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Expansion of Native Plant *Stellera chamaejasme* L. Alters the Structure of Soil Diazotrophic Community in a Salinized Meadow Grassland, Northeast China

Ying Zhang, Zhenbo Cui, Tingting Wang and Chengyou Cao *

College of Life and Health Sciences, Northeastern University, Shenyang 110169, China; zhangying@mail.neu.edu.cn (Y.Z.); cuizhenbo@mail.neu.edu.cn (Z.C.); wangtingting828@berryoncology.com (T.W.)
* Correspondence: caochengyou@mail.neu.edu.cn; Tel.: +86-24-83656232

Abstract: The invasion of native plants has posed a serious risk to species diversity and ecosystem function. How they modify underground community and facilitate successful invasion remain unknown. Soil diazotrophs may play an important role in invasion by native plants. *Stellera chamaejasme* L. has expanded within around the heavily degraded Horqin Grassland in northeast China in recent decades. This study aims to detect the effect of the expansion of *S. chamaejasme* L. on soil diazotrophic community structure through high-throughput sequencing and examine the relationship between diazotrophic community structure and soil physicochemical properties. An extensive increase in *S. chamaejasme* population induced significant changes in soil diazotrophic community and marked shifts in the relative abundances of *Bradyrhizobium* and *Desulfuromonas*. Soil organic matter (SOM), total nitrogen, NO_3^--N, and electrical conductivity (EC) increased, whereas NH_4^+-N and pH significantly decreased in soil invaded by *S. chamaejasme*. The diazotrophic community structure was correlated with SOM, nitrogen content, EC, and pH. The relative abundances of *Bradyrhizobium* and *Desulfuromonas* were significant negatively and positively correlated with soil EC, respectively. This study suggests that the interaction between *S. chamaejasme* and soil diazotrophic microbes and the durative increase in soil EC may facilitate invasion by this *S. chamaejasme* population.

Keywords: native plant expansion; soil diazotroph; soil physicochemical properties; *Stellera chamaejasme*

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1. Introduction

A biological invasion is a phenomenon in which exotic species expand rapidly in a novel ecosystem and its abundance has been increasing [1]. The mechanisms for a successful invasion of exotic species have been investigated and reviewed in decades [2]. Recently, the invasive behavior of some native species similar to that of exotic species has attracted considerable interest [3–5]. Likewise, native-invasive species can cause harm to ecosystems and social economies [6,7]. Therefore, the overexpansion of native species is an emerging problem in biodiversity conservation and ecological function. However, the mechanism of native invasion remains unknown.

Many studies about the invasion of exotic plant species in terrestrial ecosystems demonstrated that invasive plant species can alter soil physicochemical properties including carbon and nitrogen nutrients, soil pH, and moisture [8–11]. Generally, soil nutrient levels are associated with soil microbes involved in nutrient cycling [12]. Therefore, plant invasion can cause shifts in soil microbial community structure [13,14], composition [15], and function [16–18]. Moreover, the composition and diversity of soil microbial communities are associated with plant characteristics and soil physicochemical properties [19]. Lau and Suwa [20] proposed that the interactions between invasive plants and soil can drive the successful expansion of invaders. For example, a successful invasion may depend on the initial soil conditions of an invaded ecosystem. Foreign species may positively affect

soil nutrient contents under the condition of low nutrient concentrations and exert no effect under the condition of high nutrient concentrations [8,21]. Regarding native-invasive plants, how they interact with soil to facilitate their invasion and what triggers their spread remain unknown.

Nitrogen (N) is a crucial determinant of plant productivity [22,23]. Generally, soil available N performs important roles in maintaining ecosystem function, particularly in arid and semiarid regions where N availability is relatively low [24]. Thus, altered soil N availability leads to a shift in species dominance and thus changes the composition of the plant community [25]. Invasive plant species can achieve absolute dominance by various strategies for nitrogen use, including the demand for quantity, allocation, and preferred forms of N [26–29]. They can also modify soil N-cycling microbial communities to meet N demand [18,28]. Ammonia, an important soil N form available for plants, is produced through biological N fixation carried out by N-fixing microorganisms (diazotrophs). Ammonia from biological N fixation constitutes most of the natural N input without anthropogenic activities [30]. The N level of the soil is related to the size and composition of the soil diazotrophic community, and some invasive plant species can induce an increase in soil diazotrophs to meet their high N demand [28,31]. Therefore, diazotrophs are probably one of the determinants for invasion by various alien plants [32]. However, reports on whether and how native invasive species affect soil diazotrophic community to facilitate its expansion are limited.

Stellera chamaejasme L., a toxic perennial plant (Thymelaeaceae), is widely distributed in the natural grasslands of northwestern and northeastern China. It has been known to contain bioflavonoids, and a few bioflavonoids have been isolated from this plant [33,34]. It resists drought through a large and deep root system and high underground biomass. In undegraded natural grassland, its population is generally limited by other original species. However, increased *S. chamaejasme* population can be observed in many heavily degraded grasslands, and it gradually became an indicator for grassland degradation. *Stellera chamaejasme* has a random cluster distribution and low seed germination rate in natural grasslands, which are unfavorable for population enlargement [35,36]. However, in degraded grassland, many *S. chamaejasme* seedlings of different ages can be observed around their mother plants. This phenomenon suggests that elder *S. chamaejasme* may alter soil microenvironments (e.g., increase in nutrient availability) to facilitate seedlings' survival and growth, especially in adverse conditions. Sun et al. [37] reported that *S. chamaejasme* can increase the concentration, availability, and turnover rate of soil N within its patch. These findings suggest that soil diazotrophs may sensitively respond to the over-expansion of *S. chamaejasme* populations in grasslands.

Horqin Grassland, one of the largest grasslands in northern China, has undergone severe degradation because of human-mediated processes in recent decades [38]. Soil secondary salinization is one of the direct factors for grassland degradation [39]. In this degradation, the role of *S. chamaejasme* shifted from being an associated species to a dominant member in many herbaceous communities. The expansion of *S. chamaejasme* resulted in decreases in productivity and forage quality because of its toxicity. At present, the over-expansion of *S. chamaejasme* in the Horqin Grassland has become a serious environmental issue threatening the sustainability of grassland ecosystems. Information on the interaction between *S. chamaejasme* and soil microbe is required for understanding the mechanism of *S. chamaejasme* invasion and for better management and utilization of the Horqin Grassland.

In this study, we aim to (1) analyze the impacts of *S. chamaejasme* over-expansion on diazotrophic community structure and (2) analyze the relationship of the diazotrophic community with soil physicochemical properties. We hypothesized that *S. chamaejasme* invasion would change soil diazotrophic community and these alterations would be closely related to changes in local soil physicochemical properties.

2. Materials and Methods

2.1. Study Location and Site Description

This experiment was conducted at the Wulanaodu Experimental Station of Desertification (43°02′ N, 119°39′ E) located in the Horqin Grassland in Northeast China. This region is characterized by a continental semiarid monsoon climate. The mean annual precipitation is approximately 284.4 mm, and most precipitation (over 70%) occurs from May to September. Additionally, the annual average temperature is 6.3 °C. The Horqin Grassland is a mosaic of gently undulating sand dunes and interdune meadows with alkali soil. The Wulanaodu region has more than 700 hm^2 of meadow grassland. The soil is classified as alkali meadow soil. Typically, the meadow grassland is a clipping pasture because the great mass of herbaceous plants can be used as fodder and is only openly grazed after the grass is harvested from late September. In recent decades, owing to climate change and overgrazing, most of the meadow has undergone severe degradation [40]. Soil salinization induced by over-grazing is the main factor leading to the substantial reduction in above-ground biomass and considerable changes in composition and distribution of vegetation. Long-term over-grazing can result in heavy degradation of the grassland, and *S. chamaejasme* has gradually become the dominant species; however, in rationally grazed sites, the grassland is only lightly degraded and the distribution of *S. chamaejasme* population is limited. In heavily degraded grassland, *S. chamaejasme* is usually associated with *Allium ramosum*, *Potentilla chinensis*, *Arundinella hirta*, *Leymus chinense*, *Setaria viridis*, and *Chloris virgata*, while in lightly degraded grassland the dominant species include *Spodiopogon sibiricus*, *Hemarthia japonica*, *Trigonella korshinskyi*, *Leymus chinense*, and *Phragmites communis*. Total coverage in the heavily degraded and lightly degraded meadow is about 70–80% and 80–90%, respectively.

2.2. Experimental Design and Soil Sampling

The soil samples were collected in May 2018. Six heavily degraded sites (*S. chamaejasme* invaded, designated as SC) and six lightly degraded sites (*S. chamaejasme* uninvaded, designated as CK) were established in Wulanaodu meadow grassland, and the distance between the two sites was 200 m. The soil types were the same. The densities of *S. chamaejasme* in SC and CK were 5–10 and $\leq$1 individual m^{-2}, respectively. In each site, one plot (size 10 m × 10 m) was set up for soil sampling. Within each plot, 15 soil subsamples at a depth of 0–15 cm were randomly collected and mixed as a sample. All samples were homogenized with a sterilized sieve after large debris removal. A portion of each sample for DNA extraction was stored at −80 °C, and the remaining sample was air-dried and stored at room temperature.

2.3. Soil Physicochemical Property Measurements

The air-dried soil was sieved using a 2.0 mm screen for the analysis of electrical conductivity (EC), pH, and available P. A subsample was ground to sieve through a 0.25 mm mesh for the determinations of organic matter (SOM), total N, and total P. Soil pH was measured in 1:2.5 soil to water ratio with a glass electrode. EC was measured in (1:5 soil to water ratio) using a conductivity meter (Leica DDSJ-308A, Shanghai, China). SOM were determined according to the $K_2Cr_2O_7$–H_2SO_4 oxidation method. Total N was analyzed through the semimicro-Kjedahl digestion method with an auto-analyzer (Hanon, Changchun, China). Total P after digesting the extractions in concentrated H_2SO_4 and H_2O_2 and available P extracted with 0.5 M $NaHCO_3$ was measured according to the molybdenum antimony colorimetric method. Soil NH_4^+-N and NO_3^--N in extraction from soil with 1 M KCl (1:5 soil to 1 M KCl) were detected using salicylic acid and hydrazine sulfate methods, respectively. Total P, available P, NH_4^+-N, and NO_3^--N were determined by an automated discrete analyzer (CleverChem 380, Hamburg, Germany). All aforementioned analyses were carried out following the procedures published by the Institute of Soil Science, Chinese Academy of Sciences [41].

2.4. Soil DNA Extraction

Microbial genomic DNA was obtained with Fast DNA SPIN extraction kits following the procedures described by the manufacturer. DNA quality was checked through agarose gel electrophoresis, and the amount of DNA was determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.5. *nifH* Gene Sequencing and Bioinformatics Analysis

The *nifH* gene was amplified with the primers polF/polR [42]. To distinguish the samples, we incorporated sample-specific barcodes (7 bp) into the forward primers for multiplex sequencing. The purification and quantity of amplicons were performed with Agencourt AMPure beads (Beckman Coulter, Indianapolis, IN, USA) and a PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA). The mixture of triplicate PCR products from a sample in equal quantity was sequenced on the Illumina MiSeq platform with paired-end 2 × 300 bp at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Low-quality reads with ambiguous bases, shorter than 150 bp, less than 20 scores were deleted. Paired-end reads that had an overlapping base length of ≥10 bp and had no mismatched bases were assembled using FLASH. The resulting sequences from the same sample were grouped according to the barcodes. Low-quality sequences and chimeras were removed again. Then, the remainders were translated into amino acid sequences with the FunGene Pipeline of the Ribosomal Database Project server and were aligned against the *nifH* gene database [43]. Sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity by UCLUST. A representative sequence of each OTU was taxonomically identified by the BLAST algorithm-based search within GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 1 October 2021).

2.6. Data Analysis

To compare the diazotrophic diversity between SC and CK, four α-diversity indices (OTU richness, Shannon index, Chao, and ACE) were calculated. Differences in α-diversity were analyzed by one-way ANOVA in SPSS software (version 13.0) and a difference at $p < 0.05$ level was considered statistically significant. Moreover, linear correlation analysis between the relative abundances of taxonomic groups and soil physicochemical properties was carried out in SPSS. The taxonomic groups as biomarkers for SC and CK were identified by the linear discriminant analysis (LDA) effect size (LEfSe) method. According to the LEfSe method, only taxa with average abundances >1% were considered significant. A significance level of 0.05 and an effect size threshold of 2 were used for all of the biomarkers. Only taxa meeting an LDA significance threshold of >2 were shown. Furthermore, the diazotrophic community structure at the genus level in SC and CK were classified by unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) using the vegan package in the R environment (Rv.3.2.0). Redundancy analysis (RDA) was used to evaluate the correlation between soil physicochemical properties and diazotrophic community structure at the genus level using CANOCO 4.5.

All raw sequences were deposited in the NCBI Sequence Read Archive under accession number SRP191706.

3. Results

3.1. Soil Physicochemical Properties

Differences in SOM, total N, NH_4^+-N, NO_3^--N, pH, and EC were observed between SC and CK (Table 1). SOM, total N, NO_3^--N, and EC in the SC site were significantly higher than those in CK ($p < 0.05$). The NO_3^--N content in the SC sites was 9.11 times that in CK ($p < 0.05$) and EC was 26.3% more than that in CK ($p < 0.05$). However, NH_4^+-N and pH value were significantly lower in the SC sites. No significant differences in total P and available P were observed ($p > 0.05$).

Table 1. Soil nutrients, pH, EC, and moisture of samples (n = 6).

Items	SC	CK	F	p
SOM ($g \cdot kg^{-1}$)	3.83 ± 0.29a	2.43 ± 0.88b	13.659	<0.01
Total N ($g \cdot kg^{-1}$)	0.91 ± 0.09a	0.79 ± 0.07b	6.357	0.03
NH_4^+-N ($mg \cdot kg^{-1}$)	1.74 ± 0.25a	2.15 ± 0.25b	8.199	0.017
NO_3^--N ($mg \cdot kg^{-1}$)	0.82 ± 0.66a	0.09 ± 0.08b	7.319	0.022
Total P ($g \cdot kg^{-1}$)	0.52 ± 0.09a	0.61 ± 0.07a	3.58	>0.05
Available P ($mg \cdot kg^{-1}$)	5.33 ± 0.93a	5.25 ± 0.61a	0.028	>0.05
pH	8.125 ± 0.137a	8.395 ± 0.227b	6.233	0.032
EC ($\mu s \cdot cm^{-1}$)	251.8 ± 19.9a	199.3 ± 48.6b	5.983	0.034

Abbreviations: SC, grasslands invaded by *S. chamaejasme* L.; CK, uninvaded grasslands. The means in lines followed by a different letter are significantly different ($p < 0.05$).

3.2. Alpha Diversity and Taxonomic Composition

After singletons were removed, 189,121 sequences were obtained and then grouped into 7601 OTUs. The differences in the average alpha diversity indexes (including OTU number, ACE, Chao 1, and Shannon) were not statistically significant ($p > 0.05$; Table 2).

Table 2. Alpha diversity of different sites (n = 6).

Samples	OTU Number Richness	ACE	Chao 1	Shannon
CK	2176.0 ± 172.9	2577.6 ± 381.2	2457.7 ± 329.3	9.6 ± 0.2
SC	2286.1 ± 376.3	2752.4 ± 739.5	2624.3 ± 637.1	9.7 ± 0.2

Values are the means ± S.D. Abbreviations: SC, grasslands invaded by *S. chamaejasme* L.; CK, uninvaded grasslands.

The OTUs were classified into 7 different phyla, 13 classes, 29 orders, 60 families, and 96 genera. All phyla and genera (the relative abundance > 1%) are shown in Figure 1 with other taxonomic levels shown in Figure S1. Proteobacteria, with relative abundance ranging from 87.36% to 91.35%, is clearly the dominant phylum, followed by Verrucomicrobia, Cyanobacteria, Actinobacteria, and Firmicutes (Figure 1a). At the class level (Figure S1a), the relative abundance of Alphaproteobacteria was the highest, followed by Deltaproteobacteria, Betaproteobacteria, Opitutae and Gammaproteobacteria. Rhizobiales, Desulfuromonadales, Rhodospirillales, Burkholderiales, Opitutales, Desulfovibrionales, Rhodobacterales, Pseudomonadales, and Rhodocyclales were the dominant orders (Figure S1b). We observed 17 families with relative abundances of >1%. Particularly, Bradyrhizobiaceae, with an average relative abundance of 44.83%, was the most dominant group in all the samples (Figure S1c). At the genus level, *Bradyrhizobium*, which belongs to Alphaproteobacteria, was the most abundant, followed by *Geobacter* and *Desulfuromonas* from Deltaproteobacteria (Figure 1b). The other dominant genera included *Azospirillum*, *Mesorhizobium*, *Rhodoplanes*, and *Sagittula* from Alphaproteobacteria; *Azotobacter* from Gammaproteobacteria; *Pseudacidovorax* from Betaproteobacteria; and unidentified genera from the Opitutaceae of Verrucomicrobia.

3.3. Correlation Analysis between the Relative Abundances of Taxonomic Groups and Soil Physicochemical Properties

Correlation analysis showed that the relative abundances of Verrucomicrobia, Opitutae, Opitutaceae, Desulfuromonadaceae, and *Desulfuromonas* were significantly and positively correlated to EC ($p < 0.05$, Table S1). Significant negative correlations among Bradyrhizobiaceae, Burkholderiaceae, *Bradyrhizobium*, and EC were observed ($p < 0.05$). The relative abundances of Alphaproteobacteria, Bradyrhizobiaceae, and *Bradyrhizobium* from Alphaproteobacteria had a negative correlation with SOM ($p < 0.05$). By contrast, Deltaproteobacteria, Desulfuromonadaceae, Geobacteraceae, *Desulfuromonas*, and *Geobacter* from Deltaproteobacteria were positively correlated with SOM ($p < 0.05$). Phyllobacteriaceae and *Mesorhizobium*, were positively correlated with pH ($p < 0.05$). No significant correlations of other taxonomic groups to soil physicochemical properties were found.

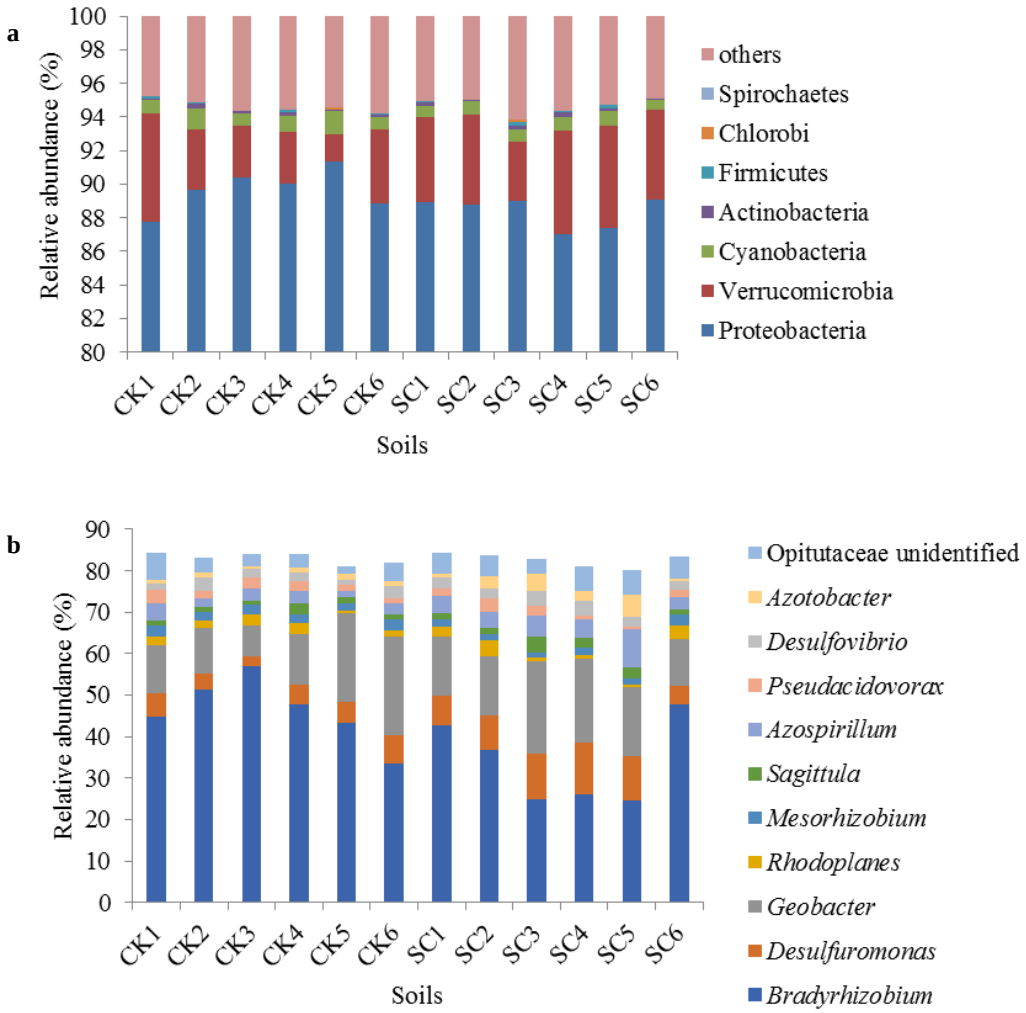

Figure 1. Relative abundances of taxonomic groups in SC and CK. Abbreviations: SC, grasslands invaded by *S. chamaejasme*; CK, uninvaded grasslands. (**a**): relative abundances of phyla; (**b**): relative abundances of genera.

3.4. Biomarkers for SC and CK

We used LEfSe to analyze the correlation of diazotrophic taxa with the SC and CK sites (Figure 2). LEfSe revealed that 20 clades showed significant and consistent differences in SC and CK. The most differentially abundant diazotrophs in CK were members affiliated with Alphaproteobacteria, including microbes from Bradyrhizobiaceae and Phyllobacteriaceae, Betaproteobacteria (*Burkholderia*), and Cyanobacteria (*Nostocales* and Rivulariaceae). In SC sites, diazotrophic taxa with different abundances included Deltaproteobacteria (Desulfuromonadaceae), Alphaproteobacteria (Rhodospirillaceae and *Rhizobium*), and several soil non-dominant taxa, which belong to Verrucomicrobia, Actinobacteria, and Firmicutes. Among these taxa, *Bradyrhizobium* and *Desulfuromonas* had the highest LDA scores, indicating marked abundances in CK and SC, respectively.

3.5. Diazotrophic Structure and Correlation with Soil Physicochemical Properties

PCA analysis was used to analyze the diazotrophic community structure of SC and CK at the genus level. PCA plots indicated that the diazotrophic communities in the SC samples were separated from those in CK by PC1, suggesting that the diazotrophic communities in the SC samples differed from those in CK (Figure 3). Moreover, we used PLS-DA analysis to classify the samples from the SC and CK sites. The PLA-DA plot showed that the samples from the SC and CK sites were divided into two groups, reflecting the structural differences in these diazotrophic communities (Figure 4). The association

between diazotrophic community structure and soil physicochemical properties was further described by RDA. Figure 5 showed that total N, NO_3^--N, NH_4^+-N, SOM, EC, and pH were closely related to the RDA1 axis, suggesting the effect of total N, NO_3^--N, NH_4^+-N, SOM, EC, and pH on diazotrophic community structure.

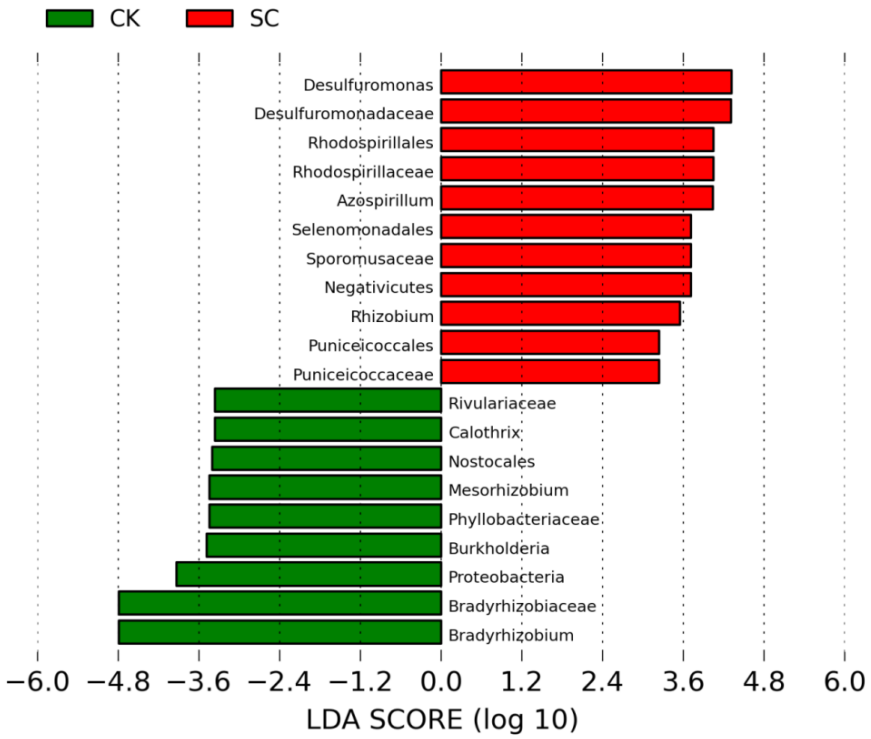

Figure 2. LEfSe method identifies the significantly different abundant taxa of diazotrophs in SC and CK. Abbreviations: SC, grasslands invaded by S. *chamaejasme*; CK, uninvaded grasslands.

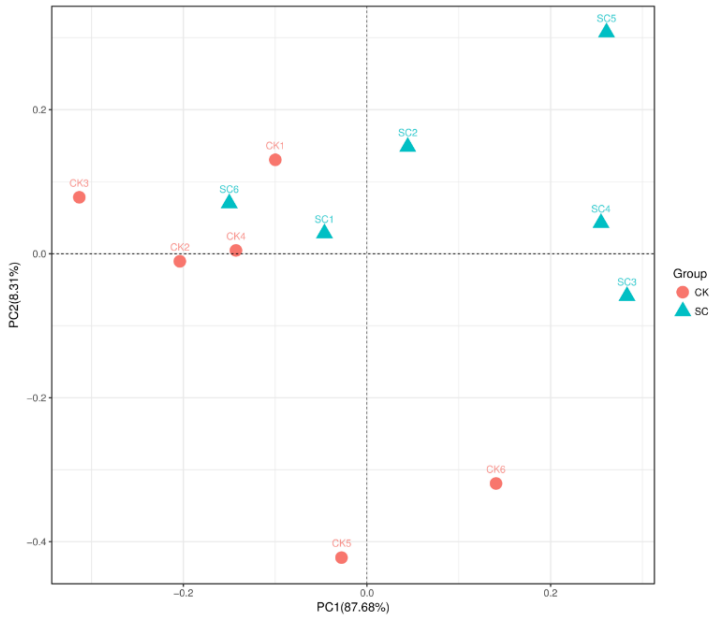

Figure 3. Principal component analysis of the *nifH* gene fragments of diazotroph at the OTU level. Abbreviations: SC, grasslands invaded by *S. chamaejasme*; CK, uninvaded grasslands.

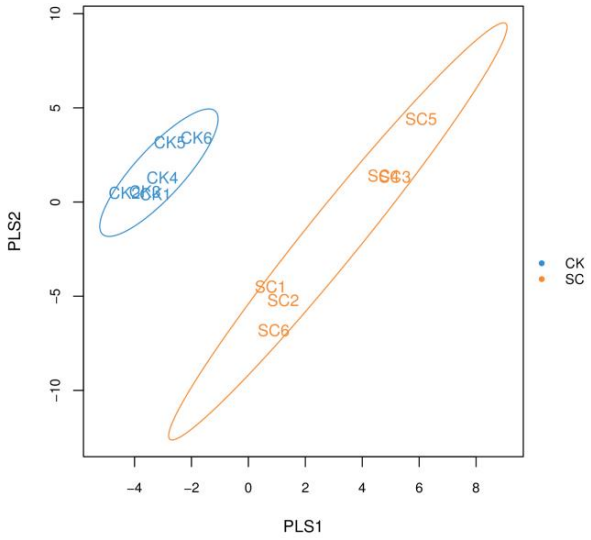

Figure 4. PLS-DA score plot of the relative abundances of diazotroph at the genus level associated with CK and SC. Abbreviations: SC, grasslands invaded by *S. chamaejasme*; CK, uninvaded grasslands.

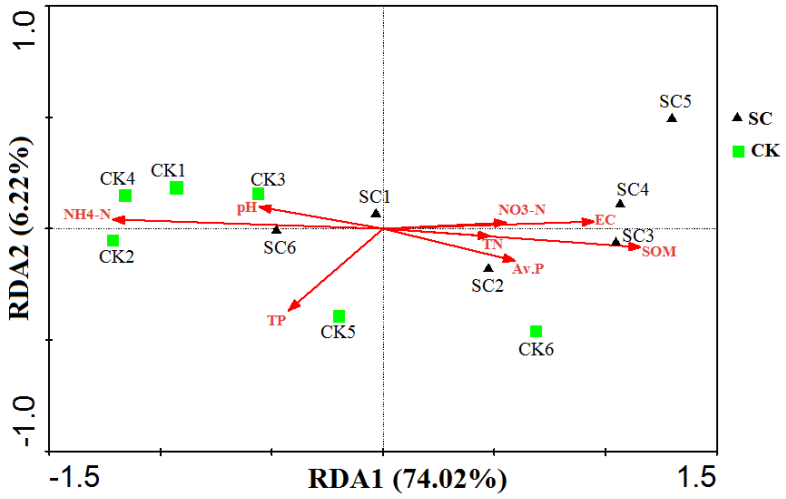

Figure 5. Redundancy analysis (RDA) of diazotrophic community structures and physicochemical properties. Abbreviations: Av. P, available P; SOM, soil organic matter; TP, total P; TN, Total N; EC, EC. Abbreviations: SC, grasslands invaded by *S. chamaejasme*; CK, uninvaded grasslands.

4. Discussion

The present study indicated that EC in SC sites was larger than that for CK sites. A similar finding was also reported in a study about the invasive plant species *Amaranthus retroflexus* L. [32]. In our experimental sites, *S. chamaejasme* expansion coincided with soil secondary salinization, characterized as soluble salt accumulation on the soil surface, high soil pH, and an increase in EC [39,40]. In a salinilized grassland, the growth of most plants was inhibited under salt stress, whereas *S. chamaejasme* was better adapted to this situation and gradually became a dominant species. Although Wu et al. [44] reported that root exudates released by alien invasive plants led to an increase in EC, higher EC observed in this study may depend on soil secondary salinization as a result of long-term over-grazing. However, the over-expansion of *S. chamaejasme* resulted in a slightly decline in soil pH in this study. This is probably because *Stellera* may have the same capability as do some invasive alien plants to absorb more NH_4^+-N than NO_3^--N because low levels of soil NH_4^+-N were detected in SC [45,46]. Another reason is the higher nitrification in SC [37], which resulted in soil acidification by releasing H^+. In addition, increases in SOM and total N in SC were observed, consistent with a previous study conducted in

an alpine meadow ecosystem that indicated that high levels of SOM and total N may be attributed to high production and litter inputs [37]. Likewise, two native invasive species, *Elymus athericus* [26] and *Brachypodium pinnatum* [47], both having high above-ground biomass, were also found to be capable of increasing soil C and N contents. High above-ground biomass was closely related to above-ground N, which is taken up by root systems and allocated to above-ground plant tissues [4,27,45,46,48]. In our study, high soil nutrients in the patch of mother *S. chamaejasme* can improve the growth of young *S. chamaejasme*, which gradually increased the *S. chamaejasme* population and its dominance in the original community.

As expected, our study showed that diazotrophic community structure in SC differed from that in CK (Figures 3 and 4) and was correlated with soil pH (Figure 5). This result highlighted that soil pH is a key factor for determining the structure of a diazotrophic community [49–51]. Changes in soil pH resulting from *S. chamaejasme* expansion likely caused shifts in diazotrophic communities. Soil EC is another determinant for shifts in soil microbial community structure [52,53]. In this study, RDA showed that soil EC was closely correlated with diazotrophic community structure characterized by the increase in the relative abundance of *Desulfuromonas* (sulfate-reducing bacteria) (Figure 2). The abundant *Desulfuromonas* implied the presence of high levels of sulfates [54]. Bui [54] suggests that soil salinity plays an important role in weed invasion in semi-arid regions. Therefore, another important factor shaping diazotrophic communities beyond simple *S. chamaejasme* expansion is grassland salinization, which then provides opportunities for *S. chamaejasme* expansion because of its tolerance to salinity. Moreover, NH_4^+, NO_3^-, total N, and SOM were all correlated to the structure of the diazotrophic community. These results were in accordance with the report of Xu et al. [55], where the significant effects of soil N fractions, especially for NH_3-N, were correlated with diazotrophic community compositions under field conditions. Similar results were observed by Chen et al. [12]. The roles of SOM in shaping diazotrophic communities may be attributed to N stocks increased by the decomposition of SOM [56]. A previous study demonstrated the joint effects of above-ground vegetation and soil physicochemical properties on soil microbial communities [57]. Hence, the shift in the diazotrophic community could be simultaneously driven by grassland salinization and *S. chamaejasme* expansion.

Because soil microbes sensitively respond to the invasion of exotic plants that alter the availability of soil nutrients [18,58,59], changes in microbial diversity induced by invasive plants are often observed [18,60,61]. However, no significant difference in alpha diversity of diazotrophic communities was found in our study. Similar findings were also reported by Wang et al. [62]. The possible reason is the homogeneity of both invaded and uninvaded soils [62].

Desulfuromonas and *Bradyrhizobium* were the most distinct biomarkers for SC and CK, respectively. Their abundances were higher and lower in SC than in CK, respectively, and were positively and negatively correlated with SOM and EC significantly ($p < 0.05$), respectively. *Geobacter*, another group from Deltaproteobacteria, also showed a similar trend to *Desulfuromonas* in SC soils. Le Roux et al. [63] reported that *Bradyrhizobium* was more dominant in legume-invaded soils than in uninvaded soils. The structure and composition of the Bradyrhizobial community are strongly influenced by soil physicochemical properties, especially soil pH and SOM [64]. Land-use history also affects diazotrophic community composition [65]. In this study, most sequences were closely related to members of *Bradyrhizobium*, and the dominance of *Bradyrhizobium* groups may be determined by soil salinization, SOM, and meadow use practice. We inferred that specific *Bradyrhizobium* groups may be sensitive to increases in EC and SOM. In this study, high EC, an indicator of high levels of soil salt, created anoxic microhabitats [53], which lead to a decline in the oxidation rate of organic matter [39]. This situation was unfavorable to the proliferation of *Bradyrhizobium*. By contrast, *Desulfuromonas* and *Geobacter* can live in oxic and anoxic microsites [66] and obtain energy by reducing sulfate and oxidizing organic compounds for their survival. Therefore, increases in SOM and EC can facilitate the growth and pro-

liferation of *Desulfuromonas* and *Geobacter*, thereby contributing to nitrogen input in this salinized soil.

The present study suggests that the expansion of *S. chamaejasme* was associated with grassland salinization. However, causal relationships involving expansion of *S. chamaejasme*, grassland salinization, and soil diazotrophic community should be further studied, and pot experiments by planting *S. chamaejasme* under this grassland soil after conditioning with a gradient of salt concentration should be conducted in the future.

5. Conclusions

The invasion of *S. chamaejasme* can increase levels of soil nutrients (SOM, total N, NO_3^--N, and NH_4^+-N.) and lower soil pH, which facilitates its expansion. The spread of *S. chamaejasme* significantly alters the structure of the soil diazotrophic community, and this effect is mainly determined by soil N, SOM, EC, and pH. The abundance of *Bradyrhizobium* and *Desulfuromonas* markedly responded to the expansion of *S. chamaejasme*. Interactions observed among soil salinization, the expansion of *S. chamaejasme*, and changes in the diazotrophic community can drive the degradation of the Horqin Grassland.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11102085/s1, Figure S1: Relative abundances of taxonomic groups in SC and CK, Table S1: Coefficient (r) for the correlations between the relative abundances of taxonomic groups and soil physicochemical properties.

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