Journal of Animal & Plant Sciences, 33(2): 2023, Page: 270-277
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.2.0618

EFFECTS OF SOIL SALT CONCENTRATION ON *nirS* AND *nosZ* GENES DIVERSITY OF PADDY SOIL DENITRIFYING BACTERIA

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ABSTRACT

To further study saline-alkali soil cultivation, the terminal-restriction fragment length polymorphism (T-RFLP) was used to analyze the effects of different salt concentrations on the denitrification functional genes *nirS* and *nosZ* after collecting different soil samples and extracting total soil DNA. The results showed that the variation range of *nirS* gene abundance was concentrated in 14 segments, the *nosZ* gene abundance was concentrated in 9 segments. The abundance of *nirS* gene in paddy soil of different concentrations was higher than *nosZ*, and the activity of the *nirS* gene was higher. Diversity index analysis showed that sample I was significantly different from III and V in the Shannon index of the *nirS*. Sample I and IV were significantly different from III in Simpson index. Except for the significant difference between III and I, II, IV, and V, there was no difference between the other four samples significant in Pielou index. The difference between sampl I and V was extremely significant in Brillouin index. The Shannon index, Simpson index and Pielou index of *nosZ* gene were not significantly different, sample II and III, IV, V were significantly different in Brillouin index. The diversity of *nirS* gene was more affected by the salt concentration, and the *nirS* gene was more widely distributed than *nosZ* in soil with different salt concentrations. It indicated that *nirS* -mediated nitrite reductase played an important role in salinity affected soil environment.

Keywords: Denitrifying bacteria, Functional denitrification gene, Salt concentration, Diversity, Soil

Published first online December 18, 2022 Published final March 24, 2023

INTRODUCTION

Denitrification of soil microorganisms was one of the main ways of nitrous oxide emission in nature, and the process led by microorganisms made an important contribution to the whole nitrogen cycle (Kool *et al*. 2011). Denitrification was a microbial process in which nitrate or nitrite was reduced to nitrogen-containing gases such as nitric oxide, nitrous oxide or nitrogen, which were mediated by nitrate reductase (Nar or Nap), cytochrome cd1-type (NirS) or copper-containing type (NirK) nitrite reductase, nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) (Han *et al*. 2021). Nitrous oxide reduction to nitrogen by the nitrous oxide reductase encoded by *nosZ* gene, which was acting as the sole sink of atmospheric nitrous oxide. The key genes that distinguish denitrifying microorganisms from other microorganisms were *nirS* and *nosZ* genes, which were usually used as marker genes for denitrifying microorganisms to study the denitrifying process in soil (Kuypers *et al*. 2018; Han *et al*. 2021).

With the continuous development of coastal areas, the land had been immersed in sea water for a long time, which resulting in a high salinity of the land (Tester *et al*. 2003). High temperature also made the land desalination, the formation and accumulation of salt in the soil, and salinization, which further affected the biological process of microorganisms in the soil (Li *et al*. 2015).The effects of salt water on ecology were receiving increasing attention from scientists (Wang *et al*., 2021). The use of salinized land was particularly important.

Soil salinity affected approximately 800 million hectares of arable lands worldwide (Yuan *et al*. 2016; Jose *et al*. 2017; Ghassemi *et al*. 1995).Total saline land was about 1125 million hectares in the world (Hossain, 2019). Saline-alkali land had seriously affected the arable land area and food security of all countries. Australia (Stein *et al*. 2019), India (De Deckker *et al*. 2019; Singh 2018), Egypt (Minhas *et al*. 2019) and other countries had carried out research and obtained good results since the 1930s. Developing strategies to make use of saline land would be crucial for addressing the problem of insufficient cropland and meeting the challenge of providing food security for the projected global population of 9.3 billion people by 2050 (Liu *et al*., 2021). The efficient exploitation of coastal saline-alkali land in agricultural production was one of the main contents of coastal saline-alkali land research for years

(Salwan *et al*. 2019).The resource utilization of saline-alkali land could be improved by improving and screening the crops suitable for growing in saline-alkali land. In recent years, the development and utilization technology of saline-alkali land had become increasingly mature. Improving saline-alkali land by physical (Li *et al*. 2008) and chemical (Zhao *et al*. 2020) ways, as well as biological comprehensive improvement methods such as the "saline-alkali tolerant rice" project led by academician Yuan Longping of the Chinese Academy of Engineering (Virto *et al*. 2015; Salwan *et al*. 2019). Popularizing and planting saline-alkali tolerant rice could increase grain yield and improve coastal saline-alkali land. Studies had shown that salt stress could inhibit denitrification rate and denitrifying enzyme activity (Cao *et al*.2008), and reduce the number of denitrifying microorganisms in soil (Li *et al*. 2009). Wang *et al*. (2018) found that the gene abundance of *nirS* and *nosZ* genes could also be inhibited by salt. Santoro *et al*. (2006) also found a significant negative correlation between the diversity of *nirS* gene and salt content in the coastal aquifer. However, some studies had found that the number of denitrifying bacteria in tidal wetland increases with the increase of salt content (Franklin *et al*. 2017). It can be seen that denitrifying microorganisms had different responses to salinity. Some studies had confirmed that salinity was one of the main factors affecting microbial community structure and diversity (Chen *et al*. 2022).

In this study, total DNA was extracted from 5 different paddy field soil samples irrigated by sea water. The denitrifying function genes *nirS* and *nosZ* were amplified and digestible. The T-RFLP was used to analyze the restriction fragment length products to explore the influence of soil environment with different salt concentrations on the abundance of denitrifying bacteria and the diversity of denitrifying functional genes.

MATERIALS AND METHODS

Collection of soil samples: Soil samples were collected from saline-alkali tolerant rice field (0cm-20cm below the surface layer) at Buchao Village in Zhanjiang City（21º8'N，110º7'E）. Fresh soil was collected from 5 different fields, 5 samples were collected from each field and dried in the shade. The soil was filtered through a 4mm-sieve after removing impurities, roots and leaves, and the physical and chemical properties were determined.

Amplification of *nirS* and *nosZ* genes: Total Soil microbial DNA was extracted from all samples using the Omega E.Z.N.A.® Soil DNA Kit. The extracted DNA was stored at -20℃. *nirS* and *nosZ* genes were amplified by PCR. The primers were synthesized by GENEWIZ Biotechnology Co., LTD. The primers were used (Throbck *et al*. 2004; Henry *et al*. 2006), *nirS*-F:5'-GTSAACGTSAAGGARACSGG(5'-FAM), *nirS*-R: 5'-GASTTCGGRTGSGTCTTGA. *nosZ*-1211:5'-CG(C/T)TGTTC(A/C)TCGACAGCCA (5'-FAM), *nosZ*-1917: 5'-CATGTGCAG(A/C/GT)GC(A/G)TGGCAGAA. A total of 25μL reaction including Taq polymerase 12.5 μL, primers 1 μL for each, DNA 2 μL and water 8.5 μL was set up with the reaction parameters of 94℃ 2 min, 36 cycles of 94℃ 30 sec, 56℃ 30 sec, and 72℃ 1 min, followed by extension for 10 min to augment *nirS* gene. Except that the annealing temperature of *nosZ* gene was 58℃, the other conditions were the same. PCR product (8 μL) was visualized after electrophoresis.

Digestion of the PCR product: Restriction enzyme HaeIII was used for the digestion of the PCR product with the following reaction: PCR product 30 μL, HaeIII 2 μL, 10×buffer 2 μL and ddH_2O 6 μL. After incubation at 37℃ for 4 hours, the digestion products were sequenced T-RFLP at Shanghai Sangon Technology Co., Ltd.

Data statistics and analysis: GeneMarker software was used to analyze the results of T-RFLP, the peaks with fluorescence signal less than 100RFU was removed, the peaks that all appeared in replicas were counted. BioDAP software was used for diversity index analysis of enzyme digestion map, and R language was used for variance analysis of diversity index.

RESULTS

Physical and chemical properties of soil: Test results of physical and chemical properties of soil in 5 different fields were shown in Table 1. Soil salinity varied from high to low in paddy fields, the salinity of sample I was obviously higher than that of the other fields, while the difference of salinity among the others was not significant. With the increase of salinity, soil organic matter, total N and available N increased in different degrees, the growth rates were 131.7%, 132% and 107.3%, respectively. Sample I was also significantly higher than others. The content of available P decreased by 34.5% with the increase of salinity, while the content of available K had no significant change.

Table 1 Analysis of soil physical and chemical properties.

Sample field	pH	Available N /mg·kg^{-1}	Total N /mg·kg^{-1}	Available P /mg·kg^{-1}	Available K /mg·kg^{-1}	Organic matter /g·kg^{-1}	Salinity /us·cm^{-1}
I	5.15±0.77^{BC}	184.33±1.17^{A}	4.27±0.29^{A}	65.32±10.99^{AB}	112.14±14.84^{B}	82.94±6.39^{A}	1394±986^{A}
II	6.28±0.38^{A}	75.05±3.56^{D}	1.47±0.03^{C}	56.93±13.58^{AB}	117.99±3.9^{B}	28.69±0.26^{C}	282±56.43^{B}
III	5.83±0.25^{AB}	111.76±19.13^{C}	2.24±0.17^{B}	24.38±15.66^{C}	81.65±15.69^{B}	42.87±3.76^{B}	261.8±101.56^{B}
IV	5.58±0.3^{AB}	88.9±6.36^{CD}	1.84±0.29^{BC}	99.69±26.54^{A}	117.32±57.65^{B}	35.79±5.62^{BC}	199.4±77.86^{B}
V	4.71±0.33^{C}	140±13.51^{B}	2.03±0.1^{B}	91.31±20.89^{A}	266.38±24.09^{A}	39.01±2.42^{B}	229.4±50.35^{B}

Note: In the same column, different capital letters indicate significant difference (P<0.01); the same or no letter superscripts indicate no significant difference (P>0.05).

Amplification of *nirS* and *nosZ* genes and T-RFLP analysis: In the electrophoretogram of enzyme digestion products, a restriction fragment (T-RFS) was defined as an Operational Taxonomic unit (OTU), the peak area corresponding to each fragment was defined as the relative number of this species (Clare *et al*. 2016), and the relative abundance value of OTU was calculated. The results obtained by calculation excluded fragments less than 50 bp and relative abundance value less than 1%, and defined the abundance value greater than 4% as dominant bacteria, less than 1% as occasional bacteria, and the rest as non-dominant bacteria (Zhang *et al*. 2010).

The size of the denitrifying function gene *nirS* was about 490 bp, and that of the *nosZ* gene was 750 bp. The partial scan electrophoresis of the enzyme digestion products of *nirS* and *nosZ* gene with fluorescence intensity on the vertical axis and fragment size on the horizontal axis was shown in Figure 1 and 2.The total number of OTU and dominant bacteria in different groups were shown in Table 2. The dominant bacteria of ranged from 1 to 5 species in each group, and the difference was not significant in *nirS* and *nosZ* gene. The distribution of dominant bacteria was shown in Figure 3 and 4. It was mainly concentrated in 104, 113, 116, 247 bp and other 10 fragments. Most of the species outside the 104-247 bp range were occasional bacteria in Fig3. They accounted for a large proportion at 52, 63, 74, 100, 105, 112, 115, 176 and 279 bp, most of the species outside the 52-279 bp range were occasional bacteria in Fig4. In addition, there was no significant difference between sample I and other samples at the total OTU number both of *nirS* and *nosZ* gene (P > 0.05).There was significant difference in the number of dominant bacteria of *nirS* gene between the sample I and III, V (P < 0.05), while significantly different of *nosZ* gene between the sample III and others (P < 0.01), no significant difference in the other samples (P > 0.05).

Table 2. Total OTUs and dominant bacteria of *nirS* and *nosZ* gene.

Gene	Sample field	Group 1		Group 2		Group 3		Group 4		Group 5		x±S	
		Total OTU	Dominant bacteria	Total OTU	Dominant bacteria	Total OTU	Dominant bacteria	Total OTU	Dominant bacteria	Total OTU	Dominant bacteria	Total	Dominant bacteria
nirS	I	21	5	30	3	22	3	13	6	16	8	20.40±6.50^{a}	5.00±2.12^{a}
	II	23	3	32	3	17	4	19	4	23	4	22.80±5.15^{a}	3.60±0.49^{a}
	III	20	2	31	3	14	1	15	1	24	3	20.80±6.24^{a}	2.00±0.89^{b}
	IV	16	4	30	4	21	4	25	2	19	3	21.20±7.49^{a}	2.60±1.02^{ab}
	V	15	1	14	2	35	3	21	4	21	3	22.20±4.87^{a}	3.40±0.80^{ab}
nosZ	I	17	5	14	4	16	4	15	2	10	4	15.60±4.39^{ab}	3.60±1.14^{a}
	II	15	4	18	5	16	4	17	3	17	4	16.60±1.14^{a}	4.00±1.00^{A}
	III	13	2	18	2	6	1	12	3	5	1	10.80±5.36^{b}	1.80±0.83^{B}
	IV	15	2	16	3	15	4	22	3	15	3	10.80±3.90^{b}	3.00±0.71^{AB}

V	11	3	7	3	11	3	17	2	8	4	16.60±3.05^{a}	3.00±0.71^{AB}

Note: In the same column, different small letter superscripts indicate significant difference (P<0.05); the same or no letter superscripts indicate no significant difference (P>0.05).

Diversity index analysis: The diversity index results of *nirS* were shown in Table 3.The sample I were significantly higher than other samples in the Shannon, Simpson and Pielou index (P < 0.01). Sample I had significant difference with sample V in the Margalef index (P < 0.01). In this study, except that the salinity of sample I was significantly higher than others, it was suggested that the salinity increased, and the diversity and richness of *nirS* denitrifying bacteria were in a fluctuation range of first decreasing and then increasing, while the uniformity showed a decreasing trend.

The diversity index results of *nosZ* were shown in Table 4.The sample I were significantly higher than other samples in the Shannon and Margalef index , and had significant difference with sample III in the Simpson index, difference with sample III to V in the Pielou index. It was suggested that the diversity, evenness and richness of *nosZ* type denitrifying bacteria were increased within a fluctuation range with the increase of salinity.

Table 3 Diversity index of *nirS* gene

Sample field	Shannon index	Simpson index	Pielou index	Margalef index
I	2.94±0.48^{A}	0.14±0.12^{B}	0.67±0.12^{A}	6.20±1.74^{A}
II	2.27±0.09^{B}	0.28±0.05^{A}	0.28±0.02^{B}	5.89±0.82^{AB}
III	1.83±0.34^{B}	0.37±0.07^{A}	0.44±0.05^{B}	4.98±1.67^{AB}
IV	2.18±0.12^{B}	0.27±0.03^{A}	0.53±0.01^{B}	4.73±1.08^{AB}
V	1.87±0.17^{B}	0.29±0.05^{A}	0.49±0.04^{B}	3.52±1.04^{B}

Table 4 Diversity index of *nosZ* gene

Sample field	Shannon index	Simpson index	Pielou index	Margalef index
I	2.40±1.36^{a}	0.54±2.47^{b}	0.55±0.28^{a}	5.94±5.21^{a}
II	1.32±0.44^{b}	0.50±0.19^{ab}	0.35±0.11^{ab}	2.91±0.57^{ab}
III	0.91±0.41^{b}	0.66±0.18a	0.27±0.11^{b}	2.12±0.44^{b}
IV	1.01±0.29^{b}	0.58±0.17^{ab}	0.30±0.08^{b}	2.02±0.39^{b}
V	0.97±0.50^{b}	0.64±0.23^{ab}	0.29±0.14^{b}	2.04±0.36^{b}

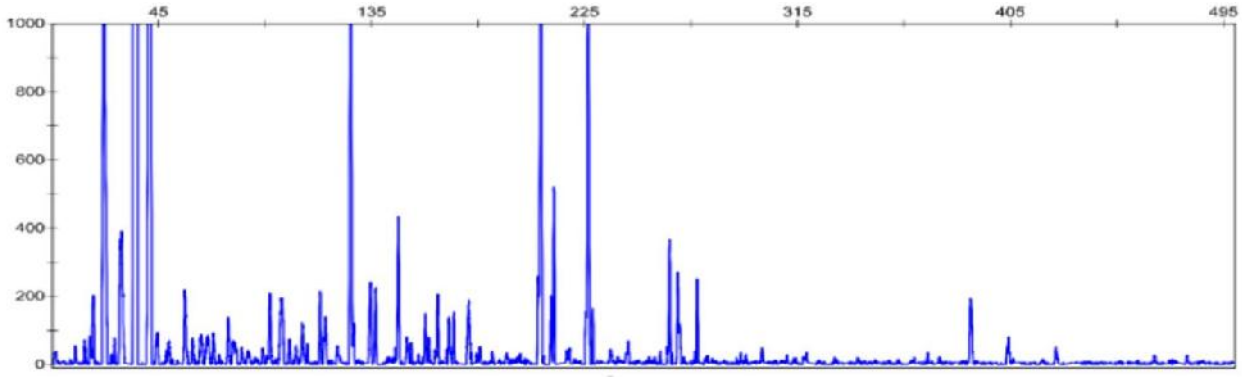

Figure 1. T-RFLP profiles of *nirS* gene (part).

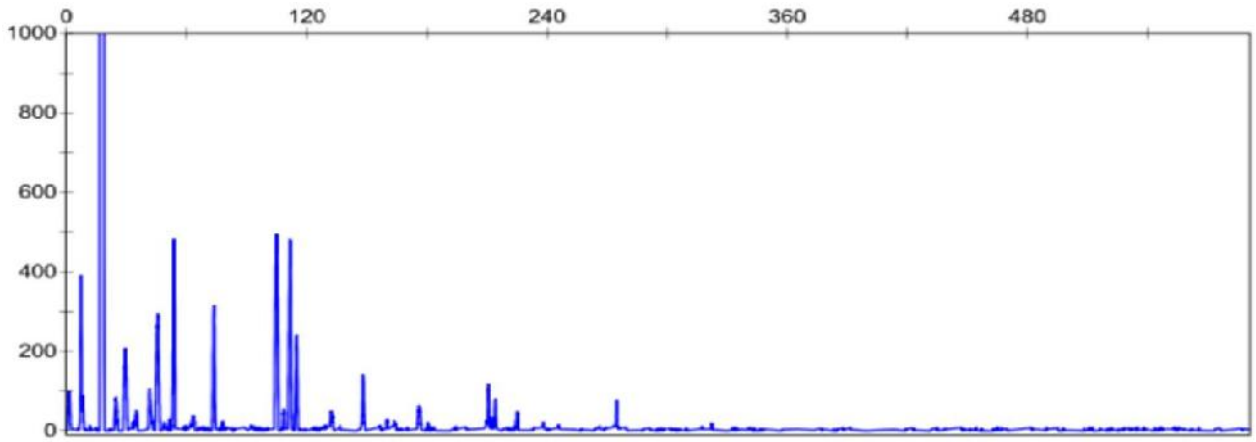

Figure 2. T-RFLP profiles of *nosZ* gene (part).

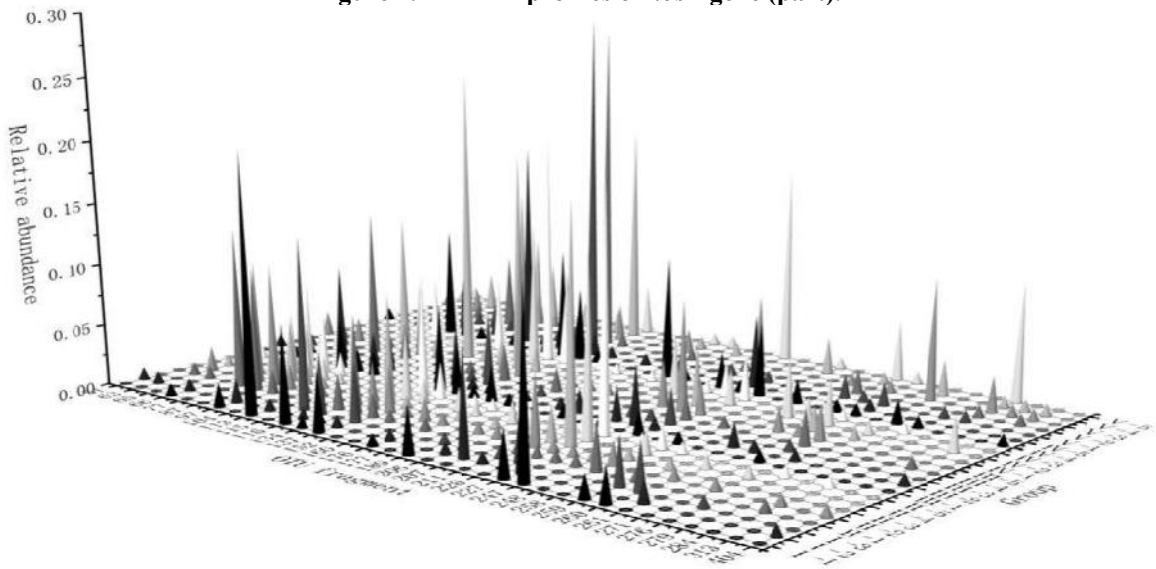

Figure 3. Relative abundance of T-RFs of *nirS* gene dominant flora.

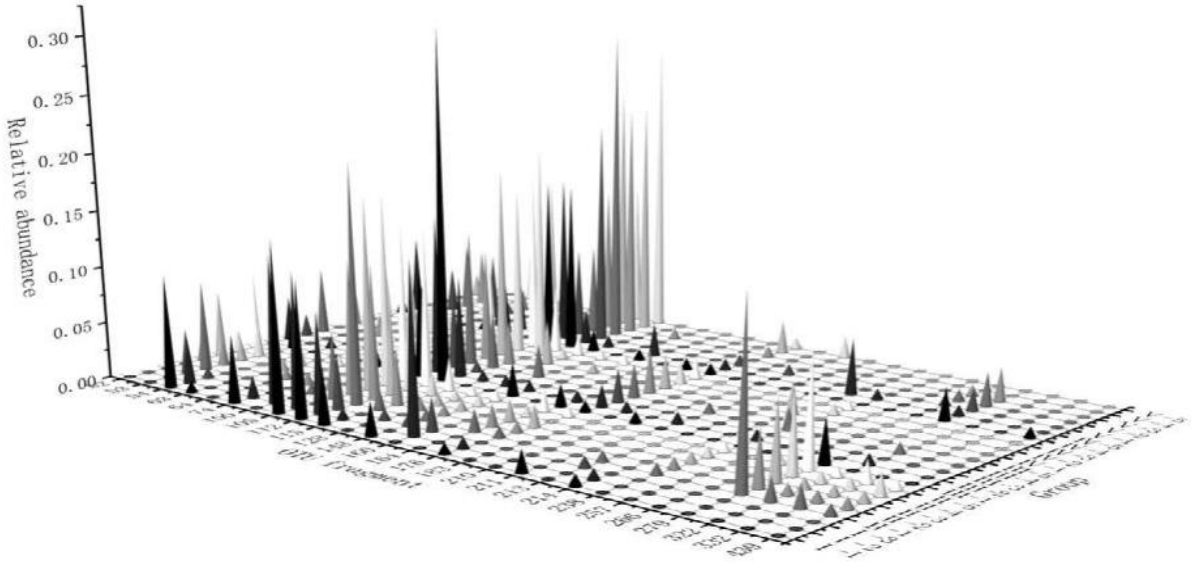

Figure 4. Relative abundance of T-RFs of *nosZ* gene dominant flora.

DISCUSSION

In this study, the effects of salinity on soil microbial denitrifying bacteria were studied by irrigating paddy fields with seawater of different salinity concentrations. Soil microorganisms played an important role in the process of material and energy exchange in coastal ecosystems, which mainly reflected in the decomposition and synthesis of soil organic matter, as well as the cycling and transformation of nutrients (Steenwerth *et al*. 2002). The microbial abundance in saline-alkali land will be correspondingly reduced (Yang *et al*. 2016), while the microorganisms adapted to coastal saline-alkali soil environment would be more saline-alkali tolerant. They influenced and promoted to change each other. Therefore, this study conducted the effects of soil salinity changes on the gene diversity of denitrifying bacteria *nirS* and *nosZ*.

The denitrifying microbial communities with different genotypes had different responses to environmental factors. In this study, T-RFLP technology was used to analyze the *nirS* and *nosZ* genes of denitrifying bacteria. It was found that the total number of OTU of *nirS* gene was higher than that of *nosZ* in soil with different salinity. When the soil salinity reached sample II, the content of total N and available N and organic matter were the lowest among all the soil samples, while the abundance and dominant bacteria of *nirS* and *nosZ* were higher in the soil samples with similar salinity. This indicates that the increase in the number of denitrifying microorganisms leaded to the decrease of nitrogen content in the soil, that is, denitrifying bacteria consumed nitrogen such as nitrite in the soil through the denitrification. The variation range of *nirS* gene abundance was concentrated in 14 segments, which was larger than the variation range of *nosZ* gene in 9 segments at 5 different groups of the same salinity sample, while there was no significant difference in the dominant bacteria of the two genes.

In this experiment, taking the sample I with the highest salinity as an example, the abundance of *nirS* gene was significantly higher than that of *nosZ* gene, indicating that the distribution of *nirS* gene was more extensive than that of *nosZ* gene in the high salinity environment, and the activity of *nirS* gene was higher (Mosier 2010). The study of Jones suggested that the *nosZ* gene was relatively stable (Jones *et al*. 2008).The abundance of *nirS*-type denitrifying bacteria may also be controlled and regulated by pH, nitrite, nitrate, and anaerobic microorganisms in the soil (Li *et al*. 2016;Wang *et al*. 2012).

The genetic diversity of denitrifying bacteria was also affected by different soil environment (Lucas *et al*. 2020). The genetic diversity of *nirS*-type denitrifying bacteria showed a slight turning point with the increase of soil salinity, which was also consistent with the results of soil physical and chemical properties analysis. The contents of total N and organic matter in the soil increased with the soil salinity, while the salinity was higher than 261.8±101.56 us·cm^{-1}, the contents of total N and organic matter began to decrease. At this time, the genetic diversity of *nirS* type denitrifying bacteria began to increase. It is suggested that soil salinity may have a certain threshold for the effect of *nirS*-type denitrifying bacteria. Microbial growth is inhibited below the threshold, than the microbes develop tolerance and multiply. Except for the effect of salinity, pH or local fertilization conditions may have an impact on the genetic diversity of *nirS*. Studies had pointed out that the genotypes of *nirS* genes in different environmental types were different (Prieme *et al*. 2002), which may be one of the factors affecting the genetic diversity of *nirS*-type denitrifying bacteria. The increase of salinity enhanced the genetic diversity of *nosZ* denitrifying bacteria. Yang (Yang *et al*. 2015) also found that *nosZ* gene was positively correlated with salt content. Studies have shown that salinity changes the soil environment due to long-term exposure to high salinity, leading to adaptive changes of *nosZ* denitrifying bacteria (Piao *et al*. 2012). Rich *et al*. (2003, 2004) found that *nosZ* gene was relatively stable in soil and little changed under the influence of environmental conditions. Similar results were obtained in this experiment. The community structure composition of *nirS* and *nosZ* denitrifying bacteria responded to soil salinity to different degrees with the increase of salinity, and the community structure composition of *nirS* responded more significantly than that of *nosZ* .

Based on the analysis results of gene abundance and diversity index of the two genes, it can be concluded that the community structure and gene diversity of denitrifying microorganisms will be changed with soil salt concentration, while the response results of denitrifying microorganisms with different genotypes are also different.

Conclusion: In this study, soil organic matter, total N and available N increased in different degrees with the increase of salinity, while the content of available P decreased, the content of available K had no significant change. The dominant bacteria of ranged from 1 to 5 species in each group, and the difference was not significant in *nirS* and *nosZ* gene. The diversity and abundance of denitrifying bacteria *nirS* gene were in a fluctuation range of first decreasing and then increasing, while the evenness showed a decreasing trend. The diversity, evenness and abundance of denitrifying bacteria *nosZ* gene were increased within a fluctuation range with the increase of salinity. The diversity of *nirS* gene was more affected by the salt concentration, and the *nirS* gene was more widely distributed than *nosZ* in soil with different salt concentrations. It indicated that *nirS* -

mediated nitrite reductase played an important role in salinity affected soil environment.

Acknowledgements: This work was supported by Key-Area Research and Development Program of Guangdong Province (2020B020219004), the Guangdong Provincial Department of Education 2021 Special Project for Key Fields of Ordinary Colleges and Universities (2021ZDZX4003), the Zhanjiang Science and Technology Bureau 2021 Provincial Science and Technology Special Funds ("Big Special + Task List") Competitive Allocation Project (2021A05231).

Conflicts of interest: The authors report that they have no conflicts of interest.

Data availability statement: All public data generated or analyzed during this study are included in this article. Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Authors contribution: Y. Ding: methodology and writing-original draft. J. He: operation of experiment. Q. Wu: sample collection and processing. D. Li, G. Liu and J. Liao: statistical analysis of data, formal analysis and investigation. Y. Ma and H. Zhou : resources, writing-reviewing & editing, supervision. All authors contributed to the article and approved the submitted version.

REFERENCES

Cao, Y.P., P.G. Green and P.A. Holden (2008). Microbial community composition and denitrifying enzyme activities in salt marsh sediments. Appl. Environ. Microb. 74(24): 7585-7595. DOI:10.1128/AEM.01221-08

Clare, E.L., F.J. Chain, J.E. Littlefair and M.E. Cristescu (2016). The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data. Genome. 59(11): 981-990. DOI:10.1139/gen-2015-0184

Chen, H., K. Ma, Y. Huang, Q. Fu, Y. Qiu and Z. Yao (2022). Significant response of microbial community to increased salinity across wetland ecosystems. Geoderma. 415: 115778. DOI: 10.1016/j.geoderma. 2022. 115778

De Deckker, P (2019). Groundwater interactions control dolomite and magnesite precipitation in saline play as in the Western District Volcanic Plains of Victoria, Australia. Sediment. Geol. 380: 105-126. DOI:10.1016/j.sedgeo. 2018.11.010

Franklin, R.B., E.M. Morrissey and J.C. Morina (2017). Changes in abundance and community structure of nitrate-reducing bacteria along a salinity gradient in tidal wetlands. Pedobiologia. 60: 21-26. DOI:10.1016/j.pedobi.2016.12.002

Ghassemi, F., A.J. Jakeman and H.A. Nix (1995). Salinisation of Land and Water Resources: Human Causes, Extent, Management and Case Studies. Cab International: Wallingford, UK. ISBN: 0-85198-906-3. https://agris.fao.org/agrissearch/search.do?recordID=GB9601091

Han, B., L.Y. Mo, Y.T. Fang, H.J. Di, J.T. Wang, J.P. Shen and L.M. Zhang (2021). Rates and microbial communities of denitrification and anammox across coastal tidal flat lands and inland paddy soils in East China. Appl. Environ. Microb. 157: 103768. DOI:10.1016/j.apsoil.2020.103768

Henry, S., D. Bru, B. Stres, S. Hallet and L. Philippot (2006). Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and compari -son of the abundances of 16S rRNA, *narG*, *nir*K, and *nosZ* genes in soils. Appl. Environ. Microb. 72(8): 5181-5189. DOI:10.1128/AEM.00231-06

Hossain, M.S (2019). Present scenario of global salt affected soils, its management and importance of salinity research. Int. J. Biol. Sci. 1: 1-3. https://www.researchgate.net/publication/333773002

Jones, C.M., B. Stres, M. Rosenquist and S. Hallin (2008). Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a comp -lex evolutionary history for denitrification. Mol. Biol. Evol. 25(9): 1955-1966. DOI:10.1093/molbev/msn146

Jose, A.M., O.O. Maria, B.V. Agustina, D.V. Pedro, S.B. Maria and H.J.A. Jose (2017). Plant responses to salt stress: Adaptive mechanisms. Agronomy. 7(1): 18. DOI:10.3390/agronomy7010018

Kool, D.M., J. Dolfing, N. Wrage and J.W. Van Groenigen (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. Soil Biol. Biochem. 43(1): 174-178. DOI:10.1016/j.soilbio.2010.09.030

Kuypers, M.M.M., H.K. Marchant and B. Kartal (2018). The microbial nitrogen-cycling network. Nat. Rev. Microbiol.16(5): 263-276. DOI:10.1038/nrmicro.2018.9

Li, C.J., J.Q. Lei, Y. Zhao, X.W. Xu and S.Y. Li (2015). Effect of saline water irrigation on soil development and plant growth in the Taklimakan Desert Highway shelterbelt. Soil Till. Res. 146: 99-107. DOI:10.1016/j.still.2014.03.013

Li, J., Z.M. Qiang, D.S. Yu, D. Wang, P.Y. Zhang and Y. Li (2016). Performance and microbial community of simultaneous anammox and denitrification (SAD) process in a sequencing batch reactor. Bioresource Technol. 218: 1064-1072. DOI:10.1016/j.biortech.2016.07.081

Li, J.L., J. Bai, H.W. Gao, X.D. Wang, J.H. Yu and G.L. Zhang (2009). Quantification of denitrifying bacteria and denitrification process in surface sediment at adjacent sea area of the Yangtze River Estuary in summer. China Environmental Science. 29: 756-761. https://www.doc88.com/p-8935507668741.html

Li, Z.G., X.J. Liu, X.M. Zhang and W.Q. Li (2008). Infiltration of melting saline ice water in soil columns: Consequences on soil moisture and salt content. Agr. Water Manage. 95(4): 498-502. DOI:10.1016/j.agwat.2007.12.001

Liu, L. and B. Wang (2021). Protection of halophytes and their uses for cultivation of saline-alkali soil in China. Biology. 10(5): 353. DOI:10.3390/biology10050353

Lucas, J.M., S.G. Mcbride and M.S. Strickland (2020). Trophic level mediates soil microbial comm-unity composition and function. Soil Biol. Biochem. 143: 107756. DOI:10.1016/j.soilbio.2020.107756

Minhas, P.S., M. Qadir and R.K. Yadav (2019). Ground - water irrigation induced soil sodification and response options. Agr. Water Manage. 215: 74-85. DOI:10.1016/j.agwat.2018.12.030

Mosier, A.C. and C.A. Francis (2010). Denitrifier abundance and activity across the San Francisco Bay estuary. Env. Microbiol. Rep. 2(5): 667-676. DOI:10.1111/j.1758-2229.2010.00156.x

Piao, Z., W.W. Zhang, S. Ma, Y.M. Li and S.X. Yin (2012). Succession of denitrifying community composition in coastal wetland soils along a salinity gradient. Pedosphere. 22(3): 367-374. DOI:10.1016/S1002-0160(12)60023-X

Prieme, A., G. Braker and J.M. Tiedje (2002). Diversity of nitrite reductase (*nirK* and *nirS*) gene fragm-ents in forested upland and wetland soils. Appl.Environ. Microb. 68(4): 1893-1900. DOI:10.1128/AEM.68.4.1893-1900.2002

Rich, J.J., R.S. Heichen, P.J. Bottomley, K. Cromack and D.D. Myrold (2003). Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Appl. Environ. Microb. 69(10): 5974-5982. DOI:10.1128/AEM.69.10.5974-5982.2003

Rich, J.J. and D.D. Myrold (2004). Community compos - ition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA. Soil. Biol. Biochem. 36(9): 1431-1441. DOI:10.1016/j.soilbio.2004.03.008

Salwan, R., A. Sharma and V. Sharma (2019). Microbes mediated plant stress tolerance in saline agricu-ltural ecosystem. Plant Soil. 442(1-2): 1-22. DOI:10.1007/s11104-019-04202-x

Santoro, A.E., A.B. Boehm and C.A. Francis (2006). Denitrifier community composition along a nitrate and salinity gradient in a coastal aquifer. Appl. Environ.Microb. 72(3): 2102-2109. DOI:10.1128/AEM.72.3.2102-2109.2006

Singh, A (2018). Salinization of agricultural lands due to poor drainage: A viewpoint. Ecol. Indic. 95: 127-130. DOI:10.1016/j.ecolind.2018.07.037

Steenwerth, K.L., L.E. Jackson, F.J. Calderon, M.R. Stromberg and K.M. Scow (2002). Soil microbi -al community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. 34(11): 1599-1611. DOI:10.1016/S0038-0717(03)00027-0

Stein, S., Y. Yechieli, E. Shalev, R. Kasher and O. Sivan (2019). The effect of pumping saline groundwa-ter for desalination on the fresh-saline water interface dynamics. Water Res. 156: 46-57. DOI:10.1016/j.watres.2019.03.003

Tester, M. and R. Davenport (2003). Na^+ Tolerance and Na^+ Transport in Higher Plants. Ann. Botlondon. 91(5): 503-527. DOI:10.1093/aob/mcg058

Throback, I.N., K. Enwall, A. Jarvis and S. Hallin (2004). Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. Fems. Microbiol. Ecolo. 49(3): 401-417. DOI:10.1016/j.femsec.2004.04.011

Virto, I., M.J. Imaz, O. Fernandez-Ugalde, N. Gartzia-Bengoetxea, A. Enrique and P. Bescansa (2015). Soil degradation and soil quality in Western Europe: Current Situation and Future Perspectives. Sustainability. 7(1): 313-365. DOI:10.3390/su7010313

Wang, C., J. Sardans, C. Tong, J. Peñuelas and W. Wang (2021).Typhoon-induced increases in porewater nutrient concentrations and CO_2 and CH_4 emiss - ions associated with salinity and carbon intrusion in a subtropical tidal wetland in China: A mesocosm study. Geoderma. 384: 114800. DOI:10.1016/j.geoderma.2020.114800

Wang, H.T., J.A. Gilbert, Y.G. Zhu and X.R. Yang (2018). Salinity is a key factor driving the nitrogen cycling in the mangrove sediment. Sci Total Environ. 631-632: 1342-1349. DOI:10.1016/j.scitotenv.2018.03.102

Wang, Y., G. Zhu, H.R. Harhangi, B. Zhu, M.S.M. Jetten, C. Yin and H.J.M. Op den Camp (2012). Co-occurrence and distribution of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in a paddy soil. Fems. Microbiol. Lett. 336(2): 79-88. DOI:10.1111/j.1574-6968.2012.02654.x

Yang, A.J., X.L. Zhang, H. Agogue, C. Dupuy and J. Gong (2015). Contrasting spatiotemporal patterns and environmental drivers of diversity

and community composition of ammonia oxidizers, denitrifiers, and anammox bacteria in estuarine sediments. Ann. Microbiol. 65(2): 879-890. DOI:10.1007/s13213-014-0929-5

Yang, W., N. Jeelani, X. Leng, X.L. Cheng and S.Q. An (2016). Spartina alterniflora invasion alters soil microbial community composition and microbial respiration following invasion chronosequence in a coastal wetland of China. Sci. Rep-UK. 6: 26880. DOI:10.1038/srep26880

Yuan, F., B.Y. Leng and B.S. Wang (2016). Progress in studying salt secretion from the salt glands in recretohalophytes: How do plants secrete salt? Front. Plant Sci. 7: 977. DOI:10.3389/fpls.2016.00977

Zhang, R., V. Thiyagarajan and P.Y. Qian (2010). Evaluation of terminal-restriction fragment length polymorphism analysis in contrasting marine environments. Fems. Microbiol. Ecolo. 65(1): 169-178. DOI:10.1111/j.1574-6941. 2008.00493.x

Zhao, Y.G., Y. Li, S.J. Wang, J. Wang and L.Z. Xu (2020). Combined application of a straw layer and flue gas desulfurization gypsum to reduce soil salinity and alkalinity. Pedosphere. 30(2): 226-235. https://doi.org/10.1016/S1002-0160 (17)60480-6