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Distinct abundance patterns of nitrogen functional microbes in desert soil profiles regulate soil nitrogen storage potential along a desertification development gradient

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ABSTRACT

With ongoing global climate change and human activities, increasing desertification plays a predominant role in increasing soil nutrient losses. Soil nitrogen (N) is the essential limiting nutrient supporting plant growth and evaluating soil nutrient content, especially in desert ecosystems. N microbial processes will ultimately restore and maintain the balance in the soil N cycle, but the damage caused by desertification to soil N functional microorganisms associated with N supply, transformation, and loss is poorly understood. We examined changes in soil N and N functional gene (NFG) abundances within vertical profiles (i.e., soil depths of 0–100 cm) throughout the desertification process. We found that the abundance of N functional genes (NFGs) (except the N-fixation gene) decreased during the desertification process, almost all NFGs were attenuated along the vertical profiles, and available phosphorus (AP), soil water content (SWC) and pH were the best explanatory variables. The sums of (*AOA* + *AOB*) (associated with available N transformation) and (*nirK* + *nirS* + *qnorB* + *nosZ*) (associated with N loss) significantly decreased as desertification progressed, leading to decreased N transformation and loss potential. The (*nifH* + *chiA*) associated with available N supply increased along the desertification process gradient, suggesting enhanced potential for the acquisition available N. The increased ratio of (*nifH* + *chiA* + *AOA* + *AOB*)/(*nirK* + *nirS* + *qnorB* + *nosZ*) (associated with available N storage) collaboratively contributed to enhanced available N storage potential throughout the desertification process. Our results lay the foundation for better understanding the distinct responses of NFGs to desertification and the process through which they ultimately regulate available N storage in the degraded soils of desert ecosystems.

1. Introduction

Desertification is one of the most severe forms of land degradation in the world (Tang et al., 2015), leading to degradation of soil conditions and a serious decline in the potential productivity of the soil (Tang et al., 2016b). Approximately 4.5 × 10^8 km^2 of land worldwide experiences some degree of desertification, affecting more than 100 countries and 850 million people (Zhu and Chen, 1994). N is an essential nutrient element for plant growth and is also a key factor for evaluating soil quality. Nitrogen storage potential may be different during desertification. Understanding the change in the N-cycle in a desert ecosystem is critical to the evaluation of the productivity and quality of desert ecosystems (Hall et al., 2011; She et al., 2016).

Soil microbiomes are the pivotal drivers of the soil N-cycle (Levy-Booth et al., 2014). Molecular marker methods have been frequently adopted in the study of soil N-cycle microorganisms (Li et al., 2018). The NFG coding the subunit of enzymes can be adopted to confirm the primary N-cycle process in soils (Song et al., 2019) because these genes integrate recent environment history and process activities (Petersen et al., 2012). The dynamics of the microbial N cycle are an interconnected network of simultaneous processes that account for soil N supply (i.e., N fixation and mineralization), transformation (nitrification) and loss (i.e., denitrification and anaerobic ammonium oxidation) (Shen et al., 2014). These simultaneous N processes involve several functional genes, including N fixation by nitrogenase (*nifH*); mineralization by chitinase (*chiA*); nitrification by ammonia monooxygenase (*amoA*); denitrification by nitrite reductase (*nirK / nirS*), nitric oxide reductase (*qnorB*), and nitrous oxide reductase (*nosZ*) (Petersen et al.,

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2012; Song et al., 2019; Wang et al., 2017). Previous studies have indicated that the abundance of functional gene groups (ratio or sum of functional genes) may provide comprehensive information that contributes to describing the dynamic N-transformation processes (Levy-Booth et al., 2014; Li et al., 2015; Wang et al., 2015). The relationships between the sums of (*nirK* + *nirS*) and underlying NO emissions have been studied in degraded grassland ecosystems and abandoned agricultural areas in karst forest ecosystems (Li et al., 2018; Song et al., 2019). The ratio of (*nirK* + *nirS*)/*nosZ* has been commonly adopted as an index for actual N_2O emissions in wetland ecosystems (Wang et al., 2014), and the ratio of the abundance of *nifH* to (*AOA* + *AOB*) has been used as a proxy for N transformation rates in agricultural soil on the Loess Plateau (Wang et al., 2017). Previous studies have quantitatively evaluated the functional microorganism groups of N-cycle process in different types of ecosystems (i.e., farmland, forest, grassland and wetland), but we have a poor understanding of the quantitative characteristics of N functional microorganism groups in desert ecosystems.

The dynamics of soil N microbial communities can be reshaped and maintained by environmental variables, such as plant species diversity, soil texture, pH, N and phosphorus (P) (Nelson et al., 2016). Liang et al. (2014) reported that compared with secondary forest vegetation, woody shrub and grass tussock, primary forest can significantly increase the diversity of ammonia oxidizing bacteria. Studies by Tang et al. (2016a) showed that N-cycle functional genes for N or/and P additions responded differently. Song et al. (2019) observed that long-term banned grazing enhanced the N emissions potential and N storage potential by increasing the N-cycle functional gene abundance. Studies by Li et al. (2018) showed that the abundance of the N-cycle functional gene increased as the vegetation recovery gradient increased. Lindsay et al. (2010) observed that in comparison to grassland soils, woodland soils can typically enhance the *nirK* gene abundance. It has long been recognized that the N microbial community plays a key role in maintaining ecosystem functioning by supporting processes such as those related to available N supply, transformation and loss. Thus, any loss in N microbial activity owing to environmental changes would likely decrease the capacity of N microbes to sustain ecosystem functions. However, we lack empirical evidence on the dynamics of the N-cycle microbial community in the soil throughout desertification processed in desert ecosystems, and few studies have quantified the relative importance of the N functional community to soil N availability. This scenario hinders our ability to formulate and implement scientific and effective measures to regulate soil N availability in desert ecosystems.

Numerous previous studies have focused on the superficial soil (~20 cm) of the soil profile, which contains the greatest microbial biomass, diversity and activity (Fierer et al., 2003). Nevertheless, the subsoil (deeper than 20 cm), a larger volume that occupies the soil profile, harbours diverse microbes and contains approximately 35% of the total microbial biomass (Hartmann et al., 2009; Jiao et al., 2018). In particular, the subsoil microbial community plays an important role in the potential cycling of soil multi-nutrients and soil formation (Hartmann et al., 2009; Jiao et al., 2018). Understanding the N cycles in the subsoil is pivotal to studying this soil zone, and compared with the microbial communities on superficial soils, the subsoil microbial communities vary between different soil ecosystems (Li et al., 2018). Microbial mass decreases with soil depth, and microorganisms tend to live in a unique vertical environmental niche (Eilers et al., 2012; Tang et al., 2018). Therefore, we cannot assume that the functions of microorganisms and metabolic activities in the superficial reflect the function and metabolic activity of the subsoil soil. There are considerable differences in microbial communities in the profile that drive the biogeochemical cycle of the whole soil profile (Wu et al., 2016). For example, Tang et al. (2018) showed that forest soil C/N ratios attenuated with increasing soil depth accompanied by a decline in the abundance of *nifH* and *chiA* genes responsible for N supply but increased the abundance of *amoA* genes associated with energy acquisition. Li et al. (2018) observed that the *chiA* gene in agricultural soil steadily increased with soil depth. All these studies primarily focused on the variation in N-cycling functional abundance with soil depth in forest soil and agriculture soil. Nevertheless, in desert ecosystems, sandy soil is characterized by high - permeability, leaching and interactions between aboveground plants and belowground soil (D'Odorico et al., 2013). Little has been determined about N-cycling functional microbial potential activity in subsoil in desert ecosystems; thus, it is important to better understand the N availability and N storage potential throughout the desertification process.

The objectives of this study were to assess the effects of desertification on NFG abundances (i.e., *nifH*, *chiA*, *AOA*, *AOB*, *nirK*, *nirS*, *qnorB* and *nosZ*) in soil profiles. We hypothesized that (1) the NFG abundances will decrease due to declining organic matter inputs from plant litter and roots during the desertification process; (2) NFG abundances will be more abundant in superficial soils owing to more organic matter content from plant litter degradation and less limited nutrient content caused by competition from plants, and (3) the key N-cycling process potential (N effectiveness, N transformed, N losses, N storage potential) will be suppressed with the desertification process and soil depth.

2. Material and methods

2.1. Study area

The study site is part of the Mu Us Desert, south of Ordos Plateau, northeast of Yanchi county, Ningxia, with an area of approximately 4×10^4 km^2 and geographic coordinates of 37°27′5″~39°22′5″N, 107°20′~111°30′E. The average altitude is between 1100 and 1300 m, the northwest is slightly higher than the other regions, and this area has a representative semiarid moderate temperate climate. The mean annual temperature at the site is 7.7 °C, and the temperature difference between day and night is large. The mean annual precipitation in this study area is 440 mm. The distribution of rainfall in time and space is uneven, mainly in summer and autumn, accounting for approximately 60% of the annual rainfall, with more precipitation in the southeast and less in the northwest. The soil of the sample area is mainly composed of sand and loessal soil. The vegetation is mainly composed of xerophytes containing *Artemisia desertorum*, *Poa annua*, *Hedysarum laeve*, *Psammochloa villosa*, and *Hedysarum scoparium*. According to the grading index of grassland desertification (Table S1), different stages of desertification were selected as sampling sites in this study area, including potential desertification (PD), moderate desertification (MD), and severe desertification (SD).

2.2. Experimental design and sampling measurements

Three desertification (PD, MD, and SD) stages were selected in July 2017, according to the primary coverage classification criteria for vegetation. Detailed information (coverage, biomass and primary plant species) on all the study sites is shown in Table 1. Ten replicate plots (20 m × 20 m) were randomly established for PD and MD stages, respectively. Five replicate plots were randomly selected for SD stage. Vegetation coverage, aboveground and belowground biomass separately quantified in each quadrat. The aboveground parts in each quadrat were clipped and dried to calculate the aboveground biomass. Leaf litter in each quadrat were collected to obtain litter biomass. Six 1 m × 1 m quadrats were randomly selected in each plot for measuring the characteristics of the vegetation. The roots in each quadrat were washed using tap-water and dried at 60 °C for 48 h to calculate belowground biomass. Nine soil cores in each plot were obtained by S-type mixed as one replicate sample from each soil depth (0–10, 10–20, 20–30, 30–60, and 60–100 cm) using a soil auger with a diameter of 5 cm. Each soil sample (125 samples) was fully mixed and divided into two parts. One part was stored at −80 °C until DNA extraction, and the other part was airdried for the physicochemical analysis.

Table 1
Characteristics of the vegetation during the desertification process.

Desertification stage	Litter biomass (g/m^2)	Aboveground Biomass (g/m^2)	Belowground biomass (g/m^2)	Coverage (%)
PD	232.78 ± 13.63 a	368.97 ± 46.19 a	1225.09 ± 124.39 a	82.25% a
MD	102.32 ± 8.35 b	201.66 ± 6.58 b	534.36 ± 60.41 b	43.8% b
SD	52.47 ± 2.51 c	65.63 ± 4.54 c	211.87 ± 19.71 c	20.22% c
F values	65.70 ***	18.66 ***	26.53 ***	211.43 ***

PD: potential desertification; MD: moderate desertification; SD: severe desertification. Values are expressed as the mean ± standard error. Different letters indicate significant differences ($p < 0.05$) among plants for the individual variables based on one-way ANOVA followed by an LSD test. The last row shows the *F* values of the general linear model and significance (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, respectively).

Table 2
Soil physicochemical properties during process of desertification.

Soil properties	PD	MD	SD	*F* values
Organic C (g kg^{-1})	1.50 ± 0.09 a	0.80 ± 0.02 b	0.76 ± 0.07 b	38.304 ***
Total N (g kg^{-1})	0.15 ± 0.01 a	0.06 ± 0.00 b	0.06 ± 0.00 b	36.089 ***
Total P (g kg^{-1})	0.23 ± 0.01 a	0.26 ± 0.01 a	0.20 ± 0.01 b	7.784 **
Available P (mg kg^{-1})	10.57 ± 0.76 b	10.01 ± 1.00 b	15.89 ± 0.36 a	9.730 ***
Available N (mg kg^{-1})	15.57 ± 0.35 a	15.99 ± 0.35 a	15.21 ± 0.47 a	0.886
pH	8.38 ± 0.06 a	7.91 ± 0.04 b	7.98 ± 0.05 b	26.419 ***
EC (uS/cm)	30.54 ± 1.41 a	19.99 ± 0.69 b	20.42 ± 0.81 b	30.938 ***
Soil water content (%)	0.30 ± 0.01 b	0.42 ± 0.01 a	0.40 ± 0.01 a	24.236 ***

PD: potential desertification; MD: moderate desertification; SD: severe desertification. Values are expressed as the mean ± standard error. Different letters indicate significant differences ($p < 0.05$) among soils for the individual variables based on one-way ANOVA followed by an LSD test. The last column shows the *F* values of the general linear model and significance (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, respectively).

2.3. Physicochemical characteristics of soil

Soil physicochemical analysis for organic C (OC), total N (TN), available N (AN), total phosphorus (TP), available P (AP), pH, electrical conductivity (EC) and soil water content (SWC) was conducted by using previously described standard methods (Bao, 2000; Zhang et al., 2016).

2.4. Quantitative polymerase chain reaction (qPCR)

Microbial DNA was extracted from 0.5 g of desert soil by using the MP Fast DNA spin kit for soil (MP Biomedicals, Santa Ana, CA, USA) and then detected with 1% agarose gel electrophoresis and stored at −20 °C until use (Zhi et al., 2015). NFG abundances were calculated by Ct value using a real-time PCR system (Quantstudio 6 Flex, Thermo Fisher, Singapore City, Singapore). The plasmid ten-fold continuously diluted method of a target gene was used to generate a standard curve using a real-time PCR system (Tang et al., 2018). Reaction mixtures (20 μL) contained 10 μL SYBR® Premix ExTaq™ (Dining), 0.5 μL DNA, 0.5 μL of primer (forward and reverse) (20 μM), and 8.5 μL sterile distilled water. At the same time, we tested the amplification specificity by performing a dissolution curve. Detailed information on the gene primers is given in Table S2.

2.5. Data analysis

One-way analysis of variance (ANOVA) and the least significant difference (LSD) test ($p < 0.05$) were performed to assess the difference in soil properties, plant cover and the abundance of NFG. Statistical analyses of the data were calculated using SPSS (IBM SPSS Statistics 25.0; International Business Machines Corporation, Armonk, NY, United States) and OriginPro 9.1 (OriginLab Corporation, One Roundhouse Plaza, Suite 303, Northampton, MA 01060, United States). A structural equation model (SEM) was performed with Amos 22.0 (International Business Machines Corporation, Armonk, NY, United States) to examine the whether the relationship between soil properties and NFG abundance. Random forest model analysis, to identify the most important predictors of varied functional gene abundance, was conducted in the R version 3.4.1 (https://www.r-project.org/).

3. Results

3.1. Vegetation and soil properties during desertification

In our study, the plant community and soil properties changed significantly with the process of desertification. ANOVA (Table 1) showed that desertification stage had a negative influence on the coverage and biomass of the plant community. The results showed that plant coverage decreased significantly with the desertification process from 77.92% to 45.72% to 12.34%. The results showed lower aboveground, belowground and litter biomass in the SD stage than in the other stages, and biomass peaked at the PD stage at 368.97 g/m^2, 1225.09 g/m^2 and 232.78 g/m^2, respectively. The dominant species of the community varied throughout the desertification process (Table S1). *Artemisia desertorum* and *Poa annua* dominated the vegetation community during the PD stage. These dominant species of the PD stage gradually decreased during the desertification process, became companion species at the MD stage, and disappeared at the SD stage. Except for *Artemisia desertorum*, *Hedysarum laeve* and *Psammochloa villosa* were the dominant species in the MD and SD stages, respectively.

Table 2 showed that the contents of SOC and TN decreased during the process of desertification and peaked at the PD stage, with the highest and lowest contents of 4.01 g/kg and 0.39 g/kg and 0.32 g/kg and 0.24 g/kg, respectively. TN and SOC significantly decreased with soil depth and peaked at the 0–10 cm layer (Table S3). The content of TP declined with the desertification process but varied slightly at different soil depths. The content of AN decreased but did not change significantly in the process of desertification, while decreased significantly with soil depth (Table S3). The pH values decreased with the desertification process, ranging from 8.38 to 7.98, while it slightly increased with soil depth (Table S3). The content of AP increased markedly during desertification and did not change markedly with soil depth. Soil EC declined during the desertification process and had different fluctuating trends at different soil depths. The soil water content increased during the process of desertification, yet it showed different fluctuation trends at different soil depths similarly to that of soil EC (Table S3).

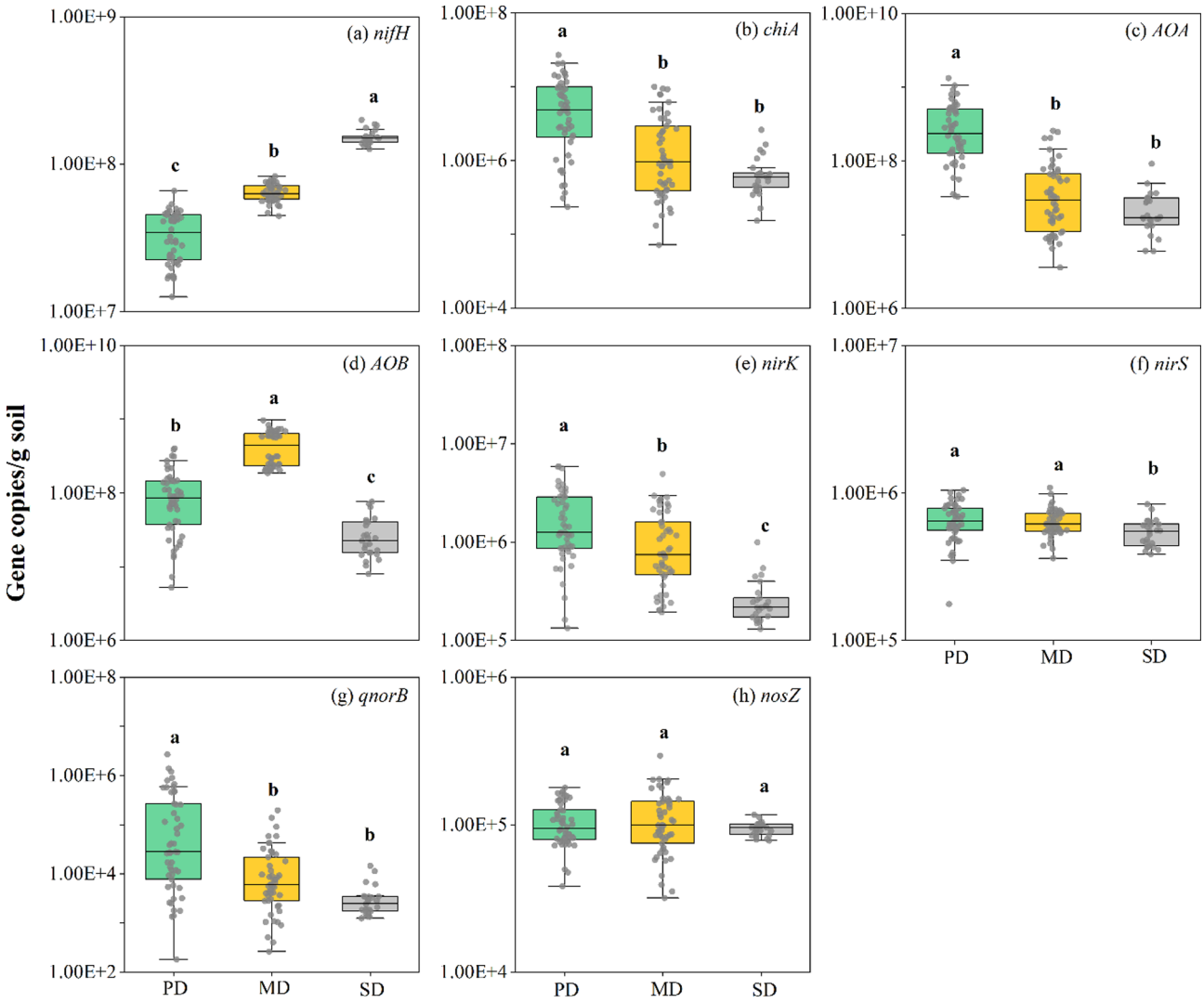

Fig. 1. The absolute abundances of NFG in different stages of desertification. PD: potential desertification; MD: moderate desertification; SD: severe desertification. Significant differences were tested by analysis of variance and post hoc Tukey's HSD test. Different lower-case letters indicate variations significant at the 0.05 level ($p < 0.05$). Error bars indicate the SE.

3.2. Abundance of NFG with the desertification process and soil profiles

The abundance of NFG changed remarkably during the different stages of desertification (Fig. 1) and varied obviously at different soil depths (Fig. S1). The abundance of the *nifH* gene, a nitrogen fixation gene ($N_2 \rightarrow$ organic N), increased significantly from 1.3×10^7 g^{-1} soil to 2.0×10^8 g^{-1} soil in the desertification process (Fig. 1a) but varied slightly with soil depth (Fig. S1). In contrast, the abundance of the *chiA* gene, which is an organic N mineralization gene (organic N $\rightarrow$ NH_4^+), decreased significantly during the desertification process and had its the highest and lowest gene abundance in the PD stage and SD stage of desertification at 2.71×10^7 copies g^{-1} soil and 7.16×10^4 copies g^{-1} soil (Fig. 1b), respectively. In addition, the abundance of the *chiA* gene decreased with soil depth (Fig. S1). Ammonia oxidation ($NH_4^+ \rightarrow NO_2^-$) as the first step in nitrification is characterized by the *AOA* gene and *AOB* gene. The abundance of the *AOA* gene decreased during the desertification process, ranging from 3.6×10^6 g^{-1} soil to 1.3×10^9 g^{-1} soil, while the *AOB* gene fluctuated from 5.2×10^6 g^{-1} soil to 9.8×10^8 g^{-1} soil (Fig. 1c). Similarly, the *AOA* and *AOB* genes decreased with soil depth (Fig. S1).

The abundance of the *nirK*, *nirS*, *qnorB*, and *nosZ* genes, which are the four functional genes associated with the (denitrification) NO_2^- deoxidize pathway ($NO_2^- \rightarrow N_2$), were significantly different among the different stages of desertification. The *nirK* and *nirS* genes both decreased significantly with desertification (Fig. 1). The *nirK* gene abundance decreased with soil depth, while the *nirS* gene fluctuated (Fig. S1). The abundance of the *qnorB* gene decreased in the process of desertification and with soil depth, but it was not significantly different in the last two stages (MD and SD) (Fig. 1g). The *nosZ* gene abundance exhibited no significant difference during the desertification process (Fig. 1h), while the abundance decreased with soil depth (Fig. S1).

The (*nifH* + *chiA*) genes indicate the supply of available N ($N_2 \rightarrow NH_4^+$) and increased significantly during the desertification process (Fig. 2a). There was a significant difference between the superficial soil (0–20 cm) and subsoil (20–100 cm) in the PD stage (Fig. 3a), while the other two stages (MD and SD) had no significant difference between the soil depth. The (*AOA* + *AOB*) genes indicating the transformation of available nitrogen ($NH_4^+ \rightarrow NO_2^-$) decreased significantly throughout the desertification process (Fig. 2b) and had the same trend as the (*nifH* + *chiA*) with soil depth (Fig. 3b). The losses of available N ($NO_2^- \rightarrow N_2$) represented by the sum of the denitrification genes (*nirK* + *nirS* + *qnorB* + *nosZ*) were significantly affected by desertification and significantly decreased during the process of desertification (Fig. 2c). There was a significant difference between the superficial soil

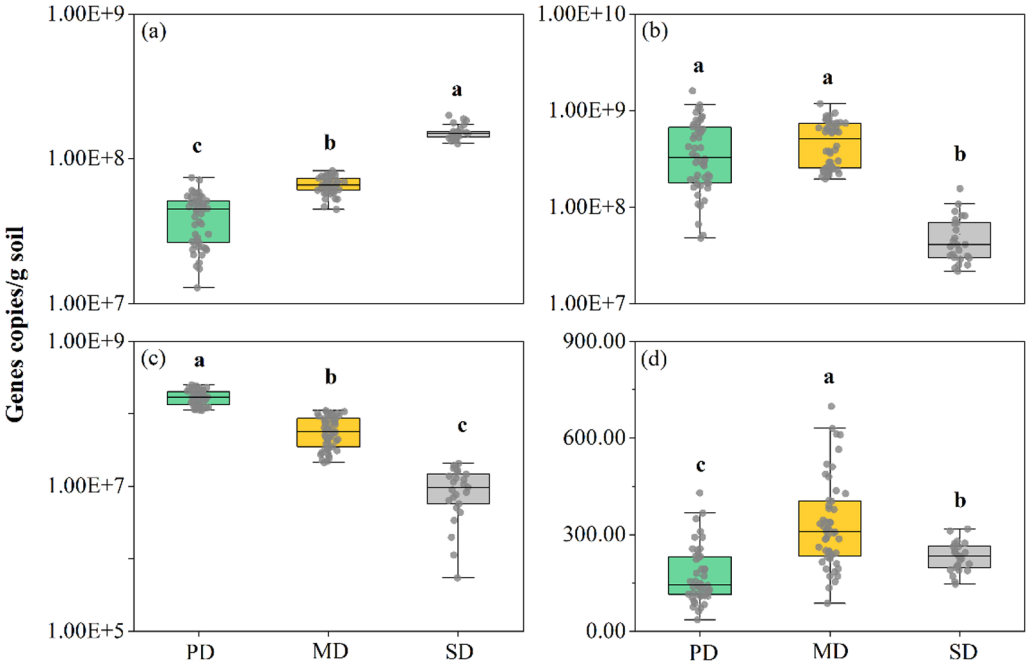

Fig. 2. The absolute abundances difference of nitrogen cycling process in different stages of desertification. (a) (*nifH* + *chiA*); (b) (*AOA* + *AOB*); (c) denitrification genes (*nirK* + *nirS* + *qnorB* + *nosZ*); (d) (*nifH* + *chiA* + *AOA* + *AOB*)/(*nirK* + *nirS* + *qnorB* + *nosZ*). PD: potential desertification; MD: moderate desertification; SD: severe desertification. Significant differences were tested by analysis of variance and post hoc Tukey's HSD test. Different lower-case letters indicate variations significant at the 0.05 levels ($p < 0.05$). Error bars indicate the SE.

and subsoil in the PD and MD stages (Fig. 3c), while there was no significant difference between them in the SD stage. The available N storage potential, represented by the ratio of (*nifH* + *chiA* + *AOA* + *AOB*)/(denitrification genes), increased significantly during the desertification process (Fig. 2d). There was a difference between the superficial soil and the subsoil only in the MD stage (Fig. 3d).

3.3. Relationships between the abundance of the NFG with soil properties

Random forest modelling was used to identify the key important predictors (SOC, TN, AN, TP, AP, SWC, EC, and soil pH) of the variation in the NFGs. Our random forest models showed that in comparison to other soil properties, TN, SOC, and pH were more important predictive factors (Fig. 4). Indeed, for the variation in *nifH* gene abundance, SOC

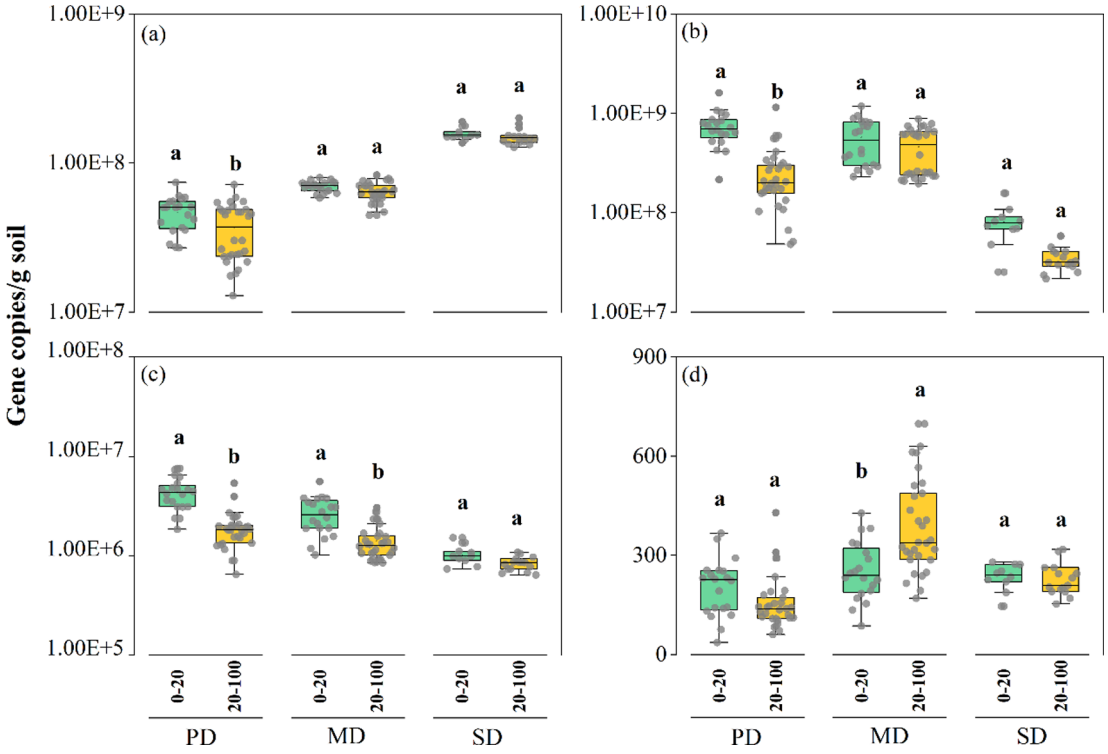

Fig. 3. The absolute abundances difference of mainly nitrogen cycling process in different soil profiles. (a) (*nifH* + *chiA*); (b) (*AOA* + *AOB*); (c) denitrification genes (*nirK* + *nirS* + *qnorB* + *nosZ*); (d) (*nifH* + *chiA* + *AOA* + *AOB*)/(*nirK* + *nirS* + *qnorB* + *nosZ*). PD: potential desertification; MD: moderate desertification; SD: severe desertification. Significant differences were tested by analysis of variance and post hoc Tukey's HSD test. Different lower-case letters indicate variations significant at the 0.05 levels ($p < 0.05$). Error bars indicate the SE.

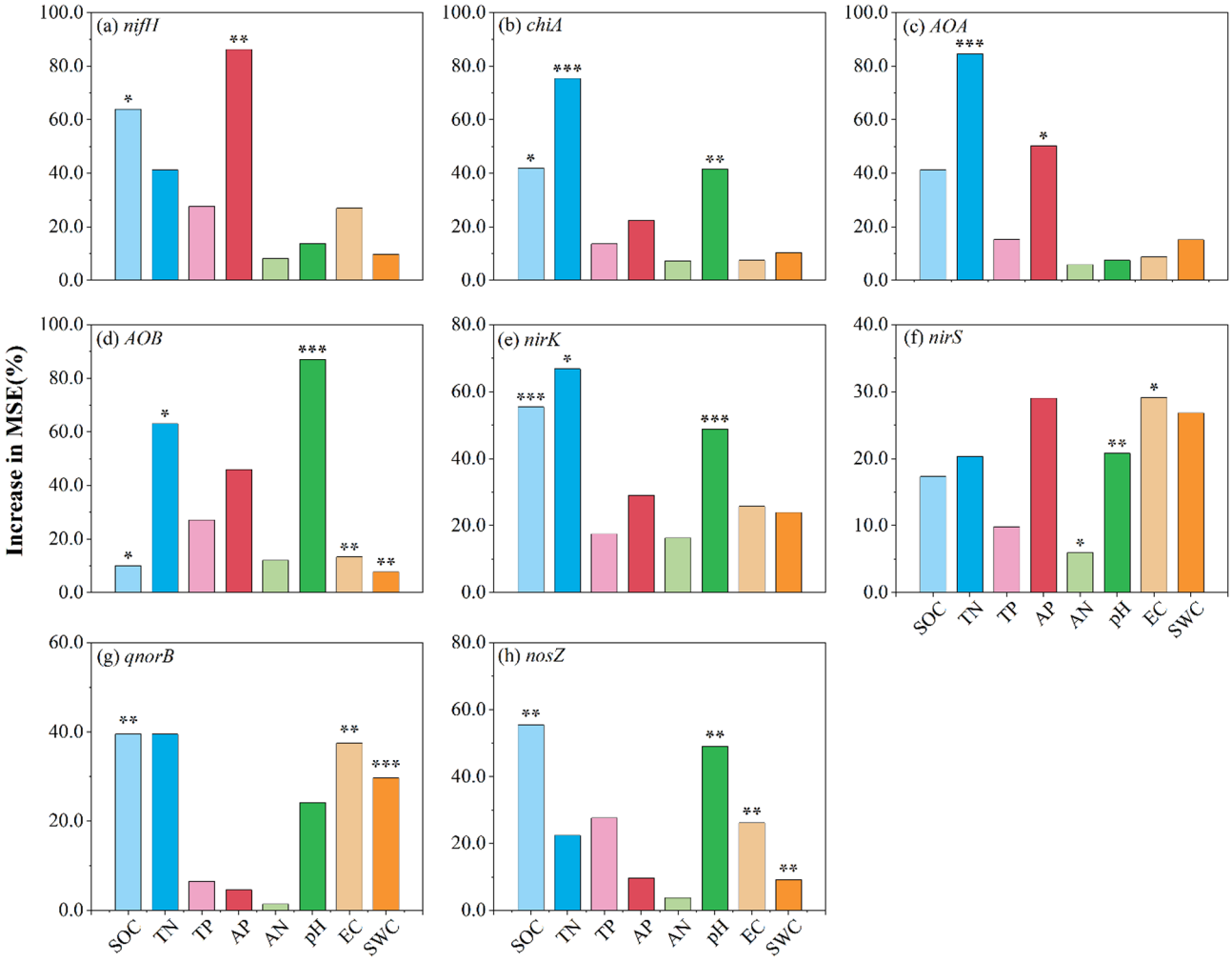

Fig. 4. Potential drivers of variation in soil nitrogen cycling functional genes (NFGs) in desertification ecosystems. SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, AP: available phosphorus, AN: available nitrogen, EC: electrical conductivity, SWC: soil water content. a Random forest (RF) mean predictor importance (percentage of increase of mean square error) of soil properties indices as drivers for the soil nitrogen cycling function genes index, in the whole profile. Percentage increases in the MSE (mean squared error) of variables were used to estimate the importance of these predictors, and higher MSE% values imply more important predictors. Significance levels are as follows: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. MSE, mean squared error.

and TN were more important predictors than other soil properties (Fig. 4a). For the *chiA* gene, SOC, pH and TN were more important predictors than other soil properties (Fig. 4b). TN and AP were more important predictors for the *AOA* gene (Fig. 4c), while for the *AOB* gene, in comparison to other soil properties, SOC, TN, pH, EC and SWC were more important predictors (Fig. 4d). For the *nirK* gene, pH, TN and SOC were the relatively more important predictors (Fig. 4e), while for the *nirS* gene, important predictors were AN, EC and pH (Fig. 4f). For the *qnorB* and *nosZ* genes, SOC, EC and SWC were the more important predictors (Fig. 4g and h). Fig. 5a shows that for the (*nifH* + *chiA*) genes, AP and SWC were the more important predictors. For the (*AOA* + *AOB*) genes, SOC, AP, pH, EC and SWC were the relatively more important predictors (Fig. 5b). For the denitrification genes (*nirK* + *nirS* + *qnorB* + *nosZ*), SOC and pH were the most important predictors (Fig. 5c). AN, pH, EC and SWC were the relatively more important predictors for the (*nifH* + *chiA* + *AOA* + *AOB*)/(denitrification genes) (Fig. 5d).

Structural equation modelling (SEM) was used to test whether the significant relationship between the abundance of NFG and environmental factors (i.e., desertification stage, soil depth and soil physicochemical properties). Fig. S2 and Fig. S3 show the correlation relationship between single N-cycling functional gene with soil properties and desertification stage. The results showed that the desertification stage was negatively correlated with the *nifH* gene and positively correlated with all the N-cycling functional genes except for the *nirS* gene. We found that soil depth negatively related to the *chiA*, *AOA*, *nirK* and *nosZ* genes. The results showed that TN was positively correlated with the *chiA* gene and *AOA* gene and negatively correlated with the *nosZ* gene. We found that TP had a negative effect on the *chiA*, *nifH*, *AOA* and *qnorB* genes, and a positive effect on the *AOB* gene. The results indicated that pH was negatively correlated with the *nirK* and *AOB* genes and positively correlated with the *nirS* gene. SOC was positively related to the *nirK* and *nosZ* genes and negatively related to the *nirS* gene. We also found positive relationships between the EC and *nosZ* and negative relationships between the EC and *nirS* and the *nifH* and *qnorB* genes. AN had a positive effect on the *nifH* and *qnorB* genes. We also found that SWC was positively related to the *nosZ* gene and negatively related to the *nifH*, *qnorB* and *nirS* genes.

Fig. 6 shows the correlation relationship between the functional gene groups (available N supply, available N transformation, available N loss, and available N storage potential) and soil properties, soil depth and desertification stage. The abundance of the (*nifH* + *chiA*) gene group was positively correlated with AN and negatively correlated with EC, SWC, TP and desertification stage. The abundance of the (*AOA* + *AOB*) gene group was positively correlated with TP, EC and SWC and negatively correlated with pH, AP and desertification stage.

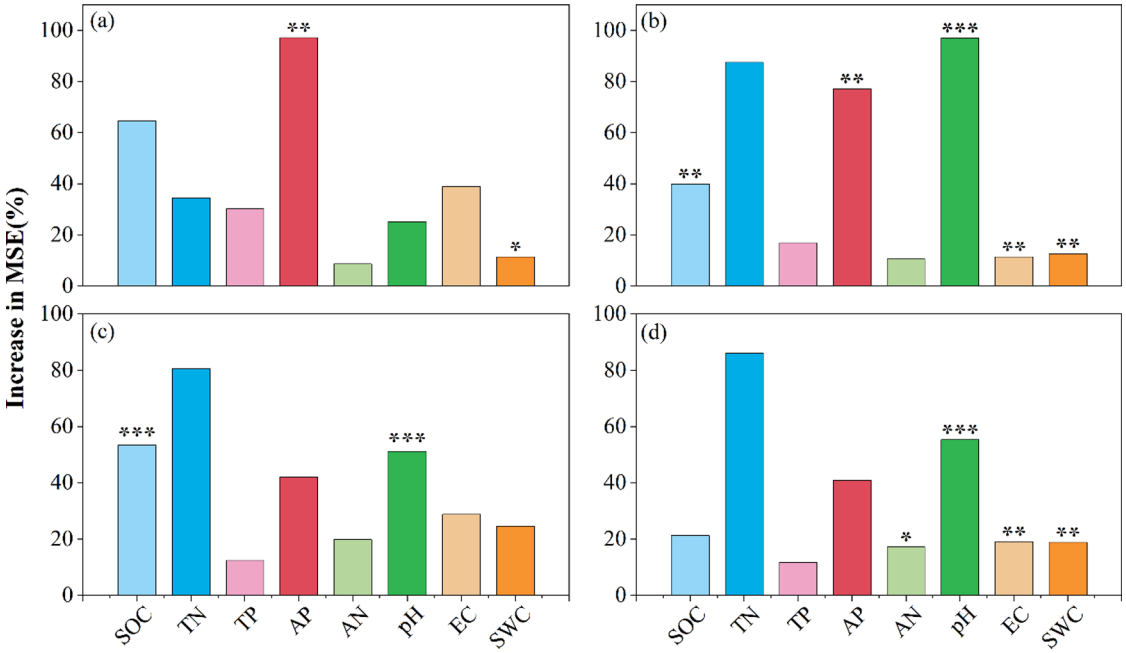

Fig. 5. Potential drivers of functional gene variation in key soil nitrogen cycle processes in desertification ecosystems. (a) (*nifH* + *chiA*); (b) (*AOA* + *AOB*); (c) denitrification genes (*nirK* + *nirS* + *qnorB* + *nosZ*); (d) (*nifH* + *chiA* + *AOA* + *AOB*)/(*nirK* + *nirS* + *qnorB* + *nosZ*). SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, AP: available phosphorus, AN: available nitrogen, EC: electrical conductivity, SWC: soil water content. A Random forest (RF) mean predictor importance (percentage of increase of mean square error) of soil properties indices as drivers for the soil nitrogen cycling function genes index, in the whole profile. Percentage increases in the MSE (mean squared error) of variables were used to estimate the importance of these predictors, and higher MSE% values imply more important predictors. Significance levels are as follows: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. MSE, mean squared error.

The abundance of the denitrification gene group (*nirK* + *nirS* + *qnorB* + *nosZ*) was positively correlated with SOC and desertification stage and negatively correlated with pH and soil depth. The ratio (*nifH* + *chiA* + *AOA* + *AOB*) / (denitrification genes) of nitrogen storage potential was negatively correlated with desertification stage, AN, and pH, and positively correlated with EC and SWC.

4. Discussion

4.1. Plant characteristics and soil properties throughout the desertification process

Land desertification, caused by aeolian soil erosion, decreased the soil N storage by removing the organic nitrogen-rich component leading to a reduction in plant production (Lowery et al., 1995; Tang et al., 2015). Our study showed that the biomass (aboveground, belowground and litter) decreased markedly during the different stages of desertification (Table 1), the result is consistent with Tang et al. (2015), indicating that desertification has a negative effect on plant productivity.

In a desert system, SOC and TN contents are the main factors that determine the sandy soil nutrition content and are also the limiting factors that affect the production of vegetation (Wezel et al., 2000). Our study showed that SOC and TN content declined as desertification progressed (Table 2), in accordance with the results of (Tang et al., 2015). At the same time, we found that SOC and TN had the same variation trend as plant biomass throughout desertification. The results demonstrated that variation in soil C and N content was consistent with the desertification process, indicating that sandy soil has a similar response to desertification as that of ground vegetation. Our results are not consistent with the results of Tang et al. (2015), who reported that compared to ground vegetation, sandy soil has a more impressible response to desertification. The results of this study are consistent with the results of Feng et al. (2002), who noted that desertification decreased the nutrient content in the soil due to reduced biomass. Moreover, as shown in previous studies, soil nutrients decrease with soil depth (Tang et al., 2015). In this study, the highest nutrient content occurred in the superficial soil, while soil water content had an inverse trend that was not consistent with the results of Cheng et al. (2016) and that may have been caused by strong wind erosion, plant uptake, and leaching in desert areas. The content of AN decreased as desertification processed, which may due to decrease of soil available nutrients caused by the increase of soil leaching. Soil pH did not vary markedly during the different stages of desertification, consistent with the findings of previous studies (An et al., 2008; Zhang et al., 2016), indicating that desertification progress had a slight effect on the pH of the soils at the study site. A more interesting thing is that soil water content increased significantly throughout the desertification process. There may be two reasons to explain that the soil water content increased with the desertification process. On the one hand, soil EC decreased significantly in the process of desertification, leading to lower salinity and less water evaporation (Harbeck, 1955). On the other hand, vegetation coverage decreased during the process of desertification, which reduced the loss of soil water due to the absorption of water by vegetation (Yang et al., 2018). This differences in soil properties may have had a strong influence on the soil microorganisms (Wang et al., 2017).

4.2. Desertification progress influences on NFGs

Numerous studies have reported that NFG abundance has a close correlation with N transformation rates and is hence generally used to evaluate the N-cycle potential of soil ecosystems (Ning et al., 2015; Tang et al., 2018). In our study, desertification decreased the microbial N turnover potential, with almost all gene abundance decreasing during the process of desertification (Fig. 1). Zhang et al. (2019) have indicated that soil properties drive variations in microbial diversity and functions in different ecosystems. Song et al. (2019) study showed that aboveground biomass and soil nutrients have a crucial effect on variation in NFGs. Our study also found that soil nutrients have a decisive effect on the variation in NFG abundance (Fig. S2 and S3).

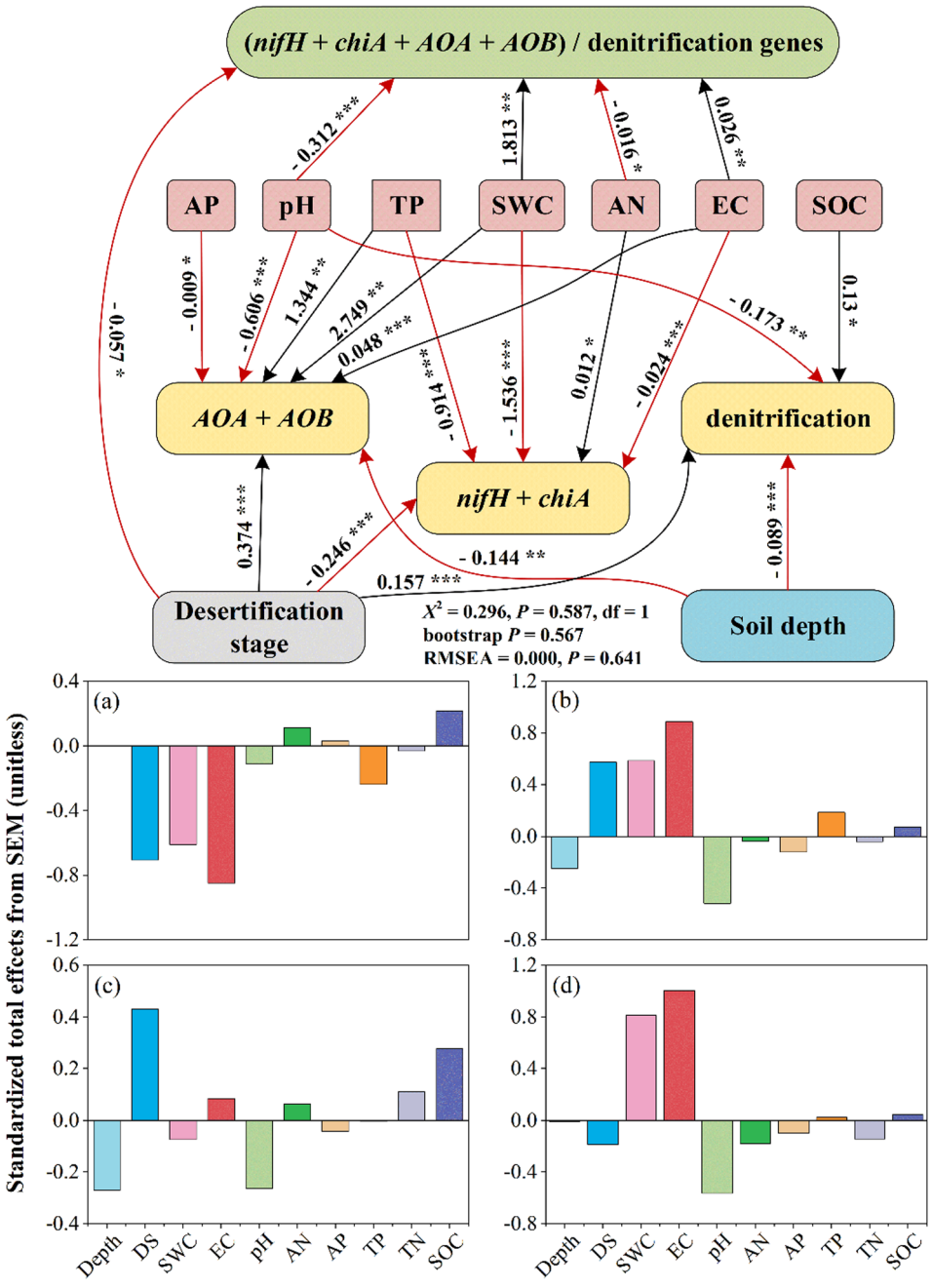

Fig. 6. Direct and indirect effects of soil properties, desertification stage and soil depth on nitrogen cycling functional genes (NFGs). (a) (nifH + chiA); (b) (AOA + AOB); (c) denitrification genes (nirK + nirS + qnorB + nosZ); (d) (nifH + chiA + AOA + AOB)/(nirK + nirS + qnorB + nosZ). DS: desertification stage; Depth: soil depth; SOC: soil organic carbon, TP: total phosphorus, AP: available phosphorus, AN: available nitrogen, EC: electrical conductivity, SWC: soil water content. Numbers adjacent to arrows are indicative of the effect-size (bootstrap p value) of the relationship. Black and red arrows indicate positive and negative relationships, respectively. R^2 denotes the proportion of variance explained. (a, b, c, d) Standardized total effects (direct plus indirect effects) derived from the structural equation models depicted above.

The abundance of the *chiA* gene continuously decreased throughout the desertification process (Fig. 1b), which attenuated the organic N decomposition activity, decreasing the NH_4^+-N supply (organic N → NH_4^+-N). The result is consistent with those of the other study of Song et al. (2019), whose result showed that grazed grassland soil has a lower abundance of the *chiA* gene than that on banned-grazing grassland soil. These results could be explained by biomass, in which a reduction in the C and N content was due to the decrease in litter decomposition. Our SEM results support this finding that the desertification stage had a significantly positive effect on the *chiA* gene (Fig. S2).

The symbiotic N_2 fixation ability of nitrogen-fixing bacteria in most soils is related to the activity of the *nifH* gene (Burgmann et al., 2003), while there may be different conditions in particular ecosystems. Interestingly, our study showed that TN content decreased during the process of desertification, while *nifH* gene abundance increased (Fig. 1a), indicating that the potential activity of nitrogen-fixing microorganisms is increased. It is consistent with Fan et al. (2019), long-term fertilization suppressed the relative abundance of key nitrogen-fixing bacteria, and the result is similar to that of Coelho et al. (2015), whose result suggests that the highest number of *nifH* gene copies was observed at low levels of applied nitrogen, the gene copies had inverse trends with the amount of fertilizer application. While, the result is inconsistent with other results of Song et al. (2019) and Wang et al. (2017), who suggested that the *nifH* gene abundance increased with banned-grazing time and revegetation. The difference may have been due to the different properties of the environment in the study area. Another possible explanation for this result is that the collected soil samples were bulk soil rather than rhizosphere soil, so the *nifH* gene detected was mainly from free-living N-fixing bacteria (Hayden et al., 2010). Existing research results indicated that contributions of free-living nitrogen-fixing bacteria to ecosystem regeneration may be necessary (Hayden et al., 2010; Li et al., 2018). And Barron (2009) noted that in dry oligotrophic deserts, autogenous nitrogen fixation is likely to

produce more nitrogen sources than symbiotic nitrogen fixation. Simultaneously, our SEM showed negative relationships between desertification stage and *nifH* gene abundance (Fig. S2), indicating that the nitrogen-fixing microorganism are mainly autotrophic autogenous nitrogen-fixing bacteria in desert ecosystem.

The marker gene *amoA* (encodes the α-subunit of the AMO enzyme) is primarily used to study nitrification, and it has an essential role in energy-generating metabolism and thus suited for molecular studies of ammonia-oxidizing microbes (bacteria and archaea) communities (Norton et al., 2002). Our study showed that the abundance of the *AOA* gene decreased significantly during the desertification process, while the *AOB* gene fluctuated (Fig. 1). This result indicates that ammonia-oxidizing microbe (*AOA* and *AOB*) community sensitivity was different due to the changed environment. In addition, we found that the soil TN concentration had a positive correlation to *AOA* abundance but had no correlation to *AOB* abundance as desertification occurred (Fig. S2). Moreover, studies by Song et al. (2019) showed that inorganic N concentrations were positively correlated with the abundance of both *AOA* and *AOB*. The above different responses may have been caused by differences in soil texture, temperature and precipitation at the study site.

Our results indicated that the abundance of denitrification genes (*nirK*, *nirS*, *qnorB*, and *nosZ*) exhibited different trends as desertification progressed. *NirK* and *nirS* gene abundance decreased during the desertification process (Fig. 1), indicating that nir-harboring microorganisms selected different habitats when soil conditions changed greatly (Levy-Booth et al., 2014). This view has been supported by previous studies (Tang et al., 2016a), which suggest that the niche of these two types of bacteria (*nirK*- and *nirS*- type) are in charge of their respective behaviours. At the same time, we found that the *nirK* gene varied more than the *nirS* gene, suggesting that the *nirK* gene was more sensitive than the *nirS* gene to varied soil environments during desertification. The results were similarly to those of Song et al. (2019), and *nirK* was always more abundant than *nirS* at every restoration stage. These results demonstrate that the *nirK* gene should be the key indicator of denitrification. Our SEM showed that SOC has a strongly positive effect on the *nirK* gene. The result is inconsistent with that of Levy-Booth et al. (2014), who found that for the increases in SOC, in comparison to the *nirK* gene, the *nirS* gene was more sensitive. This difference may have been caused by differences in the soil environment, including nutrition content, pH and EC. We observed that the abundance of the *qnorB* gene decreased during the desertification process (Fig. 1), indicating that the last step of the denitrification activity was suppressed. The SEM showed that the abundance of the *qnorB* gene was positively correlated with AN and desertification stage, and the random forest showed that among the variables, SOC was the most important predictor (Fig. 4). Available resources are gradually reduced as desertification proceeds, while denitrification is an energy consumption process. Therefore, the potential denitrification activity gradually weakens with the gradual decrease in the available resources as desertification proceeds. The abundance of the *nosZ* gene did not differ significantly as the process of desertification occurred (Fig. 1), suggesting that variation in the soil properties has little effect on the *nosZ* gene. However, this result is not consistent with that of Wang et al. (2017), and his study showed that the *nosZ* gene steadily increased during the vegetation recovery process. The difference may result in different soil properties from different study sites.

A single nitrogen transformation process may be indicated by the varied single functional gene activity, while functional gene groups (the sum or proportion) are helpful in describing the dynamics of the process of the N-cycle (Petersen et al., 2012; Wang et al., 2015; Zhi and Ji, 2014). Our results demonstrated that (*nifH* + *chiA*), (*AOA* + *AOB*), (*nirK* + *nirS* + *qnorB* + *nosZ*), and (*nifH* + *chiA* + *AOA* + *AOB*)/(denitrification genes) were the pivotal functional gene groups that account for the available N turnover process and storage potential throughout the desertification process. The first variable, (*nifH* + *chiA*) associated with the process of available N supply, increased during the desertification process (Fig. 2) and had a positive correlation with AN (Fig. S2); both the *nifH* and *chiA* genes were primarily involved in N fixation and N mineralization. The second variable, (*AOA* + *AOB*) and identified as for available N transformation in the nitrification processes, decreased significantly as the process of desertification occurred (Fig. 2), indicating that the potential of nitrogen transformation was significantly reduced. The third variable, (*nirK* + *nirS* + *qnorB* + *nosZ*) and the sum of the four denitrification genes identified as available N losses, decreased significantly as desertification progressed (Fig. 2). Denitrification is a process of energy consumption with a positive relationship with SOC and desertification stage. Finally, (*nifH* + *chiA* + *AOA* + *AOB*)/(denitrification genes) including available nitrogen increase ($N_2 \rightarrow NH_4^+$), transformation ($NH_4^+ \rightarrow NO_2^-$), and loss ($NO_2^- \rightarrow N_2$), identified as available nitrogen storage potential, increased significantly during the desertification process (Fig. 2), and showed a negative relationship with AN and desertification stage (Fig. 6). The result indicated that available nitrogen storage potential enhanced as the process of desertification occurred.

4.3. Changes in functional gene abundance with soil depth

The distribution of soil microbes in the soil profile is tightly tied to the soil properties (i.e., SOC, TN, AP and pH) (Castellano-Hinojosa et al., 2018). Most studies have shown that microorganisms are not active in the subsoil with a decrease in the C and N substrate of microorganisms as soil depth increases (Stone et al., 2015). Our study showed that the abundance of the *nifH* gene decreased with soil depth (Fig. S1). This result is consistent with results of previous studies (Tang et al., 2018), indicating that N-fixation substantially exists in the superficial soil (Song et al., 2019). The abundance of heterotrophic decomposers may be the reason for prevalent N-fixation in the superficial soil, whose N requirements are higher than that of aboveground litter as it is biodegraded (Hayden et al., 2010). Our study indicated that the abundance of the *chiA* gene decreased with soil depth (Fig. S1), and random forest showed that of the variables, SOC and TN were the best predictors of variation in *chiA* gene abundance (Fig. 4), indicating that the strategy of resource acquisition for N microbial enrichment is strongly affected by resource abundance. The result is consistent with that of Song et al. (2019). However, our results were contradictory to those of Stone et al. (2015), who found that in tropical soils, the abundance of the *chiA* gene increased with depth. The reason for the different responses may be the different conditions (i.e., climate, soil properties, and vegetation composition) in the study areas. Our study emphasizes the specificity of the desert ecosystem, and the response of NFGs involved in N-cycle processes to desertification should be unique. The studies of Song et al. (2019) showed that in grasslands where long-term grazing is banned, the *AOB* abundance decreased with depth while the abundance of *AOA* increased. In contrast, our study found that the abundance of *AOA* and *AOB* decreased with depth (Fig. S1). The results demonstrated that the ammonium oxidizing archaea and the ammonium oxidizing bacteria may occupy distinctive habitats in the soil profile of desert ecosystems (Li et al., 2018).

Our study determined that the *nirK* gene abundance declined with soil depth, while the *nirS* gene abundance increased in the (0–30 cm) soil profile and decreased in the (30–100 cm) soil profile (Fig. S1). The result was not consistent with those in previous studies (Castellano-Hinojosa et al., 2018), which found that the gene abundance of *nirK* and *nirS* increased with soil depth. The difference may be due to variations in soil environmental conditions and nir-harbouring microorganisms (*nirK* and *nirS*) occupying different niches (Levy-Booth et al., 2014). This concept is supported by Tang et al. (2016a), who showed that *nirK*- and *nirS*-type bacteria are control their different behaviours. The abundance of the *qnorB* gene decreased with soil depth (Fig. S1), suggesting that NO (not measured in this study) gradually accumulated with soil depth. Our study showed that a steady decrease in *nosZ* gene

abundance with soil depth weakened the last step of the denitrification process and enhanced the emissions potential of N_2O (not measured in this study).

We studied the variation in the N-cycle process potential in the soil profile. Previous studies of the N-cycle process focused only on superficial soil (0–20 cm), but there has been little research on subsoil (20–100 cm), particularly in desert ecosystems. Our study found that the abundance of (*nifH* + *chiA*) and (*AOA* + *AOB*) genes decreased remarkably with soil depth in PD stage, while the other stages (MD and SD) of desertification showed no significant difference between the superficial soil and subsoil (Fig. 3). This result is explained by the fact that the amount of litter in the PD stage is relatively high, and there is essentially no litter in the other stages (MD and SD). The difference in superficial soil and subsoil nutrition in the PD stage is more obvious. The results showed that in the MD and SD stages, the potential of supplying available N and transforming available N were not significantly different within the soil profile, suggesting that the subsoil microorganisms are non-negligible and that theories also play an important role. The abundance of denitrification genes (*nirK* + *nirS* + *qnorB* + *nosZ*) was significantly different between the superficial soil and subsoil, except for that in the SD stage (Fig. 3). The denitrifying bacterial heterotrophic microorganisms, with their consumption of nutrients and progress of denitrification, suppressed the subsequent process. The available N storage potential represented by (*nifH* + *chiA* + *AOA* + *AOB*)/(denitrification genes) was significantly different between the superficial soil and subsoil in only the MD stage (Fig. 3). The reason for this scenario was that the process included several processes, such as the supply of available N, the transformation of available N and the loss of available N. The factors that affect the available N storage potential are more complex.

In general, our study demonstrated that NFG abundance responded distinctively to the different stages of desertification and soil profiles. In addition, with changes in different environmental factors, NFG activity exhibited differing sensitivities, which likely reflects diverse environmental adaptation and ecological interactions among the various microbial functional groups in the N cycle (Wang et al., 2015).

5. Conclusion

Our study found that soil NFGs respond differently to the differences in desertification processes and soil profiles, suggesting that N-cycle microbes showed sensitivity differences to changes in plant communities and soil properties during desertification. These differences are closely related to the decline in ecological functions during the desertification process. First, the potential of supplying available N increased throughout the desertification process and was not significantly different at different soil depths (0–20 cm and 20–100 cm) in the latter two stages (MD and SD). Second, the potential of transforming available N decreased during the desertification process, and was not significantly different with increasing soil depth in the latter two stages (MD and SD). Third, the potential of losing available N decreased during the desertification process and was significantly different at different soil depths in the first two stages (PD and MD). Finally, the storage potential of available N increased as desertification progressed and showed significant differences in the different soil profiles only in the MD stage. This study broadens our understanding of the process related to available N turnover and N storage potential in the soil profile during different desertification stages.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2020.104716.

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