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

Raven Reitstetter · Ben Yang · Aaron D. Tews · Albert Barberán

RESEARCH ARTICLE

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Abstract

Purpose Cheatgrass invasion of Intermountain sagebrush steppe in the western United States poses increasing challenges to the function and survival of this native ecosystem. The invasive success and persistence of cheatgrass has been attributed to the increasing soil total nitrogen, but mechanisms behind remain inconclusive. We hypothesized that soil microorganisms play a role in soil nitrogen associated with cheatgrass invasion.

Methods We collected soil samples from the root zone of cheatgrass, native bunchgrass, and sagebrush at two depths and from two adjacent sites in April. We examined soil chemical properties (pH, moisture content, and $\text{NH}_4^+$ and $\text{NO}_3^-$ concentration) and soil microbial communities.

Results We found that cheatgrass invasion was associated with different soil microbial community composition compared to native bunchgrass and sagebrush. In particular, we observed higher relative abundances of N2 fixers and ureolytic bacteria and lower relative abundance of denitrifiers providing a potential mechanistic belowground explanation of raising soil nitrogen.

Conclusions Overall, our results indicate the importance of soil microorganisms in the dominance and persistence of invasive species. Targeted microbiome interventions should be considered to control cheatgrass invasion.

Keywords Cheatgrass · Soil microbial communities · Nitrogen cycling · Plant invasion · Sagebrush steppe

Introduction

Cheatgrass ($Bromus tectorum$) is an exotic annual grass that has invaded large parts of the western United States since the late nineteenth 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; Reistetter and Rittenhouse 2017). The increasing soil nitrogen, in return, enhances the competitive advantage of cheatgrass over native species (Vasquez et al. 2008a; Larson et al. 2018). Despite the importance of soil nitrogen in cheatgrass invasion, the drivers for increasing soil nitrogen still need to be identified.

Soil microorganisms play a central role in soil nitrogen cycles (Schimel and Bennett 2004), and cheatgrass invasion is often associated with significant changes in the community composition of bacteria (McLeod et al. 2021, Weber 2015) and fungi (Hawkes et al. 2006; Owen et al. 2013; Gehring et al. 2016; Dierks et al. 2019). The large turnover rate of cheatgrass root biomass at the upper soil horizon can explain the above-described changes in microbial communities (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). While cheatgrass has not been identified as a N₂ fixing species, we hypothesized that cheatgrass indirectly causes nitrogen accumulation through manipulating soil microorganisms that mediate the soil nitrogen cycle. This indirect microbial mechanism then might contribute to cheatgrass’ ability to invade and dominate.

We studied cheatgrass invasion in a sagebrush/bunchgrass community in an intermountain shrubland in Rush Valley, Utah (USA). We determined the amount of soil nitrogen ion species, soil microbial community richness and composition, and relative abundance of microbial functional groups at two depths. We predicted that: 1) cheatgrass invasion would increase soil ammonium (NH₄⁺) and nitrate (NO₃⁻) concentrations, 2) cheatgrass invasion would promote different richness and composition of microbial communities compared to those from the native vegetation types, and 3) cheatgrass invasion would increase the relative abundance of microbial functional groups that control the influx of soil nitrogen pools (e.g., N₂ fixers) and/or decrease the relative abundance of functional groups that control the efflux (e.g., denitrifiers), 4) these impacts would be larger at depth of 0–10 cm, where the majority of cheatgrass fine root biomass is located (Norton et al. 2004).

**Methods**

Experimental design and soil sampling

Soil samples were collected from two adjacent sites (site A and B), 70 m apart, to incorporate local-scale spatial heterogeneity. We sampled along 7 m transects reaching out from uninvaded sagebrush (SB)/bunchgrass (BG) islands into surrounding monotypic cheatgrass (CG) vegetation in Rush Valley, Tooele County, Utah (site A: 40.180747 N, 112.452441 W; site B: 40.180445 N, 112.451753 W, Fig. 1). Both sites have been invaded by cheatgrass for 30 years. Soils were classified according to the USDA Web Soil Survey as Taylorsflat loam (Xeric Haplocalcids, Aridisols). We sampled our soil in April 2020, during the spring growing season for all three vegetation types. Samples (3 soil cores per vegetation site=9 soil cores per site) 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 to 10 cm and lower sample from 10 to 20 cm (giving a total of 18 soil samples per site, Fig. 1). Soil core probes and spatulas were cleaned with 90% isopropyl alcohol (removing attached soil and disinfecting) in between each sample collection to avoid cross-contamination (Cando-Dumancela et al. 2021). 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 a 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 nitrogen ion concentration, 3 g of soil was extracted in 50 ml of Acid Extracting Solution (aqueous solution of 3% HCl, 2% H$_2$SO$_4$, LaMotte #6361) for 60 min while shaking, followed by centrifugation at 930 relative centrifugal force for 5 min and gravity filtration through #1 filter paper (Whatman). Concentration of NO$_3^-$ was determined using the protocol adapted from Miranda et al. (2001) and Doane and Horwáth (2003). Concentrations were converted to µg nitrogen ion per gram dry soil.

Molecular analysis

Soil microbial communities were assessed using 16S rRNA and internal transcribed spacer (ITS) amplification 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 (GTG GCCAGCMGCC GCGGTAA) and 806-R (GGA CTA CHVGGG TWT CTAA) to amplify the 16S rRNA V4 hypervariable region as the marker gene for bacteria and archaea (Caporaso et al. 2012), and ITS1-F (CTT GGT TAGAAGTAA) and ITS2 (GCT- CGTTCT TCGATGC) primer pairs to amplify 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 2×150 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 (140 bp). 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 chemical 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 paired-sample Wilcoxon rank sum tests were used to examine their differences across vegetation types and depths, 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 repeated measures 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 envfit 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 (FDR) correction (Benjamini and Hochberg 1995).

We also used compositional analyses due to the growing concern of the compositional nature of amplicon sequencing data (centered log-ratio transformation instead of rarefaction; Aitchison distance instead of Bray-Curtis; Gloor et al. 2017). The patterns were strongly correlated (Pearson correlation, r = 0.994, 0.945 for bacterial and fungal richness, respectively; distance correlation t-test, r = 0.960, 0.885 for bacterial and fungal distance matrices, respectively).

Results

Site A had significantly and on average 122% higher NH$_4^+$ concentration than site B (Wilcoxon test: P = 0.043; Table 1). Concentrations of NH$_4^+$ and NO$_3^-$ were significantly different between vegetation types (Kruskal-Wallis test: Chi-squared = 8.452, P = 0.015 for NH$_4^+$, Chi-squared = 18.507, P < 0.0001 for NO$_3^-$) but not depths (paired Wilcoxon test for depths: P = 0.171 for NH$_4^+$, P = 0.124 for NO$_3^-$; Table 1 and Fig. 3A, B). The post-hoc Dunn's test
Table 1 Comparison of soil chemical properties between sites and vegetation. Variability is assessed with a standard deviation from three samples

| Site    | Coordinates | Vegetation | Depth (cm) | Moisture content (%)b | pHb | NH₄⁺ (μg/g dry soil)a | NO₃⁻ (μg/g dry soil) |
|---------|-------------|------------|------------|-----------------------|-----|----------------------|----------------------|
| Site A  | N 40.180747 | Sagebrush  | 10         | 7.6 ± 0.50            | 8.63 ± 0.06 | 1.58 ± 1.25         | 0.16 ± 0.28          |
|         | N 40.180747 | Bunchgrass | 20         | 9.00 ± 0.87           | 9.03 ± 0.06 | 0.54 ± 0.03         | 0.00 ± 0.00          |
|         | W 112.452441| 10         | 7.13 ± 0.55 | 8.87 ± 0.06 | 1.33 ± 1.70           | 1.28 ± 0.55         |
|         | W 112.452441| 20         | 9.23 ± 0.21 | 9.29 ± 0.02 | 0.84 ± 0.57           | 0.41 ± 0.34         |
|         | Sagebrush  | 10         | 7.77 ± 0.42 | 9.37 ± 0.38 | 0.40 ± 0.20           | 0.00 ± 0.00         |
|         | W 112.452441| 20         | 8.77 ± 0.38 | 9.70 ± 0.10 | 0.23 ± 0.12           | 0.00 ± 0.00         |
| Site B  | N 40.180445 | Sagebrush  | 10         | 7.33 ± 0.55            | 8.77 ± 0.12 | 0.46 ± 0.15         | 0.30 ± 0.51          |
|         | N 40.180445 | Bunchgrass | 20         | 8.37 ± 0.40            | 9.07 ± 0.12 | 0.31 ± 0.27         | 0.14 ± 0.25          |
|         | W 112.451753| 10         | 6.93 ± 0.25 | 8.93 ± 0.12 | 0.37 ± 0.28           | 0.60 ± 0.35         |
|         | W 112.451753| 20         | 8.67 ± 0.45 | 9.40 ± 0.10 | 0.67 ± 0.43           | 0.60 ± 0.59         |
|         | Sagebrush  | 10         | 7.80 ± 0.35 | 9.13 ± 0.21 | 0.30 ± 0.15           | 0.00 ± 0.00         |
|         | W 112.451753| 20         | 9.40 ± 0.82 | 9.73 ± 0.21 | 0.16 ± 0.10           | 0.19 ± 0.33         |

a means significantly different between Site A and Site B (Wilcoxon test, p < 0.05)
b means significantly different between 0 and 10 cm and 10–20 cm in paired comparison (paired-samples Wilcoxon test, p < 0.05)

Bold values represent significant differences across vegetation types in post-hoc test (Kruskal-Wallis test and Dunn’s test, p < 0.05)

showed that CG had significantly lower NH₄⁺ than that in BG and SB. BG had significantly higher NO₃⁻ than that in CG and SB. Overall, soil pH was significantly different across both vegetation types and depth (Kruskal-Wallis test for vegetation: Chi-squared = 15.905, P = 0.0004; paired Wilcoxon test for depth: P = 0.0003).

The total richness (i.e., number of unique ASVs) was 9755 and 2045 for 16S and ITS, respectively. The average richness per soil sample was 1540 (sd = 306) for 16S and 213 (sd = 28) for ITS, after rarefaction (Fig. 2A, B). At the phylum level, the soil prokaryotic communities (Supplementary Fig. 3) were dominated by Actinobacteria (47.4%), Proteobacteria (19.4%), Chloroflexi (7.2%), Thaumarchaeota (6.1%), and Acidobacteria (5.2%). The fungal communities (Supplementary Fig. 4) were dominated by Ascomycota (72.1%), Basidiomycota (11.7%), Mortierellomycota (9.8%), Olpidiomyctca (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 the two sites were not analyzed separately. Prokaryotic richness showed no significant differences among vegetation or depths (ANOVA: F = 0.494, P = 0.628 for vegetation, F = 0.036, P = 0.859 for depth, F = 0.498, P = 0.625 for vegetation and depth interaction; Fig. 2A), and the same pattern was observed for fungi (ANOVA: F = 1.504, P = 0.279 for vegetation, F = 0.004, P = 0.955 for depth, F = 0.460, P = 0.647 for vegetation and depth interaction; Fig. 2C). Shannon diversity showed a similar nonsignificant pattern (ANOVA for 16S: F = 0.083, P = 0.921 for vegetation, F = 0.031, P = 0.869 for depth, F = 0.367, P = 0.704 for vegetation and depth interaction; ANOVA for ITS: F = 0.579, P = 0.583 for vegetation, F = 1.093, P = 0.355 for depth, F = 0.416, P = 0.673 for vegetation and depth interaction; Supplementary Fig. 1).

Prokaryotic community composition was significantly different between vegetation types and soil depths (PERMANOVA: R² = 0.143, P < 0.001 for vegetation, R² = 0.144, P < 0.001 for depth, R² = 0.06, P = 0.141 for vegetation and depth interaction; Fig. 2B; Supplementary Figs. 3 and 4). Fungal communities were only significantly different across vegetation types but not depths (PERMANOVA: R² = 0.145, P < 0.001 for vegetation, R² = 0.029, P = 0.296 for depth, R² = 0.04, P = 0.965 for vegetation and depth interaction; Fig. 2D). Soil moisture and pH were the most important predictors of the composition of prokaryotic communities (envfit, r = 0.513, p = 0.001 for soil moisture, r = 0.388, p = 0.003 for pH,
Fungal community composition was mainly determined by pH (envfit, \( r = 0.488, p = 0.001 \), Fig. 1D).

Among the seven nitrogen cycle-related bacterial groups identified by FAPROTAX, the relative abundance of three functional groups (\( N_2 \) fixers, denitrifiers, and ureolytic bacteria) were significantly different among vegetation types (Kruskal-Wallis test; Fig. 3). \( N_2 \) 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 \( N_2 \) fixers: \( P = 0.037 \) between CG and BG; Dunn’s test for ureolytic bacteria: \( P = 0.005 \) between CG and SB). Denitrifiers (*Nitrobacter* spp, *Pseudomonas fluorescens*, and *Rhodoplanes* spp) had lower relative abundance in CG (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 fungal functional groups, the relative abundances of dung saprotrophs and leaf saprotrophs were significantly different among vegetation types after FDR correction (Kruskal-Wallis test; Supplementary Fig. 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;
Soil microorganisms may play a central, but previously overlooked role, in the dominance and persistence of cheatgrass. By comparing the chemical parameters and microbial community metrics of root zones between native sagebrush, native annual bunchgrass, and invaded cheatgrass vegetation types, we found that: 1) cheatgrass invasion did not increase soil ammonium and nitrate concentrations in April, 2) compared to native flora, cheatgrass invasion led to different soil microbial community composition but not richness, 3) cheatgrass invasion was associated with microbial functional groups that lead to nitrogen accumulation as predicted, and 4) the interaction between vegetation types and depths was not significant for soil nitrogen ion species, microbial richness, and microbial community composition.

Soil nitrogen levels during the spring growing season

The lack of increased $\text{NH}_4^+$ and $\text{NO}_3^-$ can be partially explained by the life history of cheatgrass. Cheatgrass germinates from fall through spring and will start utilizing soil nutrients during this period, well ahead of

**Fig. 3** Relative abundance of putative nitrogen cycle-related microbial functional groups that are significantly different among vegetation categories (SB for sagebrush, BG for bunchgrass and CG for cheatgrass). Three out of eight nitrogen related groups were significantly different but ureolytic bacteria was the only significant group after false discovery rate correction (FDR, $^* = P < 0.05$, $^{**} = P < 0.01$, A). Ammonium (B) and nitrate (C) concentrations were significantly different across vegetation types. Relative abundance of $\text{N}_2$ fixers (D) and ureolytic bacteria (E) were higher in the cheatgrass vegetation. Denitrifiers (F) had lower relative abundance in the cheatgrass vegetation. Different letters indicate significant mean difference for vegetation types for post hoc Dunn’s tests.
bunchgrasses and sagebrush (Thill et al. 1984). This might lead to reduced levels of these nitrogen ions at the time of our sampling regime in spring. 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 plant growth (Bilbrough and Caldwell 1997; Blank 2010). Stark and Norton made similar observations during a study with spring sampling regime, however, in an early fall study after cheatgrass senescence, they reported elevated $\text{NH}_4^+$ and $\text{NO}_3^-$ concentrations in cheatgrass plots compared to native vegetation (Norton et al. 2004; Stark and Norton 2015). Soil nitrogen might be used to support cheatgrass growth in the next growing season and maintain its dominance over native species.

Soil microbial richness and community composition

We found neither phylotype richness nor Shannon diversity to be different across vegetation types or depths. 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. 2020), 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).

Cheatgrass invasion drastically modified bacterial and fungal community composition as previous studies have reported (Hawkes et al. 2006; Owen et al. 2013; Weber 2015; Weber et al. 2015; Gehring et al. 2016). However, increased turnover rate of root biomass of cheatgrass was unlikely the driver of these microbial shifts. Our prediction that the impact of cheatgrass invasion on microbial communities should be more notable in the shallow soils was not supported. For other environmental factors, 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 distinct microbial community composition between the invasive CG and the native BG and SB might be explained by the transition from intermittent plant cover combined with bare interstitial spaces in native sagebrush and perennial bunchgrass communities, to a homogeneous, mono-dominant plant cover in the annual cheatgrass community (Liao et al. 2008); whereas the variation between the native BG and SB might be explained by the different structures between shrubs and grasses (Yannarell et al. 2014). Combined with previous observations that failed to build the link between microbial communities and plant traits (Porazinska et al. 2003; Barberán et al. 2015; Leff et al. 2018; Yang et al. 2021), our findings suggest that plant cover changes driven by plant invasion might be a stronger factor for soil microbial communities than direct plant-microbe interactions.

Soil microbial functional groups

We found an increasing relative abundance of putative $N_2$ fixers and ureolytic bacteria, and a decrease in the relative abundance of denitrifiers in CG soil samples compared to the native vegetation types. Assuming that microbial community structure can predict the corresponding processes to a certain extent (Graham et al. 2016), our results shed light on how cheatgrass might manipulate the soil nitrogen cycles towards a nitrogen rich environment.

An increase in $N_2$ fixation has been previously associated with cheatgrass invasion through the evidence of decreasing $\delta^{15}N$ (indicator of recalcitrant N pool from soil organic matter) in plant tissues with longer invasion history (Blank and Morgan 2011). Amongst the potential $N_2$ fixers, CG had higher abundance of *Bradyrhizobium* spp., which is known for forming $N_2$-fixing symbioses with legumes (Laguerre et al. 2001), and in rare cases, being free-living $N_2$ fixers (Wongdee et al. 2018). *Nostoc* spp., another $N_2$ fixing microorganism identified in our study has been associated with biological soil crusts (Rosentreter and Belnap 2003). However, neither legumes nor biological soil crusts were identified in monotypic cheatgrass stands. Further investigation is needed to identify the exact interactions involved.

Increased ureolytic bacteria in cheatgrass invaded soils points to an increase in functional groups that contribute to the mineralization and subsequent ammonification of the higher root biomass (Hasan...
We would have expected a concomitant increase in soil NH$_4^+$ concentrations but the increased levels of NH$_4^+$ may have been masked by cheatgrass utilization during its active growth. Taken together our results suggest a potential microbial mechanism of higher nitrogen availability in cheatgrass invaded soils, that is, a potential elevated soil nitrogen influx via N$_2$ fixation and ammonification together with reduced efflux via denitrification in cheatgrass dominated soils compared to native vegetation types. However, due to the limitation of inferring microbial functional groups from taxonomy (Hart et al. 2020), future studies with more direct observations on soil biological processes (e.g., extracellular enzymatic essays) will be required to validate this mechanism.

Saprotrophic fungi and mycorrhizal fungi were important mediators in nitrogen cycling in forests (Tatsumi et al. 2020), but their role in supporting cheatgrass invasion might be trivial. We found a higher relative abundance of leaf saprotrophs in SB compared to CG and BG, possibly due to the presence of leaves and leaf litter in sagebrush year-round, compared to the perennial leaf presence in grasses. Hooker et al. (2008) found that C:N ratios of leaves are not different between sagebrush, bunchgrasses and cheatgrass, but lignin concentrations and lignin:N ratios are significantly higher in sagebrush compared to the other two vegetation types. Lignin is a complex recalcitrant polymer whose degradation needs saprotrophic fungi (Kamimura et al. 2019), which can explain their higher abundance in SB. We also found a higher relative abundance of dung saprotrophs in BG. This might be an indirect sign of higher small mammal activity in the native bunchgrass plots (Freeman et al. 2014; Bachen et al. 2018).

Conclusion

We observed an increase in N$_2$ fixers and ureolytic bacteria in soil underlying monotypic cheatgrass stands while denitrifiers were lower compared to soil in native mixed sagebrush/perennial bunchgrass plant associations. Although ammonium and nitrate concentrations were not increased with cheatgrass invasion in our spring sampling, the identification of microbial groups involved in nitrogen cycling suggests a mechanistic explanation for potential increased nitrogen availability in cheatgrass dominated soils.

Our results might inform the development of management tools aimed at controlling cheatgrass invasion in the Intermountain West. The seasonal fluctuation of soil nitrogen suggest that nitrogen management (e.g., high C:N ratio soil amendment) should be deployed after cheatgrass senescence, to immobilize the accumulated soil nitrogen (Vasquez et al. 2008b). Targeted microbiome intervention to suppress nitrogen cycling-bacteria should be considered to minimize the persistence of cheatgrass invasion. For example, inoculation with soil collected from uninvaded sites has previously resulted in a reduction of cheatgrass and an increase in native plants (Rowe et al. 2009). Antagonists of N$_2$ fixers and ureolytic bacteria might be an indirect solution to cheatgrass invasion (Shahrtash and Brown 2021). Future experimentation is required to disentangle the feedbacks between cheatgrass and soil microbial communities at invaded sites and how to overcome legacy effects of cheatgrass invasion.

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

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.

Conflict of interest The authors declare no conflict of interest.

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