Science of the Total Environment 722 (2020) 137849

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The mechanism of soil nitrogen transformation under different biocrusts to warming and reduced precipitation: From microbial functional genes to enzyme activity

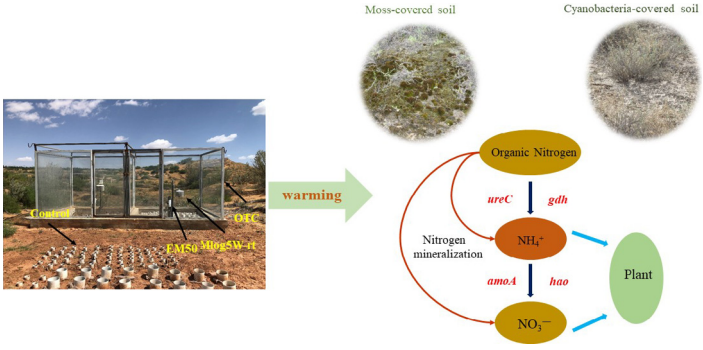
Rui Hu [a,*], Xin-ping Wang [a], Jun-shan Xu [b], Ya-feng Zhang [a], Yan-xia Pan [a], Xue Su [b]

[a] Shapotou Desert Research and Experiment Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China
[b] Northwest normal university, China

HIGHLIGHTS

- The mechanisms of N transformation in biocrusted soils to warming at different levels (functional genes→enzymes) were studied.
- Warming significantly inhibited soil Ra and Rm due to the decrease in the abundance of functional genes and enzyme activities.
- The Rn and related genes were increased in cyanobacteria-covered soil after warming.
- Warming affects the N transformation process selectively instead of regulating every process due to the mediation of biocrusts.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 July 2019
Received in revised form 5 March 2020
Accepted 9 March 2020
Available online 10 March 2020

Editor: Darrel Jenerette

Keywords:
Warming
N transformation
Biocrusts
Microbial functional genes
Enzyme activity

ABSTRACT

Soil nitrogen (N) mineralization is a microbially-mediated biogeochemical process that is strongly influenced by changing climates. However, little information is available on the mechanisms behind the response of N mineralization to prolonged warming coupled with drought in soils covered by biocrusts. We used open top chambers to investigate the rate of soil N transformation (ammonification, nitrification and mineralization), enzyme activity and gene abundance in response to warming coupled with reduced precipitation over three years (2016–2018). Warming and drought significantly reduced the N transformation rate, extracellular enzyme activity, and gene abundance in moss-covered soil. For cyanobacteria-covered soil, however, it inhibited enzyme activity and increased the abundance of the nitrification-related genes and therefore nitrification rate. Our treatments had no obvious effects on N transformation and enzyme activity, but reduced gene abundance in bare soil. Biocrusts may facilitate N transformation while the degradation of moss crust caused by climate warming will dampen any regulating effect of biocrusts on the belowground microbial community. Furthermore, belowground microbial communities can mediate N transformation under ongoing warming and reduced precipitation by suppressing ammonification- and nitrification-related gene families, and by stimulating nitrification-related gene families involved in cyanobacteria-covered soil. This study provides a basis for identifying the functional genes involved in key processes in the N cycle in temperate desert ecosystems, and our results further highlight the importance of different biocrusts organisms in the N cycle in temperate deserts as Earth becomes hotter and drier.

* Corresponding author at: 320 Donggang West Road, Lanzhou 730000, China.
E-mail address: hurui@Lzb.ac.cn (R. Hu).

https://doi.org/10.1016/j.scitotenv.2020.137849
0048-9697/

1. Introduction

Global terrestrial surface temperature will likely increase by 2.6–4.8 °C at the end of this century (IPCC, 2014). Temperature has increased over the past century and is predicted to increase further (Xiao et al., 2016). Earth will also experience uncertainty in the amount and intensity of rainfall (Li et al., 2018a), with declines in some years and increases in others according to long-term climatic records (Li et al., 2016; Li et al., 2018a).

Nitrogen (N) is one of the most limiting nutrients in desert ecosystems. It plays an important role in the carbon (C) cycle and climate change (Gruber and Galloway, 2008). Nitrogen transformation, particularly N mineralization (Nm) is closely linked to temperature (Brzostek et al., 2012; Hu et al., 2017; Song et al., 2018), and ongoing climate warming will profoundly affect the mineralization process and alter ecosystem N cycling (Bai et al., 2013; Bhatti et al., 2018; Liu et al., 2017a). Previous studies have shown that warming has increased (Hovenden et al., 2017; Li et al., 2018b; Melillo et al., 2002), reduced (Allison and Treseder, 2008; Hagedorn et al., 2010) or had no effect on Nm (Fu et al., 2019; Wang et al., 2012), depending on ecosystem type. To date, however, most studies have investigated warming and drought effects on Nm in grassland, forest and agroecosystems (Lang et al., 2010; Schutt et al., 2014; Wei et al., 2013), but its effects in moisture-limited ecosystems such as deserts remains largely unexplored (Dijkstra et al., 2010). Consequently, further information on the process of N transformation in response to warming is necessary to understand N cycling process in deserts under future climatic change scenarios.

Arid and semiarid lands account for approximately 41% of Earth's land surface and are involved in the release of 30% of gaseous N (N_2, N_2O, NOx, and NH_3) from the global N cycle (Bowden, 1986). Vegetation cover in these high temperatures, low rainfall environments is characterized by discrete patchiness (Valentin et al., 1999). The unvegetated open areas are covered by a community of highly specialized organisms collectively known as biocrusts, which comprise cyanobacteria, algae, mosses, and lichens that regulate many functional processes of desert ecosystems (Eldridge, 2000; Li et al., 2016; Li et al., 2017), They also play major roles in soil hydrology (Belnap, 2003; Belnap, 2006; Kidron et al., 2003; Li et al., 2018a; Maestre et al., 2002; Pan et al., 2010), N and C cycling (Billings et al., 2003; Hu et al., 2015; Li et al., 2012; Su et al., 2011) and microbial interactions (Liu et al., 2018; Liu et al., 2017b; Louge et al., 2013) depending on different successional stages (e.g., from cyanobacteria-to lichen- or moss-dominated crusts). Biocrusts multifunctionality, particularly in relation to N cycling, is closely linked to temperature and soil water availability, but there is little information about the dynamics of N transformation under global warming in biocrusts-covered temperate deserts, particularly among different crusts types. Several studies have examined the response of Nm to warming in laboratory experiments (Hu et al., 2014). Nevertheless, Nm exhibits substantial spatial heterogeneity in temperature and precipitation in arid regions on an interannual scale. Additionally, to our knowledge, few studies have examined the mechanisms of Nm from different perspectives (e.g., enzyme activities and gene function) in biocrusts-covered soils. Finally, there are still considerable gaps in our understanding of the linkage between enzyme activities, microbial functional genes, and Nm under global warming associated with reduced precipitation.

Extracellular enzymes secreted by soil microbes play a key role in degrading soil organic matter and might therefore be a principal driver of the within-system cycling of soil N (Gallet-Budynek et al., 2009; Nannipieri et al., 2012). Enzyme pools and activity could provide insights into the biochemical controls on Nm (Awad et al., 2018; Hu et al., 2016). Higher temperatures increase the activity of extracellular enzymes that convert large dissolved organic molecules to a form that can readily be assimilated by microbes that contribute to Nm (Allison and Treseder, 2008; Hu et al., 2014). Furthermore, warming-induced soil water shortage (Butenschoen et al., 2011) could induce diffusion limitations on enzymes and substrates (Allison, 2005; Brzostek et al., 2012). These contradictory effects might be due to the inconsistent response of enzyme activity to changes in temperature (Steinweg et al., 2013). Urease, *N*-acetyl-glucosaminidase (NAG) and L-leucine aminopeptidase (LAP) are involved in Nm, from chitin and proteins, as well as hydrolyzing urea to ammoniacal N. Zhou et al. (2013) and Hu et al. (2016) have shown that elevated temperatures increase the activity of these enzymes by enhancing soil microbial activity. L-leucine aminopeptidase is also sensitive to temperature, increasing markedly with warming (Ayo et al., 2017), but few effects of warming on NAG within soil aggregates have been reported (Fang et al., 2016). However, conditions of low water availability, such those in the arid and semiarid regions, combined with the regulation of biocrusts on enzyme activity is more complicated and has not been clarified for soil in desert ecosystem (Miralles et al., 2012; Steinauer et al., 2015). Studying the enzymatic activity in biocrusts-covered areas may yield important insights into the mechanism of N cycling under global warming on the level of enzyme kinetics in desert ecosystems.

Soil microorganisms are key components of below-ground ecosystems (Fang et al., 2016; Streit et al., 2014), and play a critical role in maintaining the function of terrestrial ecosystems and soil biogeochemistry (Canarini et al., 2017; Mangan et al., 2010; Waldrop et al., 2017). Previous studies have shown that soil microbial functional genes and their related microbial processes serve a crucial role in N transformation (Weedon et al., 2012). Although microbes are important players in N cycling, and sensitive to global warming, there is still a paucity of information needed to understand the response of microbial functional genes to environmental changes (Allison and Treseder, 2008). Changes in microbial community structure and function do not always co-occur. The large diversity of soil microbial communities may not always equate with high functional diversity due to functional redundancy among taxa (Fierer et al., 2012; Nie et al., 2013; Six et al., 2006). It is crucial, therefore, to examine microbial functional signatures such as characterizing functional genes involved in multiple N cycling processes, and relate genetic information to ecosystem functioning (Petersen et al., 2012). The rapid development of high-throughput DNA sequencing technology has enabled scientists to conduct detailed investigations of microbial communities in different ecosystems (Liu et al., 2018; Xue et al., 2016). This has coincided with a growing tendency to investigate relationships between microbial communities and N cycling processes by analyzing N-related gene families, particularly in response to changing climate (Tu et al., 2017). Nevertheless, we still have a poor understanding of how Nm-related gene functions change in arid regions in response to global warming, and whether changes in key microbial functional genes can explain the mechanism of microbe-mediated N transformation processes to warming.

In this study, we aimed to examine enzyme activity and microbial functional gene diversities of biocrusts along a succession sequence under increased temperatures and reduced precipitation in the Tengger Desert, China, and to determine the mechanisms underlying changes in soil net Nm rates in biocrusts-covered ecosystems. To accomplish this, we conducted a series of experiments with simulated warming coupled with reduced precipitation using open top chambers (OTCs). Specifically, we addressed two main questions: (i) How will a combination of warming and lower precipitation affect rates of N transformation (ammonification, nitrification and mineralization) under different biocrusts types (moss and cyanobacteria); and (ii) How will soil enzymes and functional genes associated with soil microorganisms change with warming and consequently affect N transformation.

2. Materials and methods

2.1. Study sites

This study was conducted in the Shapotou–Yiwanquan region at the southeastern margin of the Tengger Desert, China (37°25′N and 104°36′

E, 1339 m above sea level). The mean annual air temperature is 10.6 °C, and the mean annual precipitation is 191 mm, 80% of which occur between July and September. According to the local meteorological station (SDRES, CAS), over the past decade, the annual mean temperature in the current decade increased by approximately 0.5 °C, while the annual mean precipitation declined by about 4 mm (Li et al., 2016; Li et al., 2018a). The major natural vegetation in the study site is arid shrubland dominated by *Artemisia ordosica* and *Caragana korshinskii*; the shrub interspaces are dominated by well-developed biocrusts, comprising cyanobacteria, algae, lichen and mosses (Li et al., 2016).

2.2. Experimental design

Three OTCs were established before the study commenced (2016) to simulate increased temperature and reduced precipitation according to the SDRES observation records. These chambers were designed as a square composed of stalinite, with sloping sides of 100 × 100 × 100 cm. The glass walls exclude part of the precipitation from the OTCs. Air temperature and humidity in the OTCs were recorded by Mlog5W-rt data logger (Germany), and they increased the average temperature by 0.7 °C and decreased average precipitation by 10 mm.

Three microhabitats dominated by moss, cyanobacteria (>80% of total biocrusts cover) and bare soil, were randomly selected, separated by distances of 5–10 m. Thirty samples were collected in each microhabitat by polyvinyl chloride (PVC) tubes (10 cm in length and 10 cm in diameter), litter removed, and the bottoms sealed with fine mesh nylon to allow gas exchange. Sample tubes were 1–3 m apart. Finally, we collected 90 samples (30 samples × 3 microhabitats). A total of five samples for each of the two biocrusts types and bare soil were placed into each OTC and control. The field plots were monitored from January 2016 to December 2018. After three years of treatment, we removed the biocrusts and the upper layer crusts-covered soil (0–5 cm) was collected and five samples obtained. These samples were then homogenized to reduce spatial heterogeneity. For each treatment (warming with reduced precipitation and control), triplicate composite samples of each soil (two biocrusts-covered soil and bare soil) were collected, preserved in an ice box, and transported to the laboratory. All the samples were immediately sieved (by 1 mm) to remove stones and plant roots, and thoroughly homogenized. For microbial DNA extraction, samples were stored at −80 °C. To measure the soil other physiochemical properties and soil enzyme activity, samples were dried at room temperature.

2.3. Soil analysis

The soil samples before and after warming and precipitation treatments were extracted with 1 M KCl, and the inorganic N (nitrate and ammonium) in the extracts measured using a continuous flow analyzer (AutoAnalyzer 3, Germany). The rates of net ammonification (Ra), nitrification (Rn) and mineralization (Rm) during warming and reduced precipitation treatments were calculated according to the previous methods (Hu et al., 2015; Kong et al., 2019). The particle size distribution was determined by the pipette method (Nanjing Institute of Soil Sciences, 1978). Soil pH was determined in a 1:5 soil and water extract. Soil total C (TC) and total N (TN) were measured with the element analyzer (Vario MACRO cube, Elementar INC., Germany).

2.4. Soil enzyme activity

All enzymes analyzed were hydrolases involved in N transformation and included *N*-acetyl-glucosaminidase (NAG), L-leucine aminopeptidase (LAP), and urease. NAG and LAP activities were measured using a fluorometric method (DeForest, 2009) with black polystyrene 96-well microplates (300 μl, SPL Life Sciences Co. Ltd., Pocheon-si, South Korea); Urease (E.C. 3.5.1.5) activity was determined using 10% urea solution as the substrate and incubation at 37 °C for 24 h. A spectrophotometer was used to determine NH_4^+-N concentration at 578 nm (Nannipieri et al., 1980).

2.5. DNA extraction, library construction and metagenomics sequencing

For each sample, a single DNA extraction was performed directly from 0.5 g of soil using the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. Concentration and purity of extracted DNA was monitored on 1% agarose gels and the concentration was measured using a Qubit® dsDNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). DNA extract was fragmented to an average size of about 300 bp using Covaris M220 (Gene Company Limited, China). Paired-end library was constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA), and it was performed on an Illumina Hiseq 4000 platform (Illumina Inc., San Diego, CA, USA) using Hiseq 3000/4000 PE Cluster Kit and Hiseq3000/4000 SBS Kit according to the manufacturer's instructions (www.illumina.com).

2.6. Sequence quality control and gene prediction

Adapter sequence was stripped from the 3′ and 5′ end of paired end Illumina reads using SeqPrep (https://github.com/jstjohn/SeqPrep). Low-quality reads (length < 50 bp or with a quality value <20 or having N bases) were removed by Sickle (https://github.com/najoshi/sickle). Metagenomics data were assembled using MEGAHIT (https://github.com/voutcn/megahit). Contigs with the length being or over 300 bp were selected as the final assembling result for further gene prediction.

Open reading frames (ORFs) from each assembled contigs were predicted using MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/). The predicted ORFs with length being or over 100 bp were retrieved and translated into amino acid sequences. All predicted genes with a 95% sequence identity (90% coverage) were clustered using CD-HIT (http://www.bioinformatics.org/cd-hit/), the longest sequences from each cluster was chosen as representative sequences to construct non-redundant gene catalog. Reads after quality control were mapped to the representative sequences with 95% identity using SOAPaligner (http://soap.genomics.org.cn/), and gene abundance in each sample were evaluated.

2.7. Statistical analysis

Two-way Analysis of Variance was used to examine the effects of warming and N transformation and enzyme activities, and microbial functional genes among different biocrusted and control soils. A heatmap was generated in the R statistical software package to illustrate the independent and interactive effects of warming and soil type. Paired-sample Student *t*-test were used to test for differences of soil N transformation, enzyme activity and functional genes between control and warming. The relationships between soil N mineralization and functional genes and enzyme activities were examined using linear regression models. Significant differences were determined using Tukey's HSD test at a probability level of 0.05. All analyses were performed using the SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), and the results are reported as the mean ± standard error.

3. Results

3.1. Soil physicochemical properties

During the study period, soil temperature and moisture were significantly affected by warming coupled with reduction in precipitation ($P < 0.01$, Fig. 1). Warming coupled with reduced precipitation can increase the soil temperature by 15%, 16% and 14% for moss, cyanobacteria and bare soil, respectively. The soil moisture at the three microsites in

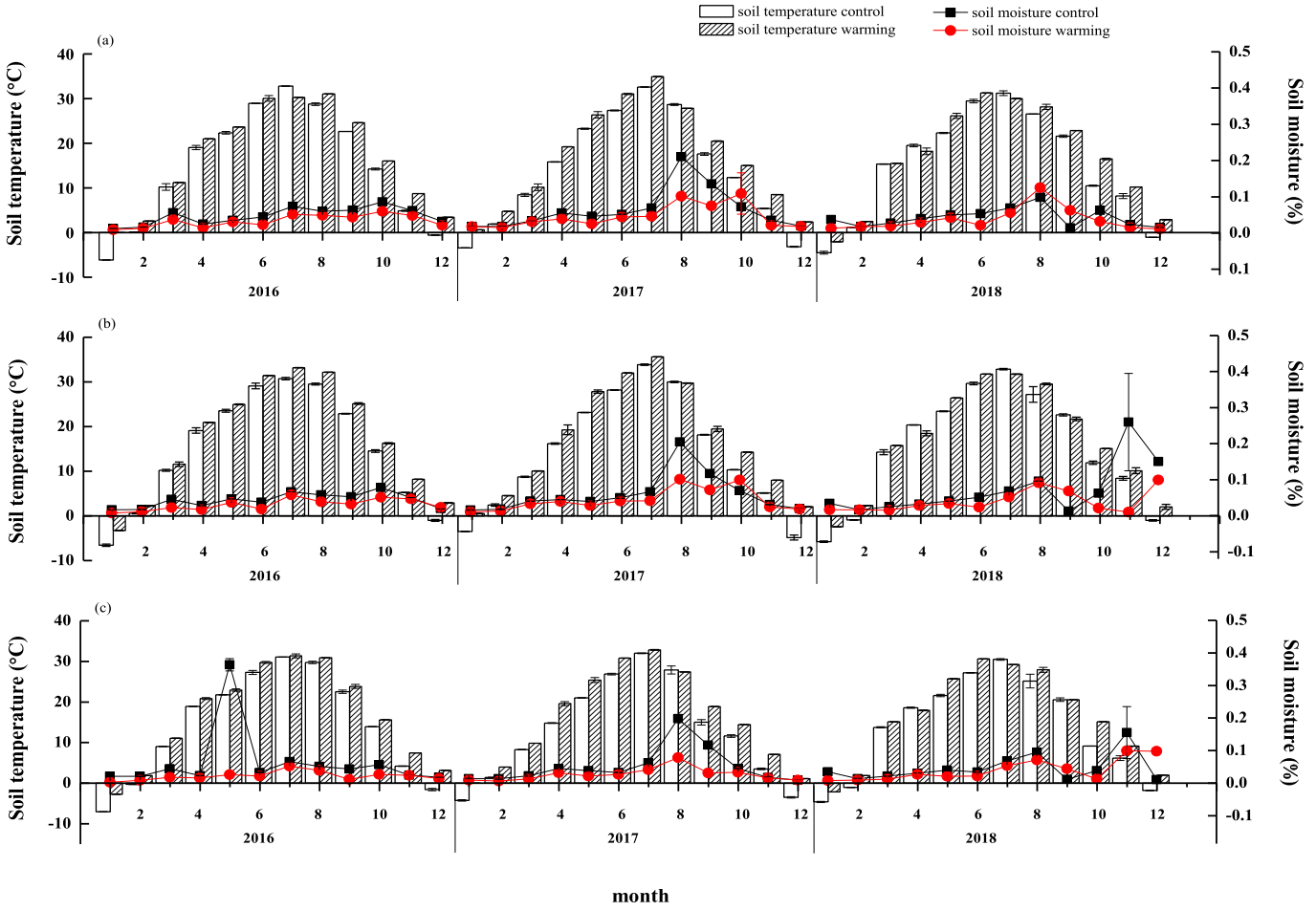


Fig. 1. The monthly variations in soil temperature and moisture from January 2016 to December 2018 in the study site. A: moss-covered soil; B: cyanobacteria-covered soil; C: bare soil.

the warming coupled with reduction in precipitation treatment was 25%, 35% and 45% lower than that of the control treatment, respectively. Although the effects of warming with lower precipitation on soil temperature and moisture were also consistent across study plots, the increases in soil temperature and decreases in soil moisture were greater in cyanobacteria-covered soil than in the other two soils.

3.2. Soil net N transformation rate

Warming coupled with reduced precipitation resulted in significant reductions in of biocrusts-covered soil net ammonification rates, with 49% and 57% reduction after warming, for moss and cyanobacteria, respectively (Fig. 2). However, no differences in Ra were observed between warming and soil types (Fig. 3). Warming resulted in a 63% reduction in Rn in moss-covered soil, and a 32% increase in that rate in the cyanobacteria-covered soil. No remarkable changes were found in Rn for bare soil (Fig. 2). The response of Rm among the three soil surfaces was similar to the Rn; The soil net Nm rate in moss-covered declined from 0.37 $mg \cdot kg^{-1}d^{-1}$ to 0.15 $mg \cdot kg^{-1}d^{-1}$ with warming and lower precipitation, but no obvious changes were found in the other two soils.

For the biocrusts-covered soil and bare soil, warming and precipitation reduction inhibited Ra, but no differences were observed among the three soils (Fig. 2). Moreover, the Rn and Rm were significantly higher in moss-covered soil under normal conditions, but after the warming treatment, cyanobacteria-covered soil exhibited higher net N turnover rates (nitrification and mineralization) than the other two soils.

3.3. Soil enzyme activities

Nitrogen-degrading enzyme activities were interactively determined by warming treatments and soil types (Fig. 3). The activities of NAG, LAP and urease were significantly lower in the warming treatment than the control for biocrusted (Fig. 4). No differences in NAG and LAP activities were found in bare soil. Moss-covered soil had greater NAG activity than the other soil types, but NAG activity declined significantly after warming. The activity of LAP declined by 23% and 31% in the moss-covered soil and bare soil, respectively, after warming. Compared with the other two soils, cyanobacteria-covered soil exhibited the largest decline in urease activity, falling from 229 $\mu g \cdot d^{-1}$ g^{-1} to 120 $\mu g \cdot d^{-1}$ g^{-1}.

3.4. Effect of warming on N transformation genes

Warming and soil types can significantly affect genes related to soil Nm processes (Fig. 3), and obvious differences in Nm genes were found after the warming treatment (Fig. 5). For example, the abundance of ammonification genes (*ureC* and *gdh*) was significantly reduced under warming, but the *gdh* genes in moss-covered soil showed no significant difference between the warming and control. Surprisingly, the *hao* gene of cyanobacteria-covered soil involved in nitrification significantly increased after warming, whereas no obvious differences were found in the *amoA* gene of cyanobacteria-covered soil and bare soil. In most cases, the genes involved in ammonification and nitrification were markedly higher in moss-covered soil than in cyanobacteria-covered soil and bare soil.

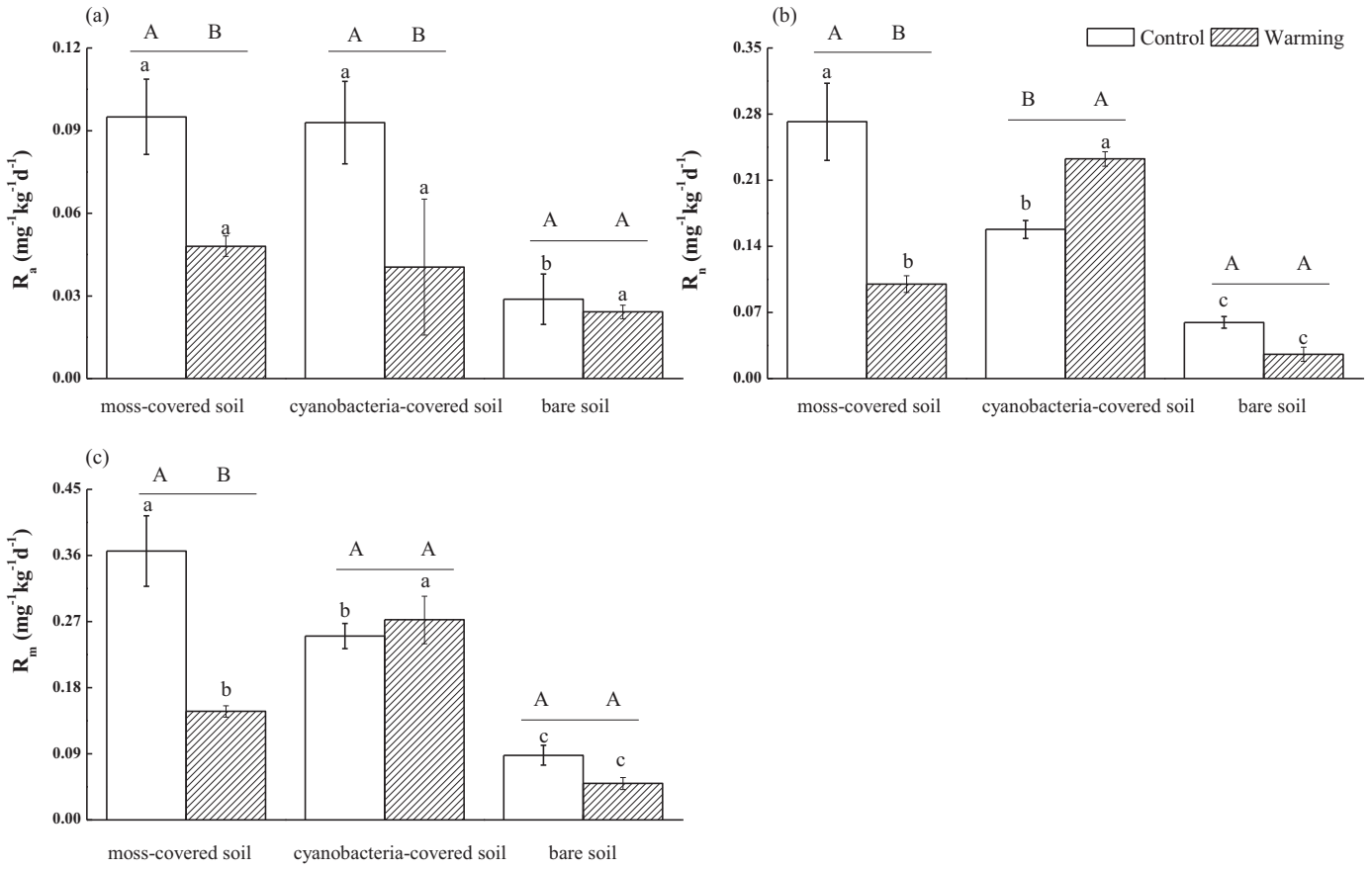


Fig. 2. Changes of the soil net N transformation rates of different biocrusts after 3-year experimental periods with warming and reduced rainfall treatments. Different capital letters indicate significant difference between control and warming; different small letters denote significant differences among three soil types ($P < 0.05$). Ra: net ammonification rate; Rn: net nitrification rate; Rm: net nitrogen mineralization.

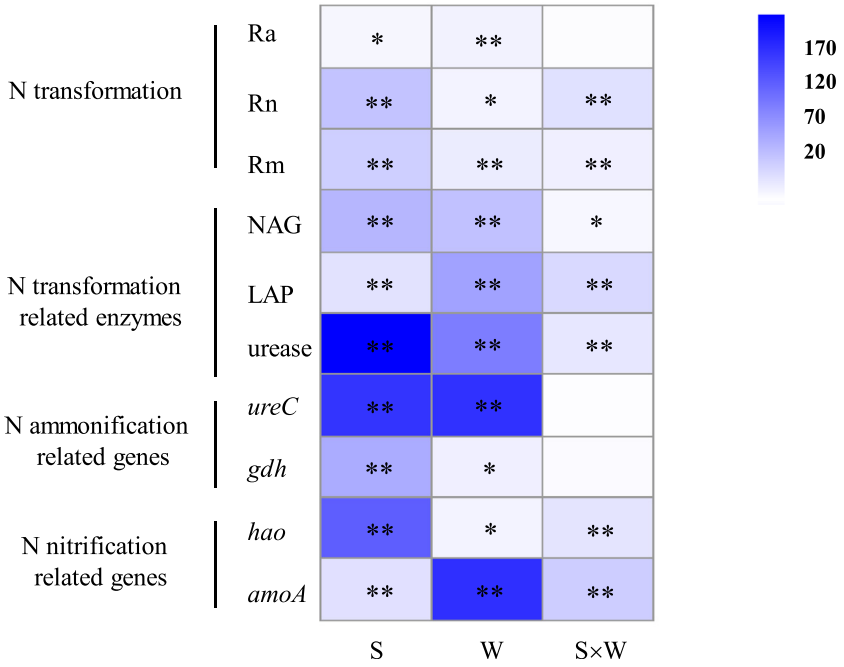


Fig. 3. The main and interactive effects of soil type (S) and warming (W) on the soil net N ammonification (Ra), net nitrification rate (Rn), net N mineralization (Rm), N-related gene abundances and enzyme activities. The gradient colors denote the F values, and the bluer the color, the bigger the F value. NAG: *N*-acetyl-glucosaminidase; LAP: L-leucine aminopeptidase. Asterisks indicate significant differences at $P < 0.05$ and $P < 0.01$ (* and **, respectively; white fields, not significant).

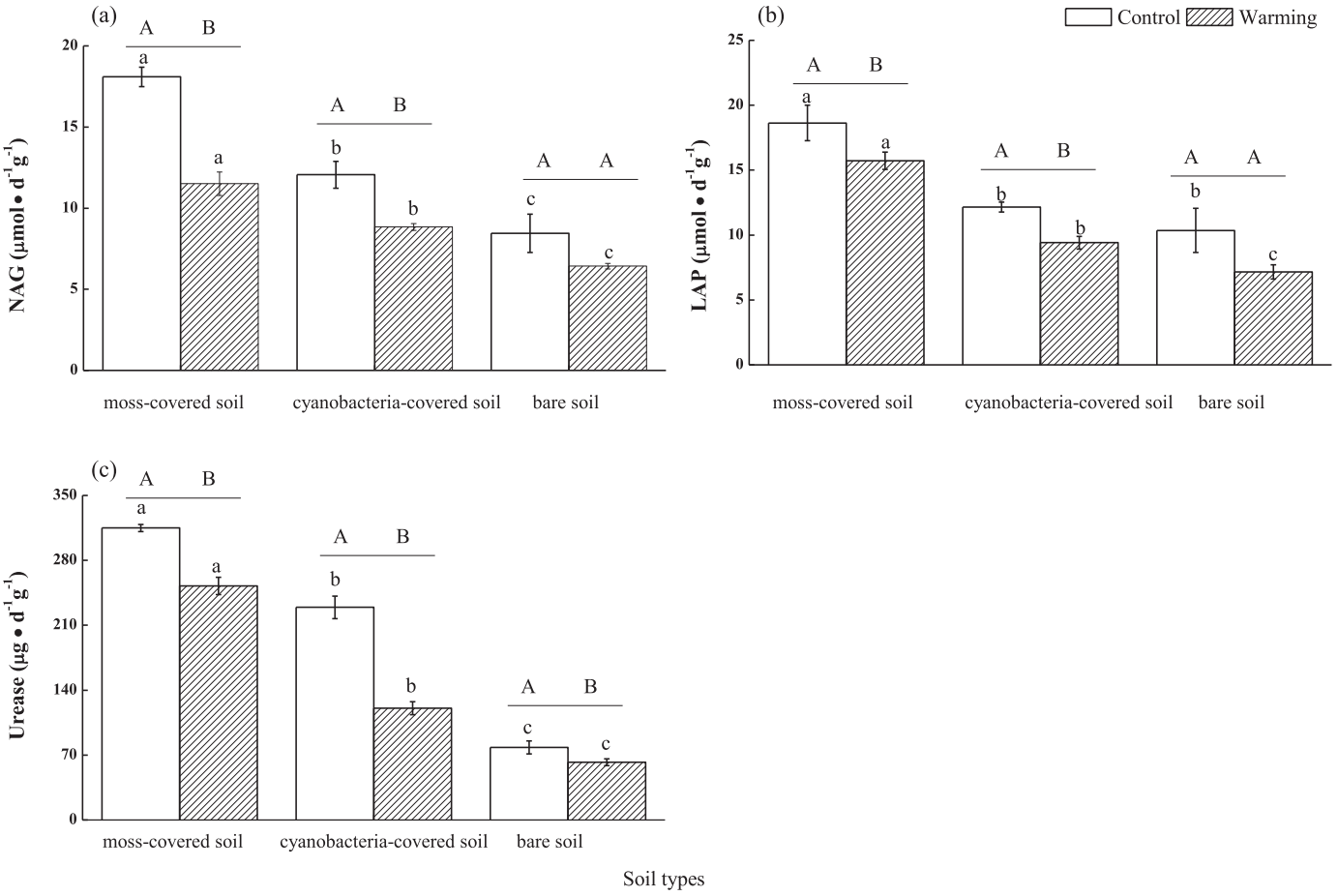


Fig. 4. Changes of the soil enzyme activities of different biocrusts after 3-year experimental periods with warming and reduced rainfall treatments. Different letters have the same meaning as Fig. 2. NAG: *N*-acetyl-glucosaminidase; LAP: L-leucine aminopeptidase.

3.5. Relationship between soil genes abundances, soil enzymes activities and N mineralization

Our modelling (Table 1) showed that soil gene abundance and soil enzyme activities were positively correlated with the rate of Nm in moss-covered soils, explaining 67%–96% and 76%–94% of variations in Rm, respectively. However, the relationships between soil gene *amoA* and Rm were not statistically significant in cyanobacteria-covered soil and bare soil. Significant negative correlations between genes (except *hao*), enzymes and Rm were found in cyanobacteria-covered soil. These results indicate that the response of Rm to warming depends upon soil gene and enzyme, and that, this relationship is regulated by biocrusts.

4. Discussion

It has been proposed that biocrusts regulate the hydrolases activities (Miralles et al., 2012), and their development directly affects the microbial functional structure (Liu et al., 2018) by adjusting the soil microenvironment. Our results demonstrate that warming with reduced precipitation would reduce the soil N transformation rates (ammonification, nitrification and mineralization) in crusts-covered soil (Fig. 2). This observation was surprising in light of previous studies reporting warming increased Nm (Dawes et al., 2017; Dijkstra et al., 2010; Fu et al., 2019). Our contrasting results may be attributable to two reasons. First, warming-induced drought may offset the effects of warming on soil Nm (Hagedorn et al., 2010; Liu et al., 2009), particularly in our OTC method, which had reduced precipitation. Soil moisture can affect soil aeration and microbial activities related to soil N turnover, and further regulating Nm (Hu et al., 2015; Zaman and Chang, 2004). Second, biocrusts play a crucial role in controlling soil water availability and regulating water redistribution. Ten years study in the same region indicated that warming, coupled with a reduction in precipitation, causes the degradation of biocrusts, especially the moss crust (Li et al., 2018a). The reduced cover and biomass of mosses in biocrusted communities may lead to maladjustment of the soil microenvironment and would not support more abundant microbial communities (Hu et al., 2014; Kandeler et al., 2006). As a result, both Ra and Rm declined rapidly after warming and reduced precipitation in moss-covered soils. Surprisingly, the soil Rn in cyanobacteria-covered soil increased after warming. This is consistent with result from Yu and Ehrenfeld (2009), who observed that lower moisture is favorable to soil nitrification, and accord with our previous study (Hu et al., 2015). The higher temperature and lower moisture conditions in cyanobacteria-covered soil facilitate aerobic microbes, a key microbial component in the nitrification process, thus stimulating soil nitrification (Corre et al., 2002). Furthermore, reduced leaching due to lower precipitation will increase the emission of nitrogen oxide. Therefore, the pronounced changes in Nm due to warming and reduced precipitation were biocrusts-mediated, and our findings apply specifically to arid and semiarid regions. However, what is the driving mechanism of Nm in biocrusts-covered soil under the warming, particularly in deserts?

In terms of enzyme activity levels, enzymatic expressions are the proximate representations of microbial nutrient demand for biogeochemical transformations, such as those of soil N, and they can provide important information on the soil's microbial status and physicochemical properties and represent a useful indicator for studying the effects of

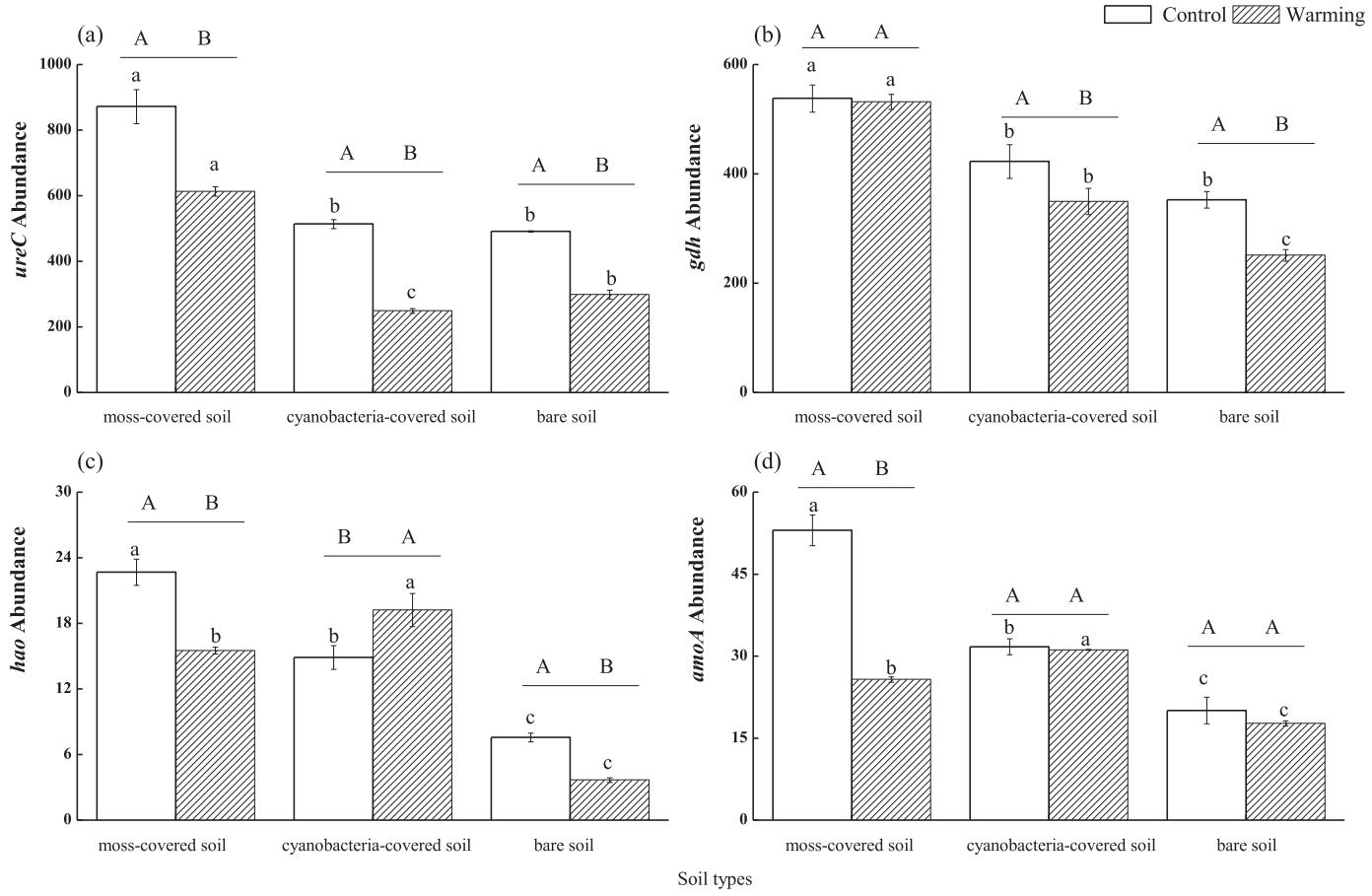


Fig. 5. Changes of the genes abundance of different biocrusts after 3-year experimental periods with warming and reduced rainfall treatments. Different letters have the same meaning as Fig. 2.

Table 1
Parameter values of the models for the relationship between the Rm and functional genes and enzyme activities in three soil types.

Soil	Rm	Y = a + bx				Y = Exp (a + b/x)			
		a	b	R^2	P	a	b	R^2	P
Moss	ureC	445.449	1157.517	0.817	0.013	7.001	−0.086	0.872	0.006
	gdh	301.047	331.929	0.669	0.047	6.181	−0.049	0.667	0.047
	hao	10.822	32.205	0.860	0.008	3.372	−0.093	0.907	0.003
	amoA	7.819	122.866	0.933	0.002	4.447	−0.177	0.962	0.001
	NAG	7.018	30.304	0.941	0.001	3.206	−0.113	0.927	0.002
	LAP	13.823	13.068	0.784	0.019	3.036	−0.041	0.756	0.024
	urease	210.736	283.910	0.896	0.004	5.903	−0.055	0.900	0.004
Cyanobacteria	ureC	2851.636	−9453.865	0.819	0.013	−0.868	1.759	0.815	0.014
	gdh	592.611	−219.850	0.753	0.025	6.176	0.028	0.743	0.027
	hao	−22.853	152.755	0.675	0.045	5.075	−0.586	0.610	0.067
	amoA	34.884	−13.135	0.146	0.454	3.346	0.027	0.129	0.484
	NAG	43.449	−126.283	0.791	0.018	−0.792	0.814	0.815	0.014
	LAP	35.048	−92.877	0.706	0.036	0.090	0.594	0.698	0.038
	urease	1091.343	−3507.516	0.635	0.048	−0.461	1.452	0.671	0.046
Bare	ureC	99.225	4296.065	0.865	0.007	6.520	−0.035	0.702	0.037
	gdh	139.847	2351.716	0.837	0.011	6.095	0.680	0.680	0.043
	hao	−0.422	87.793	0.848	0.009	2.490	−0.051	0.691	0.040
	amoA	195.721	1541.039	0.502	0.115	6.058	−0.022	0.552	0.090
	NAG	4.576	41.507	0.312	0.249	2.269	−0.017	0.295	0.265
	LAP	4.641	59.656	0.549	0.092	2.455	−0.029	0.325	0.238
	urease	41.772	413.171	0.627	0.046	4.533	−0.018	0.501	0.115
All	ureC	322.200	942.284	0.249	0.035	6.355	−0.026	0.170	0.089
	gdh	271.761	695.240	0.528	0.001	6.255	−0.034	0.698	0.000
	hao	3.138	55.135	0.845	0.000	3.193	−0.087	0.870	0.000
	amoA	20.039	67.470	0.434	0.003	3.714	−0.032	0.408	0.004
	NAG	5.493	27.623	0.638	0.000	2.654	−0.039	0.604	0.000
	LAP	31.388	6.069	0.771	0.000	2.763	−0.098	0.598	0.000
	urease	53.988	625.011	0.528	0.001	5.582	−0.071	0.595	0.000

environmental changes (Allison et al., 2007; Chen et al., 2010). The enzyme NAG and LAP are involved in the mineralization of N from chitin and proteins and soil urease is also a key enzyme for N transformation, as it can hydrolyze carbamide to ammonium (Bai et al., 2014; Zhou et al., 2013). Theoretically, warming will increase the rate of enzymatically catalyzed reactions due to an increase in kinetic energy up to an optimum of catabolic activity (Wallenstein and Weintraub, 2008). However, our study revealed that enzyme activity significantly declined with warming and reduced precipitation, consistent with the results showing that the activities of NAG and LAP were highest in moister environments (Guenet et al., 2012). Nevertheless, soil microorganisms may reduce enzyme synthesis and secretion due to warming and lower moisture (Vogel et al., 2012). Furthermore, the higher nutrition level under biocrusts enhances enzyme activity (Table S1), which is consistent with the findings of previous studies (Hu et al., 2014; Liu et al., 2014). Therefore, a potential shift in the soil enzyme activity could primarily be affected by soil moisture and soil nutrient content regulated by biocrusts under climate warming, and further modulated N transformation.

At the functional genes level, genes related to the ammonification process were sensitive to warming with reduced precipitation. The genes encoding urease and glutamate dehydrogenase declined in abundance with warming. One of the family *ureC* genes converts urea into ammonia, and *gdh* gene decomposes organic N to NH_4^+. The decline in their abundance could be ascribed to reduced abundance of several dominant bacterial taxa. However, Zhou et al. (2012) studied the genes involved in N cycling to climate warming and indicated that warming increased the abundance of N metabolism gene families. This discrepancy is likely due to differences in the soil moisture conditions. The OTC method used in our study can identify the combined impact of warming coupled with a reduction in precipitation, while soil moisture content was not affected by warming in their study. Conversely, Szukics et al. (2010) showed examined microorganisms performing nitrification and denitrification to warming and changed precipitation in forest soil, and found that lower soil water content with higher temperature will increased archaeal *amoA* abundance. These differences might be attributable to microbial functional genetic types. Higher water conditions that were likely oxygen limited will inhibit the archaeal *amoA* abundance. Additionally, the abundance of the gene family *hao*, which serves as the key gene in transforming NH_2OH to NO_2^- in the nitrification process, increased in cyanobacteria-covered soil. Moreover, the gene encoding ammonium monooxygenase was unreceptive to warming combined with reduced precipitation in cyanobacteria crust. This indicates that warm and dry environments (Fig. 1) may facilitate the N nitrification process. Both findings were consistent with our metadata showing that warming with reduced precipitation decreased the net ammonification rates while increasing net nitrification rates in cyanobacteria-covered soil. This suggests that warming with reduced precipitation disrupts the dynamic equilibrium of ammonia metabolism by changing the abundance of functional genes, reducing soil N availability and N metabolic potential, but increasing the nitrification process, which can promote N emission. The deterioration of moss-covered crusts resulting from warming will alter the microenvironment of the soil and further accelerate this process.

Soil functional genes and enzymes regulate the response of Nm to warming and our results highlight plausible mechanisms from different levels. The first mechanism is related to changes in the abundance of genes, which are driven largely by warming-induced changes in the soil microenvironment (temperature and moisture) and biocrusts structure. The abundance of functional genes indicates the potential of soil microbial functional groups (Sinsabaugh et al., 2010), which control the N transformation process. Warming coupled with reduced precipitation decreased the abundance of microbial functional genes for the N ammonification process, but not for the N nitrification process in cyanobacteria-covered soil. This indicates that biocrusts are able to affect N cycling by regulating the soil microenvironment. The increased nitrification process will increase the emission of nitrogen oxide, and further affect the climate environment. The second mechanism is by reducing enzyme activity, which inhibits the N transformation process. The microbial functions are achieved by catalytic activity. Shifts in enzyme synthesis under disturbance can lead to changes in microbial functions (Sinsabaugh et al., 2010), and further affect the soil N transformation processes. In brief, these interrelated mechanisms jointly regulate the effect on the soil Nm process under climate warming in biocrusts-covered soil. Whether the response of the microbially-mediated process is positive or negative depends on which associated functions are affected. Thus, to fully understand the response of soil N cycling in biocrusts-covered soil to climate change in arid regions, it is necessary to consider the response mechanisms of soil N transformation to climate warming in different seasonal dynamics, at least at the level of microbial structure and function.

5. Conclusions

We conclude that warming with reduced precipitation significantly inhibited the soil Ra and Rm due to the decline in abundance of microbial functional genes and related enzyme activities. The increased gene abundance of cyanobacteria-covered soil observed in soil N transformation promoted microbial metabolic potential, and changes in metabolic potential were primarily associated with the nitrification process. These results indicate that warming under condition of lower precipitation could reduce soil nutrient availability by inhibiting the ammonification process in temperate deserts, and that microbial functional genes were the major determinants of soil N transformation. Furthermore, colonization of the cyanobacterial crust may accelerate the nitrification process under these conditions, increasing N loss. These results demonstrate that the development of soil crusts, together with changes in soil abiotic properties, play key roles in driving the functional shifts in the soil microbial function and affect the N cycling. This may exacerbate climate warming (increase the emission of nitrogen oxide) and influence the ecological health of desert ecosystem.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137849.

CRediT authorship contribution statement

Rui Hu: Conceptualization, Methodology, Software, Investigation, Writing - original draft.**Xin-ping Wang:**Writing - review & editing, Supervision.**Jun-shan Xu:**Methodology, Software.**Ya-feng Zhang:**Writing - review & editing.**Yan-xia Pan:**Writing - review & editing.**Xue Su:**Resources, Investigation.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "The mechanism of soil nitrogen transformation under different biocrusts to warming and reduced precipitation: from microbial functional genes to enzyme activity"

Acknowledgements

We thank three anonymous reviewers for their constructive comments and David J Eldridge for help with the manuscript editing. This work was financially supported by National Key Research and Development Program of China (2016YEF0203400), the Strategic Priority Research Program of Chinese Academy of Sciences (No. XDA2003010301), the National Natural Science Foundation of China (Grant no. 31971749, 31971529) and the Youth Innovation Promotion Association, CAS (Grant no. 2019415).

References

Allison, S.D., 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecol. Lett. 8, 626–635.

Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob. Chang. Biol. 14, 2898–2909.

Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner, B.L., 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biol. Biochem. 39, 1770–1781.

Awad, Y.M., Lee, S.S., Kim, K.H., Ok, Y.S., Kuzyakov, Y., 2018. Carbon and nitrogen mineralization and enzyme activities in soil aggregate-size classes: effects of biochar, oyster shells, and polymers. Chemosphere 198, 40–48.

Ayo, B., Abad, N., Artolozaga, I., Azua, I., Bana, Z., Unanue, M., et al., 2017. Imbalanced nutrient recycling in a warmer ocean driven by differential response of extracellular enzymatic activities. Glob. Chang. Biol. 23, 4084–4093.

Bai, E., Li, S.L., Xu, W.H., Li, W., Dai, W.W., Jiang, P., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytol. 199, 441–451.

Bai, J.H., Xu, H.F., Xiao, R., Gao, H.F., Zhang, K.J., Ding, Q.Y., 2014. Path analysis for soil urease activities and nutrient contents in a Mountain Valley Wetland, China. Clean – Soil, Air, Water 42, 324–330.

Belnap, J., 2003. The world at your feet: desert biological soil crusts. Front. Ecol. Environ. 1, 181–189.

Belnap, J., 2006. The potential roles of biological soil crusts in dryland hydrologic cycles. Hydrol. Process. 20, 3159–3178.

Bhatti, A., Ahmad, J., Qasim, M., Riaz, M., Cresser, M.S., 2018. Effects of anthropogenic nitrogen deposition on soil nitrogen mineralization and immobilization in grassland soil under semiarid climatic conditions. Environ. Monit. Assess. 190.

Billings, S.A., Schaeffer, S.M., Evans, R.D., 2003. Nitrogen fixation by biological soil crusts and heterotrophic bacteria in an intact Mojave Desert ecosystem with elevated CO2 and added soil carbon. Soil Biol. Biochem. 35, 643–649.

Bowden, W.B., 1986. Gaseous nitrogen emissions from undisturbed terrestrial ecosystems - an assessment of their impacts on local and global nitrogen budgets. Biogeochemistry 2, 249–279.

Brzostek, E.R., Blair, J.M., Dukes, J.S., Frey, S.D., Hobbie, S.E., Melillo, J.M., et al., 2012. The effect of experimental warming and precipitation change on proteolytic enzyme activity: positive feedbacks to nitrogen availability are not universal. Glob. Chang. Biol. 18, 2617–2625.

Butenschoen, O., Scheu, S., Eisenhauer, N., 2011. Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. Soil Biol. Biochem. 43, 1902–1907.

Canarini, A., Kiaer, L.P., Dijkstra, F.A., 2017. Soil carbon loss regulated by drought intensity and available substrate: a meta-analysis. Soil Biol. Biochem. 112, 90–99.

Chen, Q., Yin, F.F., Cao, J., Lu, H.Y., Zhang, S.Z., 2010. Variation and spatial characteristics of soil enzyme activities in Pinus tabulaeformis and Larix kaempferi plantation at different development stages in Western Qinling Mountains. Journal of Ecology and Rural Environment 26, 466–471.

Corre, M.D., Schnabel, R.R., Stout, W.L., 2002. Spatial and seasonal variation of gross nitrogen transformations and microbial biomass in a northeastern US grassland. Soil Biol. Biochem. 34, 445–457.

Dawes, M.A., Schleppi, P., Hattenschwiler, S., Rixen, C., Hagedorn, F., 2017. Soil warming opens the nitrogen cycle at the alpine treeline. Glob. Chang. Biol. 23, 421–434.

DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biol. Biochem. 41, 1180–1186.

Dijkstra, F.A., Blumenthal, D., Morgan, J.A., Pendall, E., Carrillo, Y., Follett, R.F., 2010. Contrasting effects of elevated CO2 and warming on nitrogen cycling in a semiarid grassland. New Phytol. 187, 426–437.

Eldridge, D., 2000. New frontiers in bryology and lichenology - ecology and management of biological soil crusts: recent developments and future challenges. Bryologist 103, 742–747.

Fang, X., Zhou, G.Y., Li, Y.L., Liu, S.Z., Chu, G.W., Xu, Z.H., et al., 2016. Warming effects on biomass and composition of microbial communities and enzyme activities within soil aggregates in subtropical forest. Biol. Fertil. Soils 52, 353–365.

Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., et al., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc. Natl. Acad. Sci. U. S. A. 109, 21390–21395.

Fu, W., Wang, X., Wei, X.R., 2019. No response of soil N mineralization to experimental warming in a northern middle-high latitude agro-ecosystem. Sci. Total Environ. 659, 240–248.

Gallet-Budynek, A., Brzostek, E., Rodgers, V.L., Talbot, J.M., Hyzy, S., Finzi, A.C., 2009. Intact amino acid uptake by northern hardwood and conifer trees. Oecologia 160, 129–138.

Gruber, N., Galloway, J.N., 2008. An earth-system perspective of the global nitrogen cycle. Nature 451, 293–296.

Guenet, B., Lenhart, K., Leloup, J., Giusti-Miller, S., Pouteau, V., Mora, P., et al., 2012. The impact of long-term CO2 enrichment and moisture levels on soil microbial community structure and enzyme activities. Geoderma 170, 331–336.

Hagedorn, F., Martin, M., Rixen, C., Rusch, S., Bebi, P., Zurcher, A., et al., 2010. Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. Biogeochemistry 97, 7–19.

Hovenden, M.J., Newton, P.C.D., Osanai, Y., 2017. Warming has a larger and more persistent effect than elevated CO2 on growing season soil nitrogen availability in a species-rich grassland. Plant Soil 421, 417–428.

Hu, R., Wang, X.P., Pan, Y.X., Zhang, Y.F., Zhang, H., 2014. The response mechanisms of soil N mineralization under biological soil crusts to temperature and moisture in temperate desert regions. Eur. J. Soil Biol. 62, 66–73.

Hu, R., Wang, X.P., Pan, Y.X., Zhang, Y.F., Zhang, H., Chen, N., 2015. Seasonal variation of net N mineralization under different biological soil crusts in Tengger Desert, North China. Catena 127, 9–16.

Hu, R., Wang, X.P., Zhang, Y.F., Shi, W., Jin, Y.X., Chen, N., 2016. Insight into the influence of sand-stabilizing shrubs on soil enzyme activity in a temperate desert. Catena 137, 526–535.

Hu, Y.W., Zhang, L., Deng, B.L., Liu, Y.Q., Liu, Q., Zheng, X., et al., 2017. The non-additive effects of temperature and nitrogen deposition on CO2 emissions, nitrification, and nitrogen mineralization in soils mixed with termite nests. Catena 154, 12–20.

IPCC, 2014. Climate Change 2014: The Physical Science Basis. Cambridge University Press, Cambridge United Kingdom and New York.

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., Philippot, L., 2006. Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. 72, 5857–5962.

Kidron, G.J., Yair, A., Vonshak, A., Abeliovich, A., 2003. Microbiotic crust control of runoff generation on sand dunes in the Negev Desert. Water Resour. Res. 39.

Kong, W.B., Yao, Y.F., Zhao, Z.N., Qin, X., Zhu, H.S., Wei, X.R., et al., 2019. Effects of vegetation and slope aspect on soil nitrogen mineralization during the growing season in sloping lands of the loess plateau. Catena 172, 753–763.

Lang, M., Cai, Z.C., Mary, B., Hao, X.Y., Chang, S.X., 2010. Land-use type and temperature affect gross nitrogen transformation rates in Chinese and Canadian soils. Plant Soil 334, 377–389.

Li, X.R., Zhang, P., Su, Y.G., Jia, R.L., 2012. Carbon fixation by biological soil crusts following revegetation of sand dunes in arid desert regions of China: a four-year field study. Catena 97, 119–126.

Li, X.R., Hui, R., Zhao, Y., 2016. Eco-Physiology of Biological Soil Crusts in Desert Regions of China. High Education Publishing House, Beijing: China.

Li, X.R., Song, G., Hui, R., Wang, Z.R., 2017. Precipitation and topsoil attributes determine the species diversity and distribution patterns of crustal communities in desert ecosystems. Plant Soil 420, 163–175.

Li, X.R., Jia, R.L., Zhang, Z.S., Zhang, P., Hui, R., 2018a. Hydrological response of biological soil crusts to global warming: a ten-year simulative study. Glob. Chang. Biol. 24, 4960–4971.

Li, Y.Q., Qing, Y.X., Lyu, M.K., Chen, S.D., Yang, Z.J., Lin, C.F., et al., 2018b. Effects of artificial warming on different soil organic carbon and nitrogen pools in a subtropical plantation. Soil Biol. Biochem. 124, 161–167.

Liu, W.X., Zhang, Z., Wan, S.Q., 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Glob. Chang. Biol. 15, 184–195.

Liu, Y., Wang, C.H., He, N.P., Wen, X.F., Gao, Y., Li, S.G., et al., 2017a. A global synthesis of the rate and temperature sensitivity of soil nitrogen mineralization: latitudinal patterns and mechanisms. Glob. Chang. Biol. 23, 455–464.

Liu, Y.B., Zhao, L.N., Wang, Z.R., Liu, L.C., Zhang, P., Sun, J.Y., et al., 2018. Changes in functional gene structure and metabolic potential of the microbial community in biological soil crusts along a revegetation chronosequence in the Tengger Desert. Soil Biol. Biochem. 126, 40–48.

Liu, Y.M., Yang, H.Y., Li, X.R., Xing, Z.S., 2014. Effects of biological soil crusts on soil enzyme activities in revegetated areas of the Tengger Desert, China. Appl. Soil Ecol. 80, 6–14.

Liu, Y.M., Xing, Z.S., Yang, H.Y., 2017b. Effect of biological soil crusts on microbial activity in soils of the Tengger Desert (China). J. Arid Environ. 144, 201–211.

Louge, M.Y., Valance, A., el-Moctar, A.O., Xu, J., Hay, A.G., Richer, R., 2013. Temperature and humidity within a mobile barchan sand dune, implications for microbial survival. Journal of Geophysical Research-Earth Surface 118, 2392–2405.

Maestre, F.T., Huesca, M., Zaady, E., Bautista, S., Cortina, J., 2002. Infiltration, penetration resistance and microphytic crust composition in contrasted microsites within a Mediterranean semi-arid steppe. Soil Biol. Biochem. 34, 895–898.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M.L., Valencia, M.C., Sanchez, E.I., et al., 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466, 752–755.

Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., et al., 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298, 2173–2176.

Miralles, I., Domingo, F., Canton, Y., Trasar-Cepeda, C., Leiros, M.C., Gil-Sotres, F., 2012. Hydrolase enzyme activities in a successional gradient of biological soil crusts in arid and semi-arid zones. Soil Biol. Biochem. 53, 124–132.

Nanjing Institute of Soil Sciences CAS, 1978. Physical and Chemical Analysis Methods of Soils. Shanghai Science Technology Press, Shanghai.

Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase, urease, proteases, organic-carbon, and nitrogen from soil. Soil Sci. Soc. Am. J. 44, 1011–1016.

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., et al., 2012. Soil enzymology: classical and molecular approaches. Biol. Fertil. Soils 48, 743–762.

Nie, M., Pendall, E., Bell, C., Gasch, C.K., Raut, S., Tamang, S., et al., 2013. Positive climate feedbacks of soil microbial communities in a semi-arid grassland. Ecol. Lett. 16, 234–241.

Pan, Y.X., Wang, X.P., Zhang, Y.F., 2010. Dew formation characteristics in a revegetation-stabilized desert ecosystem in Shapotou area, northern China. J. Hydrol. 387, 265–272.

Petersen, D.G., Blazewicz, S.J., Firestone, M., Herman, D.J., Turetsky, M., Waldrop, M., 2012. Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. Environ. Microbiol. 14, 993–1008.

Schutt, M., Borken, W., Spott, O., Stange, C.F., Matzner, E., 2014. Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures. Soil Biol. Biochem. 77 (315-315).

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., et al., 2010. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.
Song, Y.Y., Song, C.C., Hou, A.X., Ren, J.S., Wang, X.W., Cui, Q., et al., 2018. Effects of temperature and root additions on soil carbon and nitrogen mineralization in a predominantly permafrost peatland. Catena 165, 381–389.
Steinauer, K., Tilman, D., Wragg, P.D., Cesarz, S., Cowles, J.M., Pritsch, K., et al., 2015. Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. Ecology 96, 99–112.
Steinweg, J.M., Jagadamma, S., Frerichs, J., Mayes, M.A., 2013. Activation energy of extracellular enzymes in soils from different biomes. PLoS One 89, B23B–0414.
Streit, K., Hagedorn, F., Hiltbrunner, D., Portmann, M., Saurer, M., Buchmann, N., et al., 2014. Soil warming alters microbial substrate use in alpine soils. Glob. Chang. Biol. 20, 1327–1338.
Su, Y.G., Zhao, X., Li, A.X., Li, X.R., Huang, G., 2011. Nitrogen fixation in biological soil crusts from the Tengger desert, northern China. Eur. J. Soil Biol. 47, 182–187.
Szukics, U., Abell, G.C.J., Hodl, V., Mitter, B., Sessitsch, A., Hackl, E., et al., 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. FEMS Microbiol. Ecol. 72, 395–406.
Tu, Q.C., He, Z.L., Wu, L.Y., Xue, K., Xie, G., Chain, P., et al., 2017. Metagenomic reconstruction of nitrogen cycling pathways in a CO2- enriched grassland ecosystem. Soil Biol. Biochem. 106, 99–108.
Valentin, C., d'Herbès, J.M., Poesen, J., 1999. Soil and water components of banded vegetation patterns. CATENA 37, 1–24.
Vogel, A., Scherer-Lorenzen, M., Weigelt, A., 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. PLoS One 7, e36992.
Waldrop, M.P., Holloway, J.M., Smith, D.B., Goldhaber, M.B., Drenovsky, R.E., Scow, K.M., et al., 2017. The interacting roles of climate, soils, and plant production on soil microbial communities at a continental scale. Ecology 98, 1957–1967.
Wallenstein, M.D., Weintraub, M.N., 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biol. Biochem. 40, 2098–2106.
Wang, S.P., Duan, J.C., Xu, G.P., Wang, Y.F., Zhang, Z.H., Rui, Y.C., et al., 2012. Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow. Ecology 93, 2365–2376.
Weedon, J.T., Kowalchuk, G.A., Aerts, R., van Hal, J., van Logtestijn, R., Tas, N., et al., 2012. Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. Glob. Chang. Biol. 18, 138–150.
Wei, X.R., Li, X.Z., Jia, X.X., Shao, M.G., 2013. Accumulation of soil organic carbon in aggregates after afforestation on abandoned farmland. Biol. Fertil. Soils 49, 637–646.
Xiao, G.J., Zhang, Q., Zhang, F.J., Ma, F., Wang, J., Huang, J.Y., et al., 2016. Warming influences the yield and water use efficiency of winter wheat in the semiarid regions of Northwest China. Field Crop Res. 199, 129–135.
Xue, K., Yuan, M.M., Shi, Z.J., Qin, Y.J., Deng, Y., Cheng, L., et al., 2016. Tundra soil carbon is vulnerable to rapid microbial decomposition under climate warming. Nat. Clim. Chang. 6, 595–600.
Yu, S., Ehrenfeld, J.G., 2009. The effects of changes in soil moisture on nitrogen cycling in acid wetland types of the New Jersey Pinelands (USA). Soil Biol. Biochem. 41, 2394–2405.
Zaman, M., Chang, S.X., 2004. Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. Biol. Fertil. Soils 39, 269–279.
Zhou, J.Z., Xue, K., Xie, J.P., Deng, Y., Wu, L.Y., Cheng, X.H., et al., 2012. Microbial mediation of carbon-cycle feedbacks to climate warming. Nat. Clim. Chang. 2, 106–110.
Zhou, X.Q., Chen, C.R., Wang, Y.F., Xu, Z.H., Han, H.Y., Li, L.H., et al., 2013. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. Sci. Total Environ. 444, 552–558.