Soil microbial communities and nitrogen associated with cheatgrass invasion in a sagebrush shrubland

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Abstract

Cheatgrass invasion of Intermountain sagebrush steppe in the Western US poses increasing challenges to the function and survival of this native ecosystem. The invasive success and persistence of cheatgrass has been attributed to its physiology, phenology, and recently to increases in soil nitrogen levels. We investigated whether cheatgrass invasion of sagebrush/native bunchgrass communities elevated soil ammonium and nitrate levels, and resulted in soil microbial community diversity and composition changes. Our results did not reveal an increase in nitrogen ions, but showed a shift in soil microbial communities towards bacterial functional groups that can promote nitrogen accumulation in cheatgrass invaded communities.

Introduction

Cheatgrass (*Bromus tectorum*) is an exotic annual grass that has invaded large parts of the western United States since the late 19th century (Knapp 1996; Eviner et al. 2010). Currently, it is estimated that over 210,000 km^2 in the Great Basin region are dominated by cheatgrass (Bradley et al. 2018). The replacement of the native sagebrush (*Artemisia tridentata*)/perennial bunchgrass shrubland community by cheatgrass has detrimental effects on biodiversity (Davies and Bates 2010). For example, several bird species of concern endemic to this ecosystem, such as greater sage-grouse *(Centrocercus urophasianus)*, sage thrasher *(Oreoscoptes montanus)*, and sagebrush sparrow *(Artemisiospiza nevadensis)* are at high risk (Dumroese et al. 2015; Rottler et al. 2015). Furthermore, cheatgrass invasion has increased the frequency and timing of wildfire events that not only contribute to the persistence of this invasive grass, but also consume more native plant communities when fires spill over to adjacent shrubland communities (Knapp 1996; Bradley et al. 2018). Once native shrubland ecosystems are dominated by cheatgrass it is very difficult for native plant species to be re-established (Knapp 1996).

The high invasion success, dominance, and persistence of cheatgrass in invaded areas has been ascribed to its physiological and phenological properties. Cheatgrass is an annual grass that can germinate from late fall through spring, depending on moisture availability, with plants maturing well ahead of native bunchgrasses (Thill et al. 1984). Cheatgrass invasion affects soil nutrient cycling by increasing total soil nitrogen and carbon stocks (Liao et al. 2008; Reitstetter and Rittenhouse 2017). However, varied results have been reported across multiple studies (Adair and Burke 2010; Belnap et al. 2005; Liao et al. 2008; Norton et al. 2004; Stark and Norton 2015). The observed general increase in soil nitrogen in cheatgrass invaded plant communities can be explained with the transition from intermittent plant cover combined with bare interstitial spaces in native sagebrush and perennial bunchgrass communities, to a homogeneous plant cover in the annual cheatgrass community (Liao et al. 2008). Although in semi-arid environments the surface litter contribution of cheatgrass biomass is negligible, the large root biomass contributed by this annual grass has been shown to turn over on an annual basis (Austin and Vivanco 2006; Norton et al. 2004). The additional sources of nutrients in the form of root exudates, sloughing off of root cells, and root hair death (Dakora and Phillips 2002) can drive shifts in soil microbial diversity and composition (Bais et al. 2006). For instance, Weber (2015) reported reduced

prokaryotic richness and different composition as a function of depth in the soil underlying cheatgrass compared to sagebrush. Furthermore, in an experimental study that treated native soil with cheatgrass soil derived dissolved organic matter, Acidobacteria and Verrucomicrobia increased in abundance, whereas Bacteriodetes decreased (McLeod et al. 2021). Fungal soil communities have also been shown to be influenced by cheatgrass invasion. In two studies, cheatgrass plots showed a decrease in mycorrhizal fungi (Hawkes et al. 2006; Owen et al. 2013) and a significantly higher colonization of dark septate fungi (Gehring et al. 2016) compared to native plant communities. However, whether these differences in soil microbial community diversity and composition contribute to the persistence of cheatgrass invasion remains largely unknown.

We investigated the amount of soil nitrogen ion species, and soil microbial community diversity and composition along a gradient of cheatgrass invasion in a sagebrush/bunchgrass community in an intermountain shrubland in Rush Valley, Utah (USA). The specific objectives of our study were to explore whether: 1) cheatgrass invasion leads to increased soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, 2) cheatgrass invasion promotes changes in microbial community diversity and composition, and 3) cheatgrass invasion increases the relative abundance of saprotrophic fungi and bacteria involved in the nitrogen cycle.

Methods

Experimental design and soil sampling

Soil samples were collected from two adjacent sites (site A and B), 70 m apart, along 7 m transects reaching out from uninvaded sagebrush (SB)/bunchgrass (BG) islands into surrounding cheatgrass (CG) in Rush Valley, Tooele County, Utah (site A: 40.180747 N, 112.452441 W; site B: 40.180445 N, 112.451753 W) during the spring growing season in April 2020 (Figure 1). Soils were classified according to the USDA Web Soil Survey as Taylorsflat loam. Samples were collected with a 2 cm diameter soil core probe to a depth of 20 cm and sub-divided into an upper sample from 0-10 cm and lower sample from 10-20 cm. Soil core probes and spatulas were disinfected with 90% isopropyl alcohol in between each sample collection. After clearing the soil surface from any litter debris, three soil cores were collected each in the root zone of sagebrush, annual bunchgrass, and cheatgrass along a transect at both sites for a total of 36 samples. Subdivided samples were transferred to sterile collection bags and kept on ice during transport until further analysis. Samples for microbial analysis were sent on dry ice to the University of Arizona and stored at -80°C until DNA extraction.

Soil chemical properties

Soil samples were sieved with a standard 2 mm soil sieve prior to determination of pH, moisture content, and NH_4^+ and NO_3^- concentration. We used a Hanna HI 2210 pH meter with a Sartorius PY-P22-2S probe to measure the soil pH, after soil and distilled water were mixed in 1:3 mass ratio. Soil moisture was

determined gravimetrically by drying the wet soil at 105 °C for 48 h and calculated as % gravimetric soil moisture.

For the analysis of N concentration, 3 g of soil was extracted in 50 ml of Acid Extraction Solution (LaMotte) for 60 min while shaking, followed by centrifugation at 930 rcf for 5 min and gravity filtration through #1 filter paper (Whatman). NO_3^- concentration was determined using the protocol adapted from Miranda et al. (2001) and Doane and Horwáth (2003). Briefly, 100 µl of the filtered soil extract and 100 µl of vanadium (III) chloride reagent (400 mg VCl_3 in 50 ml HCl) were added to a 96-well plate, followed by 100 µl of N-(1-naphtyl) ethylenediaminehydrochloride (NEDD)/sulfanylamide reagent (50% of 2% sulfanilamide in 5% HCl and 50% of 0.1% NEDD in distilled water, by volume). The solution was incubated at 60°C for 30 min while shaking. Reaction products were read with an BioTex Elx 800 microplate reader at 540 nm. NH_4^+ concentration was determined using a Hach Nitrogen-Ammonia Reagent Set using the salicylate method. Briefly, 200 µl of filtered soil extract was added to each plate well, followed by 40 µl salicylate reagent and 40 µl of cyanurate reagent. The solution was incubated for 45 min at room temperature with subsequent reading at 620 nm. Each plate was run with calibration standards at 0 µM, 6.25 µM, 12.5 µM, 25 µM, 50 µM,100 µM,200 µM, and 300 µM of NH_4Cl for NH_4^+ and KNO_3 for NO_3^-, respectively. Concentrations were converted to µg per gram dry soil.

Molecular analysis

Soil microbial communities were assessed using 16S rRNA and ITS amplicon sequencing. DNA was extracted from 0.25 g of soil using QIAGEN DNeasy PowerLyzer PowerSoil kits according to manufacturer instructions. We used primer pairs 515-F (GTGCCAGCMGCCGCGGTAA) and 806-R (GGACTACHVGGGTWTCTAAT) to amplify the 16S rRNA V4 hypervariable region as the marker gene for bacteria and archaea (Caporaso et al. 2012), and ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCT-CGTTCTTCATCGATGC) primer pairs to amplify the first internal transcribed spacer (ITS1) region of the rRNA operon for fungi (Schoch et al. 2012). Marker genes, Illumina adapters, and unique error-correcting 12-bp barcodes were amplified using PCR with a Roche LightCycler 96 system. Negative controls without DNA template were included to check for possible contamination. Amplicon concentration was measured fluorescently with ThermoFisher Scientific Quant-iT PicoGreen dsDNA Assay Kit. Amplicons were pooled together in equimolar concentrations and sequenced on a 2 x 150 bp MiSeq Illumina platform at the Microbiome Core, Steele Children Research Center, University of Arizona.

Sequence Processing

Raw reads were demultiplexed using idemp (https://github.com/yhwu/idemp). The 16S reads were trimmed to a uniform length (140bp). Cutadapt was used to remove primers in ITS reads due to the highly variable length of the ITS region (Martin 2011). The reads were then subject to quality filtering, dereplication, chimera detection, and merging of paired-end reads using dada2 (Callahan et al. 2016). The dada2 algorithm uses a parametric error model based on the entire dataset to correct and group sequences into unique amplicon sequence variants (ASVs; Callahan et al. 2016). Taxonomic identities were determined using the RDP classifier (Wang et al. 2007) trained on the SILVA nr version 132 database

(Quast et al. 2013) for the 16S ASVs and the UNITE database (Nilsson et al. 2019) for ITS ASVs. Those 16S ASVs classified as chloroplasts, mitochondria, or unclassified at the domain level and ASVs that were detected in the control blank samples were removed. Those ITS ASVs without a fungal domain assignment were removed. Functional groups of prokaryotes and fungi were assigned using FAPROTAX (Louca et al. 2016) and FUNGuild (Nguyen et al. 2016), respectively.

Statistical analysis

All statistical analyses were performed in R version 4.0.5. We assessed the impact of different vegetation (i.e. CG, BG, and SB) and soil depth (i.e. 10 cm and 20 cm) on four soil physiochemical parameters (NH_4^+, NO_3^-, soil moisture, and pH) and four metrics of microbial communities (richness (number of observed ASVs per sample), Shannon diversity, microbial community composition, and relative abundances of functional groups). Due to the non-normality of soil chemical parameters, the non-parametric Kruskal-Wallis tests and Wilcox rank sum tests were used to examine their differences across vegetation and depth, respectively. Microbial richness and Shannon diversity were calculated after the sequencing depth was rarefied to 54,000 for 16S and 27,000 for ITS. We then used two-way ANOVA to examine if microbial richness and diversity were significantly different between vegetation types and soil depths. Microbial community dissimilarity pattern (Bray-Curtis metric) was visualized by non-metric multidimensional scaling (NMDS). To examine the impacts of soil chemical properties on microbial community composition, we fitted soil chemical parameters onto the NMDS axes ordination using the envift function from the vegan package (Oksanen et al. 2020). Permutational multivariate analysis of variance (PERMANOVA) in the vegan package was used to examine the effects of vegetation types and soil depths on microbial community composition (Anderson 2008). The Kruskal-Wallis rank sum test was performed to examine the differences of relative abundances of nitrogen cycle related bacteria and saprotrophic fungi among vegetation types, followed by a post hoc Dunn's test. We corrected for multiple testing in functional group differences using the false discovery rate correction (Benjamini and Hochberg 1995).

Results

Site A and site B did not have significantly different NO_3^-, soil moisture, or pH (Wilcoxon test: P = 0.898, P = 0.530, P = 0.654, respectively, Table 1). NH_4^+ was significantly and on average 122% higher in site A than site B (Wilcoxon test: P = 0.029; Table 1). Concentrations of NH_4^+ and NO_3^- did not differ across vegetation types or depths (Kruskal-Wallis test for vegetation: Chi-squared = 1.754, P = 0.416 for NH_4^+, Chi-squared = 2.799, P = 0.247 for NO_3^-; Wilcoxon test for depths: P = 0.457 for NH_4^+, P = 0.380 for NO_3^-; Table 1 and Figure 3A, B). Soil moisture was significantly different between depths (Wilcoxon test: P < 0.0001) but not vegetation types (Kruskal-Wallis test: Chi-squared = 0.692, P = 0.708, Table 1). Soil pH was significantly different across both vegetation types and depth (Kruskal-Wallis test for vegetation: chi-squared = 10.672, P = 0.005; Wilcoxon test for depth: P < 0.001). The post-hoc Dunn's test showed that BG and CG had significantly higher pH than SB (P = 0.041 and 0.005, respectively).

Table 1
Comparison of soil chemical properties between sites and vegetation.

Site	Coordinates	Vegetation	Moisture content (%)	pH	NO_3^- (µg/ g dry soil)	NH_4^+ (µg/ g dry soil)
Site A	N 40.180747 W 112.452441	Bunchgrass	8.17 ± 1.20	9.02 ± 0.21	0.84 ± 0.63	1.08 ± 1.16
		Cheatgrass	8.35 ± 0.71	9.60 ± 0.28	0.00 ± 0.00	1.08 ± 1.16
		Sagebrush	8.29 ± 1.01	8.83 ± 0.19	0.08 ± 0.20	1.06 ± 0.98
Site B	N 40.180445 W 112.451753	Bunchgrass	7.79 ± 1.01	9.16 ± 0.25	0.60 ± 0.43	0.52 ± 0.37
		Cheatgrass	8.59 ± 1.05	9.43 ± 0.37	0.10 ± 0.24	0.23 ± 0.14
		Sagebrush	7.85 ± 0.70	8.90 ± 0.21	0.22 ± 0.37	0.37 ± 0.21

The total richness (i.e., number of unique ASVs) was 9,755 and 2,045 for 16S and ITS, respectively. The average richness per soil sample was 1,540 (sd = 306) for 16S and 213 (sd = 28) for ITS, after rarefaction (Figure 2A, B). At the phylum level, the soil prokaryotic communities (Supplementary figure 3) were dominated by Actinobacteria (47.4%), Proteobacteria (19.4%), Chloroflexi (7.2%), Thaumarchaeota (6.1%), and Acidobacteria (5.2%). The fungal communities (Supplementary figure 4) were dominated by Ascomycota (72.1%), Basidiomycota (11.7%), Mortierellomycota (9.8%), Olpidiomycota (3.5%), and Glomeromycota (2.0%).

Since microbial diversity metrics did not significantly differ between both sites (*t*-test: P = 0.549 for 16S richness, P = 0.840 for 16S Shannon, P = 0.409 for ITS richness, P = 0.384 for ITS Shannon), samples from two sites were not analyzed separately. Prokaryotic richness showed no significant differences among vegetation or depths (ANOVA: F = 0.9442, P = 0.401 for vegetation, F = 0.047, P = 0.829 for depth; Figure 2A), and the same pattern were observed for fungi (ANOVA: F = 1.635, P = 0.212 for vegetation, F = 0.010, P = 0.928 for depth; Figure 2C). Shannon diversity showed a similar nonsignificant pattern (ANOVA for 16S: F = 0.159, P = 0.854 for vegetation, F = 0.220, P = 0.642 for depth; ANOVA for ITS: F = 1.279, P = 0.294 for vegetation, F = 1.341, P = 0.255 for depth; Supplementary Figure 1).

Prokaryotic community composition was significantly different between vegetation types and soil depths (PERMANOVA: R^2 = 0.143, P < 0.001 for vegetation, R^2 = 0.144, P < 0.001 for depth; Figure 2B; Supplementary Figures 3 and 4). Fungal communities were only significantly different across vegetation types but not depths (PERMANOVA: R^2 = 0.145, P < 0.001 for vegetation, R^2 = 0.029, P = 0.296 for depth; Figure 2D). Soil moisture and pH were the most important predictors of the composition of prokaryotic communities (Figure 1B). Fungal community composition was mainly determined by pH (Figure 1D).

Among the eight nitrogen cycle-related bacterial groups, the relative abundance of four functional groups (nitrogen fixers, denitrifiers, nitrogen respiratory bacteria, and ureolytic bacteria) were significantly different among vegetation types (Kruskal-Wallis test; Figure 3). Nitrogen fixers (mainly *Herbaspirillum*

spp., *Bradyrhizobium spp.*, *and Nostoc spp.*) and ureolytic bacteria (mainly *Roseomonas spp.*, *Mesorhizobium spp.*, *Singulisphaera spp.*, *Massilia spp.*, and *Methylobacterium spp.*) had higher relative abundance in CG samples (Dunn's test for nitrogen fixers: P = 0.037 between CG and BG; Dunn's test for ureolytic bacteria: P = 0.005 between CG and SB). Nitrogen respiratory bacteria (*Rhodoplanes spp.*, and *Nitrobacter spp*) and denitrifiers (*Pseudomonas fluorescens*, and *Rhodoplanes spp*) had lower relative abundance in CG (Dunn's test for nitrogen respiratory bacteria: P = 0.012 between CG and SB; Dunn's test for denitrifiers: P = 0.021 between CG and SB). After FDR correction for multiple testing, only ureolytic bacteria was significantly different across vegetation. For fungi, the relative abundances of dung saprotrophs and leaf saprotrophs were significantly different among vegetation types after FDR correction (Kruskal-Wallis test; Supplementary Figure 2).

Discussion

Cheatgrass invasion is a major environmental concern in the western US because it leads to altered soil nutrient cycling, reduced biodiversity, frequent wildfires, and degraded rangelands. (Knapp 1996; Dumroese et al. 2015; Bradley et al. 2018). It has been hypothesized that cheatgrass can create a nitrogen-rich environment which further enhances its competitiveness over native grasses (Larson et al. 2018). Soil microorganisms have been shown to be a major driver of the soil nitrogen cycle (Schimel and Bennett 2004). Interestingly, distinct soil microbial composition associated with cheatgrass invasion has been identified in multiple studies (Hawkes et al. 2006; Owen et al. 2013; Weber 2015; Weber et al. 2015; Gehring et al. 2016). Soil microorganisms may play a central, but previously overlooked role, in the dominance and persistence of cheatgrass. In this study, we found cheatgrass invasion correlated with unique soil microbial communities and nitrogen cycle-related functional groups compared to the native flora.

Cheatgrass invasion does not affect soil nitrogen levels during the spring growing season

Although soil NH_4^+ and NO_3^- have been previously shown to increase in cheatgrass invaded sites (Norton et al. 2004; Stark and Norton 2015), we did not find significant differences in these ions between SB, BG, and CG communities at our study location. A possible explanation for this finding is our decision to sample in spring, when cheatgrass has the highest nutrient uptake (Malhi et al. 2006). Furthermore, in interspecific growth studies, cheatgrass has been shown to take up more nitrogen than native grasses which could offset any increased nitrogen ion availability in cheatgrass soils during active growth (Bilbrough and Caldwell 1997; Blank 2010). A similar observation was made by Stark and Norton (2015) during their spring sampling. In comparison, samples that were collected after cheatgrass senescence in fall reported elevated NH_4^+ and NO_3^- concentrations in cheatgrass plots compared to native vegetation (Norton et al. 2004). Seasonal variation in abiotic and biotic processes can explain why our results contradict previous findings that cheatgrass invasion led to higher nitrogen levels in soil (Norton et al. 2004; Stark and Norton 2015). The similar concentrations of soil NH_4^+ and NO_3^- between cheatgrass and

native vegetation demonstrates that these ions were not variables that affected soil microbial community composition and functional groups in our experiment (Liang et al. 2020); therefore, we are confident that the deviations in microbial communities are caused by plant invasion.

Soil microbial diversity does not differ among different vegetation types, but composition does

We found neither phylotype richness nor Shannon diversity to be different across vegetation types. Our findings are similar to previous observations related to cheatgrass invasion (Weber 2015; Weber et al. 2015; Gibbons et al. 2017). In general, diversity metrics do not tend to be reliable indicators of functional differences (Louca et al. 2018). For instance, plant invasions often lead to disparate soil microbial community composition (Gornish et al. 2016, 2020; McTee et al. 2017; Parsons et al. 2019), but a recent meta-analysis revealed they generally do not have a significant impact on microbial richness (Custer and van Diepen 2020). Additionally, diversity metrics can be obscured due to the persistence of relic DNA from dead microorganisms (Carini et al. 2016).

In contrast to our findings that soil microbial diversity is similar across vegetation types, cheatgrass invasion drastically modified bacterial and fungal community composition as expected (Weber 2015). However, cheatgrass invasion may not be the only variable affecting the microbiome. There are multiple environmental factors that could play a role in changing microbial community composition. Soil moisture was strongly associated with prokaryotic community composition only, while pH was associated with both prokaryotic and fungal communities as typically observed (Lauber et al. 2009; Rousk et al. 2010). The contrasting responses to soil moisture and depth between prokaryotic and fungal communities can be explained by the higher tolerance to water stress of soil fungi compared to bacteria (Manzoni et al. 2012). While environmental factors explain the distinct microbial community composition between shrub and grass (i.e., CG and BG), the dissimilarities between CG and BG samples cannot be explained by current soil chemical parameters. Instead, they may be controlled by more direct plant-microbe interaction such as plant litter qualities or soil exudates (Eisenhauer et al. 2011; Milcu et al. 2013; Steinauer et al. 2016). Our results suggest that cheatgrass invasion can alter the microbiome composition of native soils, although the mechanisms involved need further investigation.

Cheatgrass invasion altered bacterial groups involved in nitrogen cycle

The relative abundances of inferred bacterial groups involved in nitrogen fixation and ureolysis were higher in CG, while groups involved in nitrogen respiration and denitrification were lower in CG compared to SB and BG. These findings indicate a potential elevated influx and reduced efflux of nitrogen into CG soils compared to the native vegetation types. The influx of nitrogen is most likely due to mineralization of organic matter derived from the large turnover rate of cheatgrass root biomass and is supported by previous observations of increased N inputs (Austin and Vivanco 2006; Booth et al. 2003; Hooker et al. 2008; Norton et al. 2004). Interestingly, an increase in nitrogen fixation capability has not been previously associated with cheatgrass invasion. We identified *Bradyrhizobium spp*. amongst the potential nitrogen

fixers with higher abundance associated with CG. *Bradyrhizobium* is known for forming nitrogen-fixing symbioses with legumes (Laguerre et al. 2001). The association of these organisms with CG underlying soil samples could point at a possible symbiotic relationship. Alternatively, some *Bradyrhizobium* species have been found to be free-living nitrogen fixers (Wongdee et al. 2018). Further investigations are required to identify the exact mechanism involved. It is also of interest to note that we observed a marginal increase in the relative abundance of *Pseudomonas fluorescens* associated with BG and SB samples. Strains of *Pseudomonas fluorescens* have been investigated for biocontrol of cheatgrass (Kennedy 2018; Tekiela 2020). Our observation may point at *Pseudomonas fluorescens* providing protection to BG and SB against cheatgrass invasion in the natural environment.

Cheatgrass invasion did not increase the relative abundance of inferred plant-related saprotrophic fungi. We found a significant increase of leaf saprotrophs in SB compared to CG and BG. Cheatgrass invasion is characterized by increased biomass turnover, while sagebrush has a higher lignin:N ratio (Hooker et al. 2008). Differences in litter quality rather than quantity are likely to lead to higher abundance of saprotrophic fungi in SB. We also found a higher relative abundance of dung saprotrophs in BG. This might be an indirect sign of higher animal activity in the native bunchgrass plots (Freeman et al. 2014).

Conclusion

We observed that cheatgrass invasion can shift microbial community composition and cause a higher relative abundance of bacterial groups which can promote nitrogen accumulation. This pattern appeared before NH_4^+ and NO_3^- rose in the cheatgrass invasion plots. Putatively, cheatgrass will modify the soil microbiome during early growth stages to create a nitrogen-rich environment at the end of its growing season. The elevated soil nitrogen promotes cheatgrass growth (Blumenthal et al. 2017) and enhances its competition over native bunchgrasses (Vasquez et al. 2008). Targeted microbiome intervention to suppress the nitrogen cycle-related bacteria should be considered to minimize the persistence of cheatgrass invasion. The addition of native soil inoculum has resulted in a reduction of cheatgrass and an increase in native plants in the past (Rowe et al. 2009). Our study adds a belowground dimension to the current invasive species management toolbox. Future experimentation is required to disentangle the initiation of reciprocity between cheatgrass and soil microbial communities at the invaded sites and how that interaction varies by spatial scale and historical land-use legacy. It is critical to manage both aboveground and belowground feedbacks and influences to maximize restoration outcomes.

Declarations

DATA AVAILABILITY

The sequence data have been deposited in the NCBI Sequence Read Archive under BioProject accession code PRJNA771000.

STATEMENTS & DECLARATIONS

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. The authors declare no conflict of interest. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

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Figures

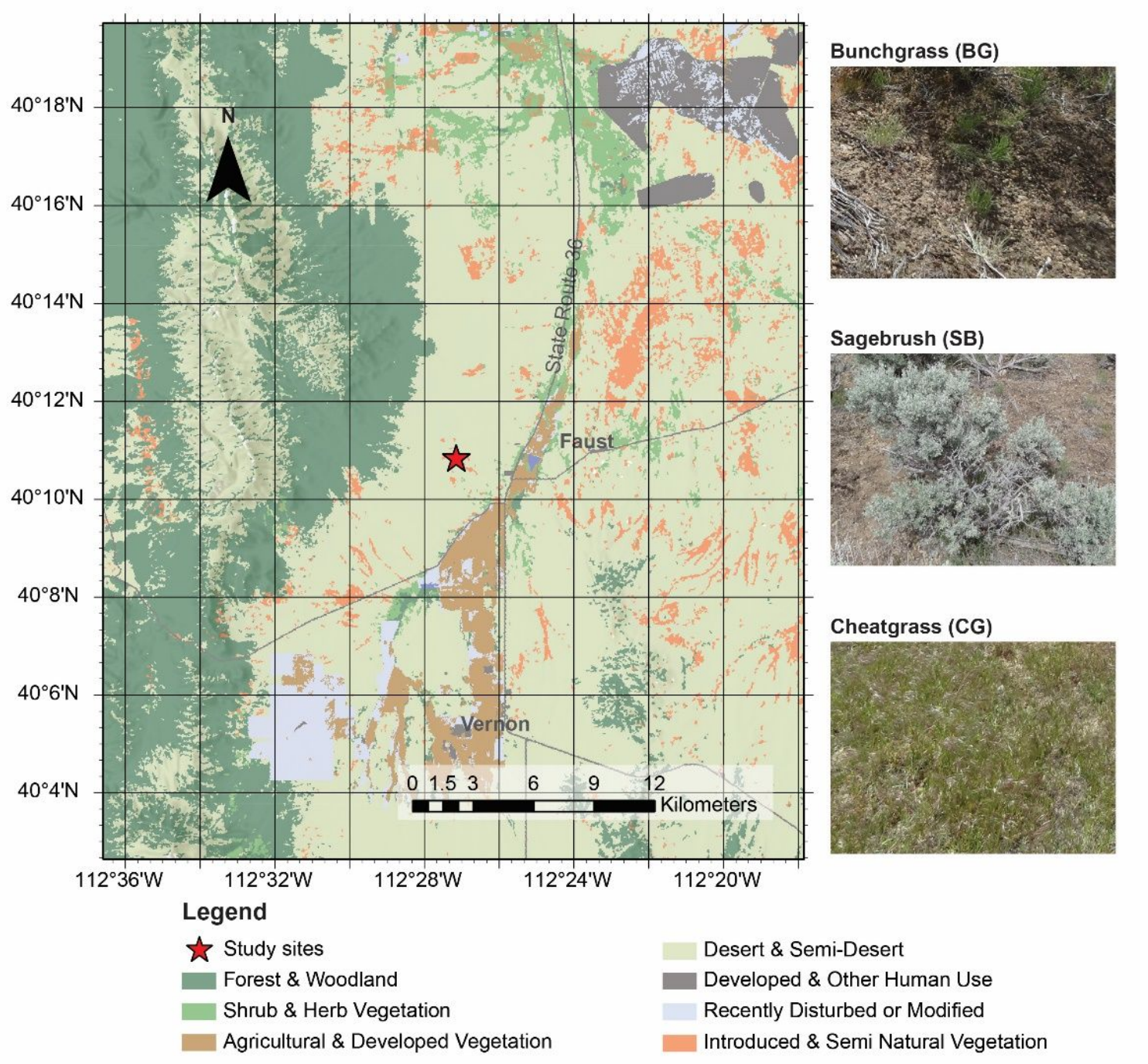

Figure 1

Map showing the location of the study site in the Great Basin physiographic region of western Utah. The three different vegetation types examined in this study are depicted to the right of the map. The base map depicts National Vegetation Classification class levels based on USGS 2001 imagery retrieved from the USGS land cover website.

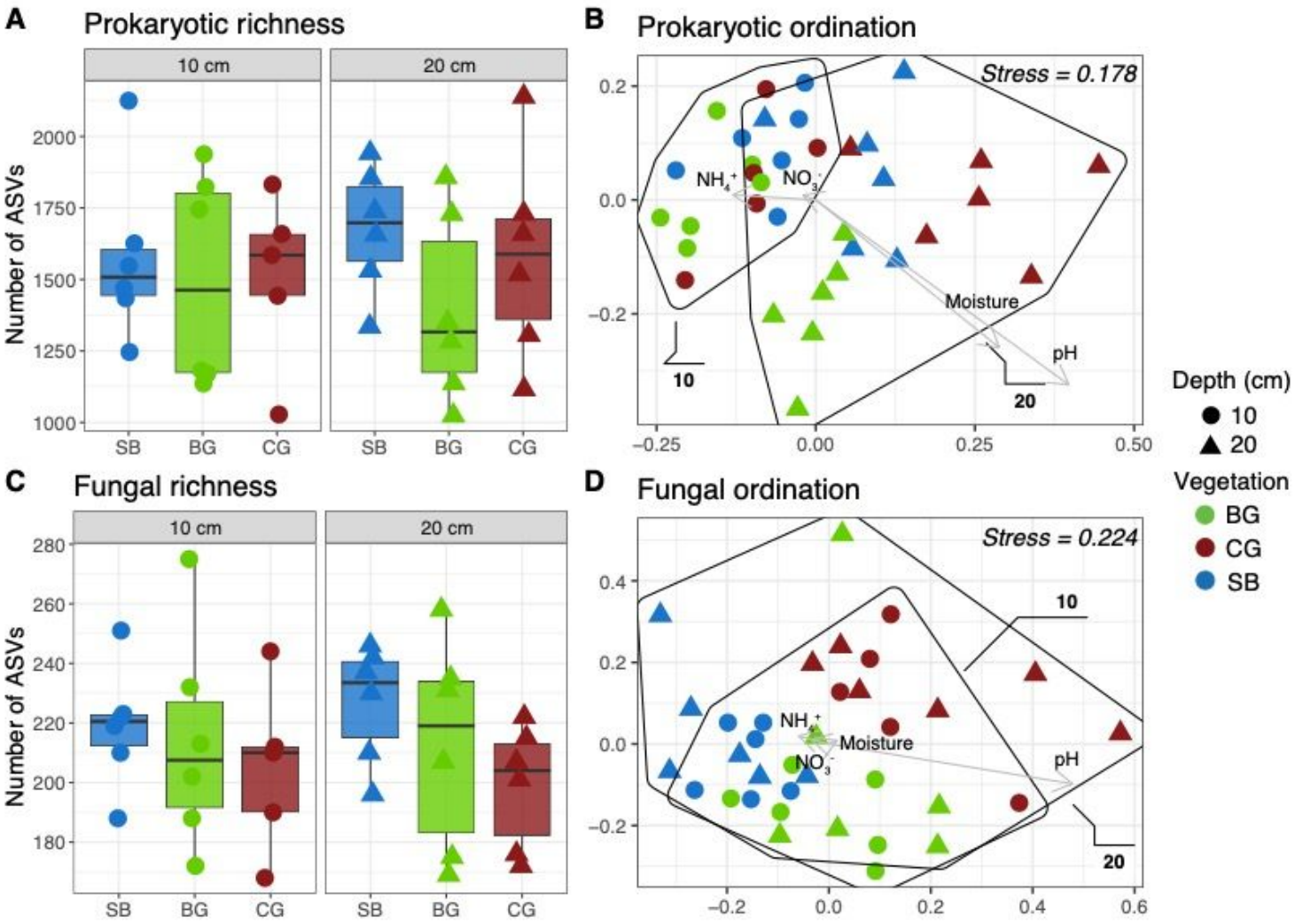

Figure 2

Soil microbial communities at different depths and vegetation categories (SB for sagebrush, BG for bunchgrass and CG for cheatgrass). Richness (number of different ASVs) of prokaryotic (bacteria and archaea) **(A)**, and fungal **(B)** communities. NMDS ordination of prokaryotic **(C)**, and fungal **(D)** communities. Environmental parameters as arrows representing the direction and strength of the gradient, and stress values are shown.

Nitrogen cycle related groups

Processes	p-value	p-value (after FDR)
Nitrogen fixers	0.029 *	0.058
Ureolytic bacteria	0.005 **	0.046 *
Nitrite oxidizers	0.514	0.685
Ammonia oxidizers	0.808	0.833
Nitrifiers	0.833	0.833
Nitrate reducers	0.230	0.368
Nitrogen respiratory bacteria	0.016 *	0.058
Denitrifiers	0.024 *	0.058

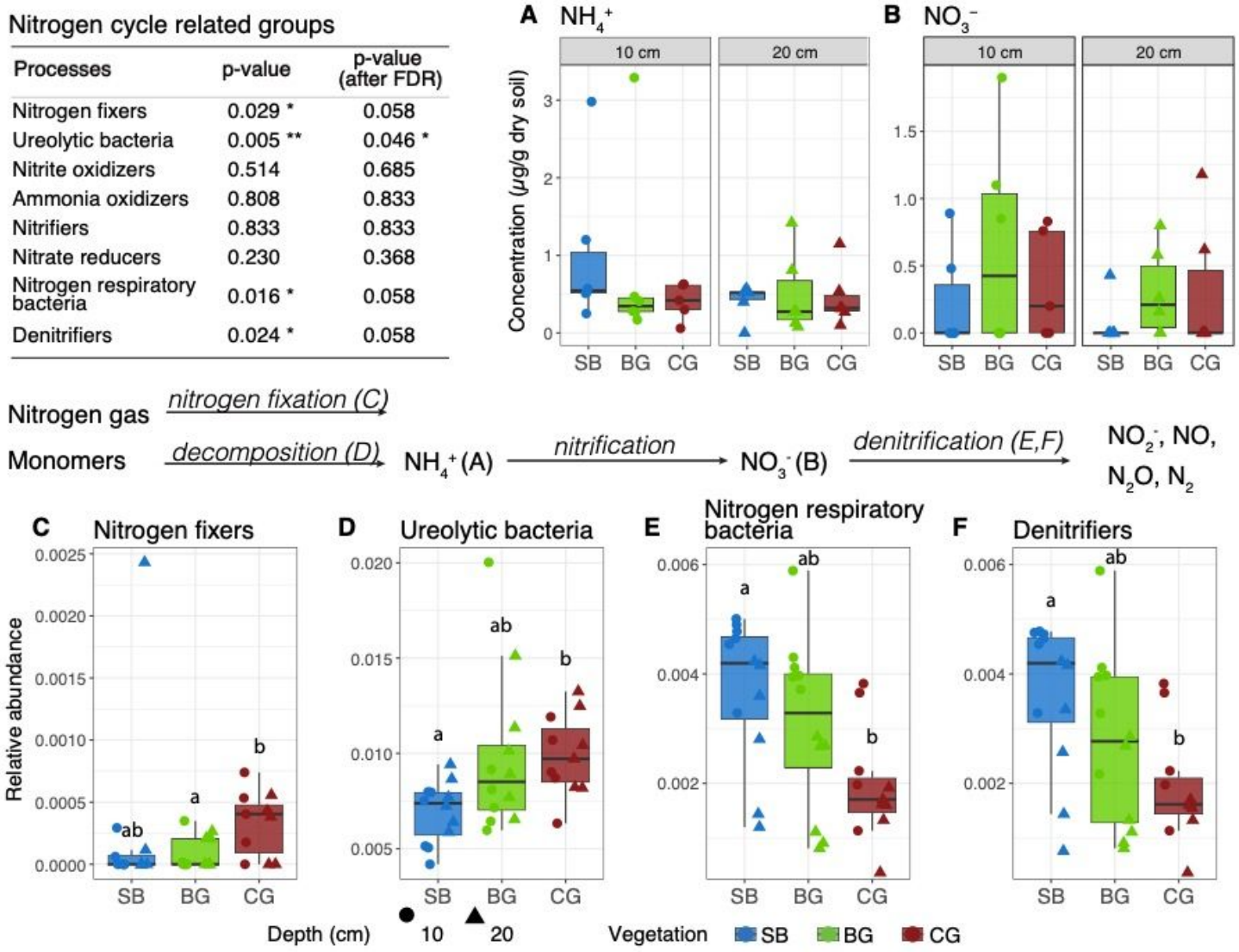

Figure 3

Relative abundance of nitrogen cycle-related microbial functional groups that are significantly different among vegetation categories (Kruskal-Wallis test; * = P < 0.05, ** = P < 0.01). Four out of eight nitrogen related groups were significantly different but ureolytic bacteria was the only significant group after FDR **(A)**. Ammonium and nitrate concentrations were not significantly different across vegetation types **(B)**. Relative abundance of nitrogen fixers **(C)** ureolytic bacteria **(D)**, nitrogen respiratory bacteria **(E)**, and denitrifiers **(F)** had lower relative abundance in cheatgrass community.

Supplementary Files

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