic properties of soils beneath dominant plant species in grassland, shrubland, and the ecotone between these systems (referred to as “ecotone” hereafter). Soils were collected on October 20, 2013 under *L. tridentata* and the three grass species along a 400 m continuous belt transect through the three habitats (shrubland, ecotone, grassland; Fig. 1). No species were present in all three systems; rather, species occurrence varied among shrubland (*L. tridentata*), ecotone (*L. tridentata, B. eriopoda, P. jamesii*) and grassland (*B. gracilis, B. eriopoda, P. jamesii*; Fig. 1). At locations where multiple plant species co-occurred (e.g., in grasslands and ecotones), replicate plants of each species were located within 1 m of each other to control for soil and microclimate conditions. Within each system, soil samples were collected from below 8 individuals of each focal species and were processed separately for a total of 56 samples (8 in shrubland, 24 in grassland, 24 in ecotone; Fig. 1). Under each plant, three separate soil samples were collected to measure soil texture/nutrients, microbial community composition via DNA sequencing, and extracellular enzyme

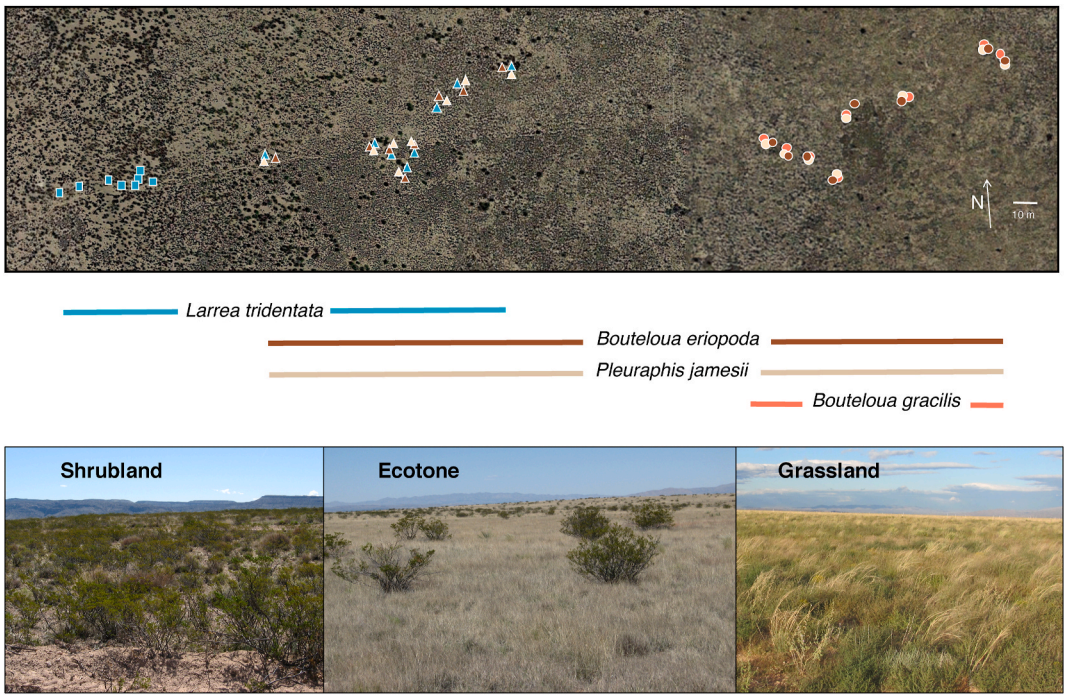


Fig. 1. Sampling locations across the 400 m transect from shrubland to grassland. Soil samples were collected under four dominant plant species, indicated by different colors, and three habitats (shrubland, ecotone, grassland). Aerial image is from Google Earth and habitat photos were taken by L.M. Ladwig.

activity.

To assess abiotic soil conditions, soil texture and nutrient content were measured. For soil collection, at least 100 g of the top 10 cm of soil were collected with a hand trowel and allowed to air dry in the lab. Once dry, samples were split in half for nutrient and texture analysis. For nutrient analysis, samples were processed at the University of Wisconsin Soil and Forage Analysis Lab (www.uwlab.soils.wisc.edu), and analysis included concentrations (ppm) of K, P (method: 1:1 water, Bray 1, Bray 1, LOI) and NO_3 (method: potassium chloride), in addition to percent organic matter (OM; loss on ignition). For texture analysis, fractions of sand, silt, and clay were measured via settling rates and a hydrometer.

Soil microbial community composition was measured by sequencing fungal DNA. For soil collection, a sterilized 15 mL plastic tube (1.5 cm diameter) was pushed into the soil at the base of the plant to a depth of 10 cm, placed in liquid N in the field, and stored at −80 °C. Samples were thawed on ice and roots and large stones were removed from the sample with forceps. DNA was extracted from 10 g of soil using a PowerMax Soil DNA Isolation Kit (Mo Bio Laboratories, Inc.). Extracted DNA was quantified using PicoGreen fluorometeric quantification (ThermoFisher) and was sent to processing facilities at the Environmental Genetics & Genomics (EnGGen) lab at Northern Arizona University (www.enggen.nau.edu) for Illumina Miseq V3 paired end sequencing of the fungal ITS2 region using the 5.8S-Fun/ITS4-Fun primer set (Taylor et al., 2016). Due to the poor quality of the reverse reads, only the forward reads were used. All sequencing processing was conducted in USEARCHv11 (Edgar 2010). The forward reads were demultiplexed using fastx_demux, resulting in 5,330,416 reads. Residual phiX reads were removed using filter_phix, and the primer sequences were removed. Reads were then quality filtered, removing any reads with expected error rate higher than 1, filtered to only unique sequences, chimera checked, clustered into operational taxonomic units (OTUs) at 97% similarity, and finally mapped back to the demultiplexed reads using the default settings in USEARCHv11 (Edgar 2013, 2016). Sequences were submitted to NCBI under BioProject PRJNA734481.

Representative fungal sequences were classified using both sintax in USEARCHv10 (UNITEv7.2) and Protax (Abarenkov et al., 2018; against UNITEv8). Classifications from sintax and Protax were mostly congruent, but those from Protax were sometimes more resolved, and so were used as the default classifications for downstream analysis. OTUs classified to fungi with less than 80% confidence according to sintax were filtered from the matrix resulting in 3,949,775 reads and 3762 OTUs. We used Kracken (Wood and Salzberg 2014) implemented on the public server at usegalaxy.org (Afgan et al., 2016) to identify OTUs that classified to bacterial species (top hits) then filtered them from the matrix.

A number of OTUs were poorly identified by both sintax and Protax, which hampered functional analyses. For example, using sintax 599 out of 2674 OTUs (22.4%) could not be identified to phylum and an additional 432 OTUs (16.2%) could not be identified to class. Results obtained from Protax with no confidence threshold were slightly better (1.9% of OTUs identified to Kingdom only and another 19.0% to Phylum only). We therefore undertook several additional steps to better identify the top 500 most abundant OTUs, which together accounted for 89.7% of the 2,321,517 reads. First, we identified the best matches in NCBI using discontiguous megablast (Johnson et al., 2008) with uncultured/environmental sequences excluded, no masking of low complexity regions, and 16 nucleotide template length with two templates for the discontinuous options. If a top hit was found that satisfied the following criteria, the manual classification was considered complete: well identified (species-level preferred, genus-level accepted if no relatively close species-level matches); >97% identity and 90% coverage; taxonomic consistency with other top hits and with any sequences from type specimens. In cases where none of the top blast matches met these criteria, we then used the “compute distance tree” option in blast to compare the 100 top hits with the query OTU. If our OTU fell within a clade surrounded by sequences from a single species, it was assigned to that species; if it fell within a clade comprised of different species from a single genus, it was assigned to that genus, and so on. In general, this corresponds to a ‘least common ancestor’ (LCA) approach as implemented in programs such as MEGAN (Huson et al., 2007). In some cases, outlier NCBI sequences that were assigned to a different class or order from the majority of the hits were ignored, as these would unnecessarily broaden the LCA assignment.

Ten OTUs were poorly identified even after the above steps were

undertaken. For these 10 OTUs, we performed full phylogenetic analyses as follows. We conducted another discontinuous megablast search, but with uncultured sequences included, with the goal of identifying closely matching sequences that were significantly longer than our 150bp reads. These longer sequences were then used in a second round of blast searches using the top, longer NCBI match as the query, once again excluding uncultured sequences in order to identify addition related GenBank entries with greater phylogenetic signal due to greater length. All well identified matches were downloaded, aligned to the NCBI query and our OTU representative sequence using MAFFT in Aliview (Larsson 2014), then subjected to maximum likelihood tree construction in Garli 0.96 (Zwickl 2006) using default parameters. The position of our OTUs within these trees were interpreted similarly to the blast distance trees to estimate OTU taxonomic placements. During these steps, we removed any OTUs that did not match fungi along with the common contaminant *Malassezia* sp. Post filtering and removal of non-target samples, the fungal community was composed of 2,321,517 reads and 2674 OTUs. To control for PCR bias and to normalize the community (McMurdie and Holmes 2014), we used variance stabilization normalization in the DESQ2 package in R (Love et al., 2014).

To test for differences in soil activity across the transition, potential extracellular enzyme activity levels (EEA) were measured. For soil collection, a sterilized 15 mL plastic tube (1.5 cm diameter) was pushed into the soil at the base of the plant to a depth of 10 cm. Samples were kept cool (cooler in the field, refrigerator in the lab) and processed within 48 h of collection to prevent enzyme degradation. The potential activity levels of four enzymes (alkaline phosphatase: AlkP – removes phosphate group from organic molecules, beta-N-acetyl-glucosaminidase: NAG – degrades chitin, alanine aminopeptidase: AAP – cleaves amino acids from proteins at the N-terminus, and beta-glucosidase: BG – separates glucose from cellooligosaccharides) were measured fluorometrically following methods of Stursova et al. (2006). Potential enzyme activity levels were calculated as nmol of product produced per hour per gram of soil (nmol h^{-1} g $soil^{-1}$) and per gram OM (nmol h^{-1} g OM^{-1}). To calculate field soil moisture, subsamples were weighed and dried at 60 °C. With a subsample, percent organic matter was calculated via loss on ignition, after samples were burned at 500 °C for 4 h.

2.3. Data processing and statistical analysis

For all statistical models, plant species (*L. tridentata, B. eriopoda, B. gracilis, P. jamesii*) and habitat (shrubland, ecotone, grassland) were included as main effects without interactions because habitat is defined by species and therefore not independent, and position along the transect was included as a random variable. All statistical analyses were performed using R (version 3.5.1, R Core Team, 2018) with an alpha of 0.05. All mixed effect models were created using lmer in the lme4 package (Bates et al., 2015) with fixed effects significance determined using a type 3 anova in lmerTest (Kuznetsova et al., 2017). Potential differences in soil texture and chemistry between habitats and species were tested using a separate mixed effect model for each soil characteristic (% sand, % clay, % silt, % organic matter (OM), and ppm of P, K, and NO_3–N).

To assess differences in soil microbial community composition, the abundances of fungal OTUs in each sample were compared via a non-metric multidimensional scaling (NMDS) approach using the Bray-Curtis dissimilarity metric. A distance based perMANOVA was used to test if soil fungal communities differed between plant species or habitats with position along the transect included as a strata variable using the 'adonis' function in the 'vegan' package (Oksanen et al., 2017). To determine which taxa were most influential in differentiating fungal communities under plants across the transition, we examined similarity percentages of taxa within the Bray-Curtis dissimilarities with the 'simper' function in the 'vegan' package. To test if community composition was related to abiotic soil properties, we ran a mantel test using the 'mantel' function in the 'ecodist' package (Goslee and Urban 2007) to examine possible correlations between the soil taxa and abiotic dissimilarity matrices [using an Euclidean distance matrix of standardized abiotic matrix (decostand function in the 'vegan' package using range standardization)] and examined the fit of soil characteristics vectors in the ordinal space of the soil community using 'envfit' function in the 'vegan' package. We also compared three diversity indices, Shannon, Simpson, and Chao1 (calculated on the non-normalized matrix), among plant species and habitats with a separate mixed effects model (described above) for each index.

To test if microbial activity varied among habitats and dominant plant species, EEA were compared among systems and species with a separate mixed effect model (described above) for each enzyme (NAG, BG, AAP, AlkP) and type of activity (per gram soil or OM), for a total of 8 tests. For some enzymes, activity levels were log transformed to meet model assumptions. To test for a correlation between enzyme activity and abiotic soil properties, a mantel test was employed to compare the two matrices (using Euclidean distance matrices), and the fit of enzyme activity vectors in the ordinal space of the soil community was tested using the 'envfit' function in the 'vegan' package. To compare potential fungal activity further, we extracted putative ecological function from each soil community using FUNGuild, which uses taxonomic information to group fungal sequences into ecological guilds (Nguyen et al., 2016). Specific functional guilds of interest included endophytes, plant pathogens, arbuscular mycorrhizal fungi, symbiotrophs, and saprotrophs at a confidence level of "Probable" or "Highly Probable". These analyses utilized the most resolved classification we were able to obtain using the multiple methods described above. In general, little or no functional annotations are possible for OTUs that are not identified to family or finer levels. A separate mixed effects model (described above) was run for each functional guild to test if number of reads differed among plant species or locations. Taxa that classified to guilds according to FUNGuild made up 45% of the OTUs and 44% of the VST normalized reads. Data and code associated with this project are publicly available (Ladwig et al., 2021).

3. Results

All measures of soil texture and chemical properties beneath plants differed significantly between at least two of the habitats along the grassland to shrubland transition (Table 1). For several soil characteristics (N, % content of clay, sand, and OM), shrubland and grassland soils had similar levels while ecotone soils were different from each of the other two (Table 1, Fig. 2). Amount of K was the only soil characteristic that varied with plant species; specifically, K concentrations were

Table 1
Statistical model results for comparisons of soil texture and chemistry under the four dominant plant species in the three habitats (grassland, ecotone, shrubland). Values in parenthesis are model and residual degrees of freedom.

	Model	Species	Habitat
Soil Texture			
Clay *p*	0.0005	0.15	0.0005
F	5.4 (5, 49)		8.9 (2, 49)
Silt *p*	0.02	0.87	0.004
F	2.9 (5, 49)		6.3 (2, 49)
Sand *p*	0.0007	0.47	0.0001
F	5.1 (5, 49)		11.1 (2, 49)
Soil Chemistry			
OM (%) *p*	<0.0001	0.75	<0.0001
F	10.1 (5, 50)		21.7 (2, 50)
N (NO_3 ppm) *p*	0.04	0.56	0.005
F	2.5 (5, 50)		5.9 (2, 50)
P (ppm) *p*	<0.0001	0.12	<0.0001
F	62 (5, 50)		102.0 (2, 50)
K (ppm) *p*	<0.0001	0.002	<0.0001
F	21.2 (5, 50)	5.8 (3, 50)	37.7 (2, 50)

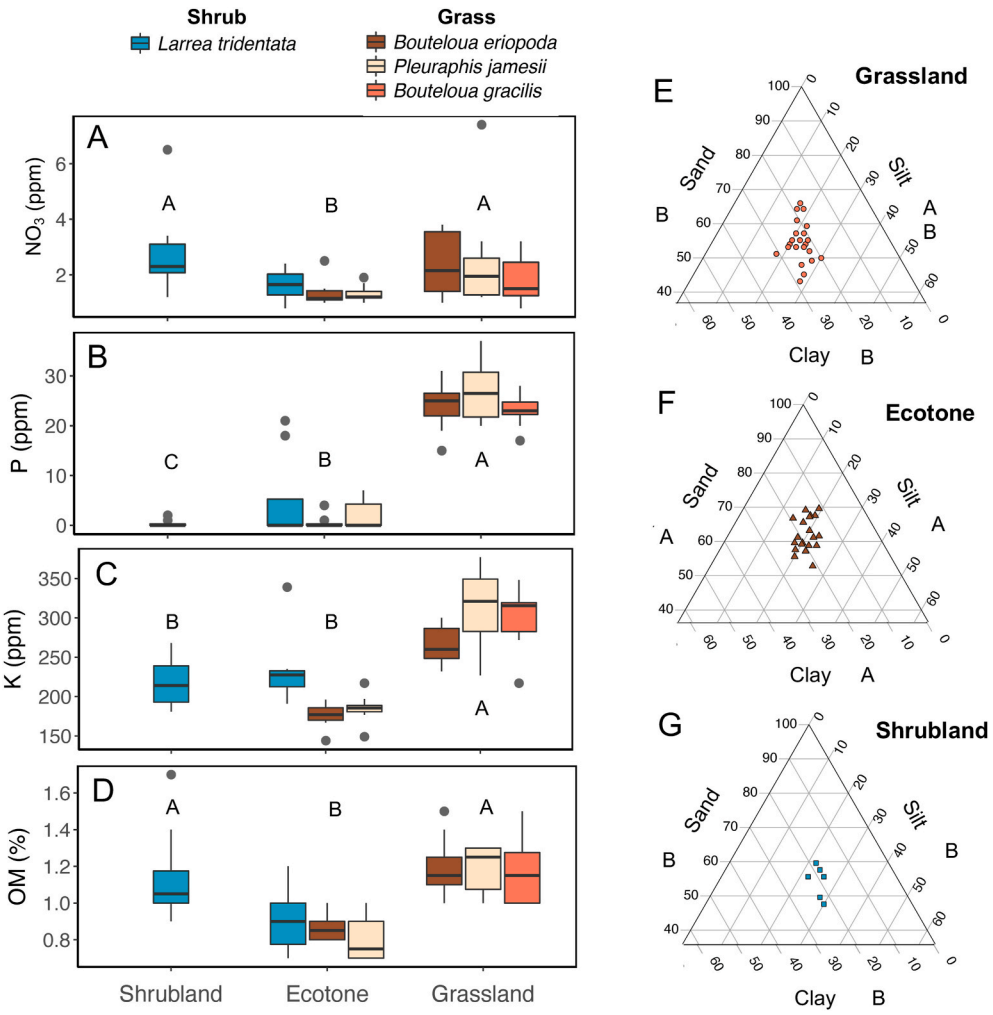


Fig. 2. Soil chemistry (A -D) and texture (E - G) under dominant plant species in the Chihuahuan Desert shrubland, ecotone, and grassland. Bars represent the first through third quartiles of the data, with the median as the center line. Different letters indicate statistically different values across habitats. For soil texture, each point represents an individual soil sample. Texture statistically differed among habitats but not species (Table 1), so all points are colored the same within each habitat. OM = organic matter.

significantly higher under *B. gracilis* than any other species. This difference among grass species could suggest a habitat by species interaction that we were unable to test statistically, since K was also significantly higher in grasslands (Table 1) and *B. gracilis* was only found in grasslands while *B. eriopoda* and *P. jamesii* were present in both grasslands and ecotone.

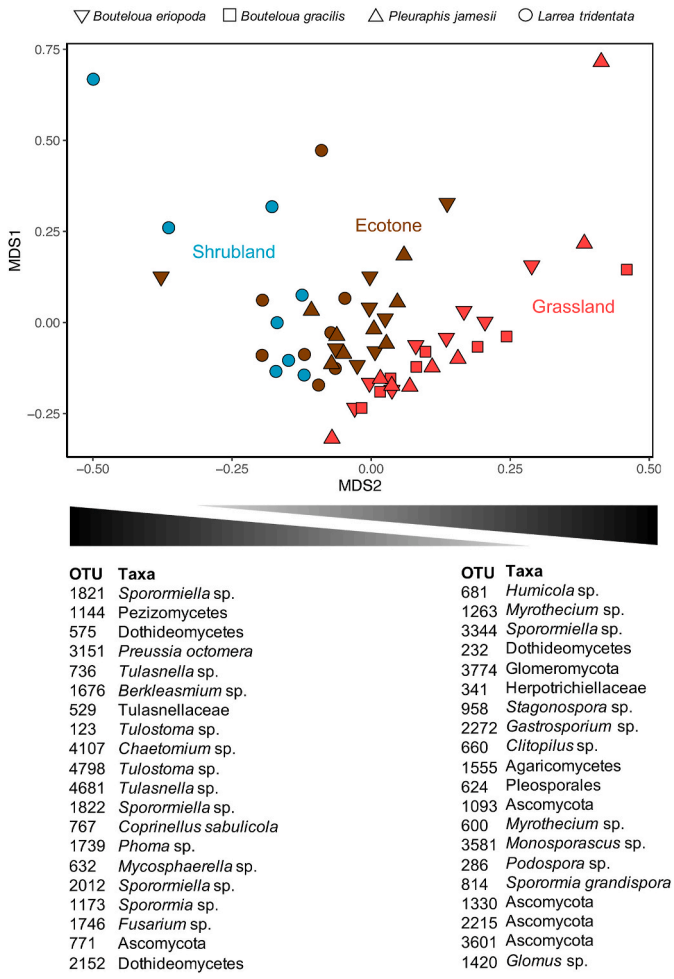
Composition of soil fungal communities varied among plant species ($p = 0.007$, $F_{3,50} = 1.24$, $R^2 = 0.06$) and habitats ($p < 0.007$, $F_{2,50} = 4.33$, $R^2 = 0.14$) based on the perMANOVA test using fungal species abundances (Fig. 3). Pairwise comparisons indicated distinct communities among all three habitats (all $p < 0.001$), and all plant species ($p < 0.01$), with the exception of *B. eriopoda* and *P. jamesii* ($p = 0.55$; Fig. 4). Based on comparisons of taxa driving differences in community composition, according to SIMPER, soil fungal communities differed more by plant species than by location (Fig. 4). Based on the Mantel test, fungal composition was also related to soil texture and nutrients ($r = 0.33$, $p = 0.001$), specifically with regard to soil clay, P, and K content ($p = 0.01$, $p < 0.001$, $p < 0.001$, respectively). Soil fungal diversity (Shannon, Simpson, Chao1) did not statistically differ between plant species or habitats ($p > 0.40$ for all indices; Figure S1).

Extracellular enzyme activity showed limited variation between habitats, plant species, or abiotic soil properties. For enzyme activity per gram soil, NAG differed between habitats, with significantly higher activity levels in grasslands than ecotones ($p = 0.01$, $F_{2,50} = 4.9$; Fig. 5), but did not vary among plant species ($p = 0.9$). For enzyme activity per gram OM, there were no differences in activities among habitats or plant species ($p > 0.1$ for all enzymes). Based on Mantel test results, enzyme activity levels were not related to soil abiotic factors ($r = 0.04$, $p = 0.3$).

When comparing FUNGuild classifications, the abundance of different functional guilds did not statistically differ among species or location. Although, the VST normalized reads abundance of arbuscular mycorrhizae trended towards having lower abundance in shrubland soils compared to ecotone soils ($p = 0.059$, $F_{2,50} = 2.99$; Fig. 6).

4. Discussion

Within the Chihuahuan Desert system, grasslands and shrublands have distinct plant and soil fungal communities, while the ecotone region shares plant and fungal taxa with both habitats (Figs. 1 and 3). Differences in fungal communities under grasses and shrubs in the ecotone were partially due to the fact that soil under grasses had unique fungal taxa not found under shrubs (Fig. 4). This could reflect differences in the amount of time these plant species were present in this system, specifically grasses have been at the site longer than shrubs. It often takes time for host plants to cultivate a more specialized microbial community, so possibly *L. tridentata* plants in the ecotone have not had sufficient time to alter soil communities to match communities in shrublands. We do not know the age of individual shrubs, so it is unclear how long these *L. tridentata* plants have been in the ecotone, but many have been there for decades. Also, shrubs may be less reliant on a specific microbial community composition and perform well in grassland soils. Changes in soil microbial composition with shrub encroachment occur in a variety of other systems (Collins et al., 2016; Li et al., 2017; Schwob et al., 2017; Xiang et al., 2018), so the response observed here is



OTU	Taxa
1821	*Sporormiella* sp.
1144	Pezizomycetes
575	Dothideomycetes
3151	*Preussia octomera*
736	*Tulasnella* sp.
1676	*Berkleasmium* sp.
529	Tulasnellaceae
123	*Tulostoma* sp.
4107	*Chaetomium* sp.
4798	*Tulostoma* sp.
4681	*Tulasnella* sp.
1822	*Sporormiella* sp.
767	*Coprinellus sabulicola*
1739	*Phoma* sp.
632	*Mycosphaerella* sp.
2012	*Sporormiella* sp.
1173	*Sporormia* sp.
1746	*Fusarium* sp.
771	Ascomycota
2152	Dothideomycetes

OTU	Taxa
681	*Humicola* sp.
1263	*Myrothecium* sp.
3344	*Sporormiella* sp.
232	Dothideomycetes
3774	Glomeromycota
341	Herpotrichiellaceae
958	*Stagonospora* sp.
2272	*Gastrosporium* sp.
660	*Clitopilus* sp.
1555	Agaricomycetes
624	Pleosporales
1093	Ascomycota
600	*Myrothecium* sp.
3581	*Monosporascus* sp.
286	*Podospora* sp.
814	*Sporormia grandispora*
1330	Ascomycota
2215	Ascomycota
3601	Ascomycota
1420	*Glomus* sp.

Fig. 3. Soil fungal communities, based on fungal OTU abundance, under plants along the shrubland to grassland transition. Plant species are designated by different shapes and location depicted with different colors. The top 20 fungi associated with each end of MDS2 are listed in rank order under the corresponding end of the axis.

not limited to this desert grassland (Fig. 3).

Over half of the fungi identified in our samples were members of the Ascomycota or Basidiomycota while >15% of the fungal community were unclassified beyond kingdom. Arbuscular mycorrhizal fungi within the Glomeraceae, including several *Glomus* spp. and *Kamienskia divaricata*, were also present. While members of the Glomeromycota are expected to be common in SNWR grasslands, early molecular studies targeting fungal ITS sequences, but using different fungal-specific PCR primers (ITS1-F/ITS4), failed to find significant numbers of sequences from this group (Porras-Alfaro et al., 2008). Detection was possible, however, employing primers specific for Glomeromycota (Porras-Alfaro et al., 2007). The abundance of Glomeraceae sequences in our samples supports results obtained with whole-metatranscriptome sequencing with SNWR soils (Hudson et al., 2015), and it suggests that the 5.8S-Fun/ITS4-Fun primer set employed here is superior to the ITS1-F/ITS4 primer set employed in early studies in terms of amplifying Glomeromycota sequences. Our sequences that match the recently-described *K. divaricata* are potentially interesting in the context of this aridland ecosystem. This species was described from sand dunes in South Africa, and sequences matching this species have been reported from soils in Texas, USA (Błaszkowski et al., 2016).

Of additional interest are sequences from species of *Monosporascus* (Ascomycota, Xylariales) found here in shrubland soils (Fig. 4). Species of *Monosporascus* are among the most common root endophytes obtained from culture and molecular studies at the SNWR (Porras-Alfaro et al. 2008, 2014; Dean et al., 2015; Robinson et al., 2020). The genus is known primarily from aridland ecosystems, and multiple species of the genus occur on diverse trees, shrubs, and grasses at the SNWR. *Monosporascus* sequences in our soil samples likely reflect the presence of spores, hyphal fragments, or contaminating root fragments. The abundance of these sequences further supports the importance of these species at the SNWR, and in this case the data suggest a skewing of abundance toward the shrub samples.

In terms of compositional differences among habitats and hosts, we note that a number of arbuscular mycorrhizal fungal taxa (*Glomus*, Glomeromycota) were more abundant in grasslands than shrublands (Figs. 3 and 4), despite the fact that many perennial plant species in the Chihuahuan Desert, including many grass species and *L. tridentata*, commonly form associations with arbuscular mycorrhizal fungi (Collier et al., 2003). In contrast, a number of decomposer basidiomycetes, such as *Conocybe*, *Crinipellis, Lepiota,* and *Tulostoma*, were more abundant in shrublands (Fig. 3) and/or in association with the shrub *Larrea tridentata* (Fig. 4). This is likely due to the higher lignin content of shrubs and suggests major functional differences in shrub versus grass-associated fungal communities, since lignin-degrading enzymes are well represented in these Basidiomycota but not in Ascomycota. Detritus can accumulate under shrub canopies leading to higher nutrient concentrations, also called “islands of fertility” (De Soyza et al., 1997), which may support a greater abundance of fungal decomposers. Furthermore, differences in litter quality not only influence decomposition rates, as leaves of *L. tridentata* contain more lignin and therefore decompose at a slower rate than dominant grasses, but also could differentially shape belowground communities (Vanderbilt et al., 2008).

Both positive and negative plant-soil feedbacks help explain temporal dynamics of dominant grass species at this study site (*B. eriopoda* & *B. gracilis*; Chung et al., 2019) and could influence the success of *L. tridentata* shrubs in the ecotone. The ecological roles of most members of the soil fungal community remain unknown, yet for the taxa that can be categorized into key functional guilds there was little variation among sampling locations (Fig. 6). It remains unclear if shrubs are moving into hospitable or hostile soil communities. Given that L. *tridentata* thrives in the ecotone despite not having the identical plant-associated fungal community found in the shrublands, shrub encroachment may not be limited strictly by the soil fungal community. It appears that shrubs initially invade the grasslands and then the shrubland-associated soil fungi follow thereafter. Further experimentation paired with these observations will illuminate the regulating power of plant-soil feedbacks on the spread of *L. tridentata* into desert grasslands. Additionally, given the differences observed here, it is possible that invading shrubs can tip the balance of competition and alter biotic conditions to their benefit by modifying both soil communities and physical properties (D’Odorico et al., 2010; He et al., 2015).

Complex interactions exist among secondary compounds, plants, and microorganisms. Examples include the reported roles of plant secondary compounds in encouraging the growth of beneficial bacteria in the rhizosphere while inhibiting plant pathogenic fungi (Schulz-Bohm et al., 2018; Stringlis et al., 2018), as well as legacy effects of soil fungal communities in shaping plant succession (Semchenko et al., 2019; Heinen et al., 2020). *Larrea tridentata* produces a host of secondary compounds, including many volatile organic compounds (Hyder et al., 2002; Jardine et al., 2010), and it is reported to be allelopathic (Mahall and Callaway 1991). In principle, secondary compounds could help facilitate *L. tridentata* encroachment by influencing microbial communities. Although the effects of secondary compounds from *L. tridentata* on microbial community composition are unknown, toxicity of *L. tridentata* compounds has been reported for fungi (Vargas-Arispuro et al., 2005), mammals (Meyer and Karasov 1989; Mangione et al., 2004), microfauna (Fowler and Whitford 1980) and bacteria (Martins et al., 2013), suggesting the potential to alter microorganism assemblages.

When using extracellular enzyme activity levels as proxies for soil

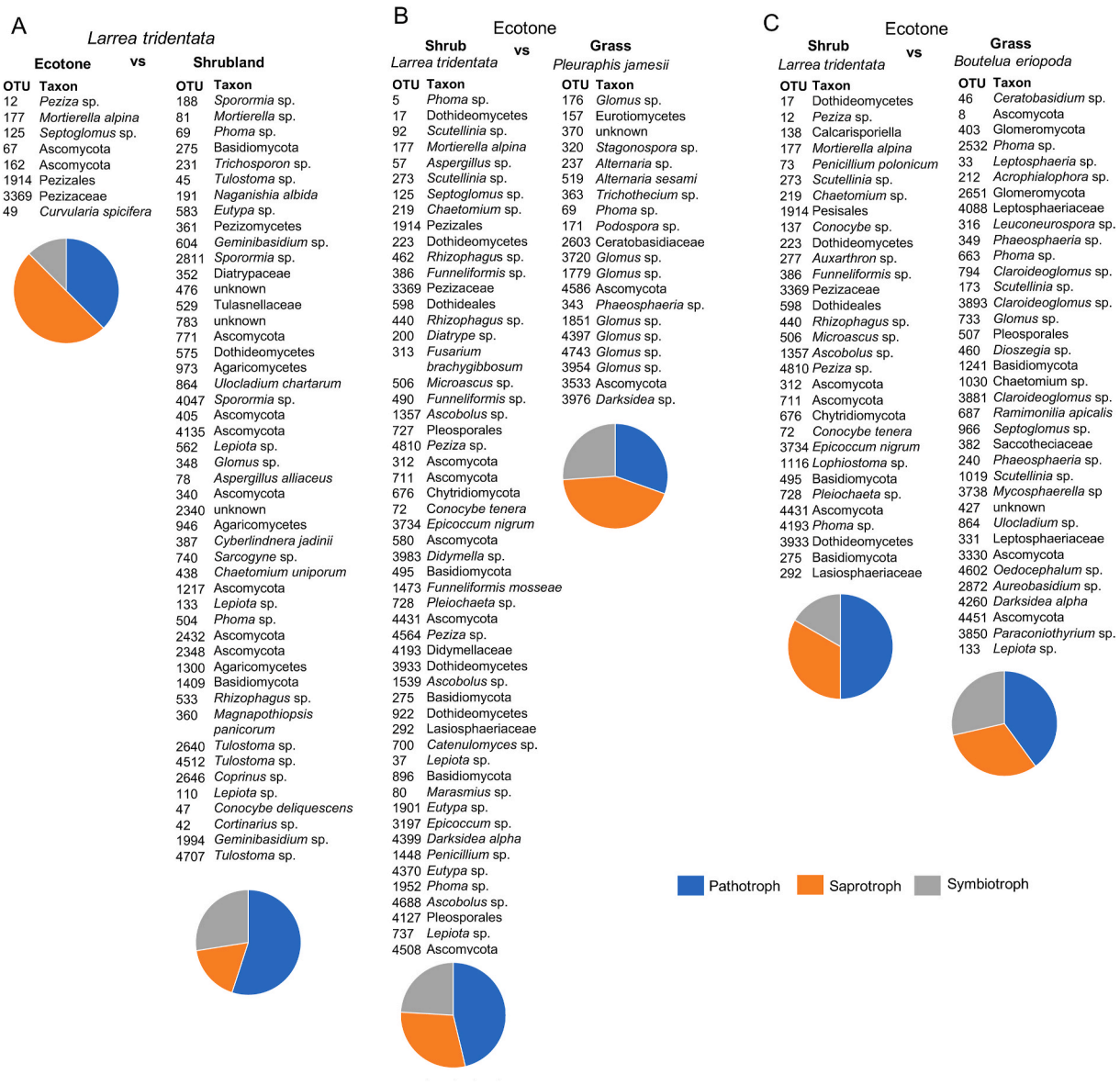


Fig. 4. Fungal taxa that mainly contributed to differences in soil communities below the shrub, *L. tridentata*, specifically (A) comparisions of *L. tridentata* soils in the ecotone and shrubland, and in the ecotone comparisions of *L. tridentata* with (B) *P. jamseii* and (C)*B. eriopoda* (C). Taxa with significantly different abundances based on SIMPER are listed under each plant/location which had the higher abundance. The pie charts depict broad functional guilds for a subset (≥60%) of the listed taxa that had functional guild assignments.

microbial activity, we found largely similar rates of nutrient processing across samples (Fig. 5), regardless of the dominant plant or habitat. Similar soil enzyme activity levels following shrub encroachment have also been observed in Mediterranean semiarid grasslands (Maestre et al., 2011). In our study, consistently low soil organic matter content (ranging from 0.7 to 1.2%, Fig. 2) could explain the similar enzyme activity levels among species and sites. Most energy and nutritional resources are gained from breaking down organic matter. Since there was low organic matter content throughout the site, enzyme activities were limited by amount of substrate, regardless of whether present in the ecotone, grassland, or shrubland. Organic matter content was lowest in ecotone soils (Table 1, Fig. 2), which may explain why one enzyme (NAG) had statistically lower activity levels per gram soil in the ecotone than in grassland (Fig. 5) and why two other enzymes showed similar trends (AlkP, AAP).

Interesting patterns occur at the ecotone – the place where ecosystems mix. In the ecotone, both plant and soil fungal communities possessed a combination of taxa from the discrete grassland and shrubland ecosystems (Fig. 3). Yet for soil abiotic conditions, some opposite patterns emerge as several soil properties were similar in grasslands and shrublands but distinct in the ecotone (Fig. 2). For example, percent sand, silt, and clay were similar under plants in grasslands and shrublands, while ecotone soils were sandier with less clay (Fig. 2). Additionally, both organic matter content and N levels were lower in ecotone soils relative to grasslands and shrublands (Fig. 2). The depletion of soil resources within the ecotone could reflect the influence of both shrubs and grass species in the system. Desert grasses and shrubs have different resource allocation strategies, such as different spatial distribution of roots (Lee and Laurenroth 1998; Gibbens and Lenz 2001), so when living together in the ecotone they may complement each other to deplete resources more completely. As species distributions and ecosystem boundaries continue shifting in response to global change (Peters 2002; Kelly and Goulden 2008; Chen et al., 2011; Ash et al., 2017), low soil resources along transitions between ecosystems may influence expansion rates and could interact with other plant-soil feedbacks influencing rates of advancement (Levine et al.,

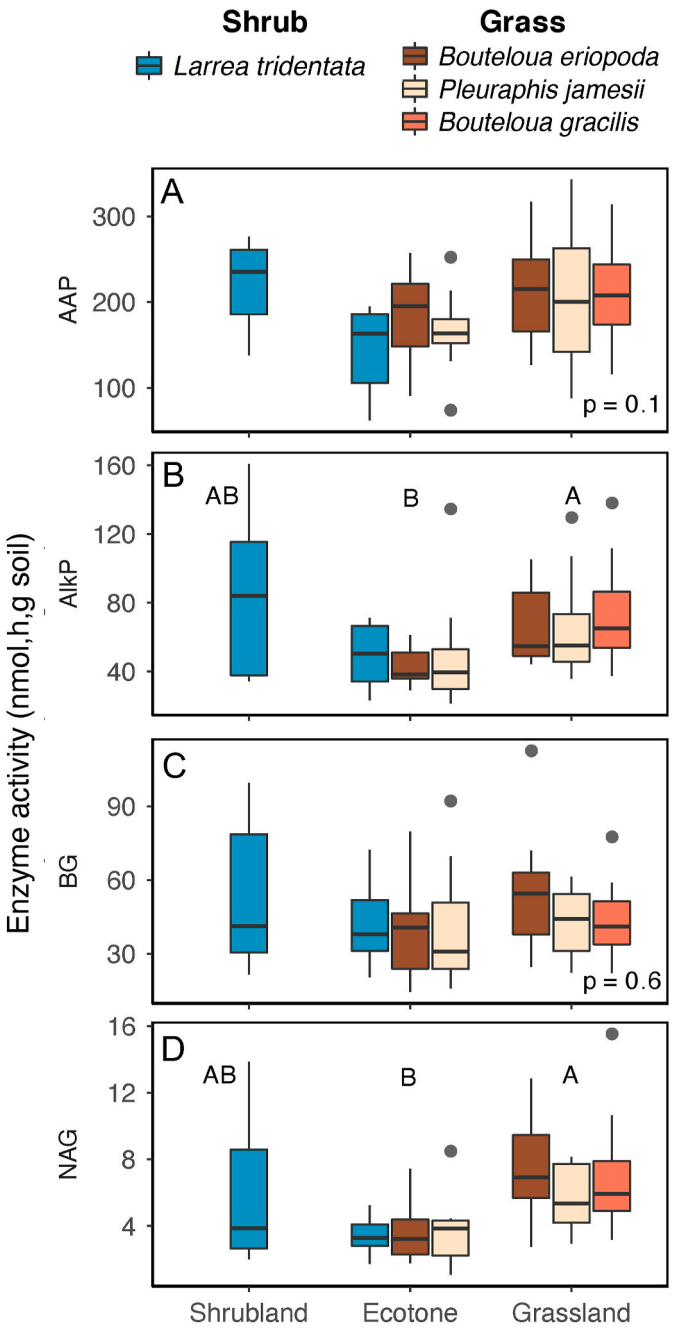


Fig. 5. Extracellular enzyme activities under dominant plants along the shrubland to grassland transition. Bars represent the first through third quartiles of the data, with the median as the center line. Different letters indicate statistical differences in enzyme levels across habitats. AP = alkaline phosphatase, NAG = beta-N-acetyl-glucosaminidase, AAP = alanine aminopeptidase, BG = beta-glucosidase.

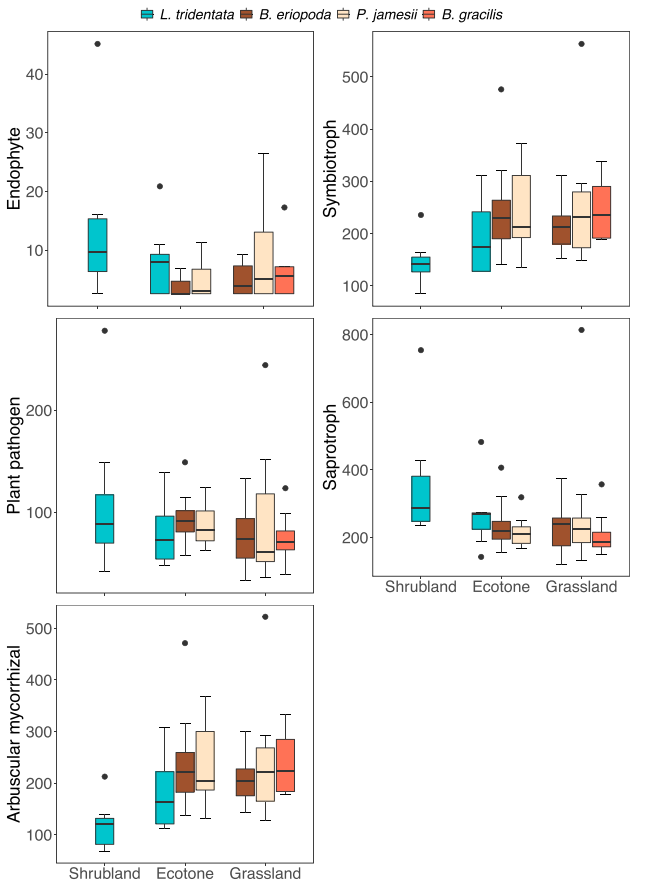


Fig. 6. Count of reads (VST normalized) for different functional categories based on FUNGuild classifications.

2006; van der Putten et al., 2010; van Grunsven et al., 2010; Suding et al., 2013; Dawson and Schrama 2016; Engelkes et al., 2018). Additional sampling across the ecotone beyond the transect sampled for this project will allow us to understand how consistent these patterns are as shrubs continue advancing into neighboring grasslands.

As climate and other global factors continue changing and influencing species range boundaries, investigations that include microbial associations can provide a more complete understanding of future species spread and changes in ecosystem function. Given the many widespread causes of shrub encroachment (Heisler et al., 2003; Archer et al., 2017), it will likely continue into the future. Combining baseline information regarding how microbial communities change across space with temporal monitoring and experimentation directly testing the regulating contributions of microbes will help us understand the full influence of microbial communities on range expansions. After several decades in the system, soil fungal communities under *L. tridentata* shrubs that successfully invaded Chihuahuan Desert grasslands reflected a mix of both grassland and shrubland fungal species.

Author contributions

LML, KCB, and SLC developed research ideas, LML, KCB, and DON collected data, LML, LPB, and DTL analyzed data, and all authors contributed to writing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2021.101096.

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