Science of the Total Environment 785 (2021) 147185

Contents lists available at ScienceDirect

Science of the Total Environment

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

Carcass decay deteriorates water quality and modifies the *nirS* denitrifying communities in different degradation stages

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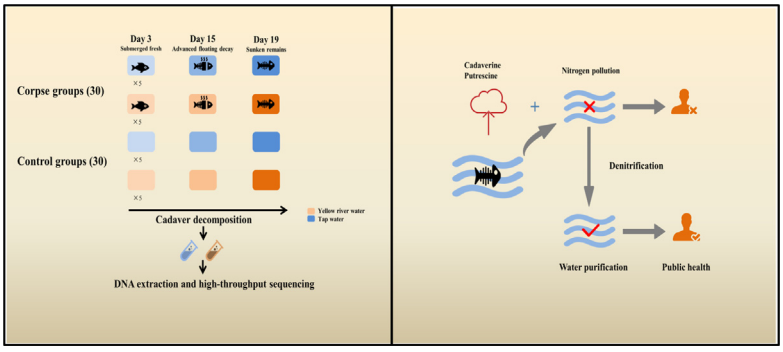
HIGHLIGHTS

- Corpse degradation deteriorates water quality.
- Water TDS, salinity, CON and phosphate primarily affect denitrifying community.
- Denitrifying community structures in corpse groups become similar finally.
- Deterministic processes dominated the denitrifying community assembly.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 February 2021
Received in revised form 19 March 2021
Accepted 12 April 2021
Available online 22 April 2021

Editor: Wei Shi

Keywords:
Cadaver degradation
Denitrification
nirS
Nitrogen contamination
Aquatic environment
Deterministic processes

ABSTRACT

Corpse degradation may release amounts of hazardous materials (e.g., cadaverine, putrescine and ammonia) into surrounding areas, which deteriorate environments and result in nitrogen contamination. Nitrate or nitrite can be reduced to nitrogen gas by denitrifying bacteria, thus alleviating nitrogen contamination and purifying aquatic environments. However, the reaction of *nirS*-encoding denitrifiers to carcass degradation is less studied. Therefore, water physiochemical analysis and high-throughput sequencing were applied to explore the successional pattern of *nirS* denitrifying communities in the Yellow River water and tap water during three stages of animal cadaver decay (submerged fresh, advanced floating decay as well as sunken remains) and relevant control group. Nitrate nitrogen (NO_3-N) and ammonia nitrogen (NH_4^+-N) concentration in corpse groups were highly elevated compared with control groups. The dominant phylum for *nirS* denitrifying communities was Proteobacteria. Abundant denitrifying genera *Paracoccus*, *Alicycliphilus* and *Diaphorobacter* were detected, and these genera have been reported to participate in the degradation of organic pollutants. Particularly, *nirS*-type community structures were remarkably influenced by corpse decay and became similar with succession. Water total dissolved solids (TDS), salinity, conductivity (CON) and phosphate were primary impacting factors driving the community structures, but the effect of water type was almost negligible. Notably, denitrifying community assembly was dominated by deterministic processes rather than stochastic processes, and the relative importance of deterministic processes among most corpse groups was higher than that in control groups, indicating that environmental filtering regulates the denitrifying communities. Our results provide new insight into environmental purification for hazardous materials produced by corpse degradation, thereby providing valuable advice to environmental administration.

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https://doi.org/10.1016/j.scitotenv.2021.147185
0048-9697/

1. Introduction

Circulatory stasis initiated the carcass decomposition as soon as the animals dead. The corpse degradation is a dynamic chemical and microbial-mediated process. Due to the action of scavenger, arthropod and microbes on the progresses of cadaver decaying, carcass condition varied along with time. Thus, the animal breakdown process in water is classified into five stages: submerged fresh, early floating, floating decay, advanced floating decay and sunken remains (Byrd and Tomberlin, 2019; Zimmerman and Wallace, 2008). The first phase submerged fresh is associated with autolysis (enzymatic degradation of soft tissue), leading to the internal oxygen depletion. Next, anaerobic microbes convert carbohydrate, protein and lipids to organic acids and gases (putrefaction), which cause the onset of the floating stages (Haglund and Sorg, 1997). Then during the stage of advanced decay, carrion mass rapidly decline and cadaveric fluids are released from carrion ruptures and cadaveric orifices into the surrounding environments (Carter et al., 2007). Sunken remains, the last stage of breakdown, is characterized by only a few bones remains (Zimmerman and Wallace, 2008). Cadaveric materials contain many nutrients and energy, which is considered as fundamental trophic level in food webs and play a vital role in nutrients and energy cycling in ecosystems (Moore et al., 2004; Moore and Schindler, 2008). Despite the positive function of carcass in ecosystem, the negative influence of cadaver on the environment quality should also not be overlooked. For example, the cadaveric flux can discharge amount of carbon (C), nitrogen (N) and phosphate (P) to the soil beneath the corpse. There is a report showed that a 68 kg human corpse leads to an elevation of about 525 μg ammonium g^{-1} in soil after death (dead for 20 days) (Vass et al., 1992). Another research found that approximately 15.6% available carcass N released in sub-cadaver soil (Parmenter and MacMahon, 2009). Fish carrion can lost about 95% of the original cadaver N as well as 60% of corpse P after 10 months postmortem (Parmenter and Lamarra, 1991). Our previous study also proved that NH_4^+-N concentration in water with fish corpse were 28 times higher than that in control groups which brought severe nitrogen pollution to the water (Zhou et al., 2021). Notably, soil near the carcass with enhanced N level can exert nitrogen toxicity to surrounding plant, thereby impacting vegetation production and growth (Parmenter and MacMahon, 2009). Besides, cadaver breakdown can also generate some detrimental and foul-smelling nitrogenous compounds: cadaverine, putrescine and indole (Vass et al., 2008), which can contaminate environment and threaten human health. Presently, in some developing countries without proper corpse disposal management rules, amounts of sick animal carcasses are discarded optionally into the water. These cadavers release excess nitrogen to the environment and gradually become pollutants that may contaminate ground and surface water, further probably impacting the supply of the drinking water and even spreading pathogens. Thus, it is of vital meaning and necessary to investigate the nitrogen removal and associated microbial mechanisms for environmental self-purification and improving water quality in areas polluted by cadavers.

Denitrifcation, a dissimilatory microbial pathway, can reduce oxidized N (nitrate or nitrite) to nitrogenous gas (nitrous oxide, nitric oxide and nitrogen gas), which has been reported to alleviate nitrogen pollution pressure and play an important role in a large shallow eutrophic reservoir (Zhou et al., 2016). This heterotrophic nitrogen removal pathway is more responsible for driving N loss in water than anaerobic ammonium oxidation (anammox), another nitrogen removal process that oxidizes ammonium to nitrogen gas (Babbin et al., 2014). Similarly, there is also a study showing that over 70% N_2 production and fixed nitrogen removal were contributed to denitrification rather than anammox in an oxygen minimum zone along ocean (Dalsgaard et al., 2012). Generally, denitrification as an environmental friendly and efficient nitrogen removal process is conducted by gradual steps accomplished by specific enzymes. For instance, nitrate reductases can reduce nitrate to nitrite, nitrite reductase can transfer nitrite to nitric oxide. Reduction of nitric oxide to nitrous gas was conducted through nitric oxide reductase. Nitrous gas turned into nitrogen gas via nitrous oxide reductase (Shrewsbury et al., 2016; Zumft, 1997). In particular, the shift from nitrite to nitric oxide catalyzed via nitrite reductase is the rate-limiting reaction, which makes soluble nitrogen exist in gaseous form for the first time during denitrification (Hou et al., 2018). Nitrite reductase is encoded by two functionally identical but structurally divergent genes, *nirK* and *nirS*. In general, there is discrepancy between *nirK*-encoding and *nirS*-encoding denitrifying community in taxonomic composition, abundance and structure, as well as the environmental driving factors (Jones and Hallin, 2010; Shi et al., 2019). Our previous study has investigated how *nirK*-type denitrifiers respond to the carcass input in water, while it is not sufficient to get knowledge about the integral denitrifying community that taking a vital part in reducing nitrite to nitric oxide during corpse decomposition (Yu et al., 2020). Therefore, our present research focuses on another gene encoding nitrite reductase, *nirS*, as a molecular marker to explore the denitrifying community across cadaver breakdown.

Community assembly processes consists of stochastic processes (e.g., drift, death, speciation and birth) and deterministic processes (e.g., species interactions and environmental filtering) (Dini-Andreote et al., 2015). Although these two processes concurrently modify the microbial communities in different aquatic systems, the relative importance for microbial communities may change and vary in different aquatic systems. For example, in landscape ponds, it has been demonstrated that stochastic processes, such as dispersal limitation, govern the assembly of prokaryotic community (Hu et al., 2018). But in Taihu Lake, the authors find that deterministic processes were the primary mode driving the free-living bacterial communities (Zhao et al., 2017) probably due to action of environmental factors (i.e., pH and nutrients). Understanding the main processes for community assembly is very helpful for regulating ecological communities to provide better functional service. If the denitrifying communities are mainly influenced by deterministic processes, we may improve nitrogen removal rate during corpse degradation by regulating the impacting factor determining denitrifying communities. However, the assembly processes for denitrifying communities during corpse degradation are still unknown.

In this study, high-throughput MiSeq sequencing for *nirS*-encoding denitrifying community was addressed to explore the succession of denitrifying microbes in water during the carcass decomposition. It is because China with vast sea areas and long coastline is endowed with broad aquaculture environment, thus there are amounts of fish carcass were abandoned into water due to diseases outbreak (Han et al., 2011). Consequently, fish was chosen for corpse degradation model and were investigated on 3rd day, 15th day and 19th day, corresponding to submerged fresh, advanced floating decay and sunken remains stages of cadaver decomposition. To get a general conclusion, we choose the Yellow River water and tap water as degradation substrate to investigate the impact of different substrate types. In addition, tap water is obtained through processed and purified water from the Yellow River in a Chinese city, Lanzhou, which is of great significance to protect and promote water quality, evaluate and manage environmental pollution. Thus, we put forward a hypothesis that corpse decomposition can change the environmental properties of the water and thus change the successional pattern of *nirS*-harboring denitrifiers. Our scientific questions are: (I) whether *nirS* denitrifying communities were influenced by cadaver decomposition and water type; (II) understand the succession of *nirS*-encoding denitrifying bacterial taxa, phylogenic clusters and core bacterial communities during the corpse decay; (III) what is the environmental driving factor for *nirS* denitrifiers across the degradation; (IV) evaluate whether the assembly processes of denitrifying communities are governed by stochastic processes or deterministic processes in different succession stages?

2. Materials and methods

2.1. Sample preparation and experimental procedure

Our experiment associated with animals has been authorized by the Ethics Committee and Animal Welfare in Lanzhou University with approval number LZU-201805-224. This study was carried on in July 2018 at Lanzhou city, China (36.3°N, 103.49°E). Fish (*Cyprinus carpio haematopterus*) were deployed as decomposition models. A total of 30 fish were booked from local wholesale seafood market, each weighting 78.52 ± 0.95 g, and then sacrificed by overusing the anesthesia MS 222 (3-Aminobenzoic acid ethyl ester methanesulfonate; Hunan Tiancheng polymer material co., Changsha, China). Observation and researches for fish corpse and the denitrifying community succession in water were initiated at different decomposition stages after fish death: submerged fresh (day 3), advanced floating decay (day 15) and sunken remains (day 19). There were 5 replicates in each group during 3 cadaver decaying stages. Total 15 plastic boxes measuring 20 × 18 × 15 cm contained 15 dead fish randomly and saturated with tap water (800 ml) collected from our laboratory. Correspondingly, 15 containers were assigned as control groups only containing 800 ml tap water without fish cadavers. We sampled the Yellow River water through Lanzhou section of the Yellow River (36.065°N, 103.815°E). Remaining 15 fish cadavers were housed in the 15 plastic boxes containing the Yellow River water. Similarly, 15 plastic boxes only with the Yellow River water were set as control groups as well. Group YF (the Yellow River water containing fish corpse) and TF (tap water containing fish corpse) were identified as experimental group. Group Y (the Yellow River water without corpse) and T (tap water without corpse) were regarded as control group. The 250 ml water samples were gathered from every container and then filtered. Membranes with core diameter of 0.45 μm and 0.22 μm were used to filter water samples from corpse group. Only 0.22 μm membranes were applied to filter water samples from control groups. After filtration, the filter membranes were immediately reserved at −20 °C before extracting DNA. Additionally, about 100–150 ml water samples were also acquired for the next measurement of water physicochemical properties. More detailed procedures can be obtained in our previous study (Zhou et al., 2021).

2.2. Water quality measurement

Water physiochemical factors, including water temperature, pH, dissolve oxygen (DO), total dissolved solids (TDS), conductivity (CON), oxidation-reduction potential (ORP), salinity, phosphate, total organic carbon (TOC), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and ammonia nitrogen (NH_4^+-N), were analyzed for each sample. A thermometer (TES1316, Shanghai Precision Instruments Co., China) and dissolved oxygen meter (YSI59, YSI Corporation in China, USA) were employed to measure water temperature and DO respectively. A bench water physicochemical property analyzer was used to detect water CON, salinity, ORP, pH, and TDS (AZ86505, Shenzhen lemaiyi electronics co., LTD.). Non-dispersive infrared absorption method was applied to detect TOC content in water (Visco et al., 2005). Nessler's reagent colorimetric method was used to analyze NH_4^+-N concentration in water (Zhao et al., 2010). Colorimetry with set of 220 and 275 nm double wavelengths was applied to measure water NO_3-N concentration (Hai et al., 2009). Colorimetry at 540 nm wavelengths was used to determine NO_2-N content (Rider and Mellon, 1946). Water phosphate content was measured according to the ammonium molybdate spectrophotometric method (Guo-Mei, 2006).

2.3. DNA extracting, sequencing and bioinformatics analyzing

All acquired filter membranes were divided into pieces by sterile scissors before extracting DNA. Soil Ezup Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China) were utilized to extract total genomic DNA. We use Nanodrop 2000 Spectrophotometer to determine obtained DNA quality and concentration (Thermo Scientific, IL, Waltham, USA). Polymerase chain reaction (PCR) amplicons of the *nirS* gene were amplified by using primer pairs *nirS* cd3aF (5′-GTSAACGTSAAGGARACSGG-3′)/ R3cd (5′- GASTTCGGRTGSGTCTTGA −3′) (Throbäck et al., 2004). PCR reaction for each sample was conducted in triplicate and detailed reaction procedures can get from our previous study (Yu et al., 2020). SanPrep DNA Gel Extraction Kit (Sangon Biotech, China) was used to purify PCR products. The PCR products content of each sample were measured based on Invitrogen Qubit3.0 Spectrophotometer (Thermo Fisher Scientific, USA). Next, sequencing library was constructed by pooling the equal amounts of PCR amplicons. Agilent 2100 Bioanalyzer (Agilent Technologies, USA) was applied to detect the size of inserted fragments. Ultimately, Illumina MiSeq systems (Illumina, USA) was employed to sequence *nirS* PCR amplicons according to 2 × 250 bp coupled end.

Quantitative Insights Into Microbial Ecology (QIIME) pipeline processed to analyze original sequencing (Caporaso et al., 2010). The coupled-terminal fastq sequences were consolidated through FLASH software (Magoč and Salzberg, 2011). Reads belonging to every sample were separated depending on their exclusive barcodes. High-quality sequences were remained for further analysis after eliminating sequences that containing six N bases or length less than 100 bp, and then Usearch 8.0 de novo mode were used to remove chimera reads (Edgar et al., 2011). Framebot was applied to correct false code shifting and diminish the amino acid reads (length below 100 bp) (Wang et al., 2013). Remaining sequences data were processed to cluster into operational taxonomic units (OTUs) based on 97% nucleotide sequence identity under UCLUST algorithm (Edgar, 2010). Taxonomic classification annotation for representative sequences of OTUs was accomplished by blastp via comparing the *nirS* database in the FunGene repository (Fish et al., 2013). For equalizing the influence of the samples with uneven reads, each sample was standardized to 13,269 reads by means of the script "daisychopper" (Gilbert et al., 2009).

Goods coverage, chao1 and observed OTUs were considered to estimate the alpha diversity for each group. Sequencing depth was assessed via OTU-level rarefaction curves. The evaluation of the beta diversity was focused on weighted (for community structures) and unweighted (for community membership) UniFrac distance metrics which consider the phylogenetic information and species existence or not (Lozupone and Knight, 2005). Visualization of the community structure difference among different groups was demonstrated by principal coordinates analysis (PCoA) plots through OriginLab 2019 (OriginLab, Northampton, USA). The *nirS* denitrifying bacterial "core community" was defined as OTUs with a presence frequency in at least 80% corpse samples. The core denitrifying communities were further analyzed to explore their number, abundance, composition and correlation with water environmental factors. Moreover, abundant OTUs with average relative abundance higher than 0.1% among all groups were selected to structure neighbor-joining (NJ) phylogenetic tree by MEGA4 (Tamura et al., 2007) and FigTree v1.4.2 (Rambaut and Drummond, 2008) after sequences were aligned by method ClustalW (Thompson et al., 2003). Original *nirS* gene sequencing data acquired was available in the European Nucleotide Archive according to approved code PRJEB41329 (http://www.ebi.ac.uk/ena/data/view/PRJEB41329).

2.4. Statistical analysis

Differentiation of alpha diversity between corpse group and control group in every sampling interval was compared by one-way analysis of variance (one-way ANOVA) in SPSS 21.0 through Tukey's post hoc test (SPSS Inc., Chicago, IL, USA). The impact of water types and decaying time on alpha diversity of *nirS* denitrifying communities was explored under two-way ANOVA analysis. To discern the discrepancy of *nirS* denitrifying community structures between different groups, analysis of similarity (ANOSIM) and permutational

multivariate analysis of variance (PERMANOVA) were applied dependent on the unweighted and weighted UniFrac distance metrics through R 'vegan' package ('anosim' and 'adonis' function) (http://www.r-project.org/) (Anderson, 2014; Chapman and Underwood, 1999). Function 'adonis' in R 'vegan' package was also utilized to explore the environmental driving factors of *nirS* denitrifying communities. Moreover, multiple regression matrices (MRM) was used to compare relative importance of water type, environmental factors, corpse treatment and decaying time to the denitrifying community based on R package 'ecodist' (Deng et al., 2016). The linkage between abundant genera and environmental factors was identified by network analysis through Gephi platform using Frucherman Reingold algorithms (Bastian et al., 2009). Only correlations with Spearman's correlation coefficient (ρ) > 0.6 and significance level (P) < 0.05 were gathered to perform final networks (Junker and Schreiber, 2011). Moreover, linkage between abundant genera (average relative abundance >1%) and environmental factors was visualized by redundancy analysis (RDA) with Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA) (ter Braak and Smilauer, 2012). Partial mantel test was used to analyze the different variables on denitrifying communities after controlling for one factor by 'mantel' function in R 'vegan' package.

2.5. Null model analysis

Null model was used to evaluate relative contribution of stochastic and deterministic processes governing the denitrifying community assembly (Z. Zhang et al., 2019). These analysis methods and procedures have been reported in the previous study (Hou et al., 2020). R code in this study can get from http://mem.rcees.ac.cn/download/. In brief, the expected similarity (E_{exp}) of null expected communities (randomly created) among samples was produced according to 1000 random shuffles of the original denitrifying community data (Chase et al., 2011). The community diversity and relative percentage of species occupancy was identical as observed similarity during this operation process. The magnitude of stochastic processes and deterministic processes was calculated according to following formula:

$$\text{Stochasticity}\ (\%) = 1 - (S_{obs} - E_{exp})/S_{obs}$$

$$\text{Deterministicity}(\%) = 1 - \text{Stochasticity}\ (\%)$$

where S_{obs} equals the observed Jaccard similarity of the actual observed communities, and E_{exp} refers to the average expected Jaccard similarity of null expected communities (H. Zhang et al., 2019). After calculating the stochasticity values, Mann-Whitney U test was utilized to compare the stochasticity discrepancy between the control and corpse groups in each decaying interval.

3. Results

3.1. Variation of water physiochemical characteristics across cadaver decomposition

Observation for changes of environmental factors was performed on the three phases of corpse degradation: submerged fresh at day 3, advanced floating decay at day 15 and sunken remains at day 19. Nearly all water physiochemical characteristics were impacted by fish corpse addition (Table S1). Water pH decreased significantly after cadaver adding with average pH values ranging from 7.916 to 8.370 in control groups and from 6.483 to 7.698 in corpse groups (all $P < 0.05$). It is worth noting that the values of pH in corpse groups slightly increased from initial stage of decomposition to later stage of decaying both in the Yellow River water and tap water. The ORP, salinity, TDS, CON and phosphate values in carrion groups were significantly higher than those in groups without carrion across three decomposition stages. The DO values declined significantly after the input of fish carrion, in the meanwhile, the values of DO in cadaver groups also decreased from 3rd day to 19th day. The concentration of the NH_4^+-N had a sharp increment in the experimental groups, averagely varying from 28.433 to 33.379, which was significantly higher than that in control groups. NO_3-N also followed similar trends that concentration approximately tripled in cadaver groups compared to control groups. In contrast, the NO_2-N concentration was not impacted by the corpse decomposition, which had no remarkable discrepancy both in cadaver and control groups across three decaying phases. Likewise, water temperature as well as TOC shows indistinctive discrepancy in corpse and control groups. Furthermore, all water physicochemical indices displayed no remarkable discrepancy between group YF (the Yellow River water with carcass) and group TF (tap water with carcass) on 3rd, 15th, 19th day, indicating that cadaver makes environmental condition similar regardless of water types.

3.2. Sequencing results

Total 60 samples were gathered to extract DNA and amplify PCR products, but 8 samples were failed to amplify the target fragments. Thus, a total of 3, 583, 672 raw reads were generated from 52 samples, and 2,774,263 high-quality reads (mean 53,351 sequences per sample, max = 142,151, min = 13,840, SD = 28,821) were obtained after removing low-quality sequences, chimeras, singletons and chloroplast. Each sample was normalized to 13,269 reads and all sequences were classified to 1, 214 OTUs with 3% dissimilarity. OTU-level Good coverage was 99.75% ± 0.01% (mean ± SE), indicating that sequencing information had fully contained most species of denitrifying communities in water. Besides, rarefaction curves plot is close to plateau reflecting that our sequencing results are enough to capture most microbial community messages (Fig. S1).

3.3. Comparison of the alpha and beta diversity of nirS denitrifying communities in diverse groups across carcass degradation

To investigate whether carcass had impact on the alpha diversity of *nirS* denitrifying community, inter-group dissimilarities of Chao 1 (evenness and richness of species) and observed OTUs (quantity of species) were compared by one-way ANOVA at control and corpse groups (Fig. 1). Our results demonstrated that alpha diversity of *nirS* denitrifiers in carcass groups almost had no significant difference compared with corresponding control groups at each decaying stage, revealing that alpha diversity of *nirS* denitrifiers were not impacted by corpse treatment. Simultaneously, two-way ANOVA was employed to investigate the impact of decaying time and water type on alpha indices (Table S2). Interestingly, water type had a significant impact on the alpha diversity of *nirS* denitrifiers, while decaying time showed no remarkable discrepancy about alpha indices among different decaying stages. Besides, all environmental factors had no significant correlation with two alpha diversity indices observed OTUs and Chao 1 (Table S3).

Principal coordinates analysis (PCoA) according to the weighted and unweighted UniFrac distance metrics was applied to profile the difference of *nirS*-type denitrifying community composition and structure in the Yellow River water and tap water across three different cadaver degradation stages (Fig. 2). At the beginning, *nirS*-type denitrifying community composition and structure among all carcass and control groups were clearly separated, which was further statistically evidenced by ANOSIM and PERMANOVA analysis (Table 1). This discrepancy proved that the carcass decaying process significantly influenced the memberships and structure of *nirS* denitrifiers in different water types. Next, the PCoA plots exhibited an overlap between YF (the Yellow River water harboring cadaver) and TF (tap water harboring cadaver) at day 3, day 15, and day 19 respectively. PERMANOVA and ANOSIM

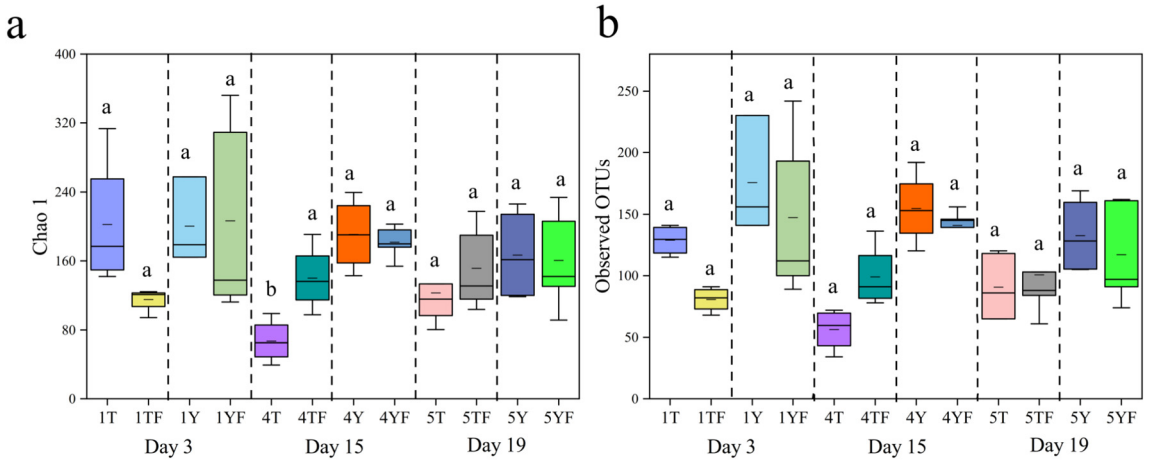

Fig. 1. The comparison of alpha diversity values (a, Chao 1; b, observed OTUs) for *nirS* denitrifying communities between control and carcass groups. Significant difference is labeled with different letters ($P < 0.05$). Abbreviations: 1T, tap water at day 3; 1TF, tap water with fish corpse at day 3; 1Y, the Yellow River water at day 3; 1YF, the Yellow River water with fish corpse at day 3; 4T, tap water at day 15; 4TF, tap water with fish corpse at day 15; 4Y, the Yellow River water at day 15; 4YF, the Yellow River water with fish corpse at day 15; 5T, tap water at day 19; 5TF, tap water with fish corpse at day 19; 5Y, the Yellow River water at day 19; 5YF, the Yellow River water with fish corpse at day 19.

analysis also provided convincing evidence that no remarkable difference occurred in two experimental groups at each corpse decomposition stage, revealing that the impact of cadaver on the *nirS*-type denitrifiers's composition and structure was similar regardless of different water types. In the end, corpse groups at initial stage of carrion decomposition (submerged fresh at day 3) were significantly

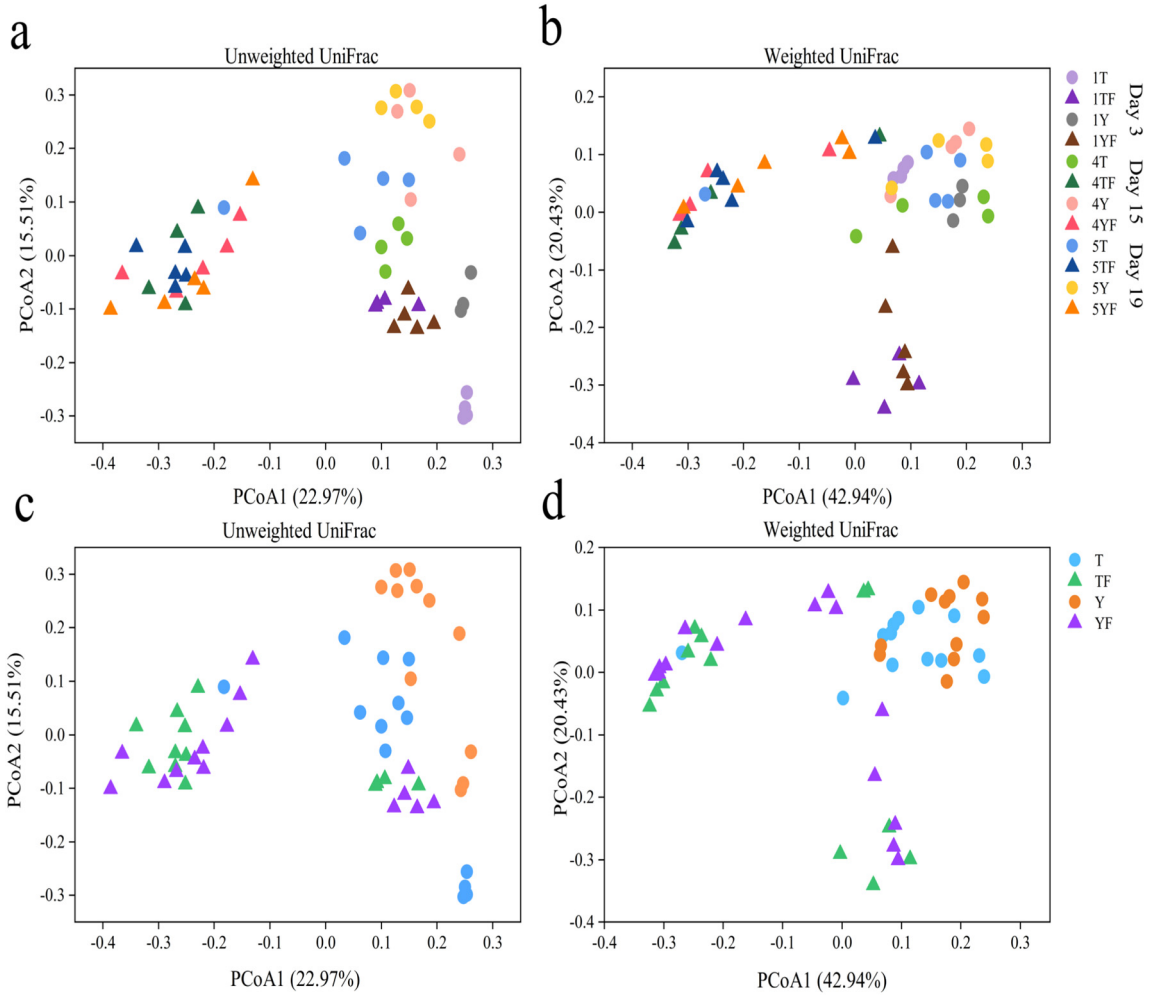

Fig. 2. The principal coordinates analysis (PCoA) plots showing the structural difference of *nirS* denitrifiers in the Yellow River water and tap water across 3 decomposition stages based on unweighted (a; c) and weighted UniFrac distance metrics (b; d). Abbreviations: 1T, tap water at day 3; 1TF, tap water with fish corpse at day 3; 1Y, the Yellow River water at day 3; 1YF, the Yellow River water with fish corpse at day 3; 4T, tap water at day 15; 4TF, tap water with fish corpse at day 15; 4Y, the Yellow River water at day 15; 4YF, the Yellow River water with fish corpse at day 15; 5T, tap water at day 19; 5TF, tap water with fish corpse at day 19; 5Y, the Yellow River water at day 19; 5YF, the Yellow River water with fish corpse at day 19.

Table 1
Permutational multivariate analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM) according to unweighted and weighted UniFrac distance metrics in comparison of community structure and membership between different groups. The bold type indicates a significant difference ($P < 0.05$). Abbreviations: 1T, tap water at day 3; 1TF, tap water with fish corpse at day 3; 1Y, the Yellow River water at day 3; 1YF, the Yellow River water with fish corpse at day 3; 4T, tap water at day 15; 4TF, tap water with fish corpse at day 15; 4Y, the Yellow River water at day 15; 4YF, the Yellow River water with fish corpse at day 15; 5T, tap water at day 19; 5TF, tap water with fish corpse at day 19; 5Y, the Yellow River water at day 19; 5YF, the Yellow River water with fish corpse at day 19.

	Weighted UniFrac					Unweighted UniFrac				
	PERMANOVA			ANOSIM		PERMANOVA			ANOSIM	
	F	R^2	*P*	R	*P*	F	R^2	*P*	R	*P*
1T vs. 1TF	25.599	0.810	**0.032**	1.000	**0.025**	8.804	0.595	**0.029**	1.000	**0.036**
4T vs. 4TF	4.613	0.435	0.050	0.615	0.052	5.206	0.465	**0.030**	1.000	**0.032**
5T vs. 5TF	3.590	0.310	**0.039**	0.372	**0.041**	4.145	0.341	**0.008**	0.784	**0.012**
1Y vs. 1YF	12.766	0.680	**0.016**	0.990	**0.022**	2.806	0.319	**0.016**	0.939	**0.022**
4Y vs. 4YF	14.251	0.671	**0.014**	0.881	**0.018**	5.840	0.455	**0.008**	0.994	**0.007**
5Y vs. 5YF	8.436	0.547	**0.011**	0.813	**0.008**	5.388	0.435	**0.007**	0.919	**0.007**
1TF vs. 4TF	8.487	0.586	**0.035**	0.771	**0.025**	5.289	0.468	**0.028**	1.000	**0.029**
1TF vs. 5TF	11.850	0.629	**0.014**	0.944	**0.011**	5.371	0.434	**0.007**	0.900	**0.008**
4TF vs. 5TF	0.162	0.023	0.774	−0.094	0.597	0.979	0.123	0.518	−0.038	0.568
1YF vs. 4YF	15.849	0.665	**0.007**	0.968	**0.007**	5.952	0.427	**0.007**	1.000	**0.009**
1YF vs. 5YF	11.405	0.588	**0.011**	0.848	**0.006**	5.596	0.412	**0.008**	1.000	**0.007**
4YF vs. 5YF	1.735	0.178	0.197	0.088	0.203	0.773	0.088	0.742	−0.104	0.781
1TF vs. 1YF	2.174	0.237	0.121	0.175	0.134	3.083	0.306	**0.010**	0.638	**0.020**
4TF vs. 4YF	0.357	0.049	0.634	−0.013	0.416	2.331	0.250	0.023	0.694	0.018
5TF vs. 5 YF	0.660	0.076	0.438	−0.052	0.525	0.847	0.096	0.705	−0.016	0.505

differentiated from those during last two stages of decaying (advanced floating decay on 15th day and sunken remains on 19th day). For example, 1TF (experimental group on 3rd day) is significantly far from 4TF (experimental group on 15th day) and 5TF (experimental group on 19th day) respectively. Interestingly, no significant difference was presented in two experimental groups during last two phases of degradation, which indicating that despite different community compositional and structural difference at early stage of degradation, the memberships and structure of the *nirS* denitrifying communities tended to resemble at last as carcass decomposition proceed.

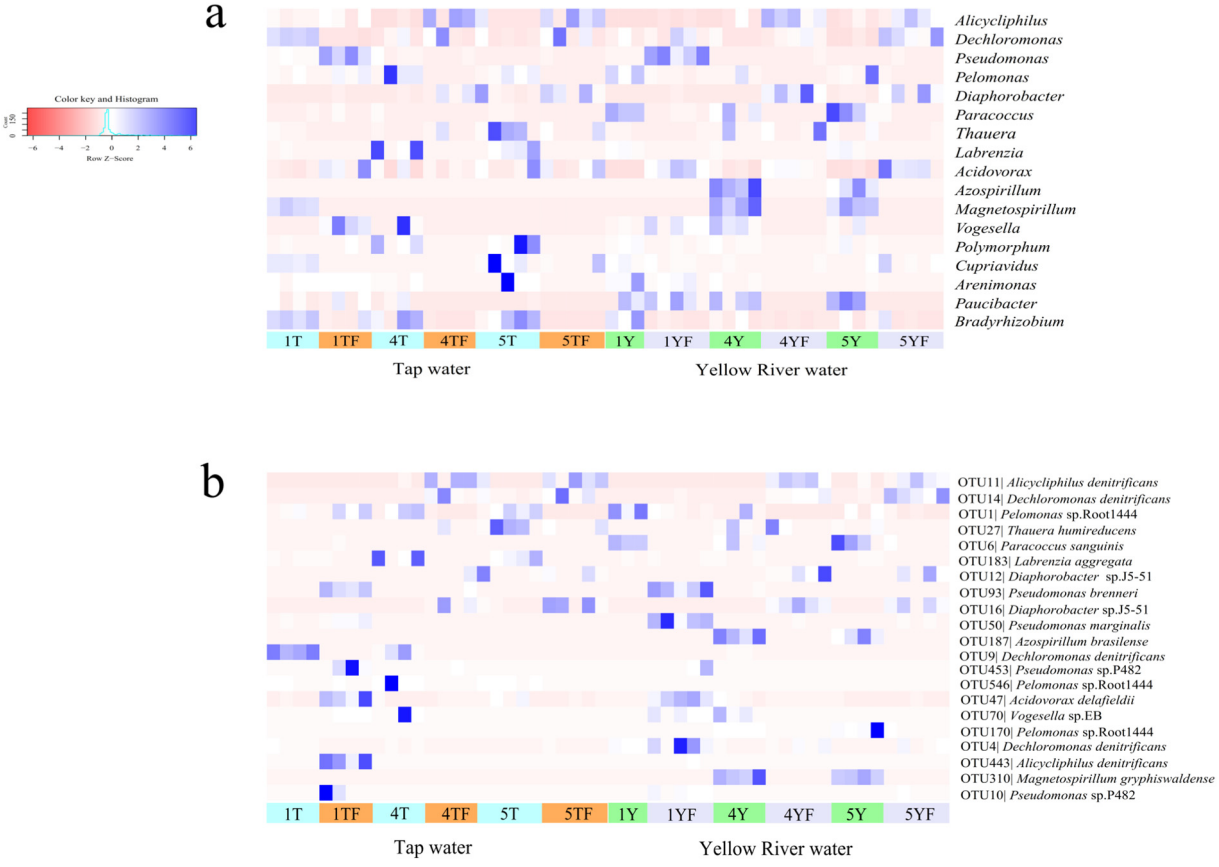

Fig. 3. *NirS* denitrifying community composition in tap water and the Yellow River water during corpse degradation at genus and OTU level. Abbreviations: 1T, tap water at day 3; 1TF, tap water with fish corpse at day 3; 1Y, the Yellow River water at day 3; 1YF, the Yellow River water with fish corpse at day 3; 4T, tap water at day 15; 4TF, tap water with fish corpse at day 15; 4Y, the Yellow River water at day 15; 4YF, the Yellow River water with fish corpse at day 15; 5T, tap water at day 19; 5TF, tap water with fish corpse at day 19; 5Y, the Yellow River water at day 19; 5YF, the Yellow River water with fish corpse at day 19.

3.4. The succession of nirS-type denitrifying community composition

At phylum level, Proteobacteria occupied overwhelming predominance across all groups of *nirS* denitrifying community in water (mean relative abundance = 99.22%; Fig. S2), showing that phylum Proteobacteria plays a major role in denitrification process during carcass decomposition. At genus level, genera with average relative abundance higher than 1% were displayed on the heatmap plot (Fig. 3a). The most abundant genus was *Alicycliphilus* (mean relative abundance = 21.84%), followed by *Dechloromonas* (14.13%), *Pseudomonas* (11.28%), *Pelomona* (7.9%), *Diaphorobacter* (6.78%), *Paracoccus* (4.87%), *Thauera* (4.48%) and others including *Labrenzia*, *Acidovorax*, *Azospirillum*, *Magnetospirillum*, *Vogesella*, *Polymorphum*, *Cupriavidus*, *Arenimonas*, *Paucibacter* and *Bradyrhizobium*. The relative abundance of *Alicycliphilus* and *Diaphorobacter* were elevated during the last two stages of degradation both in tap water and the Yellow River water, while genus *Pseudomonas* increased from control groups to experimental groups only on 3rd day. Heatmap plots also exhibited 21 abundant OTUs (mean relative abundance >1%) across all groups (Fig. 3b). OTU11 belonging to *Alicycliphilus denitrificans* predominated *nirS* denitrifying communities (average relative abundance = 17.63%), with OTU14| *Dechloromonas denitrificans* (7.98%), OTU1| *Pelomonas* sp. Root1444 (4.8%) and other OTUs coming after. Ternary diagrams showed the succession of abundant genera (mean relative abundance >0.1%) in corpse groups across different cadaver decaying stages (Fig. 4). In TF group (tap water with corpses), genera *Pseudomonas*, *Paucibacter*, *Arenimonas*, *Bradyrhizobium*, *Paracoccus*, *Pelomonas* and *Polymorphum* enriched on stage submerged fresh at 3rd day, and *Acidovorax*, *Alicycliphilus*, *Diaphorobacter*, and *Candidatus Accumulibacter* occurred in last two stages on day 15 and 19. In YF group (the Yellow River water with corpses), *Arenimonas*, *Steroidobacter* and *Pelomonas* were more abundant at day 3, and *Thauera* and *Diaphorobacter* were enriched on 15th day. Besides, *Paracoccus*, *Bradyrhizobium* and *Sulfuritalea* had a higher abundance on 19th day.

3.5. Core bacterial community of nirS denitrifying bacteria during cadaver decaying process

Core bacterial community of *nirS* denitrifying bacteria was defined as the presence frequency of OTUs over 80% across all carcass samples (Fig. 5). The largest relative abundance, 74.79%, was only occupied by 13 core OTUs taking up 1.06% of all OTUs (Fig. 5a). Over 40% of OTUs belonged to genus *Alicycliphilus*, others were assigned to *Dechloromonas*, *Pseudomonas*, *Diaphorobacter*, *Acidovorax*, *Pelomonas* and uncultured bacteria. Besides, the correlation between core bacterial community and environmental factors were demonstrated on the Fig. 5c. OTU11| *Alicycliphilus denitrificans* and OTU14| *Dechloromonas denitrificans* were both positively related with phosphate, TOC, salinity, TDS, CON and pH, but negatively linked with ORP. OTU453| *Pseudomonas* sp. P482 had a negative correlation with salinity, TDS and pH, while a positive correlation with ORP. OTU16 and OTU12 affiliated to *Diaphorobacter* sp. J5-51 were positively associated with phosphate, salinity, TDS and CON. OTU50| *Pseudomonas marginalis* was negatively correlated with pH. OTU16 was negatively linked with NH_4^+-N. OTU93, OTU50, OTU47 and OTU1 had negative correlation with NO_2-N, while OTU14, OTU16 and OTU22 positively associated with NO_2-N.

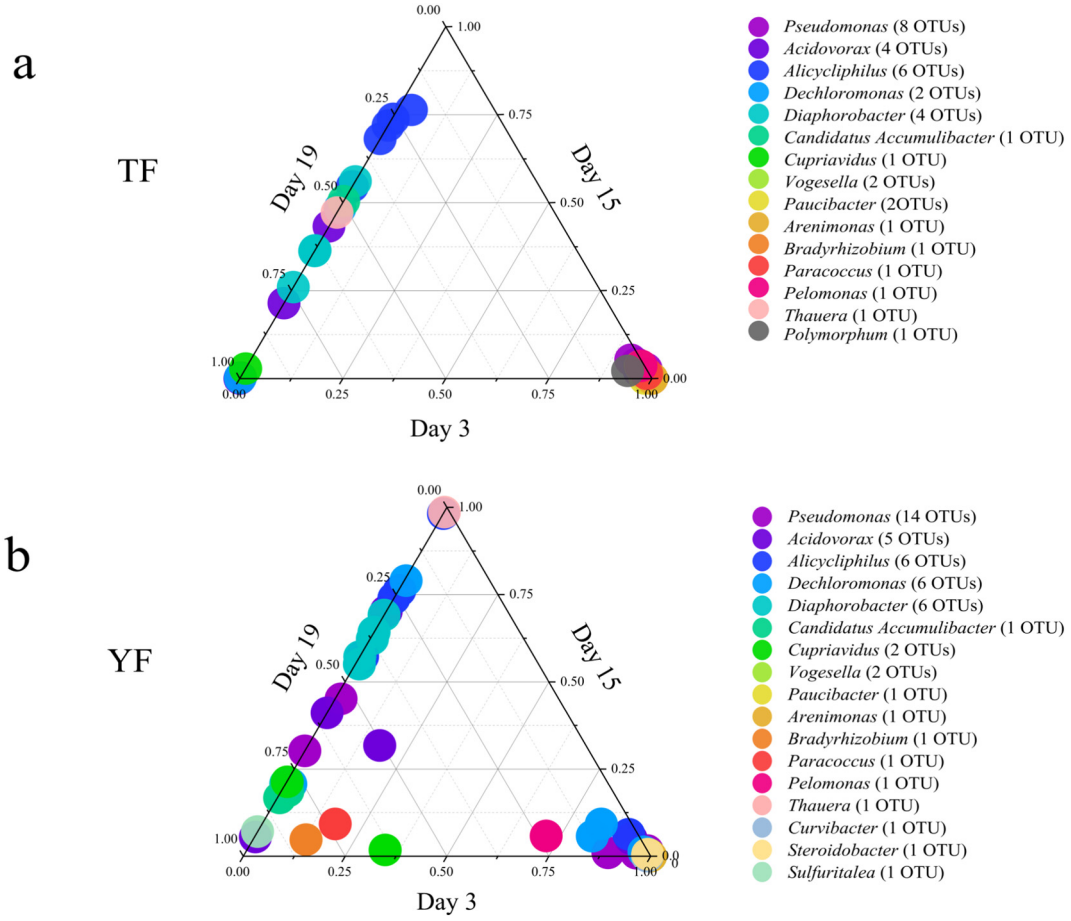

Fig. 4. Ternary diagrams show the succession of abundant genera (mean relative abundance >0.1%) in corpse groups across different cadaver decaying stages. Abbreviations: TF, tap water with corpse; YF, the Yellow River water with corpse.

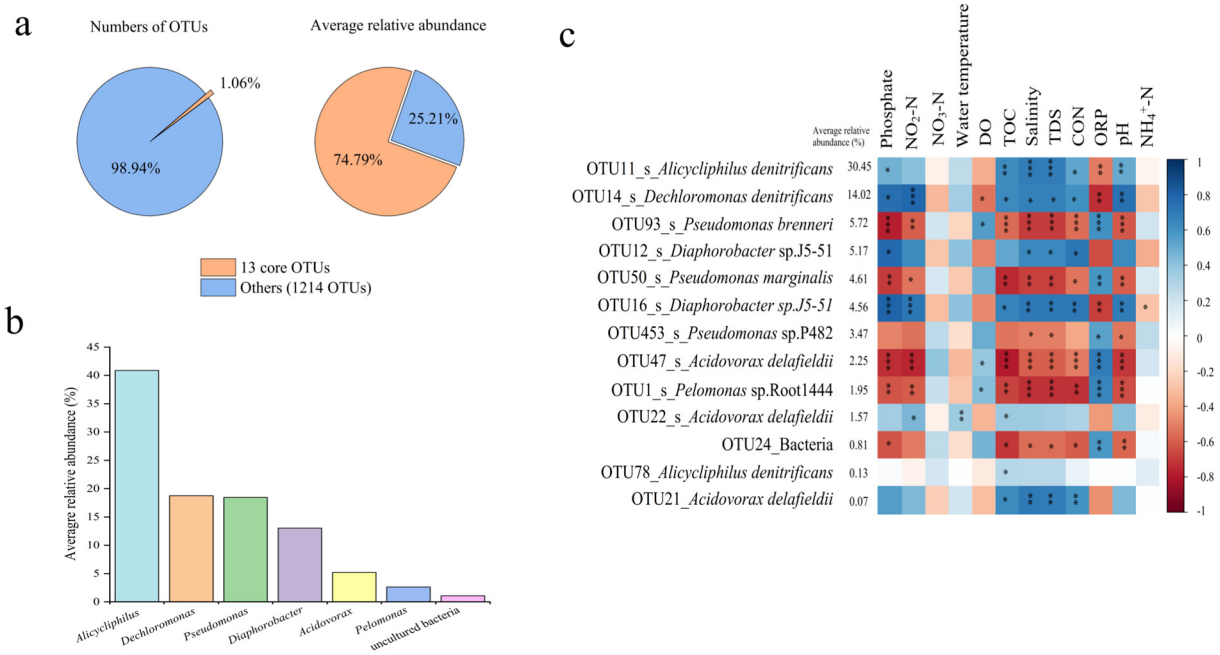

Fig. 5. Comparison of numbers and relative abundance between *nirS* core denitrifying OTUs and remaining OTUs in carcass groups (a); Genus-level composition for the *nirS* core denitrifying OTUs in corpse groups (b); Correlation between *nirS* core denitrifying OTUs and the environmental factors (c).

3.6. Phylogenetic clusters of nirS denitrifying bacteria

Neighbor-joining phylogenetic trees were generated to show that the OTUs (mean relative abundance more than 0.1%) were assigned into 4 clusters (Fig. 6). Each cluster was also classified to different genera displaying on the pie plot. Cluster 1 was dominated by *Dechloromonas* (52.64%). Cluster 2 was dominated by *Pseudomonas* (33.80%) and *Pelomonas* (25.73%). In cluster 3, *Paracoccus* (33.50%) and *Labrenzia* (29.94%) were dominant genera. Cluster 4 contained most dominant OTUs, which was dominated by *Alicycliphilus* (41.74%), followed by *Pseudomonas* (15.09%), *Diaphorobacter* (13.52%) and *Pelomonas* (12.77%). Only the abundance of cluster 4 has a significant discrepancy in corpse groups and control groups, showing a sharp increase in carcass groups compared with control groups.

3.7. The network analysis of the environmental factors and nirS denitrifying bacteria

The correlation of water physiochemical indices and abundant genera was visualized on the network plots with absolute value of correlation coefficient over 0.7 and significance lower than 0.05 (Fig. 7). Network plot contained 40 nodes representing 28 abundant genera and 12 water physiochemical indices, and 121 sides. The size of the nodes was ranked by PageRank algorithm which was used to measure the importance of the node in whole network. Phosphate, salinity, TDS, CON, NH_4^+-N and NO_3-N were the most important factors that had many interactions with genera, while NO_2-N and water temperature has no strong correlations with genus. Phosphate is positively correlated with *Diaphorobacter*, negatively correlated with *Pelomonas*, *Labrenzia*, *Arenimonas*, *Bradyrhizobium* and

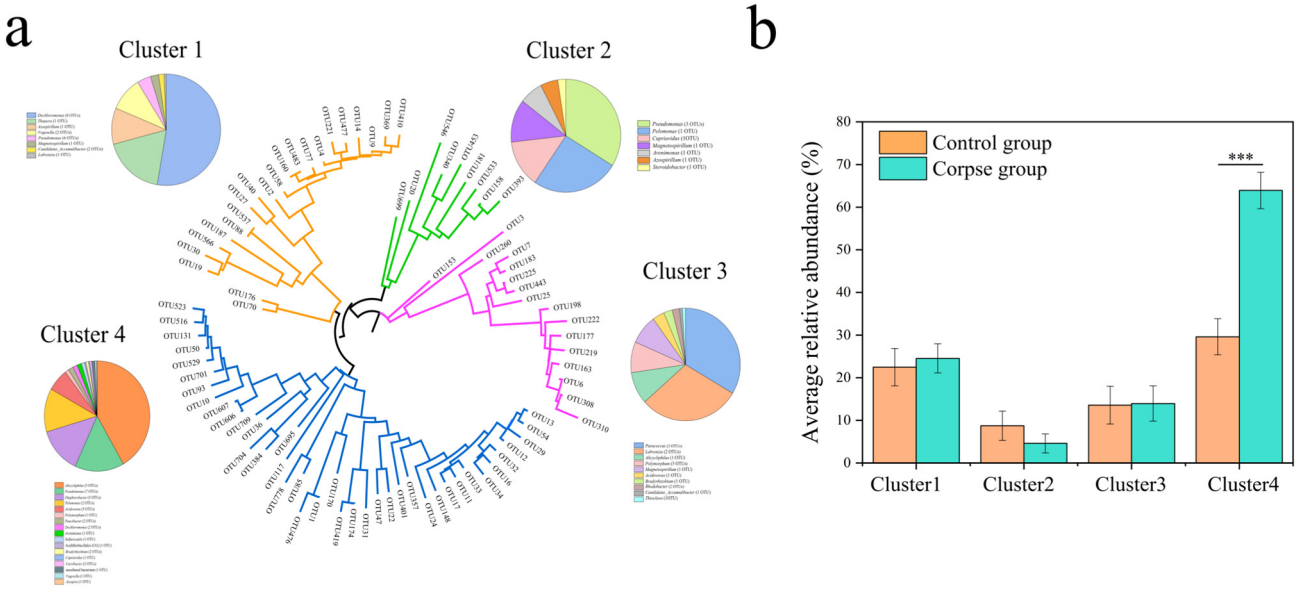

Fig. 6. Phylogenetic neighbor-joining (NJ) trees of partial *nirS* sequences (select the OTUs with average relative abundance >0.1%) (a); The proportion of four clusters in the corpse groups and control groups (b).

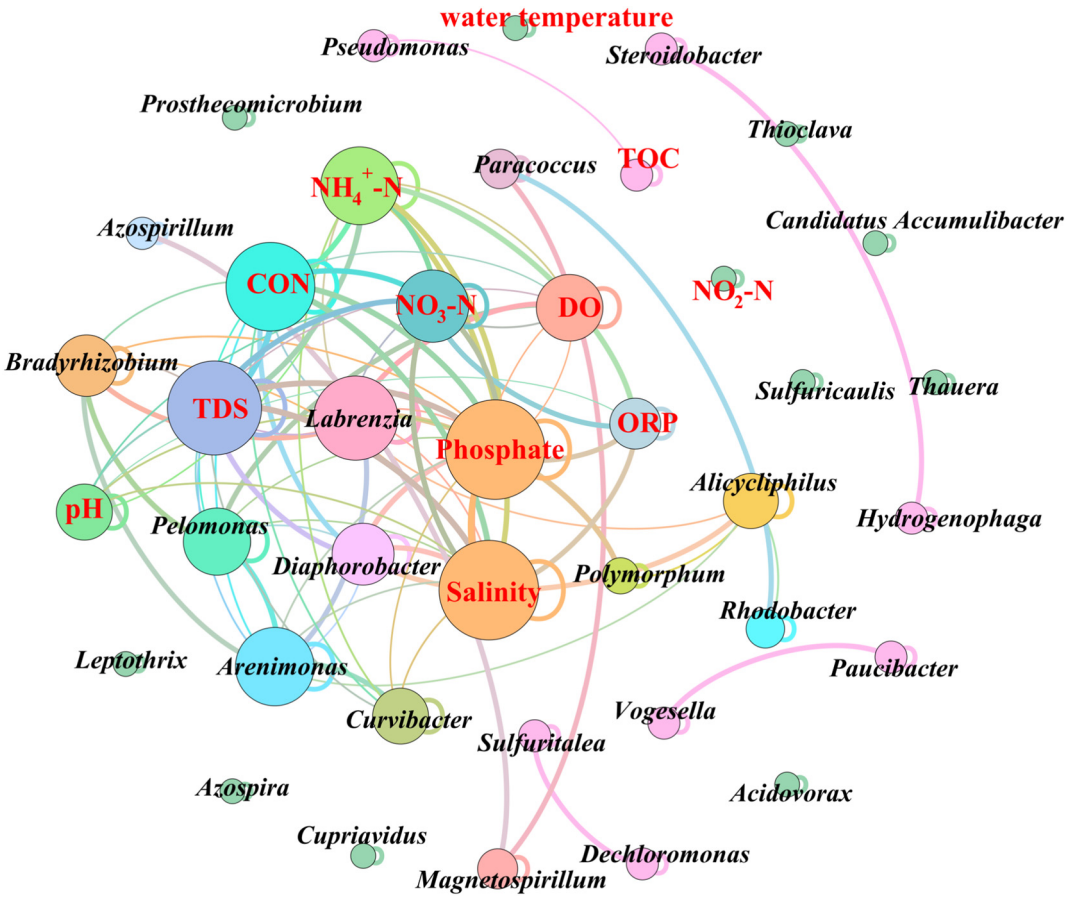

Fig. 7. Network analysis showing the co-occurrence patterns between abundant genera and environmental factors (significance level $P < 0.05$ and Spearman's correlation coefficient (ρ) > 0.6).

Curvibacter. NH_4^+-N had strong negative correlation with *Labrenzia* and *Curvibacter*. NO_3-N was negatively correlated with *Labrenzia*. DO was positively linked with *Pelomonas* and *Labrenzia*. TOC was negatively assosicated with *Pseudomonas*. Salinity, TDS and CON were positively correlated with *Diaphorobacter*, negatively correlated with *Pelomonas*, *Labrenzia*, *Arenimonas*, *Bradyrhizobium* and *Curvibacter*. Importantly, some abundant genra has significant correlations. *Alicycliphilus* is positively correlated with *Diaphorobacter*, while *Diaphorobacter* is negatively correlated with *Pelomonas*. Additionally, RDA plots also provided visualized linkage between environmental factors and genera, which proved our results again (Fig. S3).

3.8. Driving factors for nirS denitrifying bacterial communities

Furthermore, to discuss the impact of water physiochemical factor on composition and structures of *nirS*-encoding denitrifying communities, PERMANOVA analysis based on unweighted and weighted UniFrac distance metrics revealed that TDS, salinity, CON and phosphate were the most primary factor, followed by treatment, NH_4^+-N, pH and NO_3-N (Table 2). MRM analysis divided all influencing factors into four parts: environmental factors, corpse treatment, decaying time and water type (Table S4). The most influential element is environmental factors, accounting for 29.24% effects on denitrifying communities. Corpse treatment and decaying time took up 13.80% and 1.42% effects on denitrifying communities respectively, while water type had no significant contributions on the variation of community. To explore entire environmental impact on bacterial community further, partial mantel tests were used to compute environmental contribution after controlling for corpse treatment and decaying time (Table S5). When controlling for corpse treatment, the environmental factors had strong correlation with denitrifying communities ($R = 0.370$, $P = 0.001$). When controlling for decaying time, the environmental factors also exhibited strong linkage with denitrifying communities ($R = 0.504$, $P = 0.001$). These results indicate that environmental factors had most important contributions for denitrifying bacterial communities.

3.9. Assembly processes for nirS denitrifying communities

The relative contribution of deterministic and stochastic processes governing the denitrifying community assembly in each group was

Table 2
PERMANOVA analysis based on unweighted and weighted UniFrac distance metrics showing the influence of environmental factors on *nirS* denitrifying communities. The significance is tested based on 999 permutations. The bold front indicates a significant difference ($P < 0.05$).

Environmental factors	PERMANOVA					
	Weighted UniFrac			Unweighted UniFrac		
	F	R^2	*P*	F	R^2	*P*
TDS	20.930	0.295	**0.001**	11.781	0.191	**0.001**
Salinity	20.811	0.294	**0.001**	11.732	0.190	**0.001**
CON	17.401	0.258	**0.001**	10.422	0.172	**0.001**
Phosphate	16.416	0.247	**0.001**	10.640	0.175	**0.001**
Treatment	15.396	0.235	**0.001**	8.178	0.141	**0.001**
NH_4^+-N	12.564	0.201	**0.001**	7.716	0.134	**0.001**
pH	11.029	0.181	**0.001**	3.613	0.067	**0.001**
NO_3-N	9.735	0.163	**0.001**	5.116	0.093	**0.001**
Time	6.356	0.113	**0.001**	6.946	0.122	**0.001**
Water type	1.046	0.020	0.358	3.384	0.063	**0.001**
DO	7.552	0.131	**0.001**	5.868	0.105	**0.001**
ORP	7.145	0.125	**0.001**	2.579	0.049	**0.006**
TOC	4.279	0.079	**0.004**	4.980	0.091	**0.001**
NO_2-N	4.101	0.076	**0.005**	3.462	0.065	**0.001**
Water temperature	1.623	0.031	0.175	1.579	0.031	0.088

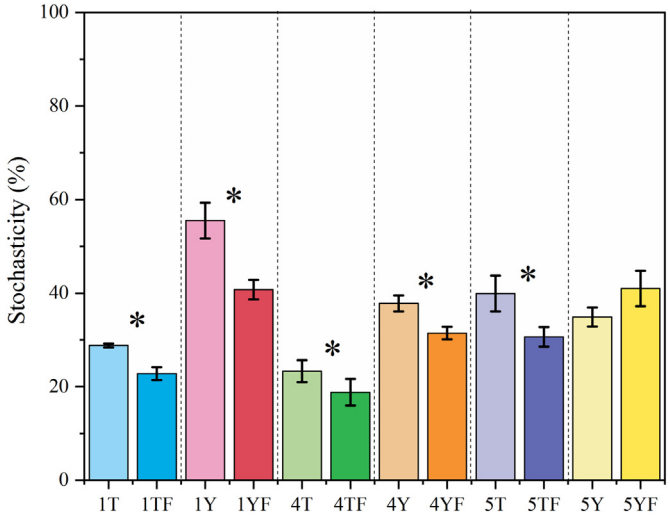

Fig. 8. The relative contribution of stochasticity in governing the assembly of *nirS* denitrifying communities in different groups. The asterisk (*) indicates significant difference (P <0.05) between the corpse and control groups in each time point based on Mann-Whitney U test.

evaluated based on the null model analysis. We found that the average percentage of stochastic processes of the experimental groups YF and TF accounted for 37.8% and 28.5% respectively. In contrast, the relative contributions of stochastic processes of the control groups Y and T accounted for 40.2% and 32.4%, respectively. These results reveal that the deterministic processes dominated the assembly of denitrifying communities. In addition, the community assembly processes for each water type in each time point were compared, we found that corpse groups had lower stochasticity than the control groups regardless of time points with the exception of 5Y and 5YF (Fig. 8), indicating that corpse increases the deterministic processes of denitrifying communities. Notably, the relative contributions of stochastic processes firstly decreased and then increased with succession.

4. Discussion

Previous researches primarily explored the whole bacterial community diversity, abundance and structure during cadaver decay in water and soil (Benbow et al., 2015; Metcalf et al., 2016). Those results often showed a succession pattern in the bacterial community across degradation, which can be an indicator of postmortem interval (PMI) or postmortem submersion interval (PMSI). However, relatively few studies focused on nitrogen pollution released by carrion resources and related functional microbes that participate in nitrogen removal. Here, we explored how denitrifying community and water quality indices respond to perturbation caused by carcass decomposition in different water types. We found that cadaver degradation significantly shifted the denitrifying community structures, which exhibit a succession patterns at different decaying stages while become similar at the final stages. In addition, deterministic processes dominated the denitrifying community assembly, and the relative contribution of deterministic processes in corpse groups was higher compared with the control groups, indicating that environmental filtering regulates the denitrifying communities. These results had important implications for improving nitrogen removal by regulating denitrifying communities in water environment polluted by corpses.

4.1. Corpse decay influences beta instead of alpha diversity of nirS-encoding denitrifying communities in water

Our previous studies concentrated on the entire bacterial community, and found that the alpha diversity decreased from 3rd to 11th day and recovered at 15th and 19th day (Zhou et al., 2021). However, our study demonstrated that corpse treatment had no remarkable impact on the alpha diversity of *nirS*-type denitrifiers, which was in consistent with our former study on *nirK* denitrifying community (Yu et al., 2020). These results indicate that species diversity of denitrifying communities in water was relative stable. Notably, it is water type rather than time that affected alpha diversity, which may be attributed to the different trophic level in the Yellow River water and tap water (e.g., different TOC and phosphate). In our study, environmental factors had no significant correlation with alpha diversity of *nirS*-harboring denitrifying communities, which is in agreement with another study in the coastal wetlands of China featured high nitrogen area (Gao et al., 2016), indicating that the alpha diversity of *nirS* denitrifying community was hardly impacted by water physicochemical properties. Notably, *nirS* denitrifying community had 118 observed OTUs per water sample, while only 84 observed OTUs per sample were found in *nirK* denitrifiers in our previous study (Yu et al., 2020). The reason for higher species diversity of *nirS* denitrifiers is possibly on account of disparate ecological niches or strategies of these two types of denitrifiers, such as diverse enzyme substrates were required (Huang et al., 2011a). In addition, the higher diversity for *nirS* denitrifying communities showed that they had stronger environmental adaptability than *nirK*-type communities. It has been exhibited that the percentage of these two gene abundance determines the potential denitrification rate in natural streams, and the denitrifying potential magnitude of *nirS*-type bacteria is higher than that of *nirK*-type bacteria (Graham et al., 2010).

In contrast, PCoA plots along with PERMANOVA and ANOSIM statistical results confirmed that carcass degradation had significant effects on the *nirS* denitrifiers' structure and membership. That can be caused by disturbance in water physiochemical quality after carcasses input, which is equal to a high nutrient resource. At onset of the corpse breakdown, the environmental stability was rapidly disrupted with a flush of cadaveric materials releasing into water. The sharp substrate variation can promote the adaptation of bacteria, which correspondingly altered the structure and assembly of bacterial community. Then, water environment began to recover and come back to a relative stable status accompany with the fact that carcass had almost finished degradation on the final stage of decay. Equally, similar and stable environmental properties can resemble the structure and assembly of bacterial communities (Zhou et al., 2021), which is responsible for the smaller dissimilarity in *nirS* community structure between shorter time interval (between day 15 and day 19) than that between longer time intervals (between day 3 and day 15 or day 19). Notably, water type had no significant contribution on driving *nirS*-encoding denitrifying community development, proving that water type was not a dominant factor that shaping denitrifying community structures. One study on mouse corpse degradation using three different soil types as substrates also confirmed that soil type was not a determinant element for the bacterial community structure variation (Metcalf et al., 2016). Altogether, cadaver resource is a powerful perturbation that can shape the bacterial community structure and membership independent of substrate type.

4.2. The succession of nirS-encoding denitrifying taxa and core community across the corpse degradation

Proteobacteria still overwhelmingly dominate the *nirS* denitrifiers at phylum level, and this result is consistent with our previous study about *nirK communities* (Yu et al., 2020). Additionally, in different environments such as river sediments, eutrophic reservoir, and wastewater treatment plants, the dominant phylum for *nirS* denitrifying communities is also Proteobacteria (Shi et al., 2019; H. Zhang et al., 2019; Zhou et al., 2016). Proteobacteria with variable morphology and versatile physiology is ubiquitous in soil, ocean and freshwater (Shin et al., 2015). This phylum had high phenotypic and phylogenetic diversity, which may explain its ability for colonizing different environmental habitats (Liu et al., 2015). In general, bacteria that capable of denitrificaiton frequently belong to

alpha and beta classes of the Proteobacteria (Zumft, 1997). However, despite *nirS* and *nirK* denitrifying communities all belong to phylum Proteobacteria, their taxonomic information is totally divergent at the genus level. *NirS*-encoding denitrifiers' abundant genera are *Alicycliphilus*, *Dechloromonas*, *Pseudomonas*, *Pelomona*, *Diaphorobacter*, *Paracoccus* and *Thauera*, while *Sinorhizobium*, *Pseudomonas*, *Achromobacter*, *Ensifer*, *Agrobacterium* and *Brucella* dominated in the *nirK* denitrifying communities (Yu et al., 2020). In particular, it has been reported that some species in the *nirS* denitrying communities may degrade contaminants in the eutrophication sediments with a large amount of nitrogen (Huang et al., 2011b). Notably, in our result, several abundant genera were reported to be pollutant-degradation species. For instance, a stain of *Diaphorobacter* isolated from wastewater treatment plant had capability of degrading phenol and performing nitrification and denitrification (Ge et al., 2015). *Alicycliphilus* strains separated from municipal sewage can degrade cyclohexanol and reduce nitrate (Mechichi et al., 2003). Species of *Paracoccus* were reported to use biodegradation of pyridine to transform nitrogen in coking wastewater treatment plant (Zhou et al., 2018). In addition, a strain isolated from *Thauera* degraded toluene with oxygen and nitrate depletion (Shinoda et al., 2004). *Candidatus Accumulibacter*, polyphosphate accumulating organisms (PAOs), was reported to be core species in wastewater treatment plant, which can enhance biological phosphorus removal (Wu et al., 2019). Nevertheless, contaminant-degradation species found in *nirS*-encoding communities during carcass decomposition were not discovered in previous study about *nirK* gene, which imply that *nirS* denitrfiers may play a more important role in the organic compounds degradation than *nirK* denitrfiers under same environments contaminated by carrion inputs. It worth to be notice that both the *nirS* and *nirK* denitrifying communities contained genera that can conduct nitrification and denitrification, implying that these genera may probably take multiple parts in nitrogen cycling during corpse decomposition.

Those genera *Pseudomonas*, *Paucibacter*, *Arenimonas*, *Polymorphum* and *Steroidobacte* in cadaver groups were enriched in the stage submerged fresh, other genera like *Thauera*, *Diaphorobacter*, *Dechloromonas* and *Cupriavidus* mainly focused on the final stage of degradation. The bacterial succession in corpse groups may be responsible for the changed environmental conditions they lived on, which probably can be used as prediction of postmortem submersion interval (PMSI) and also predict the environmental changes. The succession of bacterial communities has been used to estimate the postmortem interval in forensic science (Metcalf et al., 2013), our results broadened new knowledge for this aspect.

Core microbiome, stable and consistent taxa in complex microbial assemblages, is critical to the function of communities. These core species often have parallel ecological function and environments preference (Shade and Handelsman, 2012). Consequently, it is of great importance to unravel the core taxa during carcass degradation and further understand how these core communities respond to habitat perturbation. In our study, only 13 core OTUs (total 1214 OTUs) taking up 74.79% relative abundance, which reveal that small quantity of core denitrifying species dominated the major habitat niches during corpse degradation. Obviously, OTUs affiliated to *Diaphorobacter*, *Alicycliphilus*, *Paracoccus* and *Thauera* had been discussed above to remove different contaminants. OTU50| *Pseudomonas marginalis* also had capability of degrading toxic organic compounds and can adapt to acid environment (Babu et al., 1995). Our results showed OTU50 is negatively correlated with pH, which proved that *Pseudomonas marginalis* is acid-tolerant species. Besides, *Pseudomonas marginalis* was a pathogen for plant (Canaday et al., 1991). It reminds us *Pseudomonas marginalis* not only plays a crucial role in nitrogen cycling and degrading toxic organic chemicals, but also pose a threat to the plant, which may put some insight into the side effects of microbial communities purifying environments. Another strain of *Pseudomonas*, OTU453| *Pseudomonas* sp. P482, was a fluorescent pseudomonad with broad-spectrum antimicrobial activity, which can mitigate plant diseases and is beneficial for the agricultural productivity (Jan et al., 2011; Krzyzanowska et al., 2014). Negative correlation between phosphate, NO_2-N and OTU93, OTU50, OTU47, OTU1 may indicate that these species may associate with environment purification. Finally, we assigned all abundant denitrifying communities into four clusters. This find is similar to previous study about *nirS* denitrifying communities in sediments. However, only cluster 4 was more abundant in carcass groups and main taxa *Alicycliphilus* is one pollutant-degrading bacteria (Mechichi et al., 2003), implying that this cluster played a more important role in corpse decomposition.

4.3. TDS was the most important factor that influenced the nirS denitrifying communities

Partial mantel test showed that the water environmental factors had close link to the denitrifying communities even after controlling the treatment or decaying time, indicating that cadaver input shaped the denitrifying communities mainly by altering the water physiochemical properties. In this study, PERMANOVA analysis showed that TDS was most dominating factors for *nirS* denitrifying communities, followed by salinity, CON and phosphate, which is totally different from our previous *nirK* study which found that the dominating factors were pH, ORP, treatment and NH_4^+-N (Yu et al., 2020). This may be accounted for different substrate required by two enzymes encoded by *nirS* and *nirK* genes (Shi et al., 2019). Notably, TDS is defined as total amount of various ions, molecules and compounds dissolved in water. In the Upper Mississippi River, TDS has been reported to be the main environmental factors impacting the diversity and the abundance of bacterial community (Staley et al., 2015). In this study, corpse degradation increased the TDS content greatly due to various organic compounds (i.e., cadaverine, putrescine) and ions (i.e., NH_4^+), thus impacting the *nirS* denitrifying communities. Due to the impact of chloride ions concentration on water buffering capacity, salinity can greatly influences bacterial community composition in water (DWAF, 1996). CON may hinder the growth of bacteria in water through which they are passed, and even cause bacterial death under certain conditions (Markx et al., 1996). Many reports have found that phosphorus was the primary driving factor altering the bacterial community structures in surface water systems (Han et al., 2016), which may influence the availability of trace metals and TOC through adsorption, co-precipitation and direct precipitation reactions (Xu et al., 2018). In addition, our results are also not different in the previous study on *nirS* communities, which indicating that NO_2-N, NO_3-N and DO were the most important factors determining *nirS*-type denitrifier communities in sediments (Shi et al., 2019). These results showed that the driving factors for *nirS*-encoding denitrifier communities are distinct in different systems. It has been reported that TOC is regarded as the main electron donor for denitrifying bacteria respiring and has an important impact in the denitrifying bacteria spatially distributing (Burgin and Hamilton, 2007; Ibekwe et al., 2016). However, TOC is not dominant driving factor that influences the denitrifying communities in our study. It may be explained that TOC was not a limiting factor influencing denitrifying communities in this study.

Network analysis has been applied to depict the co-occurrence patterns of *nirS* denitrifying communities and environmental factors. However, some abundant genera showed significant negative correlations with NH_4^+-N and NO_3-N. For example, NH_4^+-N had strong negative correlation with *Labrenzia* and *Curvibacter*. NO_3-N was negatively correlated with *Labrenzia*. This finding reveals that excessive nitrogen may inhibit the growth of these denitrifying bacteria. Notably, the coexistence and interaction of abundant denitrifying genera may be essential for managing the nitrogen removal and reliving nitrogen contamination during cadaver degradation.

4.4. Deterministic assembly of nirS denitrifying communities

One previous study on community assembly of *nirS* denitrifying communities revealed that both niche-based (or deterministic processes) and

neutral processes (or stochastic processes) had an important role in structuring *nirS* denitrifier communities (Jones and Hallin, 2010), but the relative importance of these two ecological processes was not solved. In this study, we found the *nirS* denitrifying communities were mainly assembled by deterministic processes regardless of the corpse groups and control groups, implying that environmental filtering may regulate the *nirS* denitrifying communities. This opinion was also supported by our data, which found that deterministic factors, such as water TDS, salinity, CON and phosphate, were the driving force for *nirS* denitrifying communities.

Another interesting finding is that corpse degradation weakened the stochastic processes of *nirS* denitrifying communities and strengthened the deterministic processes in the corpse groups compared with the control groups, indicating that carcass degradation caused denitrifying communities changed more directionally and orderly. This point was supported by our data, which showed that the denitrifying communities between two cadaver groups were more similar. Another possible reason is that the corpse groups had similar water quality parameters (i.e., NH_4^+-N and pH), which leads to similar denitrifying communities. These results have important significance for improving denitrification rate in polluted water caused by corpse degradation through regulating environmental factors or denitrifying communities.

5. Conclusion

In this study, using one microcosm experiment, we explored the harmful effects of corpse degradation on the Yellow River water and tap water, and also resolved the related community assembly of *nirS* denitrifiers, which may reduce nitrite or nitrate to nitrogen gas, thus alleviating nitrogen contamination and purifying aquatic environments. It has been concluded that corpse degradation causes water quality deterioration, such as the dramatic increase of NH_4^+-N, NO_3-N and phosphate. In addition, corpse decomposition leads to the *nirS* denitrifying community structure separation compared with the control groups, but the communities becomes more similar with succession. TDS, salinity, CON and phosphate were the four important environmental factors determining the *nirS* denitrifying communities, but water type has little impact on the communities. Notably, deterministic processes rather than stochastic processes dominated the *nirS* denitrifying community assembly, and the relative contribution of deterministic processes in most corpse groups was higher compared with the control groups, indicating that environmental filtering regulates the denitrifying communities. These results have important implications for improving denitrification rate to purify polluted water caused by corpse degradation by controlling water quality or denitrifying communities. However, our microcosm experiment in laboratory is different from running rivers in nature, which may influence the denitrifying communities. Therefore, future research in open environments is needed to confirm whether our conclusions can be applied to running river systems.

CRediT authorship contribution statement

Qiaoling Yu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Writing – review & editing. **Rui Zhou:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Yijie Wang:** Writing – review & editing. **Wanghong Su:** Writing – review & editing. **Jiawei Yang:** Writing – review & editing. **Tianshu Feng:** Writing – review & editing. **Yaqi Dou:** Writing – review & editing. **Huan Li:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by the Special Funds for Talent Team Construction of Lanzhou University (225000-824601).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147185.

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