Heliyon 9 (2023) e20860

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Invasion by *Cenchrus spinifex* changes the soil microbial community structure in a sandy grassland ecosystem

Baihui Ren [*,1], Meng Meng [1], Jianxin Yu, Xinwei Ma, Daiyan Li, Jiahuan Li, Jiyun Yang, Long Bai, Yulong Feng [**]

Shenyang Agricultural University, Shenyang, Liaoning, 110866, China

ARTICLE INFO

Keywords:
Cenchrus spinifex invasion
Soil nitrogen cycle
Soil microbial communities
Functional genes
Environmental factor

ABSTRACT

Continuous nitrogen deposition increases the nitrogen content of terrestrial ecosystems and alters the soil nitrogen cycling process. Invasive plants have strong environmental adaptability, which can not only affect the composition and diversity of soil microbial community but also significantly affect the transformation process of soil nitrogen, leading to successful invasion. Currently, research on invasive plant soil ecosystems mainly focused on changes in soil nutrients and soil microorganisms. As an invasive annual grass weed with strong ecological adaptability, the impact of *Cenchrus spinifex* at different growth periods on soil environment and soil microbial structure composition and diversity in sandy grassland ecosystems is still unclear. In this study, soil samples were collected from four habitats with different degrees of invasion in situ during the vegetation and reproductive growth periods of *Cenchrus spinifex*. High-throughput sequencing and qPCR technology were used to analyze the changes in the composition, structure and diversity characteristics of the soil microbial communities during *Cenchrus spinifex* invasion. The results indicated that *Cenchrus spinifex* invasion had different effects on the soil environment at different growth periods, and *Cenchrus spinifex* had a preference for the utilization of ammonium nitrogen during vegetation growth period. Moreover, *Cenchrus spinifex* invasion significantly changed the composition and structure of soil bacterial communities, and the response of soil bacterial and fungal communities to the invasion was inconsistent. Additionally, the bacterial network was more stable than the fungal network. At different growth periods, *Cenchrus spinifex* had a significant impact on the key microbial communities of soil nitrogen cycling. The invasion increased the abundance of *nifH* and AOA-*amoA*, while decreased the abundance of AOA-*amoB*. Alkaline hydrolyzed nitrogen, total nitrogen and total phosphorus content were key factors that affect vegetation growth period and change the key microbial communities of nitrogen cycling. Alkaline hydrolyzed nitrogen, total phosphorus and organic carbon were key factors in reproductive growth period that alter the nitrogen cycling of key microbial communities.

* Corresponding author. No. 120 Dongling Road, Shenhe District, Shenyang, Liaoning, 110866, China.
** Corresponding author. No. 120 Dongling Road, Shenhe District, Shenyang, Liaoning, 110866, China.
E-mail addresses: bhren@syau.edu.cn (B. Ren), fyl@syau.edu.cn (Y. Feng).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2023.e20860
Received 9 September 2023; Received in revised form 7 October 2023; Accepted 9 October 2023
Available online 20 October 2023

1. Introduction

With the acceleration of globalization, the problem of biological invasion has become a ecological and economic problem [1]. Invasive plants not only alter the diversity of aboveground plant communities in the invaded land but can also have profound effects on the corresponding underground ecosystems [2].

Soil microorganisms are the most important part of the soil ecosystem, directly involved in nutrient cycling and play an important role in the improvement of soil quality and the growth and competition of plants [3,4]. Changes in the soil microbial community are one of the ecological effects of alien plant invasion [5]. Invasive plants can change the availability of nutrients in the soil and lead to changes in biodiversity [6,7]. After an invasion by *Spartina alternifora*, the diversity of soil fungi significantly decreased [8], whereas after an invasion by *Solidago canadensis* L, the soil biodiversity of the invaded areas increased [9]. The invasion by *Flavoria bidentis (L.) Kuntze* increased the number of selected functional bacteria and biodiversity in the soil, leading to a more complex community structure [10]. In addition, changes in the structure and diversity of microbial communities can affect invasive plants and ecosystems. The exotic plant *Centaurea cyanus* L. can significantly increasd the diversity and abundance of AMF in the soil thereby accelerating its degree of invasion [11]. Invasive plants may create a favorable soil environment for their own growth and competition by altering the soil microbial community, especially by increasing the number of soil microbial communities closely related to soil nutrient metabolism. Therefore, research on the effect of invasive plants on underground microbial diversity has become increasingly important.

Soil microbial communities are closely related to the soil environment and their functions could change with variations in environmental conditions [12]. In the process of competing for survival space between invasive and local plants, changes to the nutrient composition, organic matter and enzyme activity of the soil can occur, causing changes in the physical and chemical properties of the soil and the composition and diversity of microbial communities in the invaded area. Invasion by the exotic plant *Phyllostachys pubescens* significantly increased soil pH, resulting in a significant decrease in the dominant genus of *Acinetobacter* [13].

Invasive plants have different resource allocation strategies at different growth periods, resulting in inconsistent changes in soil environment and soil microorganisms [14]. Studies had shown that the absorption and utilization capacity of inorganic nitrogen by *Bidens pilosa* L. during its vegetative growth period was enhanced. During reproductive growth period, *Bidens pilosa* L. preferred phosphorus more [15]. Therefore, more attention should be taken to explore the differences in soil environment and microorganisms during the different growth stage of plant invasion.

The increase in atmospheric nitrogen deposition can improve the availability of soil nitrogen, alter the species composition structure and function of ecosystems, and thereby affect nitrogen cycling processes [16]. Invasive plants are more likely to utilize excess resources due to their growth characteristics, so an increase in soil available nitrogen because of intensifying nitrogen deposition could promote the invasion [17]. In addition, the form of nitrogen in soil exhibits spatiotemporal heterogeneity [18], and invasive plants can affect soil nitrogen cycling by transforming nitrogen pools [19]. Under the dual influence of species characteristics and invasive site properties, invasive plants can change the direction and size of their “nitrogen budget” [20]. Studies have shown that *Rhus Typhina* L. and *Solidago canadensis* L. invasion prefer to absorb and utilize soil ammonium nitrogen [21,22].

Soil microorganisms are key drivers of nitrogen cycling in ecosystems. Invasive plants affect nitrogen cycling in ecosystems by changing the community structure and diversity of key microorganisms involved in soil nitrogen transformation [23]. After the successful invasion by alien plants, the nitrogen fixation ability of nitrogen-fixing microorganisms and soil nitrogen level can be improved, which is conducive to their further invasion. The invasion by *Ageratina adenophora* not only multiplied the number of native nitrogen-fixing bacteria in the soil but also changed the species of dominant nitrogen-fixing bacteria, thereby resulting in sufficient nitrogen sources to achieve rapid expansion and growth [24]. The ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) are closely related to soil nitrification, and have a significant impact on the reaction speed of nitrification [25]. Invasion by alien plants can significantly change the community structure of soil ammonia-oxidizing microorganisms, thereby affecting the nitrogen cycle process [26]. The increase in the nitrification rate was mainly due to an increase in the abundance and change in composition of soil AOB and AOA caused by the invasion of *Spartina alterniflora*, which drived nitrification in the soil of the invasive system [27]. Invasion by alien plants can change the community structure and function of denitrifying bacteria, thereby reducing the activity of denitrifying enzymes in the soil. This can reduce the leaching of nitrate and the escape of nitrogen oxides from the invaded ecosystem [28]. However, further research is needed on how invasive plants affect key microorganisms involved in nitrogen cycling.

Cenchrus spinifex is a worldwide invasive annual grass weed with strong ecological adaptability and reproductive ability [29]. According to the survey data, *Cenchrus spinifex* was first found in Horqin district of China in 1983. Since then, it has invaded many grasslands and farmland from point to surface, and has become one of the invasive weeds with serious harm in Horqin Sandy Land so far [30]. Currently, research on *Cenchrus spinifex* mainly focuses on morphological characteristics, distribution of adaptive regions, and invasion mechanisms; however, there are few reports on the interactions among exotic plants, soil organisms, and soil ecosystem processes. We need to further comprehensively study whether the invasion by *Cenchrus spinifex* can change soil conditions, directly or indirectly change the microbial community structure, and form a self-promoting invasion mechanism at the population, community, and ecosystem levels.

In summary, we hypothesized that: (1) *Cenchrus spinifex* would reduce competition for soil nitrogen by absorbing and utilizing specific soil nitrogen forms, which is beneficial for promoting its invasion. (2) During the growth process, *Cenchrus spinifex* will have different impacts on the soil environment and soil microorganisms, which is more conducive to its further reproduction and invasion. (3) The invasion of *Cenchrus spinifex* may change the soil microbial community, thereby affecting the soil nitrogen cycling process, but different microbial communities have inconsistent responses to the invasion. We used high-throughput sequencing technology and fluorescence quantitative PCR (qPCR) technology to explore the characteristics and influencing factors of soil microbial communities in different invasive habitats during nutrient and reproductive growth periods of *Cenchrus spinifex*. Additionally, we conducted an in-

depth analysis of the internal relationship between changes in soil microbial communities with soil nutrients, which would not only provide a theoretical basis for comprehensively evaluating the impact of *Cenchrus spinifex* invasion on grassland ecosystems but also could have an important significance for exploring the soil microbiological mechanism of alien plant invasion.

2. Methods and materials

2.1. Description of study area

The research area is located in the Baiyinhua Farm (122° 33′ E, 42° 48′ N), Zhangwu County, on the southern edge of the Horqin Sandy Land. It has a northern temperate continental monsoon climate. The main soil type of the sandy land is windy sand, and the soil organic matter and nutrient contents are very low, with poor physical properties. *Cenchrus spinifex* in this area is very harmful. *Cenchrus spinifex* distributed in the northwest of Liaoning generally undergo jointing and tillering for vegetative growth period in July, flowering and fruiting for reproductive growth period in September, and stop developing after severe frost in October [31]. The main local plants include *Digitaria sanguinalis*, *Roegneria kamoji*, *Agropyron cristatum*, *Chenopodium acuminatum*, and *Lespedeza daurica*. Based on comprehensive research, an area of 100 m × 300 m with the four fllowing invasive habitats was selected in a similar landform and terrain characteristics, similar soil origin and soil type, and less human and animal interference Horqin sandy grassland ecosystem. Among them, bare land habitat (LD) represents areas where there is no vegetation growth in the sample land. The coverage of *Cenchrus spinifex* is more than 90 % in the invasive plant monodominant community sample habitat (SY), approximately 60 % in the mixed community sample habitat (HD) with invasive and local plants, and less than 10 % in the local plant community sample habitat (BY).

2.2. Experimental design and sample collection

We respectively collected plant rhizosphere soil from four different invasive habitats in June and September 2021. Four sample plots of 1 m × 1 m were selected in each polt, and 0–10 cm rhizosphere soil samples with an inner diameter of 3.6 cm were obtained. The soil samples were mixed taken from the five points and sieved through a 2-mm sieve into a self-sealing bag. The root system and gravel were removed and the samples were divided into two parts. One subsample was air-dried and sieved through a 0.25-mm mesh for physicochemical analysis. The other subsample was placed in bags with ice and immediately transferred to a super-cold refrigerator (−20 °C) for DNA extraction.

Table 1
Impact of *Cenchrus spinifex* invasion on soil physicochemical properties.

		LD	BY	HD	SY
Total nitrogen ($g \bullet kg^{-1}$)	Vegetative growth	$0.17 \pm 0.01B^{a}$	0.16 ± 0.01^{a}	$0.16 \pm 0.01B^{a}$	$0.09 \pm 0.01B^{b}$
	Reproductive growth	$0.20 \pm 0.01A^{ab}$	0.18 ± 0.01^{b}	$0.21 \pm 0.01A^{a}$	$0.20 \pm 0.01A^{ab}$
NH_4^+-N ($mg \bullet kg^{-1}$)	Vegetative growth	4.56 ± 0.57^{ab}	1.45 ± 0.49^{b}	16.28 ± 8.49^{a}	1.38 ± 0.32^{b}
	Reproductive growth	1.80 ± 1.05	0.69 ± 0.04	2.17 ± 0.39	1.11 ± 0.30
Alkali-hydrolyzable nitrogen ($mg \bullet kg^{-1}$)	Vegetative growth	17.92 ± 0.68^{bc}	$19.08 \pm 0.08B^{b}$	15.33 ± 0.67^{c}	$38.33 \pm 1.67A^{a}$
	Reproductive growth	20.67 ± 2.05^{ab}	$21.33 \pm 0.33A^{a}$	$16.92 \pm 0.65A^{b}$	$23.92 \pm 1.17B^{a}$
NO_3^+-N ($mg \bullet kg^{-1}$)	Vegetative growth	3.66 ± 1.60	2.57 ± 0.54	$1.29 \pm 0.18B$	2.66 ± 0.69
	Reproductive growth	1.75 ± 0.74	1.51 ± 0.16	3.06 ± 0.53	3.02 ± 0.30
Total phosohorus ($g \bullet kg^{-1}$)	Vegetative growth	$0.12 \pm 0.01B^{d}$	$0.17 \pm 0.01B^{c}$	0.23 ± 0.01^{a}	$0.19 \pm 0.01B^{b}$
	Reproductive growth	$0.42 \pm 0.01A^{a}$	$0.32 \pm 0.05A^{ab}$	0.32 ± 0.04^{ab}	$0.27 \pm 0.02A^{b}$
Available phosohorus ($mg \cdot kg^{-1}$)	Vegetative growth	$0.19 \pm 0.03B^{b}$	$0.12 \pm 0.03B^{b}$	0.34 ± 0.01^{a}	0.27 ± 0.01^{a}
	Reproductive growth	$0.39 \pm 0.02A^{ab}$	$0.47 \pm 0.03A^{a}$	0.43 ± 0.04^{ab}	0.32 ± 0.05^{b}
Soilorganic carbon ($g \bullet kg^{-1}$)	Vegetative growth	$0.82 \pm 0.06B^{ab}$	$0.59 \pm 0.11B^{b}$	1.21 ± 0.21^{a}	$0.48 \pm 0.02B^{b}$
	Reproductive growth	$1.50 \pm 0.06A^{a}$	$1.03 \pm 0.06A^{bc}$	0.99 ± 0.11^{c}	$1.36 \pm 0.15A^{ab}$
Electrical conductivity ($S \bullet m^{-1}$)	Vegetative growth	12.15 ± 0.83^{a}	$8.84 \pm 0.18B^{b}$	11.57 ± 0.51^{a}	$8.75 \pm 0.22B^{b}$
	Reproductive growth	13.34 ± 1.63^{ab}	$15.21 \pm 1.11A^{a}$	10.73 ± 0.95^{b}	$10.36 \pm 0.42A^{b}$
pH	Vegetative growth	5.84 ± 0.11^{ab}	5.93 ± 0.08^{a}	$6.11 \pm 0.07A^{a}$	5.65 ± 0.03^{b}
	Reproductive growth	5.76 ± 0.18	5.65 ± 0.11	$5.56 \pm 0.04B$	5.59 ± 0.15
NN_{nit} ($mg \cdot g^{-1} \cdot d^{-1}$)	Vegetative growth	$1.49 \pm 0.06A^{a}$	1.22 ± 0.24^{ab}	0.78 ± 0.06^{b}	0.80 ± 0.32^{ab}
	Reproductive growth	$0.40 \pm 0.12B^{b}$	0.96 ± 0.16^{a}	0.92 ± 0.12^{a}	0.79 ± 0.14^{ab}
NN_{amm} ($mg \cdot g^{-1} \cdot d^{-1}$)	Vegetative growth	$0.43 \pm 0.12B$	0.81 ± 0.29	$0.88 \pm 0.03B$	0.91 ± 0.09
	Reproductive growth	$1.22 \pm 0.06A$	1.04 ± 0.14	$1.23 \pm 0.06A$	1.58 ± 0.35
NN_{min} ($mg \cdot g^{-1} \cdot d^{-1}$)	Vegetative growth	$-1.06 \pm 0.17B^{b}$	-0.41 ± 0.30^{ab}	0.10 ± 0.08^{a}	0.10 ± 0.40^{a}
	Reproductive growth	$0.83 \pm 0.06A^{a}$	0.08 ± 0.24^{b}	0.31 ± 0.18^{ab}	0.79 ± 0.29^{ab}

Note: LD refers to bare land, BY refers to the sample land of the local plant community, HD refers to the sample land of mixed communities of *Cenchrus spinifex* and local plants, and SY refers to the invasion sample land composed of patches inlaid by monodominant communities of *Cenchrus spinifex*. The figures in the table represent the average value ± standard error. Different lowercase letters indicate significant differences between different invasive habitats during the same growth period ($P < 0.05$). Different capital letters indicate significant differences between different growth periods in the same invasive habitat ($P < 0.05$).

2.3. Soil physicochemical analysis

The total soil nitrogen (TN) was measured using the Kjeldahl method. The content of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in the soil was measured using a potassium chloride solution extraction flow analyzer (QC8000). The soil pH and electrical conductivity (EC) were measured using a glass electrode method (soil water mass ratio 1: 2.5). The total phosphorus in the soil (TP) was determined via molybdenum antimony resistance colorimetry. The available phosphorus within the soil (AP) was extracted via sodium bicarbonate molybdenum antimony resistance colorimetry. The total organic carbon of the soil (SOC) was determined through external heating with potassium dichromate. The soil nitrogen mineralization was measured using indoor incubation methods. Thirty grams of the screened air-dried soil sample was weighed and placed in a 250-mL plastic wide-mouthed bottle. The soil water content was adjusted to 20 % (mass water content) with deionized water. The bottle mouth was wrapped with polyethylene film, and two small holes were made within the film. The samples were placed in a 25 °C incubator for pre-incubation for 7 days. After pre-incubation, they were divided into three groups, and N50 mg·kg^{-1} (calculated based on dried soil) ammonium sulfate, N50 mg·kg^{-1} sodium nitrate solution, and constant weight deionized water (CK) were added, respectively. The humidity of the three soil groups was adjusted to 40 % of the water-holding capacity of the soil. All culture bottles were sealed again and incubated in the dark at 25 °C for 14 days. Changes in soil moisture were determined by weighing and replenishing the soil moisture with deionized water. On the 0th and 14th days of incubation, 75 mL of 2 mol L^{-1} KCL solution was added, and samples underwent vibration in a constant temperature oscillating machine at 25 °C for 1 h. The filtrate was measured using a continuous flow analyzer (Auto Analyzer 3 System, SEAL Analytical GmbH, UK) to determine the concentration of nitrate and ammonium nitrogen in the soil.

2.4. DNA extraction, illumina MiSeq high-throughput sequencing, and sequence processing

A Powersoil DNA Isolation Kit (MoBio, USA) was used to extract the genomic DNA of 24 samples, and the purity and concentration of DNA were detected using agarose gel electrophoresis and a Nanodrop spectrophotometer, respectively. Based on the selection of sequencing regions, the diluted genomic DNA is used as a template for PCR using specific primers with barcodes and efficient high fidelity enzymes. The reaction system was as follows: Soil bacteria and fungi were used as the quantitative amplification primers (Table A1). Library using TruSeq ® The DNA PCR-Free Sample Preparation Kit library was constructed using a kit. Quantitative analysis of the constructed library using quantum bits and QPCR. After the library is qualified, use v2 sequencing kit (2 × 250 bp) and Miseq sequencer for onboard sequencing.

The Quantitative Insights into Microbial Ecology (QIIME v.1.9.1) control program was used to obtain the initial sequences and to extract high-quality cleaning labels [32] (https://docs.qiime2.org/2019.4/tutorials/). Using the USARCH tool, which is based on the UCHIME algorithm [33], the sequence was clustered into operational taxons based on 97 % of pairing identities. Alpha-diversity metrics Chao1 [34], Observed species, Shannon [35], Simpson [36], Pielou's evenness [37] and Good's coverage [38], beta diversity metrics (Bray-Curtis dissimilarity) were estimated using the diversity plugin with samples were rarefied to sequences.

The DNA sequences in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession number PRJNA960835.

2.5. Fluorescence quantitativePCR technique

Four typical N-cycling microbial communities were investigated in this study: N-fixing bacteria (*nifH*) of nitrogen fixation, Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) with ammonia monooxygenase genes (*amoA*), and denitrifying bacteria (*nirK*) of nitrite reduction [39].

Quantitative fluorescence PCR was used to determine the abundance of soil microorganisms. The reaction system was as follows: soil *nifH*, AOB-*amoA*, AOA-*amoA*, and *nirK* were used as the quantitative amplification primers (Table A1). The total volume of the amplification reaction system was 20 μL, and included 10 μL 2 × GoTaq® qPCR Master Mix, 10 μmol/L upstream and downstream primers (0.5 μL each), 2 μL DNA template (1–10 ng), and 7 μL sterilized ultra-pure water. The enhanced 96-PCR plate was amplified on a quantitative fluorescence PCR instrument, with three replicates per sample. The amplification reaction conditions were as follows: predenaturation at 95 °C for 30 s, denaturation at 95 °C for 5 s, annealing at 60 °C for 40 s, extension at 72 °C for 30 s, and 40 cycles. The amplification efficiency can be seen from Table A2 qPCR algorithm：Absolute quantification.

The formulas as follows : $\text{copies} = 10^{\frac{Ct-b}{k}}$

2.6. Statistical analysis of data

Data were statistically analyzed using Excel 2010 and the SPSS 19.0 software (version 22.0; IBM Corporation, Armonk, NY, USA).

Table 2
Network topology parameters of soil bacterial and fungal communities.

	Average nearest neighbor degree	Average path lenghth	Number of vertice	Number of edge	Modularity
Bacterial communities	72.491	3.204	19325	488373	0.518
Fungal communities	4.021	5.916	1484	2208	0.844

After verifying for normal distribution, a two-way analysis of variance (ANOVA) was performed using the growth period and different invasion levels of the sample plots as processing factors, and a one-way ANOVA was performed for all indicators. Multiple comparisons were performed using the LSD and Tukey methods ($P < 0.05$). The Origin 9.0 software was used for drawing. The data in the chart are represented as the average ± standard error.

The soil nitrogen conversion rate was calculated using the following equation :

$$NN_{min} = \frac{(M_t + N_t) - (M_0 + N_0)}{t}$$

$$NN_{nit} = \frac{N_t - N_0}{t}$$

$$NN_{amm} = \frac{M_t - M_0}{t}$$

where NN_{min} is the net nitrogen mineralization rate ($mg \cdot g^{-1} \cdot d^{-1}$), NN_{nit} is the net nitrogen nitrification rate ($mg \cdot g^{-1} \cdot d^{-1}$), NN_{amm} is the net nitrogen ammonification rate ($mg \cdot g^{-1} \cdot d^{-1}$), N_0 and N_t are the nitrate concentrations at the beginning and after t of culture, respectively, and M_0 and M_t are the ammonium concentrations at the beginning and after t of culture, respectively [40].

The "ggplot 2" package in the R (Version 4.2.1) software was used to draw a box diagram and to visualize the soil microbial community at taxonomic levels to determine the differences in the alpha diversity and composition of soil microbial communities in sample plots with different degrees of invasion. The "ape" package in R and principal coordinate analysis based on the Bray–Curtis heterogeneity matrix were used to visualize the structural composition of soil microbial communities in the sample plots with different degrees of invasions. The SparCC algorithm was used together with the "ggraph" and "igrraph" software packages in R to construct a network topology index table to observe differences in different soil microbial communities. In addition, the OmicShare tool was used to create network heatmaps of environmental factors and the diversity of soil bacterial, fungal, and nitrogen transformation-related microbial communities. To examine the effect of *Cenchrus spinifex* Invasion on microbial β-diversity, Permutational multivariate analysis of variance (PERMANOVA) analysis was used. Finally, the effects of environmental factors on microbial community structure were analyzed by constrained ordinal redundancy analysis (RDA) in CANOCO 5.0 software (Microcomputer Power, Ithaca, NY, USA). The effect of each variable was assessed using an RDA-based Monte Carlo test (999 permutations).

3. Results

3.1. Effects of Cenchrus spinifex invasion on soil physicochemical properties

We used double factor variance analysis of variance to compare the interaction between two periods (Table 1). The content of EC, TN, SOC, TP in the soil of the monodominant community invaded by *Cenchrus spinifex* were significantly lower during the vegetative growth period than during the reproductive growth period ($P < 0.05$) (Table 1). The soil pH during the vegetative growth period of the mixed plant community was significantly higher than that during the reproductive growth period ($P < 0.05$), while the NN_{amm} rate during the reproductive growth period was higher than that during the vegetative growth period ($P < 0.05$).

During the reproductive growth period, with the increase in the invasion degree of *Cenchrus spinifex*, the content of TN decreased significantly, while the content of AN increased significantly ($P < 0.05$). At the reproductive growth period, the TN of mixed plant community and the AN of monodominant community are significantly increased ($P < 0.05$). During the vegetative growth period, the NH_4^+-N content in the mixed community was significantly higher than that in the monodominant community ($P < 0.05$). Additionally, the TP content in the monodominant community significantly increased in vegetative growth period, while the reproductive growth period content was significantly lower than that of bare land ($P < 0.05$). In vegetative growth period, the AP content increases with the increase of invasion degree ($P < 0.01$). During the reproductive growth period, the AP content in the monodominant community was significantly lower than that in the local plant community ($P < 0.05$). The SOC content of mixed community was significantly higher in vegetative growth period than in local plant community, whereas, during the reproductive growth period, it was significantly lower

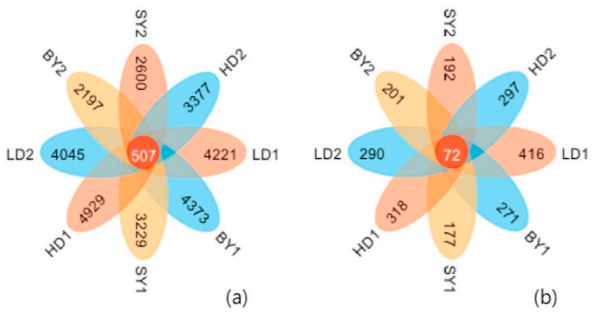

Fig. 1. OTU Venn map of bacterial (a) and fungal (b) communities in different invasive habitats at different growth periods.

than in bare land ($P < 0.05$). The EC of mixed community in vegetative growth period was significantly higher than that of local plant community ($P < 0.05$), and during the reproductive growth period, the EC of *Cenchrus spinifex* invasive sites significantly decreased ($P < 0.05$). In vegetative growth period, the soil pH in the monodominant community was significantly lower than that of local community ($P < 0.05$). With the invasion by *Cenchrus spinifex*, NN_{nit} significantly decreased during the vegetative growth period but significantly increased during the reproductive growth period ($P < 0.05$). With an increase in the invasion degree of *Cenchrus spinifex*, NN_{min} significantly increased during the vegetative growth period ($P < 0.05$).

3.2. Effects of Cenchrus spinifex invasion on soil microbial communities

3.2.1. Operational taxonomic unit (OTU) cluster comparison of bacterial and fungal communities

The analysis of OTU clustering comparison results of soil bacteria and fungi showed that the total number of soil bacterial OTUs in different growth periods and invasion levels of sample plots was significantly higher than the total number of soil fungal OTUs. The total number of OTUs of soil bacteria in different invasion levels of vegetative and reproductive growth periods in the sample plots was 33027 (Fig. 1a). The total number of OTUs in soil fungi with different degrees of invasion in vegetative and reproductive growth periods plots was 2738 (Fig. 1b). And there were 507 total OTUs of soil bacteria in different invasion levels of plots during two growth periods. In terms of reproductive growth period, the local plant community had the lowest number of bacterial communities, with only 2704 OTUs. During the vegetative growth period, mixed community had the highest number of bacterial communities, with 4929 unique OTUs. There were 72 total OTUs of soil fungi in different invasion levels of plots during two growth periods. During the vegetative growth period, with the lowest number of fungal communities and 177 unique OTUs in a single dominant community. During the vegetative growth period, with the highest number of naked fungal communities and 416 unique OTUs.

3.2.2. Structural composition of bacterial and fungal communities

The distribution differences of soil bacteria at the "phylum" classification level after the invasion by *Cenchrus spinifex* were shown in Fig. 2 (a). The dominant phyla of soil bacterial communities in all samples were Actinobacteria, Proteobacteria and Acidobacteria, with the average relative abundance of 36.43 %, 26.07 % and 11.36 %, respectively. The relative abundance of Patescibacteria during the reproductive growth period was significantly higher than that during the vegetative growth period (25.74 %; 23.91 %) ($P < 0.05$). During the vegetative growth period, the relative abundance of Actinobacteria in the mixed and monodominant communities was significantly lower than that in the bare land (33.71 %; 33.66 %; 42.69 %) ($P < 0.05$), whereas the relative abundance of Proteobacteria in the monodominant community was significantly higher than that in the bare land and local plant communities (31.00 %; 21.89 %; 23.21 %) ($P < 0.05$). During the reproductive growth period, the relative abundance of Proteobacteria in the mixed community was significantly lower than that in the bare land (22.61 %; 30.14 %) ($P < 0.05$), whereas the relative abundance of Planctomycetes in the mixed and local plant communities was significantly higher than that in the bare land (2.76 %; 2.25 %; 2.16 %) ($P < 0.05$).

The dominant phyla of soil fungal communities in all samples were Ascomycota, Bassidiomycota, and Mortierellomycota, with the average relative abundance of 83.30 %, 3.91 % and 1.50 %, respectively (Fig. 2b). There was no significant difference in the relative abundance of soil fungal communities between the vegetation and reproductive growth periods or between different habitats during the vegetation and reproductive growth periods.

3.2.3. Beta-diversity analysis and association network diagram of bacterial and fungal communities

The sum of PCo1 and PCo2 explained 33.9 % and 31.8 % of the changes in soil bacterial and fungal communities, respectively (Fig. 3). According to the PERMANOVA analysis, there was a significant difference in the beta diversity of bacterial communities ($F = 1.345$, $P = 0.013$) between two different growth periods, while there was no significant difference in the beta diversity of fungal communities. The distribution of bacterial communities in different invasive habitats is relatively independent, with significant differences between bare land and mixed communities ($F = 1.869$, $P = 0.025$) (Fig. 3a). The distance matrix of the four invasive fungal communities is relatively dense, with high similarity and small differences in the community structure (Fig. 3b).

The network coexistence relationship constructed for bacterial communities was relatively complex, whereas the network coexistence relationship constructed for fungal communities was relatively simple (Fig. 4a and b). In addition, the topology parameters of the network showed that the soil bacterial community network contained 19,325 nodes and 488,373 edges, whereas the soil fungal

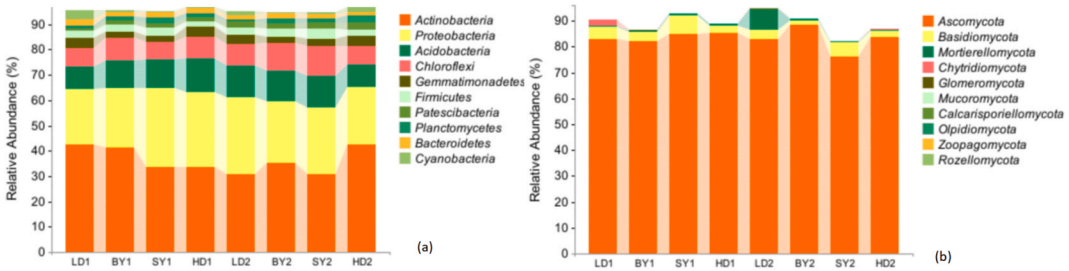

Fig. 2. Proportion of the main microbial communities at the "phylum" level in the soil invaded by *Cenchrus spinifex*.

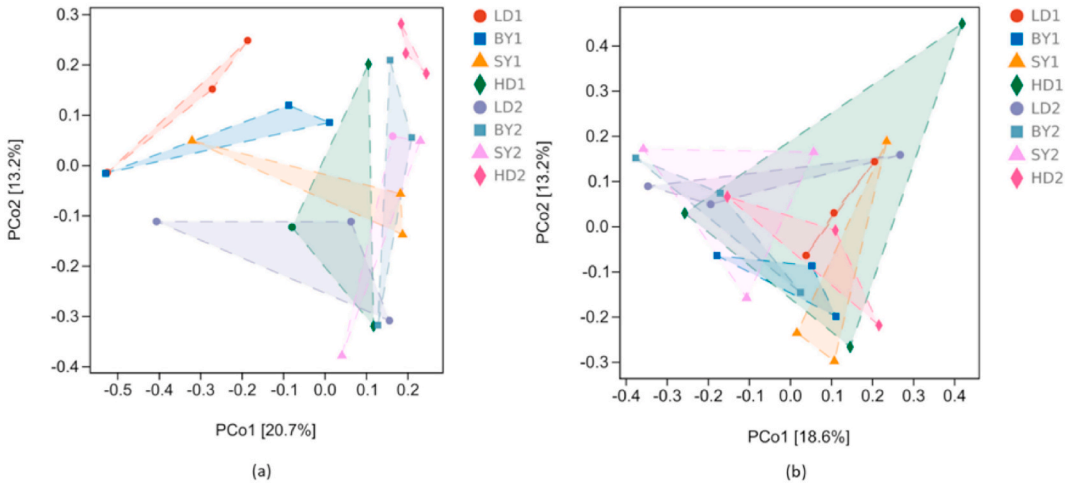

Fig. 3. PCoA of bacterial and fungal communities based on bray_curtis distance.

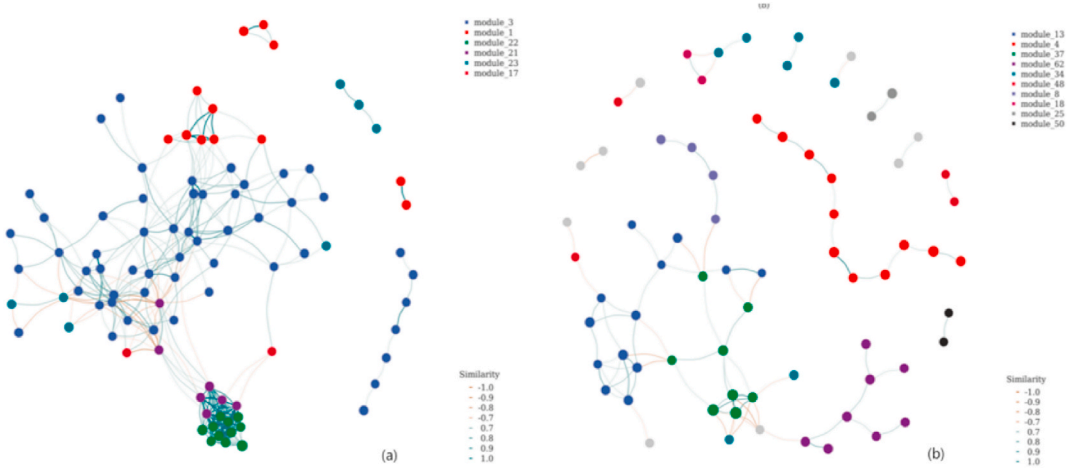

Fig. 4. Association network diagram of bacterial and fungal communities. Note: Different colors were used to identify the modules with the most nodes of the top 10 nodes.

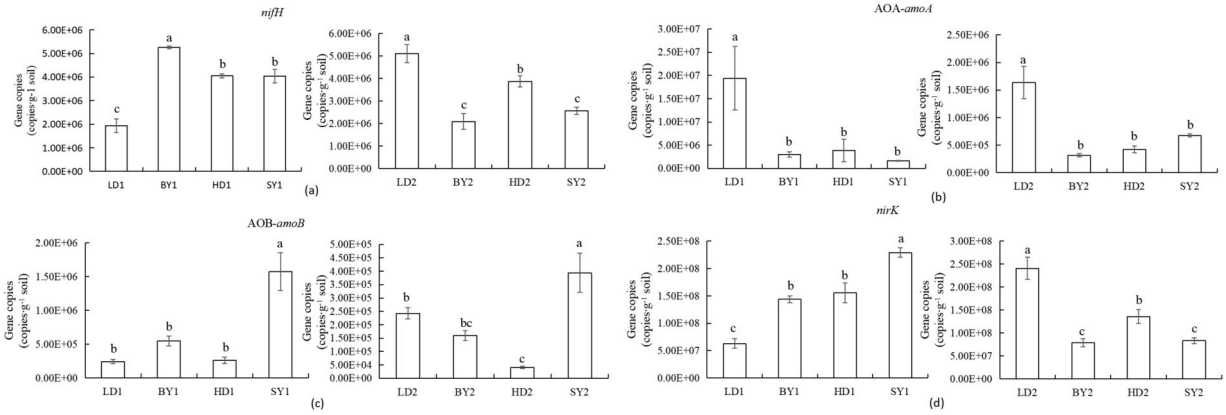

Fig. 5. Gene copy numbers of soil nitrogen transformation-related microorganisms in different invasive habitats at different growth periods.

community network only contained 1484 nodes and 2208 edges (Table 2). And the nodes of the soil bacterial network were more tightly connected than the nodes of the soil fungal network.

3.3. Response of soil functional genes to the invasion by Cenchrus spinifex

The abundance of nifH gene in monodominant and mixed communities during the vegetative growth period was significantly lower than that in the local community ($P < 0.05$). The abundance of *nifH* gene in the monodominant community was significantly lower than that in the mixed community during the reproductive growth period ($P < 0.05$) (Fig. 5a). The abundance of AOA-*amoA* in the bare land during the vegetative and reproductive growth periods was significantly higher than that in the other habitats ($P < 0.05$) (Fig. 5b), whereas the abundance of AOB-*amoB* in the monodominant community was significantly higher than that in other habitats (Fig. 5c). The abundance of *nirK* gene significantly increased with an increase in invasion degree during the vegetative growth period ($P < 0.05$), and the gene abundance in the monodominant community during the reproductive growth period was significantly lower than that in the bare land ($P < 0.05$) (Fig. 5d).

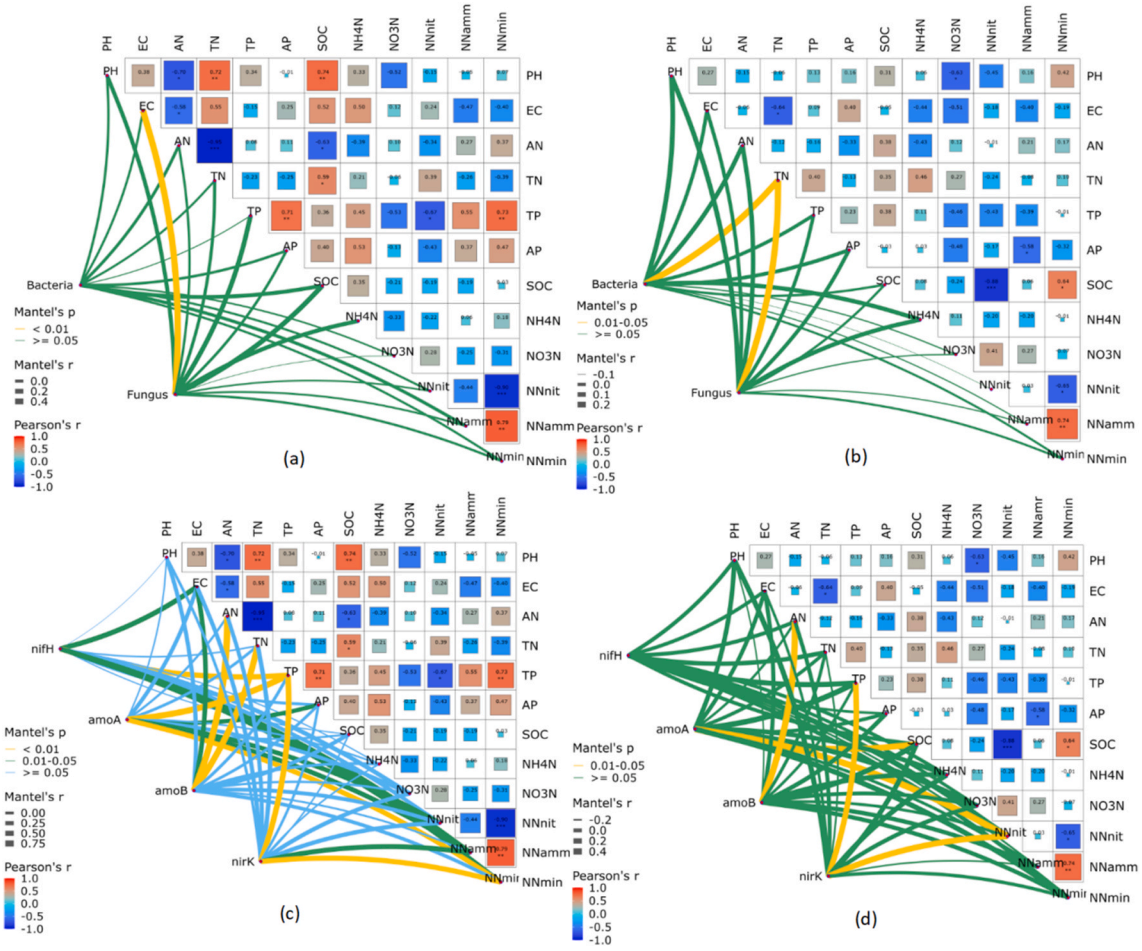

Fig. 6. Correlation between environmental factors, soil nitrogen mineralization, diversity of soil bacteria and fungi, and abundance of nitrogen transformation related microorganisms.

Note: The right triangle diagram shows Pearson correlation coefficients between the physical and chemical properties of different soils and nitrogen conversion rates. See the left line diagram for Mantel test results. For mantel analysis, we used proportional values of soil physicochemical properties and nitrogen conversion rates. Before calculating the Euclidean distance, the alpha diversity was scaled. The meanings of abbreviations for soil physical and chemical properties, functional genes, and nitrogen conversion rate are shown in the text. The line width corresponds to the partial Mantel r statistic, and the line color represents statistical significance based on 999 permutations. The environmental factors were compared in pairs. Color gradients are used to express Pearson correlation coefficients. The asterisk indicates statistical significance.

3.4. Correlation between soil microbial community and environmental factors

3.4.1. Correlation between environmental factors, soil nitrogen mineralization, and diversity soil bacterial, fungal, and nitrogen transformation-related microbial communities

During the vegetative growth period of *Cenchrus spinifex* invasion, there was a negative correlation among physicochemical properties such as soil pH, EC, SOC, TN, and AN and a positive correlation among soil pH, SOC, and TN. There was a positive correlation between soil pH and SOC ($r = 0.74$, $P < 0.01$). TP was positively correlated with AP and NN_{min} ($r < 0.73$, $P < 0.01$) and negatively correlated with NN_{nit} ($r = -0.67$, $P < 0.05$). NN_{min} and NN_{nit} were negatively correlated with each other ($r = -0.90$, $P < 0.001$) and positively correlated with NN_{amm} ($r = 0.79$, $P < 0.01$). The Mantel test showed a significant positive correlation between the soil fungal community diversity and EC ($P < 0.01$) (Fig. 6a). During the reproductive growth period, soil pH, NO_3^+-N and EC were negatively correlated with TN, AP was negatively correlated with NN_{amm} and SOC was negatively correlated with NN_{nit}. NN_{min} and NN_{nit} were negatively correlated with each other ($r = -0.65$, $P < 0.05$) and positively correlated with NN_{amm} ($r = -0.65$, $P < 0.05$). The Mantel test showed that the diversity of soil bacterial and fungal communities was significantly positively correlated with TN ($P < 0.05$) (Fig. 6b).

During the vegetative growth period of *Cenchrus spinifex*, the abundance of *nifH*, AOA-*amoA* and *nirK* was significantly positively correlated with TP ($P < 0.01$) (Fig. 6c). The abundance of AOA-*amoA* and *nirK* was significantly and positively correlated with NN_{min} ($P < 0.01$). The abundance of AOB-*amoB* was significantly and positively correlated with AN and TN ($P < 0.01$). The abundance of *nifH* was significantly and positively correlated with EC and NN_{amm} ($P < 0.05$). The abundance of *amoA* was significantly and positively correlated with AP, NN_{nit} and NN_{amm} ($P < 0.05$). The abundance of AOB-*amoB* was significantly and positively correlated with EC ($P < 0.05$). The abundance of *nirK* was significantly and positively correlated with NN_{amm} ($P < 0.05$). During the reproductive growth period, the abundance of *amoA* was significantly and positively correlated with SOC and NN_{nit} ($P < 0.05$) (Fig. 6d). The abundance of AOB-*amoB* was significantly and positively correlated with AN ($P < 0.05$). The abundance of *nirK* was significantly and positively correlated with TP and NN_{nit} ($P < 0.05$).

3.4.2. Redundancy analysis (RDA) of environmental factors and soil microbial communities

The cumulative explanatory variances of environmental factors and nitrogen conversion rates reached 47.14 % and 9.21 % for changes in the bacterial community structure and 52.94 % and 7.41 % for changes in the fungal community structure, respectively. The first axis can better reflect the relationship between the soil bacterial community structure and the basic physical and chemical properties of soil and soil nitrogen mineralization.

AN, SOC, TN, NN_{min} and NN_{nit} were the main driving factors that affected the change in bacterial communities in different invasive habitats samples (Fig. 7a). The abundance of Proteobacteria was significantly and positively correlated with AN, NN_{min} and NN_{amm} ($P < 0.05$). The abundance of Actinomycetes was significantly and negatively correlated with NO_3^+-N, NH_4^+-N and soil pH ($P < 0.05$), whereas it was significantly and positively correlated with NN_{nit} ($P < 0.05$). The abundance of Acidobacteria and Chloroflexi was significantly and positively correlated with SOC, EC, AP and TP and negatively correlated with TN ($P < 0.05$).

EC and NN_{nit} were the main driving factors that affected the changes in fungal communities in different types of soil samples (Fig. 7b). The abundance of Ascomycota was significantly and positively correlated with AP and SOC and negatively correlated with NO_3^+-N ($P < 0.05$). The abundance of Mortierellomycota was significantly and positively correlated with EC, soil pH, TN and NN_{min} and negatively correlated with TP, SOC and AP ($P < 0.05$). The abundance of Bassidiomycota was significantly and positively correlated

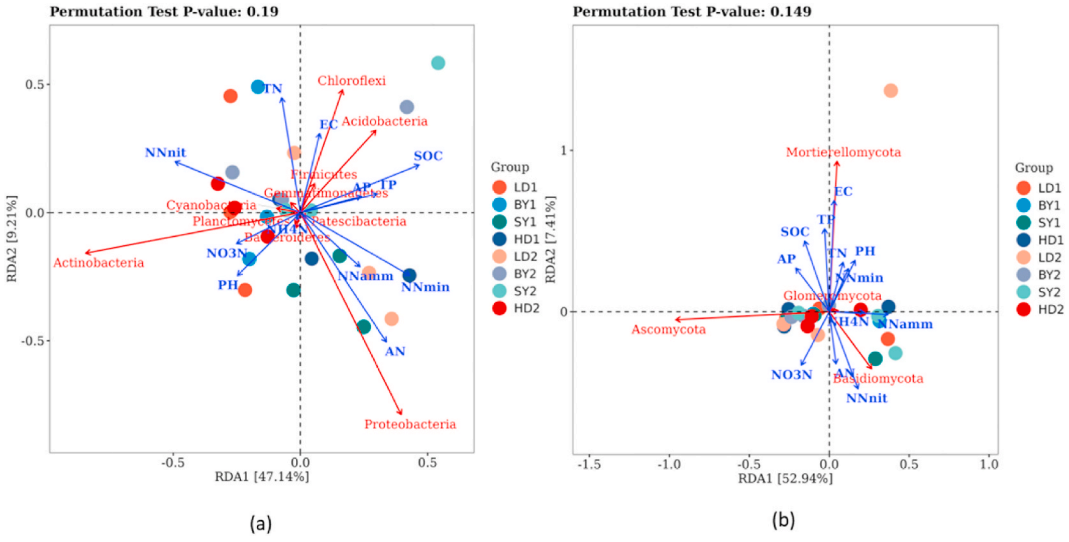

Fig. 7. RDA analysis of soil bacterial and fungal communities and environmental factors.

with NN_{nit}, AN and NN_{amm} and negatively correlated with NO_3^+-N ($P < 0.05$).

4. Discussion

4.1. Preference of Cenchrus spinifex invasion for nitrogen form utilization

Analysis of soil physicochemical properties showed that *Cenchrus spinifex* invasion significantly decreased TN content ($P < 0.05$) (Table 1). Plant invasion could alter factors such as soil chemical properties and soil moisture conditions, which may affect the adsorption, desorption, fixation, and release of nitrogen in the soil, thereby affecting the content and utilization efficiency of total nitrogen in the soil [41]. In this study, *Cenchrus spinifex* intrusion significantly increased the AN content ($P < 0.05$). The invasion of *Spartina alterniflora* had changed the soil nitrogen environment, increased the total nitrogen content of the soil, and increased the organic nitrogen content of the soil [42].

Rhus typhina L. preferred soil NH_4^+-N, and the content of NH_4^+-N in the soil decreased after invasion [21]. Research by Zhang et al. suggested that *Cenchrus spinifex* invasion preferred NH_4^+-N in soil [43]. Invading plants converted into amino acids they need, and assimilating NH_4^+ consumes more energy than NO_3^- [44]. And invasive plants could promote their own growth and colonization by changing the nutrient status of the invaded area [45]. The change of soil nitrogen pool caused by *Cenchrus spinifex* invasion was beneficial for its invasion, which may be a mechanism for *Cenchrus spinifex* to enhance ecological adaptability and achieve rapid spread and reproduction in the Horqin area.

4.2. The impact of Cenchrus spinifex on soil environment and related microbial communities at different growth periods

As the invasion degree of *Cenchrus spinifex* in vegetative and reproductive growth periods increased, soil pH and EC significantly decreased ($P < 0.05$) (Table 1). The soil pH value of *Rhus typhina* L. invasion decreased [21]. The root system of invasive plants was distributed in an extended pattern in the soil, and the soil structure was also affected by this, resulting in an increase in soil nutrients and promoting the dissolution of anions and cations, resulting in a lower pH value [46]. The content of TP and AP in vegetative growth period soil significantly increased, while the content of TP in reproductive growth period soil significantly decreased ($P < 0.05$). *Solidago canadensis* could promote the concentrated distribution of phosphorus in the soil towards the rhizosphere, thereby increasing the phosphorus absorption efficiency of plants [47]. Invasive plants had different phosphorus requirements and utilization rates at different growth stages [48].

We found significant differences in the relative abundance of individual bacterial phyla among different categories, while there was no significant difference in the relative abundance of individual fungal phyla, which is similar to previous studies [47]. The relative abundance of Actinomycetes was significantly lower than that bare land ($P < 0.05$), which may be due to their enzyme inhibitor and antibacterial properties, which were beneficial for protecting local plant growth and soil conditions, through eliminating harmful microorganisms [49,50]. The invasion of *Cenchrus spinifex* increased the abundance of Proteobacteria ($P < 0.05$) (Fig. 2). Proteobacteria has the ability to degrade cellulose and chitin, which can alter the nutrient status of the soil [51]. The changes in the relative abundance of Actinomycetes and Proteobacteria may to some extent reflect the impact of less invasion on the soil environment.

In vegetative growth period, the abundance of *nifH* gene significantly increased with the increase of *Cenchrus spinifex* invasion degree (Fig. 5). This was consistent with previous research findings that the invasive plant *Eupatorium adenophorum* significantly increased the abundance of *nifH* genes in soil [24]. But during reproductive growth period, *Cenchrus spinifex* invasion can reduce the abundance of *nifH* genes in soil. A study showed that after the invasion of *Acacia*, the abundance of nitrogen fixing bacteria in the soil surface (0–5 cm) decreased [52]. The distinct reaction of nifH genes to invasionmay be related to the interaction between the root exudates of invasive plants and rhizosphere microorganisms. The nutrients and energy in the root exudates can promote microbial reproduction and growth, but they may also lead to a decrease in the metabolic activity of nitrogen fixing bacteria in the soil, thereby affecting the abundance and community structure of nitrogen fixing bacteria encoded by the nifH gene [26]. In this study, the abundance of AOB-*amoB* in *Cenchrus spinifex* vegetative growth period was significantly higher than that in reproductive growth period, and the trend of AOB-*amoB* abundance also increased with *Cenchrus spinifex* invasion. Some studies had also found that the root exudates of invasive plants could promote the growth and activity of nitrogen transforming microorganisms in soil, thereby improving soil nitrogen cycling ability and further increasing the abundance of AOB-*amoB* ($P < 0.05$) [53]. The abundance of AOA-*amoA* in *Cenchrus spinifex* during vegetative growth period was significantly higher than that in reproductive growth period. The abundance of AOA-*amoA* in the rhizosphere soil decreased withthe *Cenchrus spinifex* invasion. A study on *Pinus tabulaeformis*, a typical invasive plant in Northeast China, found that the abundance of AOA-*amoA* in the vegetative growth period soil was about twice higher than that in reproductive growth period. This may be related to the role of plant root exudates, which can promote the reproduction and growth of microorganisms in the soil, thereby increasing the abundance of AOA-*amoA*. Besides, the invasion of *Cenchrus spinifex* in vegetative growth period increased the abundance of *nirK*. However, invading during reproductive growth period reduced the abundance of *nirK*. Studies have shown that invasive plants can alter the nitrogen cycling process in soil, thereby affecting the abundance and diversity of denitrifying bacteria in the soil [54,55]. The invasion of *Typha orientalis Presl* increased the nitrogen content in the soil, thereby increasing the abundance of denitrifying bacteria [56].

4.3. The impact of Cenchrus spinifex invasion on soil nitrogen cycling and the response of microbial communities to invasion

Nitrogen deposition could reduce the diversity of soil microbial communities and change their original main microbial community

structure; however, most research results were inconsistent [57,58]. The soil nitrification process in the early growth season is the main process of soil nitrogen transformation; additionally, the soil ammonification process in the late growth season and the non-growth season is the main process of soil nitrogen transformation [59]. In this study, soil TP during the vegetative growth period and SOC during the reproductive growth period were positively correlated with NN_{min} and negatively correlated with NN_{nit}. During the vegetative growth period, NN_{nit} was significantly positively correlated with the abundance of AOA-*amoA*. NN_{amm} was significantly positively correlated with the abundance of *nifH*, AOA-*amoA* and *nirK*. NN_{min} was significantly and positively correlated with the abundance of AOA-*amoA*, *nifH* and *nirK* ($P < 0.05$). During the reproductive growth period, NN_{nit} was significantly and positively correlated with the abundance of AOA-*amoA* and *nirK* ($P < 0.05$). Atmospheric nitrogen deposition is mostly dominated by ammonia and nitrate, and microorganisms can directly utilize it, which in turn accelerates the soil nitrogen cycle, leading to the active growth of soil microorganisms related to the nitrogen cycle during the vegetative and reproductive growth periods [60].

Microbial interactions play an important role in maintaining soil functions, such as plant nutrient acquisition and soil formation in the microbial environment [61,62]. Co-occurrence network analysis can unlock complex microbial interactions [63], and network topology features can be used to define interactions between microbial communities [64]. In this study, the topological characteristics of soil bacterial communities (including the number of nodes, edges, and the average degree of bacterial network) were higher than those of fungal communities, and the relationship between bacterial communities was closer, while the relationship between fungal communities was looser, with more negative correlation edges for bacteria than for fungi (Fig. 4; Table 2). This means that the main behavior of the bacteria in *Cenchrus spinifex*-invaded soil should be competitive interaction, while the main behavior of the fungi would be cooperative interaction to adapt to environmental changes [65], which indicated that bacterial communities have a strong resistance to invasion. Some scholars have reported that there is no significant difference in the alpha diversity of bacterial and fungal communities with the intensification of the invasion by *Deyeuxia angustifolia* [65], which was consistent with the results obtained in this study during the vegetative and reproductive growth periods. However, the results of the principal component analysis of bacterial and fungal communities showed that the invasion by *Cenchrus spinifex* significantly affected the composition of soil bacterial community structure ($P < 0.05$), though had no significant impact on fungal communities (Fig. 3), which indicated that bacterial communities would be more sensitive to invasion. Studies had shown that *Moso bamboo* invasion led to significant changes in soil microbial activity and community structure [66]. This is also consistent with our hypothesis that the invasion by *Cenchrus spinifex* would significantly affect the soil microbial community, and the response of bacteria and fungi to invasion is inconsistent.

Environmental factors could significantly affect relatively abundant microbial communities [67], though the dominant factors affecting bacterial and fungal communities were inconsistent. RDA analysis showed that soil AN, SOC, TN, NN_{min} and NN_{nit} were the main driving factors that affected the changes in bacterial communities in soil samples with different invasion degrees. Soil EC and NN_{amm} were the main driving factors that affected the changes in fungal communities in different types of soil samples (Fig. 7). Soil AN can be directly absorbed and utilized in soil and is an important indicator to measure soil nitrogen supply capacity and reflect soil nitrogen availability [68]. The key parameter of the soil organic carbon cycle is microbial carbon utilization efficiency, and the soil organic carbon content significantly affects the activity of soil microorganisms [69]. The TN content in soil has multiple effects on the growth, composition, and function of microorganisms [70]. Soil EC affects the transformation and cycling of phosphorus and carbon in the soil, thereby affecting microbial communities [71]. Correlation analysis indicated a significantpositive correlation between the soil TN content and the diversity of soil bacterial and fungal communities due to the invasion by *Cenchrus spinifex* during the reproductive growth period (Fig. 6). The analysis within this study indicated that soil TN was the main driving factor affecting the soil microbial communities. Soil bacteria use TN as a nutrient, and nitrogen is a limiting factor for biological growth. Many studies have shown that changes in soil nitrogen could cause changes in soil microbial activity, biomass, and community composition [72,73].

5. Conclusion

This study provided a comprehensive analysis on the diversity and structural composition of soil bacterial and fungal communities under the background of nitrogen deposition, key microbial community abundence related to nitrogen cycling, and the corresponding influencing environmental factors, which were affected by *Cenchrus spinifex* invasion in different growth periods. *Cenchrus spinifex* invasion reduced competition and chaged soil nitrogen transforming by the preference of ammonium soil nitrogen. The invasion reduced soil pH, EC and TN content, while increasing AN content and net nitrogen mineralization rate, further altering the structure and composition of microbial communities. However, the response of soil bacterial and fungal communities to invasion was different. The composition and structure of soil bacterial communities showed a more significant response to invasion. At the same time, the relationship between vegetative growth and reproductive growth of *nirK* abundance showed different patterns. The invasion resulted in a significant increase in the abundance of *nifH* and AOA-*amoA*, while the abundance of AOB-*amoB* decreased significantly, thereby affecting soil nitrogen cycling. These observation results would provide new insights into the relationship between microbial community structure and environmental factors during the invasion process of *Cenchrus spinifex* at different growth periods, thus providing clues for us to understand the microbial ecological functions in sandy grassland ecosystems.

Data availability statement

The DNA sequences in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession number PRJNA960835.

CRediT authorship contribution statement

Baihui Ren: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Resources, Validation, Data curation. **Meng Meng:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, Resources. **Jianxin Yu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software. **Xinwei Ma:** Data curation, Formal analysis, Investigation, Methodology, Software. **Daiyan Li:** Data curation, Formal analysis, Investigation, Methodology, Software. **Jiahuan Li:** Methodology, Supervision, Validation, Visualization. **Jiyun Yang:** Investigation, Supervision, Validation, Visualization. **Long Bai:** Supervision, Validation, Conceptualization. **Yulong Feng:** Supervision, Validation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the China Postdoctoral Science Foundation (program, 2021M693865) and the Liaoning Provincial Scientific Research Fund (LJKZ0638).

References

[1] W. Yang, N. Jeelani, X. Leng, et al., Spartina alterniflora invasion alters soil microbial community composition and microbial respiration following invasion chronosequence in a coastal wetland of China, Sci. Rep. 6 (1) (2016), 26880, https://doi.org/10.1038/srep26880.
[2] S.L. Flory, J.T. Bauer, Experimental evidence for indirect facilitation among invasive plants, J. Ecol. 102 (1) (2014) 12–18, https://doi.org/10.1111/1365-2745.12186.
[3] G. Zeng, Z. Hua, G. Huang, et al., Physicochemical and microbiological effects of biosurfactant on the remediation of hoc-contaminated soil, Prog. Nat. Sci.: Mater. Int. 15 (7) (2005) 9, https://doi.org/10.1080/10020070512331342590.
[4] E. Marchante, A. Kjciller, S. Struwe, H. Freitas, Short- and long-term impacts of acacia longifolia invasion on the belowground processes of a mediterranean coastal dune ecosystem, Appl. Soil Ecol. 40 (2) (2008) 210–217, https://doi.org/10.1016/j.apsoil.2008.04.004.
[5] N. Torres, I. Herrera, L. Fajardo, et al., Meta-analysis of the impact of plant invasions on soil microbial communities, BMC Ecol Evo 21 (2021) 172, https://doi.org/10.1186/s12862-021-01899-2.
[6] R.A. Lankau, Coevolution between Invasive and Native Plants Driven by Chemical Competition and Soil Biota, 2012, https://doi.org/10.1073/pnas.1201343109.
[7] M.A. Shah, Z.A. Reshi, D. Khasa, Arbuscular mycorrhizal status of some Kashmir Himalayan alien invasive plants, Mycorrhiza 20 (2009) 67–72, https://doi.org/10.1007/s00572-009-0258-x.
[8] G. Zhang, J. Bai, C.C. Tebbe, et al., Spartina alterniflora invasions reduce soil fungal diversity and simplify co-occurrence networks in a salt marsh ecosystem, Sci. Total Environ. 758 (2021), 143667, https://doi.org/10.1016/j.scitotenv.2020.143667.
[9] M. Liao, Xm Xie, Y. Peng, et al., Characteristics of soil microbial community functional and structure diversity with coverage of Solidago Canadensis L, J. Cent. South Univ. 20 (2013) 749–756, https://doi.org/10.1007/s11771-013-1544-5.
[10] Z. Song, Q.F. Ji, W.D. Fu, et al., Effects of Flaveria bidentis invasion on the diversity of functional bacteria in rhizosphere soil, Chin. J. Appl. Ecol. 27 (2016) 2636–2644, https://doi.org/10.13287/j.1001-9332.201608.013.
[11] Y. Lekberg, S. Gibbons, S. Rosendahl, et al., Severe plant invasions can increase mycorrhizal fungal abundance and diversity, ISME J. 7 (2013) 1424–1433, https://doi.org/10.1038/ismej.2013.41.
[12] W. Wan, G.M. Gadd, Y. Yang, et al., Environmental adaptation is stronger for abundant rather than rare microorganisms in wetland soils from the Qinghai-Tibet Plateau, Mol. Ecol. 30 (10) (2021) 2390–2403, https://doi.org/10.1111/mec.15882.
[13] H. Qin, L. Niu, Q. Wu, et al., Bamboo forest expansion increases soil organic carbon through its effect on soil arbuscular mycorrhizal fungal community and abundance, Plant Soil 420 (2017) 407–421, https://doi.org/10.1007/s11104-017-3415-6.
[14] H. Liu, Y. Zhou, C. Qi, et al., Effects of Mikania micrantha invasion on soil nutrient contents and enzyme activities, Ecology and Environment | Ecol Environ 21 (12) (2012) 1960–1965.
[15] X. Luo, Q. Shi, Y. Jia, et al., Dynamics of soil enzyme activities and soil nutrients in the rhizosphere at different growth stages of three invasive plants[J], Journal of Biosafety 26 (4) (2017) 8, https://doi.org/10.3969/j.issn.2095-1787.2017.04.007.
[16] J. Cui, J. Zhou, Y. Peng, et al., Atmospheric wet deposition of nitrogen and sulfur in the agroecosystem in developing and developed areas of Southeastern China, Atmos. Environ. 89 (2014) 102–108, https://doi.org/10.1016/j.atmosenv.2014.02.007.
[17] N. Wang, H. Chen, Increased nitrogen deposition increased the competitive effects of the invasive plant Aegilops tauschii on wheat, Acta Physiol. Plant. 41 (10) (2019) 1–9, https://doi.org/10.1007/s11738-019-2968-9.
[18] L. Gao, X.Y. Cui, Hill, et al., Uptake of various nitrogen forms by co-existing plant species in temperate and cold-temperate forests in northeast China, Appl. Soil Ecol. 147 (2020), 103398, https://doi.org/10.1016/j.apsoil.2019.103398.
[19] F. Song, Phillips Jonathan, Shouse Michael, Biogeomorphic impacts of invasive species, Annu. Rev. Ecol. Evol. Systemat. 45 (1) (2014) 69–87, https://doi.org/10.1146/annurev-ecolsys-120213-091928.
[20] E. Philip, P.E. Hulme, Belinda Gallardo, et al., Bias and error in understanding plant invasion impacts, Trends Ecol. Evol. 28 (4) (2013), https://doi.org/10.1016/j.tree.2012.10.010.
[21] Q. Huang, H. Xu, Z. Fan, et al., Effects of Rhus typhina invasion into young Pinus thunbergii forests on soil chemical properties[J], Ecol. Environ. (7) (2013) 1119–1123, https://doi.org/10.3969/j.issn.1674-5906.2013.07.005.
[22] N. Scott, S. Saggar, D. Mcintosh, Biogeochemical impact of Hieracium invasion in New Zealand's grazed tussock grasslands: sustainability implications[J], Ecol. Appl. 11 (5) (2001) 1311–1322, https://doi.org/10.1890/1051-0761.
[23] H. Yu, J.J. Le Roux, Z. Jiang, et al., Soil nitrogen dynamics and competiti.on during plant invasion: insights from Mikania micrantha invasions in China, New Phytol. 229 (6) (2021) 3440–3452, https://doi.org/10.1111/nph.17125.
[24] L. Dai, H. Li, Z. Jiang, et al., Invasive effects of Ageratina adenophora (Asteraceae) on the changes of effective functional bacteria, enzyme activity and fertility in rhizosphere soil ecosystem[J], Ecol Environ Sci 21 (2) (2012) 237–242, https://doi.org/10.3969/j.issn.1674-5906.2012.02.007.
[25] Y. Zhang, L. Chen, W. Wang, et al., Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea, Front. Microbiol. (2019), 102731, https://doi.org/10.1016/j.soilbio.2016.05.014.

[26] C.L. Piper, E.G. Lamb, S.D. Siciliano, Smooth brome changes gross soil nitrogen cycling processes during invasion of a rough fescue grassland, Plant Ecol. 216 (2015) 235–246, https://doi.org/10.1007/s11258-014-0431-y.

[27] H. Wang, J. Su, T. Zheng, et al., Insights into the role of plant on ammonia-oxidizing bacteria and archaea in the mangrove ecosystem, J. Soils Sediments 15 (2015) 1212–1223, https://doi.org/10.1007/s11368-015-1074-x.

[28] N. Dassonville, N. Guillaumaud, F. Piola, et al., Niche construction by the invasive Asian knotweeds (species complex Fallopia): impact on activity, abundance and community structure of denitrifiers and nitrifiers, Biol. Invasions 13 (2011) 1115–1133, https://doi.org/10.1007/s10530-011-9954-5.

[29] Z. Sun, Q. Shu, K. Gao, et al., Invasion status ,adaptive mechanism and control strategy of field sandbur:a review[J], Acta Agrestia Sinica 28 (5) (2020) 1196–1202, https://doi.org/10.11733/j.issn.1007-0435.2020.05.003.

[30] J. Ma, Y. Zhang, X. Tian, et al., Studies on physiological adaptability of Cenchrus pauciflorus in different growth periodsof Horqin sandy land[J], Grassland and Turf 40 (6) (2020) 52–64, https://doi.org/10.13817/j.cnki.cyycp.2020.06.008.

[31] X. Gao, X. Yang, The distribution, harm, and prevention and control measures of Cenchrus pakaciflorus benth. In fuxin[J], Plant Quarantine (1) (2008) 64–65, https://doi.org/10.3969/j.issn.1005-2755.2008.01.030.

[32] E. Bolyen, J.R. Rideout, M.R. Dillon, et al., Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, Nat. Biotechnol. 37 (2019) 852–857, https://doi.org/10.1038/s41587-019-0209-9.

[33] R. Edgar, B. Haas, J. Clemente, et al., UCHIME improves sensitivity and speed of chimera detection, Bioinformatics 27 (16) (2011) 2194–2200, https://doi.org/10.1093/bioinformatics/btr381.

[34] A. Chao, Non-parametric estimation of the number of classes in a population, Scand. J. Stat. 11 (1984) 265–270.

[35] C.E. Shannon, A mathematical theory of communication, The Bell System Technical Journal 27 (1948) 379–423.

[36] E.H. Simpson, Measurement of diversity, Nature 163 (1949) 688.

[37] E.C. Pielou, An Introduction to Mathematical Ecology, John Wiley, New York, 1969, p. 286.

[38] I.J. Good, The population frequencies of species and the estimation of population parameters, Biometrika 40 (1953) 237–264.

[39] X. Ke, R. Angel, Y. Lu, et al., Niche diferentiation of ammonia oxidizers and nitrite oxidizers in rice paddy soil, Environ. Microbiol. 15 (8) (2013) 2275–2292, https://doi.org/10.1111/1462-2920.12098.

[40] J. Li, L. Sha, J. Wang, et al., Seasonal variation of soil nitrogen mineralization in a mount moist evergreen broad leaved forest in Ailao Mountains, SW China[J], J. Mt. Sci. 24 (2) (2006) 186–192, https://doi.org/10.1038/s41587-019-0209-9.

[41] Y. Liu, J. Xu, Y. Jin, et al., Relationship between herbaceous plant invasion and soil properties in the Changbai Mountain Tundra[J], Acta Ecol. Sin. 38 (4) (2018) 1235–1244, https://doi.org/10.5846/stxb201701050046.

[42] B. Jin, H. Yan, W. Zhang, et al., Contents and storages of various forms of nitrogen in soils of wetlands in the min river estuary under Spartina alterniflora invasion[J], Wetland Science 15 (3) (2017) 10.

[43] T. Zhang, W. Fu, R. Zhang, et al., Effects of invasive Cenchrus spinifex on nitrogen pools in sandy grassland[J], Chin. J. Appl. Ecol. 28 (5) (2017) 1522–1532, https://doi.org/10.13287/j.1001-9332.201705.040.

[44] X. Han, L. Li, J. Huang, An Introduction to Biogeochemistry (in Chinese), Higher Education Press, Beijing, 1999, 7-04-007757-4.

[45] M. Hejda, K. Štajerová, P. Pyšek, Dominance has a biogeographical component: do plants tend to exert stronger impacts in their invaded rather than native range? J. Biogeogr. 44 (1) (2017) 18–27, https://doi.org/10.1111/jbi.12801.

[46] Y. Hou, L. Liu, H. Chu, et al., Effects of exotic plant Rhus typhina invasion on soil properties in different forest types[J], Acta Ecol. Sin. 35 (16) (2015) 5324–5330, https://doi.org/10.5846/stxb201404160740.

[47] J. Lu, W. Qiu, J. Chen, et al., Impact of invasive species on soil properties: Canadian goldenrod (Solidago canadensis) as a case study[J], Biodivers. Sci. 13 (4) (2005) 10, https://doi.org/10.3321/j.issn:1005-0094.2005.04.008.

[48] R. Wang, Soil microecological mechanism of efficient utilization of phosphorus elements by Cenchrus spinifex[D], Chinese Academy of Agricultural Sciences (2020), 3-09-07.

[49] K. Amrita, J. Nitin, C.S. Devi, Novel bioactive compounds from mangrove derived actinomycetes, Int. Res. J. Pharm. 3 (9) (2012) 25–29.

[50] T. Arumugam, P. Senthil Kumar, R. Kameshwar, K. Prapanchana, Screening of novel actinobacteria and characterization of the potential isolates from mangrove sediment of south coastal India, Microb. Pathog. 107 (2017) 225–233, https://doi.org/10.1016/j.micpath.2017.03.035.

[51] H. Lin, T. Yao, Y. Ma, et al., Soil bacterial community changes across a degradation gradient in alpine meadow grasslands in the central Qilian Mountains[J], Acta Prataculturae Sinica 28 (8) (2019) 170–179, https://doi.org/10.11686/cyxb2018245.

[52] E. Pankhurst, S. Yu, G. Hawke, et al., Impact of Acacia auriculiformis invasion on microbial abundance and community structure in a coastal forest soil in southeastern China, Appl. Soil Ecol. 99 (2016) 7–16.

[53] L. Maron, M. Marler, Field assessment of mycorrhizal responsiveness of exotic plant species that are invading natural areas, Appl. Soil Ecol. 39 (2) (2008) 172–181.

[54] Q. Zeng, S.S. An, L. Chen, Effects of plant invasion on soil nitrogen-cycling microbial communities in a desert ecosystem, PLoS One 7 (7) (2012), e41046.

[55] Y. Li, X.R. Li, S. Wan, et al., Effects of invasive plant solidago canadensis on soil nitrogen mineralization and microbial community functional diversity, J. Soils Sediments 16 (1) (2014) 364–373.

[56] L. Angeloni, J. Jankowski, C. Tuchman, J. Kelly, Effects of an invasive cattail species (Typha × glauca) on sediment nitrogen and microbial community composition in a freshwater wetland, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 263 (2006) 86–92, https://doi.org/10.1111/j.1574-6968.2006.00409.x.

[57] M. Lu, Y. Yang, Y. Luo, et al., Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis, New Phytol. 189 (2011) 1040–1050, https://doi.org/10.1111/j.1469-8137.2010.03563.x.

[58] K. Button, J. Ioannidis, C. Mokrysz, et al., Erratum: Power failure: why small sample size undermines the reliability of neuroscience, Nat. Rev. Neurosci. 14 (2013) 365–376, https://doi.org/10.1038/nrn3502, 2013.

[59] M. Liu, Z. Ma, Effects of experimental warming on soil nitrogen transformation in alpine scrubland of eastern Qinghai-Tibet Plateau, China, Chin. J. Appl. Ecol. 32 (6) (2021) 2045–2052, https://doi.org/10.13287/j.1001-9332.202106.007, 2021 Jun.

[60] B. Chen, H. Wei, W. Chen, et al., Effects of Plant Invasion on Soil Nitrogen Transformation Processes and its Associated microbes[J], vol. 42, 2018, pp. 1071–1081, 11.

[61] U. Effmert, J. Kalderás, R. Warnke, et al., Volatile mediated interactions between bacteria and fungi in the soil, J. Chem. Ecol. 38 (2012) 665–703, https://doi.org/10.1007/s10886-012-0135-5.

[62] T. Bell, S. El-Din Hassan, A. Lauron-Moreau, et al., Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny, ISME J. 8 (2014) 331–343, https://doi.org/10.1038/ismej.2013.149.

[63] L. Chen, Y. Jiang, C. Liang, et al., Competitive interaction with keystone taxa induced negative priming under biochar amendments, Microbiome 7 (2019) 77, https://doi.org/10.1186/s40168-019-0693-7.

[64] B. Ma, H. Wang, M. Dsouza, et al., Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China, ISME J. 10 (2016) 1891–1901, https://doi.org/10.1038/ismej.2015.261.

[65] Y. Liu, J. Xu, Y. Jin, et al., Relationship between herbaceous plant invasion and soil properties in the Changbai Mountain Tundra[J], Acta Ecol. Sin. 38 (4) (2018) 1235–1244, https://doi.org/10.5846/stxb201701050046.

[66] E. Chang, C. Chiu, Changes in soil microbial community structure and activity in a cedar plantation invaded by moso bamboo, Appl. Soil Ecol. 91 (2015) 1–7, https://doi.org/10.1016/j.apsoil.2015.02.001.

[67] K. Furtak, A. Gałązka, J. Niedźwiecki, Changes in soil enzymatic activity caused by hydric stress, Pol. J. Environ. Stud. 29 (4) (2020) 2653–2660, https://doi.org/10.15244/pjoes/112896.

[68] Z. Zhu, T. Zhu, L. Yang, et al., The spatial relationship between soil alkeline-nitrogen content and environmental factors in China[J], Ecology and Environmental Sciences 28 (11) (2019) 9, https://doi.org/10.16258/j.cnki.1674-5906.2019.11.008.

[69] G. Wu, G. Ren, Q. Dong, et al., Above- and belowground response along degradation gradient in an alpine grassland of the qinghai-Tibetan plateau, Clean Soil Air Water 42 (2014) 319–323, https://doi.org/10.1002/clen.201200084.
[70] B. Zhang, J. Zhang, Y. Liu, et al., Co-occurrence patterns of soybean rhizosphere microbiome at a continental scale[J], Soil Biol. Biochem. (2018) 118178–118186, https://doi.org/10.1016/j.soilbio.2017.12.011.
[71] N. Yan, P. Marschner, Response of soil respiration and microbial biomass to changing EC in saline soils, Soil Biol. Biochem. 65 (2013) 322–328, https://doi.org/10.1016/j.soilbio.2013.06.008.
[72] S. Sarathchandra, A. Ghani, G. Yeates, et al., Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils, Soil Biol. Biochem. 33 (7–8) (2001) 953–964, https://doi.org/10.1016/s0038-0717(00)00245-5.
[73] Y. Dai, Z. Yan, J. Ji, et al., Soil bacteria diversity in rhizosphere under two types of vegetation restoration based on high throughput sequencing, Acta Pedol. Sin. 2017 (3) (2017) 735–748, https://doi.org/10.11766/trxb201603150062.