Saline and alkaline stresses alter soil properties and composition and structure of gene-based nitrifier and denitrifier communities in a calcareous desert soil

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Abstract

**Background:** Saline and alkaline stresses damages the health of soil systems. Meanwhile, little is known about how saline or alkaline stress affects soil nitrifier and denitrifier communities. Therefore, we compared the responses of gene-based nitrifier and denitrifier communities to chloride (CS), sulfate (SS), and alkaline (AS) stresses with those in a no-stress control (CK) in pots with a calcareous desert soil.

**Results:** Compared with CK, saline and alkaline stress decreased potential nitrification rate (PNR) and NO$_3$-N; increased pH, salinity, water content, and NH$_4$-N; and decreased copy numbers of *amoA*-AOA and *amoA*-AOB genes but increased those of denitrifier *nirS* and *nosZ* genes. Copies of *nirK* increased in SS and AS but decreased in CS. There were more copies of *amoA*-AOB than of *amoA*-AOA and of *nirS* than of *nirK* or *nosZ*. Compared with CK, SS and AS decreased operational taxonomic units (OTUs) of *amoA*-AOB but increased those of *nirS* and *nosZ*, whereas CS decreased *nirK* OTUs but increased those of *nosZ*. The numbers of OTUs and *amoA*-AOB genes were greater than those of *amoA*-AOA. There were positive linear relations between PNR and *amoA*-AOA and *amoA*-AOB copies.

Compared with CK, the Chao 1 index of *amoA*-AOA and *amoA*-AOB decreased in AS, that of *nirK* increased in SS and SS, but that of *nirS* and *nosZ* increased in all treatments. The Shannon index of *amoA*-AOB decreased but that of *nirS* increased in CS and SS, whereas the index of *nirK* decreased in all treatments. Saline and alkaline stress greatly affected the structure of nitrifier and denitrifier communities and decreased potential biomarkers of *nirS*-type; however, AS increased those of *nirK* and *nosZ*-type, and SS decreased those of *nosZ*-type. Soil water content, pH, and salinity were important in shaping *amoA*-AOA and denitrifier communities, whereas soil water and pH were important to *amoA*-AOB communities.

**Conclusion:** These results indicate that the nitrifier and denitrifier communities respond to saline and alkaline stresses conditions. Communities of *amoA*-AOA and *amoA*-AOB contribute to nitrification in alluvial gray desert soil, and those of *nirS* are more important in denitrification than those of *nirK* or *nosZ*.

**Keywords:** Chloride stress, Sulfate stress, Alkaline stresses, Bacterial community diversity, High-throughput sequencing, Potential nitrification rate

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Background
Salt stress is a primary threat to environmental resources and human health and also decreases crop yields and restrains the use of agricultural land [1]. Estimates suggest there are 1128 Mha of salt-affected land worldwide [2], accounting for more than 20% of total cultivated and 33% of irrigated agricultural lands [3]. In China, there are approximately 8.11 × 10^7 ha of saline or alkaline soils, accounting for 8 to 9% of the total land area [4]. In general, salt-affected soils are either saline or alkaline. Alkaline salt stress and neutral salt stress are different and therefore should be correctly distinguished as alkaline stress and saline stress, respectively [5]. The effects of saline stress (NaCl or Na₂SO₄) are generally osmotic and ionic, whereas alkaline stress (NaHCO₃ or Na₂CO₃) results from higher pH. Saline or alkaline stress adversely affects soil physicochemical properties, soil microbiological processes, and plant growth [6, 7].

Nitrification is the oxidation of ammonium (NH₄⁺) to nitrite (NO₂⁻) and then to nitrate (NO₃⁻). The nitrification process primarily involves ammonia-oxidizing bacteria (AOB) or archaea (AOA) and nitrite-oxidizing bacteria; ammonia oxidation is the rate-limiting step in nitrification and is driven by AOA and AOB. Denitrification is the stepwise reduction of NO₃⁻ and NO₂⁻ to the gases nitric oxide (NO), nitrous oxide (N₂O), and nitrogen (N₂) under the catalysis of enzymes [8]. The reduction of NO₂⁻ to NO is the rate-limiting step in denitrification. Nitrification and denitrification may occur simultaneously in different microsites of the same soil, and are affected by changes in salinity, pH, mineral N, soil water content (SWC), and temperature [9–13]. Soil salinity and pH affects nitrification and denitrification, primarily by inhibiting the activity of nitrifying and denitrifying bacteria. However, the response of N microbes to alkalinity or salinity stress is indeterminate.

Both AOA and AOB have amoA genes that encode ammonia monooxygenase to oxidize NH₄-N. Ammonia oxidation is dominated by AOA in acidic environment or low nutrient content environment but by AOB in alkaline soils, because of the low survival of AOB at low pH [14–17]. Shi et al. found that AOB copies were positively correlated with soil water content, NH₄-N, and NO₃-N and negatively correlated to soil pH, indicating that AOB was mainly affected by soil water content, pH, NH₄-N, and NO₃-N [18]. In addition, previous studies found that copies of the amoA gene of AOB and AOA are negatively correlated with soil salinity [19, 20]. However, according to Wang and Gu, high soil salinity can promote the growth of AOB and AOA [21], and Mosier and Francis found that copies of amoA of AOB increase with an increase in soil salinity [22]. Therefore, how soil salinity affects the relative contributions of AOB and AOA to nitrification remains debatable. There is also little information on how pH and salinity affect the distributions of AOA and AOB in saline or alkaline soils.

The genes nirK, nirS, and nosZ are frequently used as functional markers to analyze denitrifier communities [23]. Soil salinity inhibits nitrification and denitrification rates [24, 25]. However, denitrifier communities respond differently to diverse environments. Salinity reduces copies of the denitrifier genes nirK, nirS, and nosZ and alters denitrifier community structure [26–28]. By contrast, Franklin et al. and Li et al. found that gene copies of denitrifying bacteria increased along gradients of increasing salinity [29, 30]. Thus, the effects of salinity on the abundance of soil denitrifying bacteria also remain unclear. Additionally, a pH-dependent mechanism is involved in regulating soil microbial community composition and function [31]. For example, Bai et al. found that increases in pH in saline soil increase the activity of denitrifying microorganisms [32]. These studies demonstrate that, owing to the complexity of soil microbial communities, the effects of saline or alkaline stress on the abundance and structure of nitrifier and denitrifier communities are poorly understood.

Nitrogen (N) is an essential nutrient needed to improve crop yields, and many farmers apply excess N fertilizer to ensure maximum yield and profit [33]. Soil microbial communities have essential roles in nutrient cycling, and many of the microbiological processes involved in N cycling in terrestrial ecosystems are altered under saline or alkaline stress, which can affect plant productivity and production of atmospherically active gases. An understanding of the effects of saline and alkaline stresses on the abundance of nitrifiers and denitrifiers is important, because nitrification and denitrification rates determine soil inorganic N concentrations, nitrate leaching, and the production of N₂O [20, 27, 34]. An increase in soil salinity may shift microbial community structure and increase the predominance of saline or alkaline adapted microorganisms. However, there are few reports focused on the effects of saline and alkaline stresses on composition and structure of gene-based nitrifier and denitrifier communities, and the results will help to guide the application of saline and alkaline soil.

In this study, the effects of saline and alkaline stresses on nitrifier and denitrifier abundance and community structure were determined. We hypothesized that (i) saline and alkaline stresses would have different effects on nitrifier and denitrifier abundance, but overall, an increase in soil salinity or alkalinity would decrease the abundance of both, and that (ii) saline and alkaline stresses would have different effects on nitrifier and denitrifier community structure, but with an increase in salinity or alkalinity, the predominance of saline or alkaline-adapted microorganisms would increase. The
hypotheses were tested in a pot experiment with cotton plants and different types of soil salinity and alkalinity stresses. We assessed nitrifier and denitrifier abundance by quantitative polymerase chain reaction (q-PCR) and community structure by 16S rRNA gene sequencing. The information obtained in this study can provide a theoretical basis for the efficient use of N fertilizers and rational N management in saline or alkaline soils in arid areas.

Results
Cotton biomass
Cotton biomass decreased significantly under salt–alkali stress (Table 1). Compared with the CK, the biomass of the leaves, stem, and root was significantly lower by 47.55, 65.68, and 32.26%, respectively, in the CS treatment, by 46.85, 50.89, and 43.01%, respectively, in the SS treatment, and by 60.14, 57.40, and 31.18%, respectively, in the AS treatment. Overall, compared with the CK, total cotton biomass was decreased by 51.94, 74.74, and 53.18% in the CS, SS, and AS treatment, respectively.

Soil physicochemical properties and potential nitrification rate
Saline and alkaline stresses significantly increased SWC (Fig. 1a) and salinity (Fig. 1b). Across all treatments, including CK, SWC was between 11.88 and 18.87%, and salinity was between 0.33 and 2.64 dS m⁻¹. Compared with CK, SWC was 44.59% higher in CS, 58.82% higher in SS, and 18.05% higher in AS, and salinity was 438.41% higher in CS 708.99% higher in SS, and 18.05% higher in AS. Compared with that in CK, Saline and alkaline stresses significantly increased soil pH (Fig. 1c). In CS and SS, the pH increased by 0.69 and 0.63 units, respectively, compared with that in CK. In AS, the pH was significantly higher than that in the other treatments and was approximately 2.00 units higher than that in CK. The NH₄⁺-N content increased significantly in CS, SS, and AS, compared with that in CK (Fig. 1d), increasing by 106.29% in CS, 73.54% in SS, and 236.74% in AS. By contrast, the NO₃⁻-N content decreased significantly in CS, SS, and AS, compared with that in CK, decreasing by 7.68% in CS, 10.68% in SS, and 13.47% in AS (Fig. 1e). Similarly, the soil PNR decreased significantly in CS, SS, and AS, compared with that in CK (Fig. 1f), decreasing by 501% in CS, 608% in SS, and 697% in AS. There were no significant differences in PNR among CS, SS, and AS.

| Treatments | Biomass (g/plant) | Leaves | Stems | roots | Total |
|------------|------------------|--------|-------|-------|-------|
| CK         |                  | 7.15 a | 7.61 a| 3.72 a| 18.48 a|
| CS         |                  | 3.75 b | 2.61 d| 2.52 b| 8.88 c |
| SS         |                  | 3.80 b | 3.74 b| 2.12 c| 9.66 b |
| AS         |                  | 2.85 c | 3.24 c| 2.56 b| 8.65 c |

Table 1 Component biomass and total biomass of cotton plants as affected by salt and alkali stresses in a calcareous desert soil

amoA-AOA, amoA-AOB, nirK, nirS, and nosZ gene copy numbers
Saline and alkaline stresses significantly decreased amoA-AOA (Fig. 2a) and amoA-AOB (Fig. 2b) gene copy numbers. Across all treatments, including CK, gene copy numbers of amoA-AOA were between 0.44 × 10⁶ and 1.48 × 10⁷ per g dry soil and those of amoA-AOB were between 3.89 × 10⁶ and 5.45 × 10⁷ per g dry soil. Thus, the gene copy numbers of amoA-AOB were higher than those of amoA-AOA. The amoA-AOA and amoA-AOB gene copy numbers in CS, SS, and AS were significantly lower than those in CK. For amoA-AOA gene copies, the number was not significantly different between CS and AS. For amoA-AOB gene copies, the number was not significantly different between CS and SS. Compared with CK, amoA-AOA gene copies were 75.18% lower in CS, 63.34% lower in SS, and 70.08% lower in AS. Compared with CK, amoA-AOB gene copies were 17.48% lower in CS, 10.87% lower in SS, and 28.46% lower in AS. The amoA-AOA/amoA-AOB ratio in CS, SS, and AS was significantly lower than that in CK (Fig. 2c), but there was no significant difference between SS and AS.

Figure 2d, e, and f show the copy numbers of the denitrification genes nirK, nirS, and nosZ, respectively. Across all treatments, including CK, the numbers of nirK were between 0.74 × 10⁷ and 1.65 × 10⁷ per g dry soil, those of nirS between 4.69 × 10⁶ and 33.34 × 10⁶ per g dry soil, and those of nosZ between 2.23 × 10⁷ and 7.49 × 10⁷ per g dry soil. Compared with CK, the nirK copies decreased in CS by 41.54% but increased significantly by 28.94% in SS and by 14.71% in AS. Saline and alkaline stresses significantly increased nirS and nosZ gene copy numbers, compared with those in CK. The nirS copy numbers increased by 201.51% in CS, 368.18% in SS, and 612.57% in AS. The nosZ copy numbers increased by 46.44% in CS, 235.86% in SS, and 78.28% in AS.

Regression analysis showed soil PNR was significantly positively related to the gene copy numbers of both amoA-AOA (R² = 0.9122, P < 0.001; Fig. 3a) and amoA-AOB (R² = 0.5533, P = 0.005; Fig. 3b). Thus, the PNR was highly linearly related to the abundances of amoA-AOA and amoA-AOB.
Correlations between soil properties and potential nitrification rate and abundances of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ genes

Looking first at the relationships between the soil’s physicochemical properties and its PNR and nitrifier communities gene abundances (Table 2). The soil PNR was significantly negatively correlated with SWC, EC1:5, pH, and NH4-N content, the abundance of amoA-AOA was significantly negatively correlated with SWC, pH, and NH4-N content, and the abundance of amoA-AOB...

Fig. 1 Effects of different types of salt and alkali stresses on soil properties and nitrification in a calcareous desert soil. a Soil water content (%), b salinity (dS m⁻¹), c pH, d NH4-N (mg kg⁻¹), e NO3-N (mg kg⁻¹), and f potential nitrification rate (μg NO2-N g⁻¹ h⁻¹). Columns and error bars represent the mean ± standard error (n = 3), respectively. CK, control treatment without salt or alkali stress; CS, NaCl stress treatment; SS, Na2SO4 stress treatment; AS, Na2CO3 + NaHCO3 stress treatment.

Correlations between soil properties and potential nitrification rate and abundances of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ genes Looking first at the relationships between the soil’s physicochemical properties and its PNR and nitrifier communities gene abundances (Table 2). The soil PNR was significantly negatively correlated with SWC, EC1:5, pH, and NH4-N content, the abundance of amoA-AOA was significantly negatively correlated with SWC, pH, and NH4-N content, and the abundance of amoA-AOB...
was significantly negatively correlated with pH and NH$_4$-N content. However, soil PNR and the abundance of amoA-AOA and amoA-AOB were significantly positively correlated with soil NO$_3$-N content.

Looking at the relationship between the soil’s physicochemical properties and denitrifier communities gene abundances, the abundance of nirS was significantly positively correlated with soil pH and soil NH$_4$-N content.
content, and the abundance of \textit{nosZ} was significantly positively correlated with SWC and EC$_{1:5}$, but the abundance of \textit{nirS} and \textit{nosZ} was significantly negatively correlated with soil NO$_3$-N content. In addition, the abundance of \textit{nirK} was not significantly correlated with any soil property.

**Venn diagrams of the operational taxonomic units of \textit{amoA-AOA}, \textit{amoA-AOB}, \textit{nirK}, \textit{nirS}, and \textit{nosZ} genes**

The sequence coverage of \textit{amoA-AOA}, \textit{amoA-AOB}, \textit{nirK}, \textit{nirS}, and \textit{nosZ} genes was greater than 99% in all samples, indicating that the depth reasonably represented the actual communities (Table 3). Saline and alkaline stresses significantly affected the number of OTUs and sequences. For \textit{amoA-AOB}, the number of OTUs decreased significantly in SS and AS, compared with that in CK. For \textit{nirK}, the number of sequences increased significantly in CS, SS, and AS. In addition, For \textit{amoA-AOB}, the number of sequences decreased significantly in CS, compared with that in CK, however, the number of sequences increased significantly in AS. For \textit{nirK}, the number of sequences increased significantly in CS, compared with that in CK. For \textit{nirS}, the number of sequences increased significantly in CS, SS, and AS, compared with that in CK.

Venn diagrams were used to compare the shared and unique OTUs among \textit{amoA-AOA}, \textit{amoA-AOB}, \textit{nirK}, \textit{nirS}, and \textit{nosZ} communities (Fig. 4). One hundred and seventeen \textit{amoA-AOA}-related OTUs were identified in all treatments, and 19 were shared among the four treatments (16.24% of the total) (Fig. 4a). Nine hundred and thirty-four \textit{amoA-AOB}-related OTUs were identified in all treatments, and 106 were shared among the four treatments (11.35% of the total) (Fig. 4b). The number of \textit{amoA-AOB} species was significantly greater than that of \textit{amoA-AOA} species. Furthermore, the saline and alkaline stresses had greater influence on the number of \textit{amoA-AOB}-related OTUs than on the number of \textit{amoA-AOA}-related ones.

As shown in Fig. 4c, 1,404 \textit{nirK}-related OTUs were identified in all treatments, with 150 shared among the four treatments (10.68% of the total). As shown in Fig. 4d, 1,026 \textit{nirS}-related OTUs were identified in all treatments, with 173 shared among the four treatments (16.86% of the total). As shown in Fig. 4e, 965 \textit{nosZ}-related OTUs were identified in all treatments,
Table 3 Number of operational taxonomic units (OTUs), sequences, and coverages of the genes used to identify nitrifier and denitrifier communities under salt and alkali stresses in a calcareous desert soil

| Treatments | OTUs  | Sequences | Coverages |
|------------|-------|-----------|-----------|
|            | amoA-AOA | amoA-AOB | nirK  | nirS  | nosZ  | amoA-AOA | amoA-AOB | nirK  | nirS  | nosZ  |
| CK         | 31 a    | 324 a     | 377 a  | 285 b  | 178 c  | 37,110 a | 42,345 b | 51,095 b | 29,293 a | 33,242 b | 0.9998   | 0.9954   | 0.9980   | 0.9979   | 0.9993   |
| CS         | 34 a    | 312 a     | 270 b  | 310 b  | 232 b  | 40,672 a | 35,221 c | 56,195 a | 28,503 a | 40,047 a | 0.9998   | 0.9948   | 0.9983   | 0.9975   | 0.9986   |
| SS         | 36 a    | 271 b     | 284 b  | 353 a  | 314 a  | 39,192 a | 48,961 a | 52,321 b | 29,144 a | 39,112 a | 0.9998   | 0.9953   | 0.9982   | 0.9973   | 0.9979   |
| AS         | 33 a    | 165 c     | 336 a  | 348 a  | 295 a  | 40,583 a | 40,662 b | 50,138 b | 28,254 a | 39,385 a | 0.9999   | 0.9978   | 0.9980   | 0.9973   | 0.9985   |

CK, control treatment without salt or alkali stress; CS, NaCl stress treatment; SS, Na₂SO₄ stress treatment; and AS, Na₂CO₃ + NaHCO₃ stress treatment. Different lowercase letters in the same column indicate significant differences among treatments (P < 0.05).
Fig. 4 Venn diagrams based on operational taxonomic units of different communities under different salt and alkali stresses in a calcareous desert soil. The communities were a amoA-AOA, b amoA-AOB, c nirK, d nirS, and e nosZ. CK, control treatment without salt or alkali stress; CS, NaCl stress treatment; SS, Na2SO4 stress treatment; AS, Na2CO3 + NaHCO3 stress treatment.
with 96 shared among the four treatments (9.95% of the total). The numbers of nirK and nirS species were significantly higher than that of nosZ species. Furthermore, the saline and alkaline stresses had greater influence on the numbers of nirK- and nirS-related OTUs than on the numbers of nosZ-related ones.

**a-Diversity of amoA-AOA, amoA-AOB, nirK-, nirS-, and nosZ-type denitrifier communities**

Table 4 shows the Chao1 and Shannon diversity indices of amoA-AOA, amoA-AOB, nirK-, nirS-, and nosZ-type denitrifier communities. Compared with CK, CS and SS had no effect on the Chao 1 index of amoA-AOA and amoA-AOB communities; however, AS significantly decreased the Chao 1 index of amoA-AOA and amoA-AOB communities. Compared with CK, CS significantly decreased the Shannon index of amoA-AOA and amoA-AOB communities. In SS the Shannon index of the amoA-AOB community decreased significantly.

Compared with CK, CS and SS significantly decreased the Chao1 index of the nirK community, whereas the same treatments significantly increased the index of the nirS and nosZ communities. In addition, the Shannon index of the nirK community decreased significantly in CS and SS, but in those same treatments, the index of the nirS community increased significantly. The Shannon index of the nosZ-type denitrifier community was not affected by any treatment.

**Nonmetric multidimensional scaling analysis**

Nonmetric multidimensional scaling analysis was performed to compare the differences in structure of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ communities among treatments (Fig. 5). The amoA-AOA, amoA-AOB, nirK, and nosZ communities were clustered into four groups (Fig. 5a, b, c, d). The structure of nosZ communities was not significant between CS and SS (Fig. 5e). However, the structure of the nosZ communities under saline and alkaline stresses was significantly different from that of those communities in CK. This result indicated that microbial community structure might be directly correlated with soil properties affected by saline and alkaline stresses.

**Composition of amoA-AOA, amoA-AOB, nirK-, nirS-, and nosZ-type denitrifier communities**

Saline and alkaline stresses significantly affected the genus-level composition of amoA-AOA and amoA-AOB communities (Fig. 6). In the amoA-AOA community, the two dominant genera were Nitrososphaera and Candidatus Nitrosococcus (Fig. 6a). In all treatments, Nitrososphaera had the highest relative abundance (76.08 to 96.94%). The relative abundance of Nitrososphaera was significantly higher in CK than that in CS, SS, and AS. However, the relative abundance of Candidatus Nitrosococcus was significantly higher in CS, SS, and AS than that in CK. Nitrososphaera (96.94%) was dominant in CK, and Candidatus Nitrosococcus (23.66%) was significantly enriched in SS (Fig. 6b).

In the amoA-AOB community, Nitrosospira and Nitrosonomas were the two dominant genera (Fig. 6c). Across all treatments, including CK, the relative abundance of Nitrosospira was between 42.41 and 99.04% and that of Nitrosonomas between 0.01 and 57.53%. The relative abundance of Nitrosonomas was significantly higher in CK than that in CS, SS, and AS. However, the relative abundance of Nitrosonomas was significantly higher in SS, SS, and AS than that in CK. Nitrosonomas (99.04%) was dominant in CK, and Nitrosonomas (57.53%) was significantly enriched in SS (Fig. 6d).

The genotypes of denitrifying communities were significantly affected by saline and alkaline stresses (Fig. 7). Figure 7a shows the composition of the nirK-type denitrifier communities. The dominant genera included Sinorhizobium and Rhizobium, which together accounted for 40.10 to 59.81% of the relative abundance in all treatments. The highest relative abundance of Sinorhizobium was in CK, with the relative abundance 89.80% higher than that in CS and SS, 25.57% higher than that in SS, and 184.82% higher than that in AS. However, the lowest relative abundance of Rhizobium was also observed in CK, with the relative abundance 75.22% lower than that in CS, 82.89% lower than that in SS, and

**Table 4** Diversity indices of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ communities under different salt and alkali stresses in a calcareous desert soil

|          | amoA-AOA | amoA-AOB | nirK   | nirS   | nosZ   |
|----------|-----------|-----------|--------|--------|--------|
| CK       | 43.51 a   | 486.03 a  | 425.56 a| 324.68 b| 194.53 c|
| CS       | 43.90 a   | 543.02 a  | 360.05 b| 372.77 a| 263.40 b|
| SS       | 41.62 a   | 516.82 a  | 362.20 b| 402.67 a| 367.40 a|
| AS       | 33.42 b   | 258.74 b  | 413.12 a| 396.40 a| 326.62 a|

|          | amoA-AOA | amoA-AOB | nirK   | nirS   | nosZ   |
|----------|-----------|-----------|--------|--------|--------|
| CK       | 2.07 ab   | 2.60 a    | 4.80 a  | 4.40 b  | 0.67 a  |
| CS       | 1.80 b    | 1.90 b    | 4.04 c  | 5.09 a  | 0.62 a  |
| SS       | 2.53 a    | 1.82 b    | 3.93 c  | 5.19 a  | 0.69 a  |
| AS       | 2.50 a    | 2.61 a    | 4.31 b  | 4.24 b  | 0.65 a  |

CK, control treatment without salt or alkali stress; CS, NaCl stress treatment; SS, Na2SO4 stress treatment; and AS, Na2CO3 + NaHCO3 stress treatment. Different lowercase letters in the same column indicate significant differences among treatments (P < 0.05)
Fig. 5  Non-metric multidimensional scaling (NMDS) analysis of different soil microbial communities involved in nitrification and denitrification under different salt and alkali stresses in a calcareous desert soil. The communities were a *amoA*-AOA, b *amoA*-AOB, c *nirK*, d *nirS*, e and *nosZ*. CK, control treatment without salt or alkali stress; CS, NaCl stress treatment; SS, Na$_2$SO$_4$ stress treatment; AS, Na$_2$CO$_3$ + NaHCO$_3$ stress treatment.
87.01% lower than that in AS. The other genera in the *nirK*-type denitrifier communities included *Azospirillum* (0.40 to 6.96%), *Brucella* (0.12 to 3.13%), *Bradyrhizobium* (0.56 to 1.83%), *Bosea* (0.19 to 1.81%), *Paracoccus* (1.03 to 3.81%), *Mesorhizobium* (0.04 to 0.74%), *Rhodopseudomonas* (0.37 to 2.52%), *Devosia* (0.01 to 0.77%), *Agrobacterium* (0.01 to 0.30%), and *Pleomorphomonas* (0.00 to 0.14%). In addition, the relative abundance of *Azospirillum* and *Brucella* in CK was significantly higher than that in CS, SS, and AS. Dominant *nirK*-type denitrifier genera appeared only in AS (Fig. 7b), and the relative abundances of *Sagittula* (17.73%), *Achromobacter* (0.01 to 2.89%), *Pseudomonas* (0.00 to 0.14%), and *Lysobacter* (0.00 to 0.01%) were significantly higher than those in other treatments.

In the *nirS*-type communities, the dominant genera included *Azospira*, *Cupriavidus*, *Azoarcus*, and *Pseudomonas* (Fig. 7c). The four genera accounted for 26.34 to 56.09% of the total relative abundance in all treatments. The composition of *nirS*-type communities varied significantly among the saline and alkaline stress treatments. Compared with CK, the relative abundance of *Azospira* decreased significantly 24.02 to 1.71% and that of *Cupriavidus* 13.95 to 4.24% under saline and alkaline stresses. By contrast, the relative abundance of *Azoarcus* increased significantly 8.80 to 21.60% under saline and alkaline stresses.

Compared with CK, the relative abundance of *Pseudomonas* increased significantly in CS and SS; however, there was no significant difference between AS and CK. The other genera in the *nirS*-type communities included *Rhodanobacter* (0.11 to 1.22%), *Azospirillum* (0.21 to 0.98%), *Pseudogulbenkiania* (0.18 to 0.78%), *Thauera* (0.57 to 2.71%), *Zoogloea* (0.51 to 5.85%), *Herbaspirillum* (0.47 to 1.68%), *Aromatoleum* (0.15 to 1.34%), *Paracoccus* (0.03 to 1.65%), *Sulfuritalea* (0.01 to 0.73%), *Bradyrhizobium* (0.08 to 0.23%), and *Magnetospirillum* (0.00 to 1.62%). Figure 7d shows the dominant *nirS*-type denitrifier taxa. The dominant genera in CK were *Azospira* (24.02%), *Cupriavidus* (13.95%), *Azospirillum* (0.98%), *Rhodanobacter* (1.22%), and *Pseudogulbenkiania* (0.78%). In CS, the dominant genera were *Sulfuritalea* (0.73%) and *Magnetospirillum* (1.62%). In SS, *Pseudomonas* (25.43%), *Thauera* (2.71%), and *Aromatoleum* (1.34%) were the dominant genera, and in AS, *Zoogloea* (5.85%) and *Paracoccus* (1.65%) were dominant.
Fig. 7 Genus-level composition (a, c, e) and linear discriminant analysis (LDA) effect size analysis (b, d, f) of bacterial communities based on denitrification functional genes under different types of salt and alkali stresses in a calcareous desert soil. Relative abundance (%) of genera in a nirK, c nirS, and e nosZ communities. LDA scores of dominant taxa in b nirK, d nirS, and f nosZ communities. CK, control treatment without salt or alkali stress; CS, NaCl stress treatment; SS, Na2SO4 stress treatment; AS, Na2CO3 + NaHCO3 stress treatment.
In the nosZ-type communities, the dominant genera included Achromobacter, Tardiphaga, Pseudomonas, Paracoccus, and Burkholderia (Fig. 7e). The five genera accounted for 46.31 to 60.18% of the total relative abundance in all treatments, with the relative abundance of all > 1%. The relative abundances of Achromobacter and Tardiphaga were higher in CK than in CS, SS, and AS. However, the relative abundance of Paracoccus was lower in CK than in CS, SS, and AS. In CS and SS, the relative abundance of Burkholderia was significantly higher than that in CK and AS, with the lowest relative abundance in AS. However, the relative abundance of Pseudomonas was not significantly different among the four treatments. The other genera in nosZ-type communities included Rhodoferax (1.40 to 5.51%), Sinorhizobium (1.54 to 3.83%), Rhodopseudomonas (0.19 to 2.93%), Azospirillum (1.72 to 9.50%), Pleomorphomonas (0.00 to 1.44%), Ochrobactrum (0.25 to 0.99%), Bradyrhizobium (0.24 to 0.96%), Cupriavidus (0.24 to 2.15%), Halomonas (0.00 to 11.08%), and Alicyciphilus (0.00 to 1.22%), which were in all treatments. By pairwise comparison among different treatments, Pleomorphomonas (1.44%) and Bradyrhizobium (0.96%) were dominant in CK, Azospirillum was dominant in CS (9.50%), Alicyci-philus was dominant in SS (1.22%), and Halomonas (11.08%) was dominant in AS (Fig. 7f).

There were some common genera among nirK-type, nirS-type, and nosZ-type denitrifier communities. The genera Achromobacter and Alcaligenes were in both nirK-type and nosZ-type denitrifier communities. The nirK and nosZ genes were found in the genera Aromatoleum, Azotococcus, Cupriavidus, and Herbaspirillum. There were six denitrifiers that had nirK and nosZ genes, including Brucella, Mesorhizobium, Ochrobactrum, Pleomorphomonas, Rhodopseudomonas, and Sinorhizobium. In addition, all three nirK, nirS, and nosZ genes were identified only in the genera Azospirillum, Bradyrhizobium, Paracoccus, Pseudomonas, and Rhodanobacter.

**Redundancy analysis**

Figure 8 shows the correlations between species with amoA-AOA and amoA-AOB genes at the genus level and soil properties. Figure 8a shows the correlation of amoA-AOA community structure with soil properties. Axis 1 and 2 explained 98.44% of the total variation. The CK samples were clearly separated from those of CS, SS, and AS along axis 1 (98.17%). However, there was no significant difference between CS and SS. The amoA-AOA community structure was significantly correlated with soil pH (variation explained, 71.31%; P = 0.001), SWC (variation explained, 19.50%, P = 0.001), and soil salinity (variation explained, 5.40%, P = 0.003). Nitrosospira was positively correlated with NO₃-N and negatively correlated with pH and NH₄-N, whereas the correlations with Candidatus Nitrososphaericus were the opposite. For amoA-AOB community structure, axis 1 and axis 2 together explained 95.95% of the total variation (Fig. 8b). The CK samples were clearly separated from those of CS, SS, and AS along axis 1 (95.42%). The amoA-AOB community structure was significantly correlated with SWC (variation explained, 35.61%, P = 0.001) and pH (variation explained, 37.18%, P = 0.001) but not with the other soil properties. Nitrosospira was positively correlated with NO₃-N and negatively correlated with pH and NH₄-N; whereas the correlations with Nitrosomonas were the opposite.

Figure 9 shows the correlations between species with nirK, nirS, and nosZ genes at the genus level and soil properties. Axis 1 and 2 explained 82.19% of the total variation in the composition of nirK-type denitrifier...
communities (Fig. 9a). The nirK-type denitrifier community structure was significantly correlated with pH (variation explained, 58.61%, $P = 0.001$), SWC (variation explained, 8.37%, $P = 0.002$), and salinity (variation explained, 7.90%, $P = 0.03$). Sinorhizobium, Rhizobium, Sagittula, and Paracoccus were negatively correlated with pH and NH$_4$-N and positively correlated with NO$_3$-N; whereas the correlations with Azospirillum and Mesorhizobium were the opposite. Bradyrhizobium, Devosia, and Agrobacterium were positively correlated with NO$_3$-N and negatively correlated with NH$_4$-N. Brucella and Bosea were negatively correlated with SWC and salinity. Achromobacter was positively correlated with NH$_4$-N and pH. The other genera did not separate and were concentrated at the original point.

For nirS-type denitrifier community structure, axes 1 and 2 explained 88.85% of the total variation (Fig. 9b). The nirS-type denitrifier community structure was significantly correlated with SWC (variation explained, 41.99%, $P = 0.001$), salinity (variation explained, 42.90%, $P = 0.009$), and pH (variation explained, 28.49%, $P = 0.001$), but not with the other soil properties. Cupriavidos, Azospira, Rhodanobacter, and Azospirillum were negatively correlated with pH and NH$_4$-N and positively correlated with NO$_3$-N, whereas the correlations with Zoogloea and Paracoccus were the opposite. Azoarcus, Pseudomonas, Thauera, Herbaspirillum, and Aromatoleum were positively correlated with SWC and salinity. Pseudogulbenkiania was negatively correlated with SWC, NH$_4$-N, and salinity and positively correlated with NO$_3$-N.

Axis 1 and 2 contributed 82.24% of the total variation in the structure of the nosZ-type denitrifier communities (Fig. 9c). The nosZ-type denitrifier community structure was significantly correlated with soil pH (variation explained, 47.42%, $P = 0.001$), salinity (variation explained, 21.61%, $P = 0.003$), and SWC (variation explained, 17.28%, $P = 0.002$), but not with the other soil properties. Paracoccus and Halomonas were positively correlated with pH and NH$_4$-N and negatively correlated with NO$_3$-N, whereas the correlations with Rhodoferax and Sinorhizobium were the opposite. Achromobacter,
**Rhodopseudomonas**, and *Pleomorphomonas* were negatively correlated with SWC, salinity, pH, and NH$_4$-N and positively correlated with NO$_3$-N. *Burkholderia* and *Alicyclobifilus* were positively correlated with SWC and salinity; whereas the correlations with *Ochrobactrum* were the opposite. *Tardigrada* was negatively correlated with SWC, salinity, and NH$_4$-N and positively correlated with NO$_3$-N. *Bradyrhizobium* was negatively correlated with SWC, pH, and NH$_4$-N and positively correlated with NO$_3$-N. *Cupriavidus* was positively correlated with NH$_4$-N and NO$_3$-N.

**Discussion**

Soil salinization is a worldwide problem and a major challenge to sustaining soil quality. It is an important factors limiting agriculture production in arid regions. Soil salinity mainly causes damage to plants through ion toxicity and osmotic stress, and the inhibition of plant growth is the most common physiological response in a saline and alkaline habitat [35]. In this study, saline and alkaline stresses significantly inhibited cotton growth. The inhibition might have been due to the toxicity of Na ions with salt stress [36] and the increase in pH and disturbance of plant nutrition and metabolism with alkaline stress [37]. Salinity adversely affects soil physicochemical properties, which in turn, affect ecosystem nutrient cycling and especially the key transformations of N [38, 39]. In this study, NH$_4$-N content increased significantly but NO$_3$-N content decreased significantly under saline and alkaline stresses (Fig. 1d, e), which might be explained by the inhibition of soil nitrification due to the increase in soil salinity [40]. Saline and alkaline stresses also significantly inhibited soil PNR in this study (Fig. 1f). Akhtar et al. and He et al. also found that nitrification rates decrease with increases in soil salinity [11, 41]. Thus, these results suggest that saline and alkaline stresses inhibit the conversion of NH$_4$-N to NO$_3$-N, the key microbial process associated with nitrification.

Salinity stress affects soil biological properties by decreasing the abundance and diversity of microbial communities [42]. Moreover, soil salinization is usually accompanied by alkalization, which causes further serious deterioration of soil properties [43]. Microbiologically mediated soil N transformations, such as nitrification and denitrification, are also influenced by changes in salinity [44, 45]. Ammonia oxidation is the first and rate-limiting step in nitrification, with AOB and AOA being the primary microbial groups involved [10]. Li et al. found that the copies of *amoA*-AOB and *amoA*-AOA are negatively correlated with soil salinity [19]. In this study, saline and alkaline stresses significantly decreased the gene copy numbers of *amoA*-AOA and *amoA*-AOB (Fig. 2a, b). This result suggests that the increases in salinity and pH caused by saline and alkaline stresses are not suitable for the growth and reproduction of AOB and AOA. However, the gene copies of *amoA*-AOB were higher than those of *amoA*-AOA in this study. One explanation is that AOB prefer neutral pH and high-N agricultural soils, whereas AOA dominate in acidic or low nutrient-content soils [14]. Others have also observed higher abundance of AOB than that of AOA in saline and alkaline soils [46, 47] and AOA as the dominant microbial group in acidic soils [48]. Moreover, Nicol et al. reported that *amoA*-AOB copies decrease with a decrease in soil pH, whereas those of *amoA*-AOA decrease with an increase in pH (from 4.9 to 7.5) [9]. Li et al. reported a pH range of 5.0 to 7.0 for AOA enriched from activated sludge, with the optimum pH at 6.0 [49]. The results collectively suggest that soil pH has different effects on the ecological sites of AOB and AOA under different environmental conditions. In this study, the higher *amoA*-AOB copy numbers indicated that AOB was the dominant group in the ammonia-oxidizing community and the major contributor to ammonia oxidation in the saline and alkaline soils. In addition, the copy numbers *amoA*-AOB and *amoA*-AOA were significantly positively related to soil PNR (Fig. 3), suggesting that AOB and AOA participated in nitrification in the saline and alkaline soils. In addition, the *amoA*-AOA/*amoA*-AOB ratio was significantly lower under saline and alkaline stresses than that in the control soil. The decrease in the *amoA*-AOA/*amoA*-AOB ratio of the ammonia-oxidizing community indicated there was selective pressure against AOA under saline and alkaline stresses. Collectively, these results support the hypothesis that AOB are adapted to alkaline to neutral pH soils, whereas AOA are adapted to acidic soils. Nevertheless, the *amoA*-AOA/*amoA*-AOB ratio alone does not provide sufficient information to determine which of the two ammonia-oxidizing groups is functionally dominant in ammonia oxidation [50, 51]. The decreases in *amoA* gene copies might indicate lower potential soil nitrification under the saline and alkaline stresses. Indeed, the copy numbers of *amoA*-AOB and *amoA*-AOA *amoA* in this study were highly related to PNR ($P < 0.001$ and $P = 0.005$, respectively; Fig. 3). The positive linear relations indicated that *amoA*-AOB and *amoA*-AOA were most likely important in explaining the variation in PNR in this soil. However, because the number of *amoA*-AOB copies was higher than that of *amoA*-AOA, the AOB community might have played a more important role in soil nitrification. Moreover, in correlation analyses, *amoA*-AOB and *amoA*-AOA copies were positively correlated with NO$_3$-N content (Table 1), further indicating that AOA and AOB contributed to nitrification.

The effects of salinity on the structure of AOB and AOA communities have been investigated in many
previous studies [19, 20]. Salinity significantly alters the structure of AOA and AOB communities in wetland soil [52]. In this study, CS decreased the Shannon index of the amoA-AOB community, and AS decreased the Chao 1 index of the amoA-AOA and amoA-AOB communities (Table 3). Dang et al. also found that salinity decreases the diversity of the AOB community [53]. The NMDS analysis (Fig. 5) showed clear separation of communities under saline and alkaline stresses from those in control soil, suggesting that the changes in amoA-AOB and amoA-AOA communities might be partially attributed to the low concentrations of soil mineral N and relatively high pH values associated with saline and alkaline stresses. In addition, in the amoA-AOA communities in this study, the main genera were Nitrososphaera and Candidatus Nitrosocaldus. Saline and alkaline stresses significantly increased the relative abundance of Candidatus Nitrosocaldus, indicating it was strongly tolerant of saline and alkaline stresses. According to Lehtovirta-Morley et al. Candidatus Nitroscococcus (AOA), in a Nitrososphaera sister cluster, was first isolated from a near-neutral pH agricultural soil, suggesting its potential contribution to ammonia oxidation in neutral pH soils [54]. Wu et al. also reported that alkaline soil is suitable for the growth of Candidatus Nitrosotalea (AOA), which shows strong adaptability to pH variation. In the amoA-OB community, the dominant genus was Nitrosospira and Nitrosomonas [55]. Saline and alkaline stresses significantly increased the relative abundance of Nitrosomonas but significantly decreased that of Nitrosospira. By contrast, Saham and Muyzer found that Nitrosospira is enriched in a high-salt environment, whereas Nitrosomonas is enriched in a low- or medium-salt environment [56]. According to the Lefse analysis, the dominant genera in CK were Nitrososphaera and Nitrosospira, whereas Nitrosomonas was significantly enriched in SS and Candidatus Nitroscococcus was significantly enriched in AS. In this study, the variations in the amoA-AOA community were only significantly correlated with SWC and pH, whereas the variations in the amoA-OB community were only significantly correlated with SWC and pH. Hu et al. also found that the communities of AOA and AOB were positively correlated with pH [57]. Nevertheless, we could not accurately determine the contributions of the amoA-AOA and amoA-OB communities to nitrification, which need to be investigated further.

Saline and alkaline stresses alter soil physicochemical properties, thereby affecting microbial processes. In this study, the copies of nirK decreased in CS; however, the copies increased significantly in SS and AS. Stress from NaCl can inhibit denitrification activity [58], and decrease the abundance of denitrifying bacteria [59]. Wang et al. also reported that salinity significantly decreases the abundance of nirK genes [28]. In this study, saline and alkaline stresses significantly increased the copies of nirS and nosZ. Franklin et al. found that the number of denitrifying bacteria also increases with salinity in a beach wetland [60]. One explanation is that saline and alkaline stresses increase soil water content and cause poor soil aeration. Because the nosZ gene is sensitive to oxygen [61], its activity can be inhibited under aerobic conditions [62]. Therefore, with reduced soil aeration after irrigation with saline water, the growth of bacteria with the nosZ genotype may be stimulated [63]. Emissions of N2O are inversely related to nosZ gene expression [12]. In addition, an increase in nosZ gene copies indicates that the denitrification process is more complete, leading to N2 as the end product [64]. Moreover, the number of copies of nirK and nosZ was significantly lower than that of nirS. Similarly, Mosier and Francis and Santoro et al. also found that copies of nirS are higher than those of nirK [65, 66]. Francis et al. found that the nirS gene can increase in richness in low or medium salinity regions, significantly changing community structure and also indicating that nirS is more important in denitrification than nirK or nosZ [60]. In our study, the copies of nirS were positively correlated with pH and NH4-N (Table 1). Morales et al. also found that nirS copies are significantly positively correlated with NH4-N content [67]. The copies of nosZ were positively correlated with SWC and EC1:5 (Table 1). The copies of both nirS and nosZ were negatively correlated with NO3-N; whereas the copies of nirK were not significantly correlated with soil properties. These results indicate that nirS- and nosZ-type denitrifiers are more sensitive than nirK-type denitrifiers to saline and alkaline stresses.

Changes in the copies of denitrifying bacteria under saline and alkaline stresses likely alter community diversity. In this study, CS and SS decreased the Chao1 index of nirK, but saline and alkaline stresses significantly increased that of nirS and nosZ (Table 3). These results suggested that neutral salt (CS and SS) stress reduced the abundance of nirK but AS increased that of nirS and nosZ. In addition, saline and alkaline stresses decreased the Shannon index of nirK, whereas CS and SS increased the Shannon index of nirS. These results suggested that saline and alkaline stresses reduced the diversity of nirK but neutral salt (CS and SS) stress increased that of nirS. Thus, saline and alkaline stresses altered the community structure of denitrifying bacteria. The nosZ gene is considered to be relatively stable [68], and in this study, saline and alkaline stresses had no significant effect on its Shannon index. However, this result is in contrast to that of Yang et al. who reported that salinity is positively correlated with the diversity of nosZ genes [69]. The NMDS analysis also showed that saline and alkaline
stresses significantly altered the community structure of denitrifying bacteria.

In this study, compared with CK, the saline and alkaline stresses altered the community structure of nirK-type denitrifiers. The dominant nirK-type denitrifiers were Sinorhizobium and Rhizobium, similar to the observations by Tang et al. [70]. Saline and alkaline stresses significantly increased the relative abundance of Rhizobium, whereas that of Sinorhizobium significantly decreased, indicating that Rhizobium was strongly tolerant of saline and alkaline stresses. In the nirS-type communities, the dominant genera were Azospira, Cupriavidus, Azorarcus, and Pseudomonas. Saline and alkaline stresses significantly decreased the abundance of Azospira and Cupriavidus, whereas that of Azorarcus significantly increased. In addition, neutral salt (CS and SS) stress significantly increased the relative abundance of Pseudomonas. These results indicated that Azorarcus was strongly tolerant of saline and alkaline stresses and that Pseudomonas was strongly tolerant of neutral salt stress. In the nosZ-type communities, the dominant genera were Achromobacter, Tardiphaga, Pseudomonas, Paracoccus, and Burkholderia. Saline and alkaline stresses significantly decreased the abundance of Achromobacter and Tardiphaga, whereas that of Paracoccus significantly increased. In addition, neutral salt (CS and SS) stress significantly increased the relative abundance of Burkholderia, whereas alkaline stress significantly decreased it. These results indicated that Paracoccus was strongly tolerant of saline and alkaline stresses and that Burkholderia was strongly tolerant of neutral salt stress. Burkholderia also imparts some degree of tolerance in plants to other abiotic stresses such as drought, metal toxicity, and high temperature [71]. The AS treatment had the most potential biomarker species of nirK and nosZ genes, whereas the CS and SS treatments had the fewest potential biomarker species of the nirS gene. The fewest potential biomarkers species of the nosZ gene were in the SS treatment. These results indicated that saline and alkaline stresses affected the structure of different denitrifying bacteria communities to varying degrees. According to RDA, the variations in denitrifier communities were largely explained by salinity, SWC, and pH. Denitrifier community structure is also significantly correlated with salinity, pH, and SWC in previous studies [27, 72].

Farmland soil is the most important source of N₂O emissions to the atmosphere, and the microbial processes involved in the N cycle are the primary drivers of those emissions [73]. Henry et al. and Zhao et al. reported that nirS and nirK genes are responsible for the microbial production of N₂O; whereas the nosZ gene is responsible for reducing N₂O to N₂ [74, 75]. Thus, the denitrifier communities are critical in regulating N₂O emissions. Moreover, nitrification may be the main source of N₂O in arid regions [76]. Therefore, soil N₂O emissions include potential contributions from ammonia-oxidizing bacteria and archaea, which release N₂O during the nitrification–denitrification process or through links to that process. Thus, the relative contributions of nitrification and denitrification to N₂O production in saline and alkaline soils should be considered in further research.

Conclusion
The results of pot experiment support our hypothesis that saline and alkaline stresses changed the abundance and composition of nitrifier and denitrifier community. Saline and alkaline stresses decreased the copy numbers of amoA-AOA and amoA-AOB but increased those of nirS and nosZ, and there were more gene copies of amoA-AOB than of amoA-AOA and more gene copies of nirS than of nirK and nosZ. The PNR was positively linearly related to the copy numbers of both amoA-AOB and amoA-AOA. In addition, saline and alkaline stresses greatly affected the richness, diversity, and structure of nitrifier and denitrifier communities. Saline and alkaline stresses led to increases in the relative abundance of Candidatus Nitrosocosmicus, Nitrosomonas, Rhizobium, Azorarcus, and Paracoccus but decreases in the relative abundance of Nitrososphaera, Nitrosospira, Sinorhizobium, Azospira, Cupriavidus, Achromobacter, Tardiphaga, and Rhodoferax. The pH and SWC were main drivers of changes in the abundance in amoA-AOA and denitrifier communities, whereas amoA-AOB community structure was only significantly correlated with SWC and pH. Therefore, amoA-AOA and amoA-AOB communities contribute to nitrification in alluvial gray desert soil and that the nirS community may have a more important role in denitrification than nirK and nosZ communities. The present study proposed that a theoretical basis for the efficient use of N fertilizers and rational N management in saline or alkaline soils in arid areas.

Methods
Experimental site and soil description
Surface soils (0 to 30 cm) were collected from a cotton field (Gossypium hirsutum L.) at the experimental station of Shihezi University in Shihezi, Xinjiang Province, China (44°18′N, 86°02′E). The climate is temperate arid zone with a mean annual temperature of 7.8 ℃, precipitation of 210 mm, and evaporation of 1660 mm, with little annual variation. The soil was collected from multiple points in an unfertilized cotton field in March 2019. The soil is classified as calcareous desert soil (Calcaric Fluvisol in the FAO/UNESCO system) with a loam texture. The soil physicochemical properties were
the following: electric conductivity (EC$_{1:5}$), 0.35 dS·m$^{-1}$; pH, 7.86; organic matter, 14.9 g·kg$^{-1}$; alkaline N, 41.2 mg·kg$^{-1}$; available P, 10.6 mg·kg$^{-1}$; and available K, 248 mg·kg$^{-1}$.

**Experimental design**

A pot experiment was performed in the experiment station greenhouse at Shihezi University. The cotton was planted on 25 April 2020, and seedlings were selected at the two-leaf stage, with four uniform seedlings kept in each pot. The pot experiment was conducted from 25 April 2020 to 10 September 2020. During the experiment, the maximum temperature and minimum temperature of the greenhouse were 15.8°C and 41.6°C, respectively.

According to the salt components and pH in most of the salt-affected soils in Xinjiang, China, three common types of salt-affected soils were obtained by adding chloride as NaCl (chloride stress, CS), sulfate as Na$_2$SO$_4$ (sulfate stress, SS), or carbonate as Na$_2$CO$_3$ + NaHCO$_3$ (alkaline stress, AS) to the sampled soil. The control (CK) soil had no saline or alkaline stress. The soil EC$_{1:5}$ and pH values of the different saline and alkaline stress treatments and their salinization or alkalization degree are shown in Table 5.

The field-collected soil was naturally dried and then crushed and sieved (2-mm pore size). Solutions of NaCl, Na$_2$SO$_4$, or Na$_2$CO$_3$ + NaHCO$_3$ (weight ratio 1:1) were added to the soil to produce a supersaturated state (the same volume of deionized water was added to the control soil). The NaCl, Na$_2$SO$_4$, Na$_2$CO$_3$ + NaHCO$_3$ addition amount were 4.0 g/kg, 6.0 g/kg, and 1.5 g/kg, respectively. After mixing evenly, the treated soil was left to stand for 1 month to ensure homogeneous distribution of salt. Then, the three treatment soils were naturally dried, crushed, and passed through a 2-mm sieve.

Non-draining soil pots with 35-cm internal diameter and 60-cm height were used. The treated soil was added to a bulk density of 1.25 g·cm$^{-1}$, with 60.0 kg per soil pot. The experiment was a completely randomized block design with three replications per treatment. The pots were drip-irrigated, and the emitters (and columns) were 0.4 m apart with a discharge rate (pressure compensated) of 2.1 L·h$^{-1}$. The drip irrigation pipe was laid flat on the surface of the soil pots, with each soil pot supplied by one emitter fixed at the center of the pot. During the cotton growing season, the pots were irrigated 12 times. The irrigation interval was seven to 10 days, and 52 L of irrigation water was added per pot. The pots were all irrigated on the same dates. A flow meter was used to measure the amount of water applied.

The same amount of N (1,350 kg·ha$^{-1}$, 13.73 g per pot), P$_2$O$_5$ (105 kg·ha$^{-1}$, 1.07 g per pot), and K$_2$O (60 kg·ha$^{-1}$, 0.61 g per pot) was applied in all treatments. The N fertilizer was applied through the drip irrigation system during the cotton growing season. Consistent with local practices, urea was the N source. The N fertilizer was applied in six equal amounts 53, 64, 72, 81, 90, and 99 days after planting. All pots were fertilized with P$_2$O$_5$ and K$_2$O before sowing.

**Cotton sampling**

To determine the dry matter of cotton, three representative cotton plants were selected in each treatment on 5 August 2020 (103 days after planting). The roots, stems, and leaves was washed with distilled water and then dried in an oven at 70°C for 48 h, weighed.

**Soil sampling**

Soil samples were collected from the 0 to 20 cm layer from three pots per treatment on 5 August 2020 (103 days after planting). The samples were stored with ice packs and transported to the laboratory. Soils were passed through a 2-mm sieve, after which each soil sample was divided into three subsamples. One subsample was immediately flash-frozen in liquid nitrogen and stored at -80°C for total DNA extraction. One subsample was stored immediately at 4°C to determine soil water content (SWC), soil mineral N content, and potential nitrification rate (PNR). The remaining subsample was air-dried to determine soil salinity and pH.

**Soil analyses**

Soil water content was determined gravimetrically by oven drying at 105°C until constant weight. Soil NH$_4$-N and NO$_3$-N were extracted with 2 mol L$^{-1}$ KCl (5 g of soil in 50 mL of KCl solution) on a horizontal shaker for 1 h at 220 rpm and then measured by a SmartChem 140 auto discrete Analyzer (Westco Scientific, Danbury, Connecticut, USA). Soil salinity and pH were determined with an MP521 lab pH/conductivity meter in a soil: water ratio of 1:5 and 1:2.5, respectively. As an

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**Table 5** Soil EC$_{1:5}$, pH values, and Na$^+$ concentration in different saline and alkaline stress treatments in a calcareous desert soil

| Treatment          | Salinity and alkalinity                | EC$_{1:5}$ (dS·m$^{-1}$) | pH (1:2.5) | Na$^+$ concentration (g/kg) |
|--------------------|---------------------------------------|--------------------------|------------|----------------------------|
| Control (CK)       | No additional salinization or alkalization | 0.35                     | 8.16       | 0.060                      |
| NaCl (CS)          | Moderate salinization                  | 1.39                     | 8.43       | 0.886                      |
| Na$_2$SO$_4$ (SS)  | Moderate salinization                  | 2.01                     | 8.19       | 0.827                      |
| Na$_2$CO$_3$ + NaHCO$_3$ (AS) | Moderate alkalization             | 0.63                     | 9.92       | 0.466                      |
index of the size of active nitrifier populations, soil potential nitrification rate (PNR) was determined using the method described by Kurola et al. [77]. In brief, 5 g of fresh soil was put into 50 mL centrifuge tubes containing 20 mL of phosphate buffer saline solution with 1 mmol L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\). To inhibit nitrite oxidation, potassium chloride (KClO\(_3\)) was added to the tubes at a final concentration of 10 mmol L\(^{-1}\). After incubation for 24 h in the dark at the room temperature of 25 °C, nitrite (NO\(_2\)-N) was extracted with 5 mL of 2 M KCl and determined spectrophotometrically at 545 nm with N-(1-naphthyl) ethylenediamine dihydrochloride.

**DNA extraction, qPCR assay, and pyrosequencing**

Soil microbial DNA was extracted using a Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., USA) following the manufacturer’s instructions and then stored at −80 °C. The DNA concentration and purity were measured using a UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. After which, the DNA was stored at −20 °C.

The abundances of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ were determined by real-time qPCR. Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) performed the qPCR on a CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories, USA). Target plasmids were constructed with PMD-18 plasmids (TaKaRa, Tokyo, Japan), and the correct gene inserts were chosen. The qPCR reaction was performed in triplicate in a 20-μL reaction system containing 10 μL of 2× SYBR Green qPCR Master Mix (Applied Biosystems, Foster City, CA, USA), 2 μL of DNA template, 1 μL of each primer, and 6 μL of ddH\(_2\)O. After qPCR, the gene copy numbers of nitrification and denitrification genes were normalized by the amount of soil based on the dilution rates and the volumes of the DNA used in the qPCR. Table 6 lists detailed conditions for PCR amplification. The numbers of copies of the target genes were calculated from standard curves.

High-throughput sequencing was used to analyze the composition and diversity of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ gene-based bacterial communities. The primers were the same as those used in the qPCR. The 25-μL reaction system included 2 μL of DNA template, 1 μL of forward and reverse primer (10 μL), 5 μL of 5× Q5 reaction buffer, 5 μL of 5× Q5 High-Fidelity GC buffer, 0.25 μL of Q5 High-Fidelity DNA Polymerase (5 U μL\(^{-1}\)), 2 μL of (2.5 mM) dNTPs, and 8.75 μL of ddH\(_2\)O. The thermal cycle reaction system for the genes used the following program: initial denaturation at 98 °C for 5 min; 35 cycles consisting of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s; and a final extension at 72 °C for 5 min. The PCR primers were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using a PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. After the individual quantification step, equivalent amounts of samples were mixed before high-throughput sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Aqu Biotechnology Co., Ltd. (Shanghai, China).

**Data analyses**

All data are expressed as the mean ± standard deviation. One-way ANOVA was conducted using SPSS (IBM Software, Chicago, IL, USA). Tukey’s test was used to identify significant differences among means (P < 0.05). Pearson’s correlation analysis was used to test the correlations between PNR, abundance of genes, and soil properties. The sequence data were analyzed using QIIME (version 1.8.0) and R packages (v 3.5.0). The diversity and richness indices were calculated using an operational taxonomic unit (OTU) table in QIIME, the

**Table 6** Primers and thermal profiles used for real-time quantitative PCR of the different nitrifying and denitrifying genes in bacterial communities in a calcareous desert soil

| Target gene | Primer | Sequence (5′–3′) | Thermal profile | References |
|-------------|--------|-----------------|----------------|-----------|
| amoA-AOA   | Arch-amoAF | 5′-STAATGCTCTGGCTTAGACG-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Hu et al. [78] |
|            | Arch-amoAR | 5′-GGGGCCCATCCATCTGATG-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 30 s. | Ebie et al. [79] |
| amoA-AOB   | amoA-1F  | 5′-GGGGTTCTTACTGGTGGT-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Kim et al. [80] |
|            | amoA-2R  | 5′-CCCCCTGGSAAGCCTTTC-3′ | 95 °C for 5 min; 35 cycles consisting of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s; and a final extension at 72 °C for 5 min. | Hallin and Lindgren [81] |
| nirK       | FluC    | 5′-ATCAGTGTGCTGGCCGCGG-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Dong et al. [82] |
|            | R3Cu    | 5′-GCTCTGACAATGRTTGTGTGTTT-3′ | 95 °C for 5 min; 35 cycles consisting of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s; and a final extension at 72 °C for 5 min. | Wu et al. [83] |
| nirS       | cd62F   | 5′-GTAACGGTSAAGGARACGCG-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Hu et al. [78] |
|            | R3cd    | 5′-GASTTCGGRTGSGTGATCG-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Ebie et al. [79] |
| nosZ       | nosZ-1126F | 5′-GGGCTBGCGCCGRTGCA-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Kim et al. [80] |
|            | nosZ-1381R | 5′-GGGGTTTCTACTGGTGGT-3′ | 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 30 s. | Hallin and Lindgren [81] |
sequences were grouped into OTUs using a definition of 95% similarity. The visualization analysis of classification and abundance results was performed in MEGAN. Nonmetric Multidimensional scaling (NMDS) was also conducted based on genus-level compositional profiles. Constrained ordination by redundancy analysis (RDA) in R (vegan, v 3.5.0) was used to elucidate relations between the structure of amoA-AOA, amoA-AOB, nirK, nirS, and nosZ gene-based communities and the soil physicochemical properties measured for each sample. Linear discriminant analysis effect size (LEfSe) was calculated in Visual Genomics to search for statistically different biomarkers between treatments.

Abbreviations
SWC: Soil water content; EC: Electrical conductivity; PNR: Potential nitrification rate; One-way ANOVA: One-way analysis of variance; OTU: Operational taxonomic unit; RDA: Redundancy analysis; NMDS: Nonmetric multidimensional scaling; LEfSe: Linear discriminant analysis effect size

Acknowledgements
We thank LetPub (www.letpub.com) for its linguistic assistance and scientific consultation during the preparation of this manuscript.

Authors’ contributions
WM, and HJG conceived and designed this experiment. JXG and YXZ collected samples and performed the study. WM, HJG, JXG, and YXZ participated in the acquisition and analysis of the data. JXG wrote the manuscript. HJG and YXZ participated in the discussion draft of the manuscript. WM revised the manuscript finally. All authors read and approved the final manuscript.

Funding
This work was supported by the Youth Science and Technology Innovation Research Foundation of Xinjiang Production and Construction Corps, China (2020CB020). The funding agencies had no role in the study design, sample collection, data collection and analysis, and manuscript preparation.

Availability of data and materials
All sequences recovered by high-throughput sequencing have been deposited into NCBI Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra). The accession number is PRJNA727758 (http://www.ncbi.nlm.nih.gov/bioproject/727758), which includes 60 accession items (SAMN 19032946–SAMN19033005).

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
No potential conflict of interest was reported by the authors.

Received: 4 May 2021 Accepted: 6 September 2021
Published online: 15 September 2021

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