

Article

Composition Characteristics of Organic Matter and Bacterial Communities under the *Alternanthera philoxeroide* Invasion in Wetlands

Qingqing Cao^{1,2}, Haijie Zhang³, Wen Ma⁴, Renqing Wang² and Jian Liu^{2,*}

- School of Architecture and Urban Planning, Shandong Jianzhu University, Jinan 250101, China; caoqingqing18@sdjzu.edu.cn
- ² Environment Research Institute, Shandong University, Qingdao 266237, China; wrq@sdu.edu.cn
- ³ State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China; zhanghj53@mail.sysu.edu.cn
- ⁴ College of Chemistry and Chemical Engineering, Qilu Normal University, Jinan 250200, China; 20177736@qlnu.edu.cn
- * Correspondence: ecology@sdu.edu.cn; Tel.: +86-532-58631969

Received: 29 June 2020; Accepted: 10 August 2020; Published: 12 August 2020



Abstract: The influence of Alternanthera philoxeroide (alligator weed) invasion on wetland organic matter (OM) accumulation and bacterial changes is rarely studied, but is possibly an important step for revealing the invasion mechanism. Thus, the distribution characteristics of light fraction organic carbon and nitrogen (LFOC and LFON), and heavy fractions organic carbon and nitrogen (HFOC and HFON) were analyzed. Sampling was done on two sediment depths (0–15 cm and 15–25 cm) of invaded and normal habitats of two natural wetlands and two constructed wetlands, and bacterial taxa and composition in surface sediments were also analyzed by high-throughput sequencing. In the surface sediments, the LFOC and LFON contents were significantly higher in the constructed wetlands $(0.791 \text{ and } 0.043 \text{ g·kg}^{-1})$ than in the natural wetlands $(0.500 \text{ and } 0.022 \text{ g·kg}^{-1})$, and the contents of the C and N fractions were also prominently higher in the invaded areas than in normal wetland habitats. The OM storage was relatively stable. Proteobacteria (55.94%), Bacteroidetes (5.74%), Acidobacteria (6.66%), and Chloroflexi (4.67%) were the dominant bacterial phyla in the wetlands. The abundance of Acidobacteria, Actinobacteria, and Gemmatimonadetes were significantly higher in the invaded areas than in the normal habitats. The relative high abundance-based coverage estimator (ACE) index in the constructed wetlands and invaded areas suggested the corresponding high bacterial diversity. The significant and positive relationship between Acidobacteria and organic nitrogen concentrations suggested their potential and positive interrelationships. This study demonstrated that the alligator weed invasion could significantly change the compositions of sediment organic matterand bacteria, thus further changing the nutrition cycle and wetland microhabitat.

Keywords: Alternanthera philoxeroide; bacterial composition; organic matter; wetland

1. Introduction

Exotic plant invasion is a tremendous threat to natural ecosystems, it was also one focus of ecological research over the last few decades [1]. As one kind of water-saturated and multifunctional ecosystem, wetland provides an ideal habitat for aquatic exotic plants to invasion, growth, and reproduction [2,3]. Especially, *Alternanthera philoxeroides* (alligator weed) was a typical invasive plant species in the world, it was first recorded in South America in the 1900s, subsequently spread to North America, and then invaded the Chinese water areas during the 1930s [4]. Its high reproductive and migration ability caused serious damage to local water environment [5], thus alligator weed invasion has attracted great



attention. However, previous studies mostly focused on the harm of alligator weed invasion, as well as how to reduce or eliminate it [1,3], while ignoring its effects on the nutrition cycle or microorganism in the wetland ecosystem.

Wetland has a great potential for carbon and nitrogen accumulation, which plays an important role in relieving global warming and purifying the water quality [6,7]. Plenty of studies showed that the exotic plant invasion might affect the accumulation of soil organic carbon (SOC) and organic nitrogen (ON) [8,9]. Soil organic matter (SOM) is the main form of OC and ON, the study on different SOM categories can further reveal the influence of alien plant invasion on the accumulation process of carbon and nitrogen [10]. The SOM can be divided into light fraction organic matter (LFOM) and heavy fraction organic matter (LFOM), through soil density difference [11]. With the soil density less than 1.7 g·cm⁻³, LFOM is mostly composed of undecomposed or partially decomposed biological residues, and is sensitive to plant types, land-use types, etc. [12]. Thus, it can be an early indicator to evaluate soil OM changes when suffering alligator weed invasion. With a soil density higher than 1.7 g·cm⁻³, HFOM is relatively stable in a terrestrial ecosystem and can be used to analyze the dynamic changes of stable OM. The influences of several exotic plant invasion on OC and ON accumulation were previously studied [8,13]. For example, the invasion of *Typha* significantly increased the concentrations of SOM, nitrate, and ammonium [8], the *Phragmites australis* invasion also increased the C stock corresponding to the increase in aboveground biomass [9]. While little research involved the effects of alligator weed invasion on SOM.

Alien plant invasion might also change the soil microhabitat through root exudates, and then change the composition of bacterial communities in the soils, which is likely to be the mechanism of plant invasion [14]. Batten et al. [15] reported that both the invasion of *Centaurea solstitialis* and *Aegilops triuncialis* changed the soil bacterial communities, and especially increased the proportions of sulfur-oxidizing bacteria. However, the effects of alligator weed invasion on bacterial composition is still unknown.

Nansi Lake (NL) is one of the largest inland lakes in the South-to-North Water Diversion Project in China. The investigation from 2013–2016 showed that part of the Nansi Lake Basin was invaded by alligator weed, among which constructed wetlands were largely invaded. In addition, previous studies suggested that different wetland types can affect the distribution of LFOM, HFOM, and microorganism [16], which might cause disturbance to the effects of alligator weed invasion. Thus, two natural wetlands and two constructed wetlands from the Nansi Lake Basin were investigated and invaded, and normal wetland sediments were sampled to study the accumulation and composition differences of SOM and bacterial communities under the alligator weed invasion. The aims of this study were—(1) to analyze the content and storage differences of OC and ON by comparing sediments from invaded areas and normal wetland habitat, and (2) to explore and compare the microbial composition under the effects of alligator weed invasion and wetland types. Our results would be important to predict the wetland function under alligator weed invasion.

2. Materials and Methods

2.1. Study Site and Sampling

The field sampling was conducted in the Nansi Lake Basin (NLB), which is located in the Jining, Shandong Province of China. NLB belongs to warm temperate monsoon climate, has a high temperature, is rainy in summer, and sunny and cold weather in winter. The annual average temperature is 13.3–14.1 °C. The average frost-free period is 199 days and the annual precipitation is 597–820 mm.

With a total area of 1266 km², the Nansi Lake (NL) is the largest inland lake in the Shandong Province. It has 53 tributaries and the Xinxue River (XR) is a large tributary that connects to NL from the southeast. The Xinxue River Constructed Wetland (XRCW) were designed and operated in 2008, downstream of XR, and were 5100-m-long and 270-m-wide [17]. The Nansi Lake estuary (NLE) is an

extensive flat wetland mouth of XRCW that transfers water from the XRCW into NL. NLE has similar characteristic and habitat with XRCW, and also receives the rush from lake water. XRCW and NLE have significantly higher plant richness and diversity than NL and XR. Concretely, the main plants in XRCW and NLE are *P. australis, Acorus calamus*, and *A. philoxeroides*, etc., while NL and XR have fewer plant cover. Sediments can be divided into sand, silt, and clay, based on the particle size. In NLB, the corresponding proportions of sand, silt, and clay in NL and XR were on average, 20.21%, 43.30%, and 36.48%, and those in XRCW and NLE were 13.16%, 76.54%, and 10.31%, respectively, based on the previous reports [18,19]. In this study, within the geographic range of 34°42 50.02–34°52 18.51 N and 117°04 45.30–117°18 29.41 E, we collected sediment samples from the XR and NL for characterizing the natural wetlands and from the XRCW and NLE, as representative of constructed wetlands.

The sediment sampling lasted for ten days from May to June of 2017. Sediments of the constructed wetlands were collected first and then the sediments of XR and NL were collected accordingly. Preliminary investigation found that NL and XR were rarely invaded by alligator weed with an invaded area of less than 5%, while XRCW and NLE were largely invaded, with the invaded area exceeding 30%. Thus, the invaded sites and non-invaded sites were selected as representative of invaded habitats and normal habitats in this study. Specifically, we selected eight invaded sites and seven non-invaded sites in XRCW, and three invaded sites and four non-invaded sites in NLE. Two and five non-invaded sites were selected in NL and XR, respectively. In total, 29 sites were selected and the details of the sampling sites are shown in Figure 1. Previous reports showed that OC and ON were sensitive to soil depths in terrestrial ecosystem [20,21], thus sediments from two depths (0–15 cm and 15-25 cm) were collected to analyze the vertical distribution pattern of C and N fractions. At each site, sediment cores of 0–25 cm were excavated, the five-point sampling method was applied to collect sediment samples of 500–1000 g wet weight from two depths, using a GRASP sediment sampler (GRASP ZYQ-WN, Beijing, China). Meanwhile, surface sediment samples of 30–50 g were collected and stored in 4 °C ice box for bacterial analysis, and surface sediments were also in-situ collected using a cutting ring of 100 cm³ to analyze soil bulk density and moisture contents.



Figure 1. The (**a**) national, (**b**) regional, and (**c**) local geographical setting of the study area. Map (**c**) showed the sampling sites from the invaded and normal habitats of the four wetlands.

2.2. Separation and Analysis of Organic Matter

Sediment samples were air-dried at room temperature (~20 °C) and then ground for further analysis. Due to sensitivity of soil OC and ON analysis, the dried soil was passed through a 0.9-mm

sieve to remove biological residues, broken roots, and detritus. The LFOM and HFOM of the weighed 10 g soils were stratified by adding 40 mL $1.70 \text{ g}\cdot\text{mL}^{-1}$ sodium iodide solution. The C and N contents of the LFOM and HFOM were determined using an elemental analyzer (Vario EL III; Elementar Analysensysteme, Hanau, Germany). From the data thus obtained, we calculated the contents of LFOC, HFOC, LFON, HFON, and the C:N ratios of the light and heavy fractions (LFOC:LFON and HFOC:HFON). Detailed procedures were reported by Zhang et al. [18]. Moisture contents and bulk density were determined and calculated by comparing sediment weight in a set volume (100 cm³), before and after drying at 105 °C [22]. In addition, the storages of C or N fractions were calculated, based on the sampling depths, bulk density, and C and N fractions contents [23].

2.3. Illumina MiSeq and Data Processing

After the field sampling, the 29 surface samples were freeze-dried for 48 h at -50 °C (F-20; NIHON Co., Shanghai, China) and sifted through a 2-mm nylon sieve to remove residual animal and plant matter, for analyzing the bacterial composition. The cetyltrimethylammonium ammonium bromide was widely used to extract the total bacterial and vegetal genomic DNA in the last decades [24], so it was used in our research. The detailed DNA extracting procedures referred to the report of Niemi et al. [25]. We quantified the extracted DNA by using Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and checked the DNA quality with 1.2% agarose gel electrophoresis [26]. The purified DNA was diluted to 25 ng/µL, and then PCR amplification of the 16S rRNA genes V4 region was performed using the primers 515F and 806R [27]. Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR was conducted using the Phusion High-Fidelity PCR Master Mix (Phusion High-Fidelity DNA Polymerase) with GC buffer (New England Biolabs Co., Ipswich, MA, USA) and concrete operations were showed in the report of Dolgova et al. [28]. The PCR products were mixed in equal amounts, according to concentration, and the mixed products were purified with 2% agarose gel electrophoresis and quantified by using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Then, the paired-end 2 × 300 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to process the sequencing data, as previously described [26,27]. The nucleotide sequences we obtained were deposited in the National Center for Biotechnology Information, under the accession number PRJNA470781, PRJNA470783, and PRJNA470794.

Raw sequencing reads with exact matches to the barcodes were assigned to the respective samples and identified as valid sequences in FLASH (version 1.2.7, Center for Computational Biology of Johns Hopkins University, Baltimore, Maryland, USA, 2011. http://ccb.jhu.edu/software/FLASH) [26]. The chimeras were identified and removed by Uchime of Mother (version 4.1, The Department of Microbiology & Immunology at The University of Michigan, Detroit, MI, USA. 2011. http://www. mothur.org/), the low-quality sequences were filtered and the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identify by UCLUST, and then representative OTU sequences were selected [16,29]. These sequences were identified at the genus level, against the SILVA rRNA database in Mothur (version 1.31.2, The Department of Microbiology & Immunology at The University of Michigan, Detroit, MI, USA. 2009. http://www.mothur.org/) [30]. On the basis of the taxonomic information obtained, we determined the community composition of each sample at the different classification levels—kingdom, phylum, class, order, family, and genus [31]. To minimize the difference of sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets, under 90% of the minimum sequencing depth.

2.4. Statistical Analysis

Before further statistical analysis, all data were checked by the normality test (Kolmogorov-Smirnova test) and the outliers were removed, based on the corresponding stem-leaf

plot. The homogeneity tests of variances were also applied to ensure that the significances were higher than 0.05. The mean concentrations and storages of the C and N fraction were calculated in the four wetlands and one-way analysis of variance (ANOVA) was conducted to indicate the OM differences in the four wetlands. A two-way ANOVA was conducted to determine the effects of two wetland types (constructed/natural wetlands) and two wetland habitats (invaded/normal wetland habitats) on the OM concentrations and storages for each sediment depth. Meanwhile, the contents of C and N fractions and LFOC LFON ratio of all sampling sites were also independently analyzed for the effects of two sediment depths. The above-mentioned steps were conducted in SPSS (version 21.0).

To evaluate microbial alpha diversity, we calculated the abundance-based coverage estimator (ACE) index, using an ACE calculator (The Department of Microbiology & Immunology at The University of Michigan, Detroit, MI, USA. 2009. https://www.mothur.org/wiki/Ace) [32]. Observed OTUs were counted in Mothur, and OTU-level ranked abundance curves were generated to compare the richness and evenness of OTUs among groups (Figure S1). Principal Component Analysis (PCA) of bacterial phyla was performed in Canoco (version 4.5, Wageningen University and Research Centre, Vakhn, Netherlands, 2002) [33]. All bacterial taxa at the phylum level and the ACE index were checked to remove the outliers, and all data used obeyed normal distribution. For the potential effects of alligator weed on bacterial composition, we conducted the mean value analysis of the bacterial phyla in the four wetlands, which differentiated between the invaded and normal habitats. A two-way ANOVA was conducted to determine the significant differences of bacterial taxa and ACE index between constructed/natural wetlands and between invaded/normal habitats. The Benjamini-Hochberg algorithm was processed to control false discovery rate at the significance level of 0.05 for the two-way ANOVA of bacterial phyla in R (version 3.2.2) [34]. Cluster analysis by the unweighted pair-group method with arithmetic means was conducted to the bacterial phyla among the four wetlands that differentiated between invaded and normal habitats in R (version 3.2.2). Pearson correlation analysis was also conducted to assess the potential associations between the bacterial taxa and OM fractions, using the SPSS software (version 21.0). Concretely, the *p*-values for the Pearson correlation analysis meant the significances between the two variables in this study indicated significant correlation, and extremely significant correlation when the *p*-values were less than 0.05 and 0.01, respectively.

3. Results and Discussion

3.1. Organic Matter in the Wetland Habitats

In this study, the contents and storage of the C and N fractions did not present noteworthy difference among the wetlands of XRCW, NLE, XR, and NL. This indicated that the different wetlands were not an important factor affecting the OM contents and storage (Table 1). This finding was in accordance with the reports of Cao et al. [35], who showed the similar distribution trend of organic carbon in XRCW and XR. Specifically, LFOC and HFON reached the maximal average values of 0.834, and 0.414 g·kg⁻¹ in the surface sediments of NLE, LFON reached the maximal average content (0.043 g·kg⁻¹) and storage (0.010 kg·m⁻²) in the surface sediments of XRCW, while HFOC contents and storage of HFOC and HFON were the greatest in NL with the average values of 15.55 g·kg⁻¹, and 4.199 and 0.078 kg·m⁻². Hogan et al. [36] indicated that natural wetlands had a higher OC concentration than the constructed wetlands. Further, Bruland and Richardson [37] showed that non-riverine organic soil flat hydrogeomorphic of natural or constructed wetlands had significantly higher OC contents. Due to the extensive flat transition area from XRCW to NL, NLE showed the highest moisture content in this study.

The distribution and storage characteristic of organic matter in the natural wetlands (XR and NL) and the constructed wetlands (XRCW and NLE) were analyzed in this study (Table 2). Results showed that contents of LFOC and LFON in the surface sediments were significantly higher in the constructed wetlands (mean values of 0.791 and 0.043 g·kg⁻¹) than those in the natural wetlands (mean values of 0.500 and 0.022 g·kg⁻¹), while HFs showed no significant difference. The finding indicated the

sensitivity of LFOC and LFON, and the effects of wetland types were mainly on the LFs. Gao et al. [38] also showed that LFOC was the best potential indicator of the OC dynamics. Nelson et al. [39] reported that high C inputs contributed to the high LFOC contents in restored grassland. In this study, the significantly higher contents of LFOC and LFON in the constructed wetlands than in natural wetlands might result from the high plant cover and the corresponding input of LFs into sediments [12,40]. The moisture content was also remarkably higher in the constructed wetlands (76%) than in the natural wetlands (45%), the result was in accordance with our previous report [21]. It was reported that moisture contents had positive and significant relationships with the composition of clay and silt, while it had negative associations with proportion of sand [41]. Thus, the higher moisture contents in the constructed wetlands might have resulted from the higher proportion of clay and silt in the constructed wetlands (86.85%) than in the natural wetlands (79.78%) [18,19]. In addition, sampling-time difference and root activities might also be important factors [42]. The storages of LFOC, LFON, and HFON in he constructed wetlands were relatively higher but not significant. A previous study showed that storages of OC and ON had no significant difference between wetland types of bogs and fens [43], suggesting that the OM storage was relatively stable and not easily affected by different wetland types.

Table 1. The concentrations $(g \cdot kg^{-1})$ and storage $(kg \cdot m^{-2})$ of C and N fractions in the surface sediments of the four wetland areas (mean values ± standard deviation).

Parameters	XRCW	NLE	XR	NL	<i>p</i> -Values
LFOC	0.771 ± 0.28	0.834 ± 0.41	0.460 ± 0.24	0.601 ± 0.04	0.173
LFON	0.043 ± 0.021	0.042 ± 0.024	0.022 ± 0.014	0.025 ± 0.005	0.182
HFOC	13.92 ± 5.05	14.80 ± 8.70	13.27 ± 5.37	15.55 ± 8.03	0.961
HFON	0.290 ± 0.21	0.414 ± 0.32	0.115 ± 0.11	0.308 ± 0.27	0.360
S _(LFOC)	0.170 ± 0.06	0.164 ± 0.07	0.147 ± 0.09	0.168 ± 0.014	0.937
S _(LFON)	0.010 ± 0.01	0.008 ± 0.005	0.007 ± 0.005	0.0068 ± 0.003	0.798
S _(HFOC)	3.20 ± 1.14	2.75 ± 1.30	3.80 ± 1.27	4.199 ± 1.58	0.350
S _(HFON)	0.061 ± 0.06	0.071 ± 0.06	0.031 ± 0.04	0.078 ± 0.09	0.619
Moisture content (%)	0.682 ± 0.17	0.925 ± 0.55	0.441 ± 0.10	0.464 ± 0.14	0.054
Bulk density (g·cm ^{−3})	0.979 ± 0.16	0.852 ± 0.29	1.146 ± 0.16	1.125 ± 0.18	0.087

 $S_{(LFOC)}$ means the storage of light fraction organic carbon (LFOC) in wetland sediments of 0–15 cm depth. The *p* values indicated the distribution differences among the four wetlands by one-way ANOVA.

The areas invaded by alligator weed showed significantly higher moisture contents than the normal wetland habitats, root growth and activities might be the main driving factors [42]. While Hossler [44] showed that the newly created (constructed) wetland had a higher soil bulk density, less moisture content, plant biomass, or SOC than the natural wetlands. The opposite results might indicate that wetland age was also an important factor affecting the soil physical properties and the OM accumulation level [45]. In the surface sediments, the contents of the C and N fractions in invaded habitats were prominently higher than those in normal wetland habitat. This was in accordance with the reports of Yang et al. [46] and Zhang et al. [47], who observed the increases in the contents of OC and ON under Spartina alterniflora invasion. On average, in this study, the LFOC, LFON, HFOC, and HFON of invaded habitats were 0.265 g·kg⁻¹, 0.029 g·kg⁻¹, 5.27 g·kg⁻¹, and 0.278 g·kg⁻¹ higher than those in normal habitats. Zhang et al. [47] showed that S. alterniflora invasion promoted the increase of OC and ON storage for 3.67–4.90 g C·kg⁻¹ and 0.307–0.391 g N·kg⁻¹, respectively. The increasing level of ON was higher than that in the invaded sediments of this study, while the increase in OC was less than that in our results. Moreover, Singh et al. [48] indicated higher pH values in the invaded soil than in the non-invaded soil and Dlamini et al. [49] showed a significantly higher depletion of OC in acidic soil (pH < 5) than in soil with high pH. Therefore, the high accumulation of OC in the invaded areas might profit from the potential high pH values in this study, relative works need to be done to confirm the deduction. Furthermore, the ON storages were also much higher in the invaded habitats than in the normal habitats (Table 2). However, the invasion of *S. alterniflora* and *Lythrum salicaria*

dramatically stimulated N mineralization and nitrification, by adding N input, thus the storage of ON was unchanged [50–52]. Therefore, this study demonstrated that alligator weed invasion could be beneficial to the net storage of N fractions. It was reported that the deposition of C could be inhibited by a low N content, given that the denitrification process was closely associated with that of OC decomposition [53,54]. Therefore, an increase of N input was considered favorable for the deposition of OC.

The contents of LFOC and LFON were 0.721 and 0.038 $g \cdot kg^{-1}$ in the surface sediments (0–15 cm), significantly higher than they were in the subsurface (15–25 cm), at 0.511 and 0.022 g kg⁻¹ respectively (Figure 2). The accumulation and mineralization of LFs were dynamic and sensitive, and easily affected by climate change, wetland hydrology, and soil physical components [55]. Zhang et al. [20] and Zhang et al. [56] also reported that the OC contents significantly decreased in the soil profiles of reed wetland, paddy field, fens, and humus marsh. As the decomposable fraction, LFs were remarkably decreased in the wetland profile [21,57], which could be caused by their physicochemical lability. In the surface sediments, the effects of wetland types or wetland habitats on the LFs contents were significant, while the effects were mostly insignificant in the subsurface sediments, indicating that the C and N fractions in the surface sediments were easily changed and affected by environmental factors. LFOC:LFON values showed no difference between natural and constructed wetlands in surface sediments, while it was notably higher in natural wetlands than in constructed wetland in the subsurface sediments. Wang et al. [43] indicated the interacting effects on OC and ON but not on the C:N ratio, indicating that the OC and ON might have different distribution patterns affected by the wetland types and depths. In this study, the changes of LFOC: LFON values between the two depths were mostly caused by the higher decreasing rate of LFON (41.66%) than that of LFOC (29.09%) from the surface to the subsurface sediments, which further indicated the sensitivity of LFON to the sampling depths.



Figure 2. The distribution characteristic of LFOC, LFON, and the LFOC:LFON values in the two sediment depths (0–15 cm and 15–25 cm). The error bars indicate the standard deviation. One-way ANOVA of two depths was conducted on LFOC and LFON, and the mean value analysis was also implemented to the LFOC:LFON ratio.

Parameters (0–15 cm)	LFOC	LFON	HFOC	HFON	S(LFOC)	S(LFON)	S(HFOC)	S(HFON)
Constructed wetlands	0.791 ± 0.32	0.043 ± 0.022	14.20 ± 6.23	0.313 ± 0.24	0.168 ± 0.07	0.010 ± 0.008	3.058 ± 1.18	0.064 ± 0.056
Natural wetlands	0.500 ± 0.21	0.022 ± 0.011	13.92 ± 5.59	0.175 ± 0.14	0.153 ± 0.07	0.007 ± 0.004	3.913 ± 1.23	0.044 ± 0.055
p values	0.012	0.004	0.912	0.146	0.633	0.255	0.138	0.431
Invaded habitats	0.885 ± 0.29	0.056 ± 0.019	17.40 ± 5.31	0.464 ± 0.26	0.174 ± 0.08	0.012 ± 0.011	3.610 ± 1.24	0.087 ± 0.060
Normal habitats	0.620 ± 0.29	0.027 ± 0.015	12.13 ± 5.598	0.186 ± 0.18	0.159 ± 0.06	0.007 ± 0.003	3.053 ± 1.21	0.042 ± 0.046
p values	0.027	0.000	0.019	0.014	0.585	0.152	0.249	0.046
Parameters (15-25 cm)	LFOC	LFON	HFOC	HFON	S(LFOC)	S(LFON)	S(HFOC)	S(HFON)
Constructed wetlands	0.572 ± 0.45	0.026 ± 0.018	15.27 ± 6.36	0.295 ± 0.18				
Natural wetlands	0.245 ± 0.19	0.006 ± 0.002	12.11 ± 4.01	0.180 ± 0.15				
p values	0.137	0.042	0.23	0.169				
Invaded habitats	0.688 ± 0.58	0.030 ± 0.02	16.29 ± 6.91	0.328 ± 0.15				
Normal habitats	0.390 ± 0.26	0.017 ± 0.01	13.42 ± 5.23	0.232 ± 0.18				
p values	0.082	0.079	0.214	0.202				

It showed the significant and extremely significant difference, when p values were less than 0.05 and 0.01, respectively (in bold). S_(LFOC) means the storage of LFOC.

3.2. Bacterial Distribution and Composition

The PCA was conducted and the first two axes explained 90.3% of the sample variation (Figure 3). Thereinto, a variance of 81.8% was attributed to principal component 1, and principal component 2 captured 8.5% of the variance. The samples, from the constructed or the natural wetland, and from the invaded area or the normal wetland habitats, were mostly decentralized and distributed, and showed no obvious aggregation. As a whole, this indicated that the factors of invaded/normal wetland habitats and constructed/natural wetlands might not cause notable changes to the sediment microflora.



Figure 3. Principal component analysis (PCA) based on the sampling sites and bacterial phyla composition.

The composition pattern of the bacterial phyla was analyzed and the cluster analysis of the four wetlands that differentiated between invaded and normal habitats was implemented (Figure 4). Cluster analysis showed that the invaded areas were gathered first, and then clustered with NLE, which followed by NL, XRCW, and XR, accordingly. The finding suggested that the alligator weed invasion was a dominant factor that affected the bacterial phyla composition, while the effect of wetland types was not obvious, due to the scattered cluster between natural and constructed wetlands.

Specifically, Proteobacteria (55.94%), Bacteroidetes (5.74%), Acidobacteria (6.66%), and Chloroflexi (4.67%) were the dominant bacterial phyla in the studied areas. Among the dominant phyla, Firmicutes was significantly higher in the constructed wetlands (2.01%) than in the natural wetlands (1.43%; adjusted *p* value of 0.044), while distribution of Gemmatimonadetes was the opposite (adjusted *p* value of 0.026). Proportion of Cyanobacteria was also very high in the natural wetlands (0.55%) than in the constructed wetlands (0.33%). The results were similar with our previous study on these wetlands, indicating the relatively stable composition of bacterial phyla [21]. Moreover, Adrados et al. [58] showed the significant differences of Bacteroidetes proportions among different wetland units, and it was mostly associated with the degradation of high molecular weight compounds and complex organic particles, such as cellulose and lignin [59,60]. The insignificant distribution of Bacteroidetes in this study indicated its stability under the effects of wetland types and the invaded/normal habitats. Cyanobacteria can reportedly degrade pyrene and other complex organics, and it was very abundant in oil-polluted sediments [61]. Thus, the high proportion of Cyanobacteria in natural wetlands might contribute to the degradation of the C and N fractions, which result in the corresponding low contents, especially in XR. In addition, as a ubiquitous colonizer, Cyanobacteria prefers a high NO₃⁻-N and NH₄⁺-N environment, thus, the higher proportion of Cyanobacteria in natural wetlands than in constructed wetlands might indicate potential eutrophication, especially in NL. Firmicutes was mainly identified as a denitrifier [62], including abundant bacterial taxa that reduce the nitrate into ammonia in anaerobic environment and improve sewage purification in constructed wetlands. Therefore, the high Firmicutes in constructed wetlands and high Cyanobacteria in natural wetlands promote the metabolism of NO₃⁻ and NH₄⁺ into ON and ammonia, respectively. Nitrospirae is one typical nitrifying bacteria that oxidize NH_3 and NO_2^- into NO_3^- [63], it showed no significant difference between the two wetland types or between the invaded and normal wetland habitats, with a mean proportion of 3.58% in this study.



Figure 4. The cluster analysis of the invaded and normal wetlands and the composition characteristic of the dominant bacterial phyla. I-XRCW/I-NLE—Invaded Xinxue River Constructed Wetland/Invaded Nansi Lake estuary.

In the level of bacterial phyla taxa, Acidobacteria, Actinobacteria, and Gemmatimonadetes were significantly higher in the invaded areas than in the normal wetland habitats (adjusted p values were 0.028, 0.041, and 0.045, respectively). It was reported that Acidobacteria and Actinobacteria were important microbial components that closely associate with the decomposition of biological

residues and carbon mineralization, respectively [21,64]; thus, their high proportions in the invaded areas might suggest the high efficiency of carbon sequestration and mineralization. The ACE index was much higher in both the invaded areas (6332.8) and the constructed wetlands (6170.0) than in normal or natural wetland conditions (5838.7 and 5574.0), with *p* values of 0.128 and 0.158, respectively. This suggested that the invaded and constructed wetland habitats were beneficial to the increase in bacterial diversity. Batten et al. [15] showed that the invasion of *C. solstitialis* and *A. triuncialis* significantly changed the soil bacterial composition pattern, and the newly invaded areas had a microbial composition similar to the original native soils. Alligator weed invasion increased bacterial diversity and the abundance of Acidobacteria, Actinobacteria, and Gemmatimonadetes. At the class level, the α -, β -, γ -, and δ -proteobacteria accounted for 9.22%, 14.61%, 12.23%, and 16.82% of the microbial communities, respectively, which were the dominant classes in this study. The Pearson correlation analysis showed that proportions of Acidobacteria were significantly and positively associated with the concentrations of LFON (p = 0.425, $R^2 = 0.187$) and HFON (p = 0.474, $R^2 = 0.224$). This indicated that the Acidobacteria activities might promote the ON accumulation.

4. Conclusions

In this study, the contents of LFs significantly differed under the effects of wetland types and alligator weed invasion. The contents of LFs were also sensitive and easily affected in the surface sediments, as compared to the subsurface sediments. The storages of OM were very stable. Proteobacteria, Bacteroidetes, Acidobacteria, and Chloroflexi were the dominant bacterial phyla and abundance of Acidobacteria, Actinobacteria, and Gemmatimonadetes were significantly higher in the invaded habitats than in normal habitats. The high ACE index in the constructed wetlands and invaded areas suggested the corresponding high bacterial diversity. In addition, Acidobacteria had significant and positive effects on ON accumulation. This study contributed to the understanding of the effects of alligator weed invasion on the composition characteristics of organic matter and bacterial communities in wetland, which is important to predict the function of wetlands, under plant invasions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/16/5571/s1, Figure S1: The OTU-level ranked abundance curves of the 29 samples in this study.

Author Contributions: Conceptualization, Q.C. and J.L.; methodology, W.M.; software, H.Z.; validation, R.W. and J.L.; formal analysis, W.M.; investigation, Q.C.; data curation, Q.C.; writing—original draft preparation, Q.C. and J.L.; writing—review and editing, Q.C., H.Z., and J.L.; visualization, H.Z.; supervision, R.W.; funding acquisition, Q.C. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key R&D Program of China (No. 2017YFC0505905), the Natural Science Foundation of Shandong Province, China (ZR2017MC013), and the Science Foundation of Shandong Jianzhu University (Grant No. X18047ZX).

Acknowledgments: We would like to thank Editage (www.editage.com) for English language editing.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bassett, I.; Paynter, Q.; Beggs, J.; Preston, C.; Watts, J.H.; Crossman, N.D. Alligator weed (*Alternanthera philoxeroides*) invasion affects decomposition rates in a northern New Zealand lake. In Proceedings of the Managing Weeds in a Changing Climate, Adelaide, South Australia, 24–28 September 2006; pp. 776–779.
- Palihakkara, C.R.; Dassanayake, S.; Jayawardena, C.; Senanayake, I.P. Floating wetland treatment of acid mine drainage using *Eichhornia crassipes* (water hyacinth). *J. Health. Pollut.* 2018, *8*, 14–19. [CrossRef] [PubMed]
- 3. Hao, W.; Juli, C.; Jianqing, D. Invasion by alligator weed, *alternanthera philoxeroides*, is associated with decreased species diversity across the latitudinal gradient in China. *J. Plant Ecol.* **2015**, *3*, 311–319.
- 4. Spencer, N.R.; Coulson, J.R. The biological control of alligator weed, *Alternanthera philoxeroides*, in the United States of America. *Aquat. Bot.* **1976**, *2*, 177–190. [CrossRef]

- 5. Tao, Y.; Jiang, M. Study on anatomical structure adaptation of stem of *Alternanthera philoxeroides (Mart.) Griseb* to various water condition. *Wuhan Bot. Res.* **2003**, *22*, 65–71.
- Chen, G.; Azkab, M.H.; Chmura, G.L.; Chen, S.; Sastrosuwondo, P.; Ma, Z.; Dharmawan, I.W.E.; Yin, X.; Chen, B. Mangroves as a major source of soil carbon storage in adjacent seagrass meadows. *Sci. Rep.-UK* 2017, 7, 1–10. [CrossRef]
- Skornia, K.; Safferman, S.I.; Rodriguez-Gonzalez, L.; Ergas, S.J. Treatment of winery wastewater using bench-scale columns simulating vertical flow constructed wetlands with adsorption media. *Appl. Sci.* 2020, 10, 1063. [CrossRef]
- 8. Geddes, P.; Grancharova, T.; Kelly, J.J.; Treering, D.; Tuchman, N.C. Effects of invasive *Typha* × *glauca* on wetland nutrient pools, denitrification, and bacterial communities are influenced by time since invasion. *Aquat. Ecol.* **2014**, *48*, 247–258. [CrossRef]
- 9. Martina, J.P.; Hamilton, S.K.; Turetsky, M.R.; Phillippo, C.J. Organic matter stocks increase with degree of invasion in temperate inland wetlands. *Plant Soil.* **2014**, *385*, 107–123. [CrossRef]
- 10. Nguyen, H.T.T.; Chao, H.R.; Chen, K.C. Treatment of organic matter and tetracycline in water by using constructed wetlands and photocatalysis. *Appl. Sci.* **2019**, *9*, 2680. [CrossRef]
- 11. Whalen, J.K.; Bottomley, P.J.; Myrold, D.D. Carbon and nitrogen mineralization from light-and heavy-fraction additions to soil. *Soil Biol. Biochem.* **2000**, *32*, 1345–1352. [CrossRef]
- 12. Tan, Z.; Lal, R.; Owens, L.; Izaurralde, R.C. Distribution of light and heavy fractions of soil organic carbon as related to land use and tillage practice. *Soil Till. Res.* **2007**, *92*, 53–59. [CrossRef]
- 13. Liao, J.D.; Boutton, T.W.; Jastrow, J.D. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biol. Biochem.* **2006**, *38*, 3184–3196. [CrossRef]
- Bertin, C.; Yang, X.; Weston, L. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil*. 2003, 256, 67–83. [CrossRef]
- 15. Batten, K.M.; Scow, K.M.; Davies, K.F.; Harrison, S.P. Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biol. Invasions.* **2006**, *8*, 217–230. [CrossRef]
- 16. Cao, Q.; Hui, W.; Chen, X.; Wang, R.; Jian, L. Composition and distribution of microbial communities in natural river wetlands and corresponding constructed wetlands. *Ecol. Eng.* **2017**, *98*, 40–48. [CrossRef]
- 17. Zhang, J.; Zhang, B.; Jing, Y.; Kang, X.; Zhang, C. Design of constructed wetland in Xinxue River Estuary into Nansi Lake in Eastern Line of South-to-North water transfer project. *China Water Wastewater* **2008**, *24*, 49–51.
- 18. Zhang, Y. Plant Diversity, Soil Mercury Risk and Eco-Sustainability of Xinxue River Constructed Wetland in Nansi Lake, China. Ph.D. Thesis, Shandong University, Jinan, China, 2014.
- 19. Liu, H. Distribution Characteristics, Bioaccumulation, and Sources of Mercury in Rice at Nansi Lake Area, Shandong Province, China. *JAPS J. Anim. Plant Sci.* **2015**, *25*, 114–121.
- 20. Zhang, W.J.; Xiao, H.A.; Tong, C.L.; Su, Y.R.; Xiang, W.S.; Huang, D.Y.; Syers, J.K.; Wu, J. Estimating organic carbon storage in temperate wetland profiles in Northeast China. *Geoderma* **2008**, *146*, 311–316. [CrossRef]
- 21. Cao, Q.; Wang, R.; Zhang, H.; Ge, X.; Liu, J. Distribution of organic carbon in the sediments of Xinxue River and the Xinxue River Constructed Wetland, China. *PLoS ONE* **2015**, *10*, e0134713. [CrossRef]
- 22. Moiwo, J.P.; Wahab, A.; Kangoma, E.; Blango, M.M.; Ngegba, M.P.; Suluku, R. Effect of biochar application depth on crop productivity under tropical rainfed conditions. *Appl. Sci.* **2019**, *9*, 2602. [CrossRef]
- 23. Bambi, P.; Rezende, R.D.S.; Feio, M.J.; Leite, G.F.M.; Alvin, E.; Quintão, J.M.B.; Araújo, F.; Júnior, J.F.G. Temporal and spatial patterns in inputs and stock of organic matter in Savannah Streams of Central Brazil. *Ecosystems* **2017**, *20*, 757–768. [CrossRef]
- 24. Nadzirah, K.Z.; Zainal, S.; Noriham, A.; Normah, I. Efficacy of selected purification techniques for bromelain. *Int. Food Res. J.* 2013, 20, 43–46.
- 25. Niemi, R.M.; Heiskanen, I.; Wallenius, K.; Lindström, K. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *J. Microbiol. Meth.* **2001**, *45*, 155–165. [CrossRef]
- Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Lozupone, C.A.; Turnbaugh, P.J.; Fierer, N.; Knight, R. Global patterns of 165 rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 2011, 108, 4516–4522. [CrossRef] [PubMed]
- 27. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef] [PubMed]

- Dolgova, A.S.; Stukolova, O.A. High-fidelity PCR enzyme with DNA-binding domain facilitates de novo gene synthesis. *Biotech* 2017, 7, 1–6. [CrossRef]
- 29. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [CrossRef]
- 30. García, A.L.; Quiroga, C.P.; Atxaerandio, R.; Pérez, A.; Recio, O.G. Comparison of Mothur and Qiime for the analysis of rumen microbiota composition based on 16s rRNA amplicon sequences. *Front. Microbiol.* **2018**, *9*, 1–11.
- 31. DeSantis, T.Z.; Hugenholtz, P.; Larsen, N.; Rojas, M.; Brodie, E.L.; Keller, K.; Huber, T.; Dalevi, D.; Hu, P.; Andersen, G.L. Greengenes, a Chimera-Checked 16S rRNA Gene Databaseand Workbench Compatible with ARB. *Appl. Environ. Microbial.* **2006**, *72*, 5069–5072. [CrossRef]
- 32. Schloss, P.D. A high-throughput DNA sequence aligner for microbial ecology studies. *PLoS ONE* **2009**, *4*, e8230. [CrossRef]
- 33. Ramette, A. Multivariate analyses in microbial ecology. FEMS Microbial. Ecol. 2007, 62, 142–160. [CrossRef]
- 34. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. B.* **1995**, *57*, 289–300. [CrossRef]
- Cao, Q.; Wang, H.; Zhang, Y.; Lal, R.; Wang, R.; Ge, X.; Jian, L. Factors affecting distribution patterns of organic carbon in sediments at regional and national scales in China. *Sci. Rep.-UK* 2017, 7, 1–10. [CrossRef] [PubMed]
- 36. Hogan, D.M.; Jordan, T.E.; Walbridge, M.R. Phosphorus retention and soil organic carbon in restored and natural freshwater wetlands. *Wetlands* **2004**, *24*, 573–585. [CrossRef]
- 37. Bruland, G.L.; Richardson, C.J. Comparison of soil organic matter in created, restored and paired natural wetlands in North Carolina. *Wetl. Ecol. Manag.* **2006**, *14*, 245–251. [CrossRef]
- Gao, J.; Lei, G.; Zhang, X.; Wang, G. Can δ¹³C abundance, water-soluble carbon, and light fraction carbon be potential indicators of soil organic carbon dynamics in Zoigê Wetland? *Catena* 2014, *119*, 21–27. [CrossRef]
- 39. Nelson, J.D.J.; Schoenau, J.J.; Malhi, S.S. Soil organic carbon changes and distribution in cultivated and restored grassland soils in Saskatchewan. *Nutr. Cycl. Agroecosys.* **2008**, *82*, 137–148. [CrossRef]
- 40. Lehmann, J.; Skjemstad, J.; Sohi, S.; Carter, J.; Barson, M.; Falloon, P.; Coleman, K.; Woodbury, P.B. Australian climate–carbon cycle feedback reduced by soil black carbon. *Nat. Geosci.* **2008**, *1*, 832–835. [CrossRef]
- 41. Ma, S.; Xie, Y.; Hu, H.; Ni, B. Relationship between soil water content and soil particle distribution in two kinds of typical community types of desert steppe. *Soil Water Conserv. China* **2019**, *7*, 61–65.
- 42. Gerke, H.H.; Kuchenbuch, R.O. Root effects on soil water and hydraulic properties. *Biologia* **2007**, *62*, 557–561. [CrossRef]
- Wang, X.; Song, C.; Sun, X.; Wang, J.; Zhang, X.; Mao, R. Soil carbon and nitrogen across wetland types in discontinuous permafrost zone of the Xiao Xing'an Mountains, Northeastern China. *Catena* 2013, 101, 31–37. [CrossRef]
- 44. Hossler, K. Accumulation of carbon in created wetland soils and the potential to mitigate loss of natural wetland carbon-mediated functions. Ph.D. Thesis, The Ohio State University, Environmental Science, Columbus, OH, USA, 2005.
- 45. De Mastro, F.; Cocozza, C.; Brunetti, G.; Traversa, A. Chemical and spectroscopic investigation of different soil fractions as affected by soil management. *Appl. Sci.* **2020**, *10*, 2571. [CrossRef]
- 46. Yang, W.; Zhao, H.; Leng, X.; Cheng, X.; An, S. Soil organic carbon and nitrogen dynamics following *Spartina alterniflora* invasion in a coastal wetland of Eastern China. *Catena* **2017**, *156*, 28–289. [CrossRef]
- Zhang, Y.; Ding, W.; Luo, J.; Donnison, A. Changes in soil organic carbon dynamics in an Eastern Chinese coastal wetland following invasion by a C4 plant *Spartina alterniflora*. *Soil Biol. Biochem.* **2010**, *42*, 1712–1720. [CrossRef]
- 48. Singh, M.R.; Theunuo, N. Variation of soil pH, moisture, organic carbon and organic matter content in the invaded and non-invaded areas of *Tithonia diversifolia* (Hemsl.) A. gray found in Nagaland, North-east India. *Eco. Env. Cons.* **2017**, *23*, 2181–2187.
- 49. Dhillon, J.; Del Corso, M.R.; Figueiredo, B.; Nambi, E.; Raun, W. Soil organic carbon, total nitrogen, and soil ph, in a long-term continuous winter wheat (*Triticum aestivum l.*) experiment. *Commun. Soil Sci. Plan.* **2018**, *8*, 1–11. [CrossRef]
- 50. Fickbohm, S.S.; Zhu, W.X. Exotic purple loosestrife invasion of native cattail freshwater wetlands: Effects on organic matter distribution and soil nitrogen cycling. *Appl. Soil Ecol.* **2006**, *32*, 123–131. [CrossRef]

- 51. Martini, J.; Orge, C.A.; Faria, J.L.; Pereira, M.F.R.; Soares, O.S.G.P. Catalytic advanced oxidation processes for sulfamethoxazole degradation. *Appl. Sci.* **2019**, *9*, 2652. [CrossRef]
- 52. Zhang, Y.; Xu, X.; Li, Y.; Huang, L.; Xie, X.; Dong, J.; Yang, S. Effects of *Spartina alterniflora* invasion and exogenous nitrogen on soil nitrogen mineralization in the coastal salt marshes. *Ecol. Eng.* **2016**, *87*, 281–287.
- 53. Moore, T.R.; Trofymow, J.; Prescott, C.E.; Titus, B.; Group, C.W. Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. *Plant Soil* **2011**, 339, 163–175. [CrossRef]
- 54. Zhao, W.; Liu, Y.; Wei, H.; Zhang, R.; Luo, G.; Hou, H.; Chen, S.; Zhang, R. NO removal by Plasma-Enhanced NH₃-SCR using methane as an assistant reduction agent at low temperature. *Appl. Sci.* **2019**, *9*, 2751. [CrossRef]
- 55. Huo, L.; Chen, Z.; Zou, Y.; Lu, X.; Guo, J.; Tang, X. Effect of Zoige Alpine Wetland degradation on the density and fractions of soil organic carbon. *Ecol. Eng.* **2013**, *51*, 287–295. [CrossRef]
- 56. Zhang, W.; Peng, P.; Tong, C.; Wang, X.; Wu, J. Characteristics of distribution and composition of organic carbon in Dongting Lake Floodplain. *Environ. Sci.* **2005**, *26*, 56–60.
- Six, J.; Merckx, R.; Kimpe, K.; Paustian, K.; Elliott, E.T. A re-evaluation of the enriched labile soil organic matter fraction. *Eur. J. Soil Sci.* 2000, *51*, 283–293. [CrossRef]
- Adrados, B.; Sánchez, O.; Arias, C.A.; Becares, E.; Garrido, L.; Mas, J.; Brix, H.; Morató, J. Microbial communities from different types of natural wastewater treatment systems: Vertical and horizontal flow constructed wetlands and biofilters. *Water Res.* 2014, 55, 304–312. [CrossRef]
- 59. Kirchman, D. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* **2002**, 39, 91–100. [CrossRef]
- Dorador, C.; Meneses, D.; Urtuvia, V.; Demergasso, C.; Vila, I.; Witzel, K.P.; Imhoff, J.F. Diversity of Bacteroidetes in high altitude saline evaporitic basins in northern Chile. *J. Geophys. Res. Biogeoences* 2015, 114, 65.
- 61. Yan, Z.; Jiang, H.; Li, X.; Shi, Y. Accelerated removal of pyrene and benzo[a]pyrene in freshwater sediments with amendment of cyanobacteria-derived organic matter. *J. Hazard. Mater.* **2014**, 272, 66–74. [CrossRef]
- Liu, X.; Gao, C.; Zhang, A.; Jin, P.; Wang, L.; Feng, L. The nos gene cluster from Gram-positive bacterium *Geobacillus thermodenitrificans* NG80-2 and functional characterization of the recombinant NosZ. *FEMS Microbiol. Lett.* 2008, 289, 46–52. [CrossRef]
- Fang, J.; Zhao, R.; Cao, Q.; Quan, Q.; Sun, R.; Liu, J. Effects of emergent aquatic plants on nitrogen transformation processes and related microorganisms in a constructed wetland in Northern China. *Plant Soil* 2019, 443, 473–492. [CrossRef]
- 64. De Vrieze, J.; Saunders, A.M.; He, Y.; Fang, J.; Nielsen, P.H.; Verstraete, W.; Boon, N. Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome. *Water Res.* **2015**, *75*, 312–323.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).