

Effect of early dry season induced by the Three Gorges Dam on the soil microbial biomass and bacterial community structure in the Dongting Lake wetland



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ARTICLE INFO

Article history:

Received 15 September 2014

Received in revised form 27 January 2015

Accepted 28 January 2015

Keywords:

Microbial biomass carbon
Bacterial diversity
Hydrologic cycle
Hydroelectric project
Downstream wetland

ABSTRACT

In this study, we used the soil microbial biomass (SMB) and the bacterial community structure as indicators to determine the potential ecological responses of the Dongting Lake wetland (China) to the early dry season that has been induced by the Three Gorges Dam (TGD), the largest hydroelectric project in the world. We measured the soil properties, SMB and bacterial community structure for samples E0, E20 and E40 (for which the dry season arrived early by 0, 20 and 40 days, respectively). The results indicated a significant increase in SMB as the dry season occurred increasingly earlier. The microbial biomass carbon (MBC) was used as a representative for the SMB and increased for the samples in the following order: E0 < E20 < E40. The bacterial 16S rDNA gene copy number changed similarly to the MBC. Significant changes were also observed in the soil bacterial community structure. The bacterial community structure of E40 was more diverse than that of E20, which was similar to that of E0. The relationship between the bacterial community composition and the soil properties was evaluated by redundancy analysis (RDA). The results indicated that the lead time of the dry season was the controlling influence on the soil bacterial community structure.

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1. Introduction

The damming of rivers has a global impact on natural wetlands (Chai et al., 2009; Wu et al., 2013). Dams inevitably change river flow regimes (Wu et al., 2013). Over the past 100 years, dramatic changes have occurred in the hydrologic cycles of the floodplains of most large rivers on the earth in terms of the flood duration and the start date of the dry season (Ligon et al., 1995). Further changes in the hydrologic cycle are expected to occur over the next 100 years because of climate change (De Jager et al., 2012; Milly et al., 2002). Changes in the hydrologic cycle affect the length of oxic and anoxic periods (Ponnamperuma, 1972), which directly influences biota, ecosystem processes, ecosystem properties and associated ecosystem services (Auble et al., 1994; De Jager et al., 2012).

The Three Gorges Dam (TGD, in China) on the Yangtze River (the Changjiang River) is the largest hydroelectric project in the world (Wu et al., 2013). The project began in 1993, and commissioning started in 2003 and was completed in 2009. This project has played an important role in controlling frequent catastrophic floods downstream, generating hydropower (with an installed capacity of 18,200 MW), and improving navigation at the upper reaches of the Yangtze (CWRC, 1997). However, the project has created environmental problems that have attracted the attention of environmental activists, researchers and communities around the world (e.g., Li et al., 2011; Nilsson et al., 2005; Stone, 2008; Wang et al., 2007; Wu et al., 2013; Xie, 2003). An early dry season in the downstream wetlands (such as Poyang Lake and Dongting Lake, the two largest freshwater lakes in China) is one of the important effects of the TGD and is the primary cause of the increasing need for sluice/dam construction to store water in the wetlands of Poyang Lake and Dongting Lake wetland (Guo et al., 2012; Li, 2009; Wang et al., 2013a). Feng et al. (2013) reported that there was a significant trend of early starting dates for the dry season of the Poyang Lake wetland from 2000 to 2009 (-7.35 days year⁻¹) and the Dongting

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Lake wetland (-3.42 days year $^{-1}$). Liang et al. (2012) found that the average starting date of the dry season for the Dongting Lake wetland in the post-TGD period (2003–2010) was 18 days earlier than that for the pre-TGD period (1981–2002). Zou et al. (2000) showed that the average starting date of the dry season for the Dongting Lake wetland arrived 2, 7 and 35 days early in a high-flow year, a median-water year and a low-flow year, respectively, during the early stages of TGD construction and that this increase in lead time would continue for 50 years after the TGD was constructed.

Several studies have been conducted on the early dry season (Feng et al., 2013; Guo et al., 2012; Li, 2009; Liang et al., 2012; Zou et al., 2000) but have primarily been focused on the prediction, verification and analysis of the early starting dates. However, little is known about the effect of the early dry season on the soil biological properties, particularly the soil microbial biomass (SMB) and the bacterial community structure. Soil microorganisms are critical to global ecosystem because of their a critical role in regulating global nutrients and carbon cycling via fundamental ecological processes such as mineralization and decomposition (Harris, 2009; Hartman et al., 2008; Hua et al., 2013; Poret-Peterson et al., 2007). The SMB and soil bacterial community structure are sensitive to local changes in the environmental conditions (Gong et al., 2009; Poret-Peterson et al., 2007) and anthropogenic activity (Card and Quideau, 2010; Wu et al., 2013) and thus serve as sensitive indicators of environmental changes (Hargreaves et al., 2003; Wu et al., 2013). Therefore, the early dry season in the two largest freshwater lake wetlands in China that has been induced by the TGD will impact soil microorganisms as well as nutrients and carbon cycling.

In this study, we used the SMB and bacterial community structure as indicators to investigate the ecological responses of the Dongting Lake wetland to the early dry season. The objectives of this study were as follows: (1) to analyze the responses of the SMB and the soil bacterial community to the early dry season induced by the TGD; and (2) to determine the relationship among the soil properties, the SMB, the bacterial community, and anthropogenic activities (damming).

2. Materials and methods

2.1. Area of study

The Yangtze River (Changjiang River) is as one of the major rivers on earth and plays a critical role in the global water cycle, the sediment cycle, the energy balance, climate change and ecological development (Li et al., 2011; Xia et al., 2006; Zeng et al., 2013a). This river is located in south-central China (Fig. 1). The river exhibits seasonal variability in the water level and area from monsoon-driven precipitation, such that there is a high water level and area in the wet season from May to October and a low water level and area in the dry season from November to the following April (Li et al., 2011). The TGD is located 44 km upstream of Yichang station (the control point of the upper Yangtze River basin). The TGD is the largest hydroelectric project in the world and was built to realize flood control, navigation and hydropower generation. Many lakes are located downstream of the TGD. Among these lakes, Poyang Lake ($28^{\circ}11' - 29^{\circ}51' \text{ N}$, $115^{\circ}31' - 117^{\circ}06' \text{ E}$) and Dongting Lake ($28^{\circ}30' - 29^{\circ}38' \text{ N}$, $112^{\circ}18' - 113^{\circ}15' \text{ E}$) (Fig. 1) are the two largest freshwater lakes in China (Feng et al., 2013; Zeng et al., 2013b). Dongting Lake receives water from four tributaries (Xiangjiang River, Zishui River, Yuanjiang River and Lishui River) and Yangtze River and then empties into the Yangtze River (Du et al., 2011; Wu et al., 2013). The water area is 2691 km 2 in the annual wet season and 710 km 2 in the annual dry season (Du et al., 2001). The hydrological data showed that the average starting date of the dry season (for 1981–2002) was November 17 (Liang et al.,

2012). In the dry season, exposed grasses and a reduced water level provides abundant food for fauna and birds. Consequently, the lake has been an important wintering habitat and pathway for many migratory birds. This wide variety of flora and fauna, especially birds resulted in the Eastern Dongting Lake Nature Reserve being listed as one of the most important wetlands at the Ramsar Convention in 1992 (Yuan et al., 2014).

2.2. Experimental design

First, 150 kg of surface soil (5–25 cm, where 0–5 cm of soil was removed to reduce the amount of humic matter and grass roots) was collected from a low-altitude shallow ($29^{\circ}14'10'' \text{ N}$, $113^{\circ}04'14'' \text{ E}$) of the Dongting Lake wetland in February 2013. The following soil particle size distribution was determined using a hydrometer method (Patrick, 1958): clay, 28.05%; silt, 46.86% and sand, 25.09%. The soil was air-dried, sieved ($< 2 \text{ mm}$), mixed and then filled into nine plastic culturing buckets (21 cm diameter, 23 cm depth). The bucket had many holes (aperture $< 0.5 \text{ mm}$) with which to exchange pore water and nutrients in the experiment that was subsequently performed. Each bucket was linked to a buoy by a nylon rope (Fig. 2a). Then, the buckets were put on the shallow near the aforementioned sampling site (Fig. 2b). The vegetation at this site was *Carex leiorhyncha*. Culturing buckets were spaced $\approx 2 \text{ m}$ apart on a line. The tops of the culturing buckets were covered with 200 g of fresh soil from the site to introduce indigenous microbes and lower the impacts of air-drying, sieving and mixing, which could have generated additional modifications/disturbances. Subsequently, the site was inundated with water with the arrival of the wet season (Fig. 2c and e). Three buckets at a time were removed at random from the water on October 8, 28 and November 17. The soil samples from each of these buckets corresponded to dry seasons where the starting date was early by 40 (E40), 20 (E20) and 0 (E0) days. These buckets were placed on the shallow (200 m away from the aforementioned sampling site) and were not waterlogged when they were taken out of water (distance of each bucket: $\approx 1 \text{ m}$, Fig. 2d and f). The middle soil layer (5–15 cm) from each bucket was collected on 25 March 2014 for subsequent testing. Visible plant particles were removed by hand from each sample, which was then placed into a plastic bag. The samples were transported in an insulated cooler to the laboratory. A portion of each sample was stored at 4°C and used to investigate the soil properties and determine the SMB. The remaining soil was stored at -20°C and used in molecular genomic analysis.

2.3. Determination of soil properties

The soil organic matter (SOM) was examined by the loss-on-ignition method after ashing at 550°C for 4 h (Wright et al., 2008). The soil pH was measured using a digital pH meter (for a water:soil ratio of 1:2.5). The soil moisture was measured from the mass loss upon drying at 105°C overnight (Wu et al., 2013). The soil cation exchange capacity (CEC) was determined by the BaCl_2 method (Hendershot and Duquette, 1986). The total nitrogen content (TN) was determined by BUCHI Kjeldahl digestion analysis.

2.4. Soil microbial biomass

The microbial biomass carbon (MBC) was used as a representative for the SMB and determined by the fumigation–extraction method (Wu et al., 1990). The amount of carbon that was extracted by K_2SO_4 was determined using a Shimadzu TOC-V CPH total organic carbon analyzer. The MBC was calculated as the difference in the extractable C between the fumigated and unfumigated samples with a conversion factor of 0.37 (Ye and Wright, 2010).

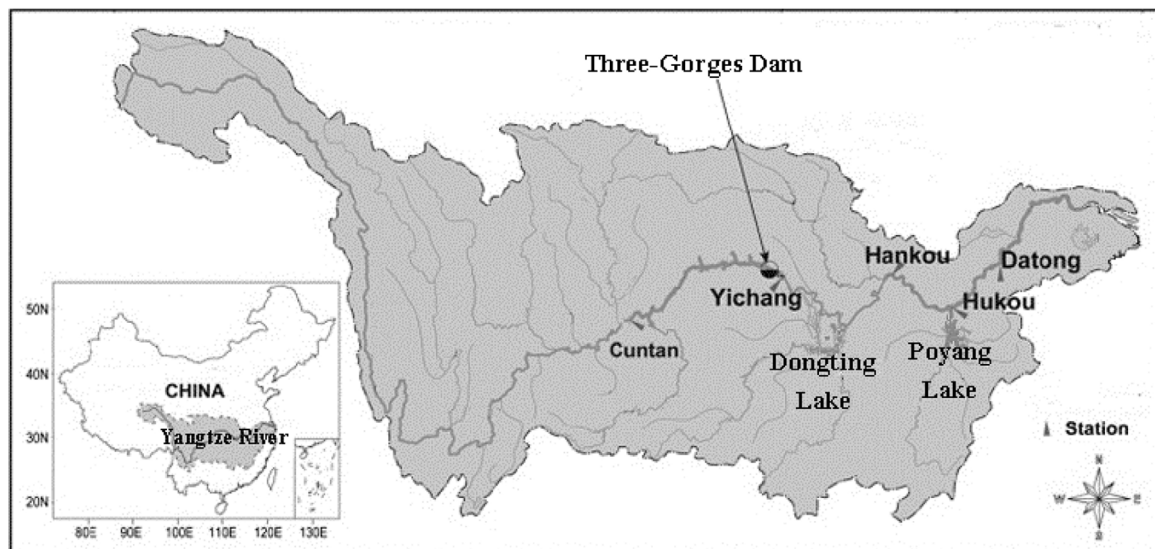


Fig. 1. Locations of Dongting Lake and Three Gorges Dam (TGD).

2.5. PCR-DGGE and qPCR

DNA was extracted from 1.5 g of soil using a method (Protocol PL) that has been previously described (Yang et al., 2007). To prevent potential inhibition effects by humic acids, the DNA extracts were diluted 10-fold (Zeng et al., 2011). The DNA stock solution (0.15 mL) was diluted with 1.5 mL of sterile water in a 5-mL centrifuge tube. The centrifuge tube was wobbled slightly and centrifuged to separate the DNA from the water containing dissolved humic acids. The extracted DNA was dissolved in 100 μ L of TE buffer and stored at -20°C before use (Wu et al., 2013).

The variable V3 region of 16S rDNA genes was amplified using the bacterial universal primers GC338F/518R (Li et al., 2008). The PCR mixture contained 1 μ L of template DNA (≈ 20 ng), 25 μ L of 2 $\times$ Power Taq PCR MasterMix (Invitrogen, USA), 1 μ L of BSA (10 mg mL $^{-1}$), 1 μ L of each primer (20 μ M) and 22 μ L of sterile water. The PCR amplification was performed as follows: initial denaturation (5 min, 94°C) was followed by 35 cycles of denaturing (45 s, 94°C), annealing (40 s, 55°C) and elongation (40 s, 72°C), single elongation (7 min, 72°C), ending at a final temperature of 4°C .

The DGGE was implemented by a Dcode Universal Mutation Detection System (Bio-Rad, USA). The PCR products containing approximately equal amounts (40 μ L) of PCR amplicons were loaded into 1-mm-thick 8% (w/v) polyacrylamide gels in a 1 $\times$ TAE buffer with a denaturing gradient ranging from 30 to 70% (Wu et al., 2013). Electrophoresis was performed at 60°C at a constant voltage of 80 V for 12 h. The gels were stained with Dured nucleic acid gel stain, scanned and analyzed using QuantityOne software (version 4.5, Bio-Rad, USA).

The qPCR was performed using an iCycler IQ5 Thermocycler (Bio-Rad, USA). The qPCR assays were conducted in a 20- μ L volume containing 1 μ L of template DNA, 0.2 μ M of primer (338F/518R) and 10 μ L of 2 $\times$ SYBR real-time PCR premixture (Biotek, Beijing), where the total volume was adjusted using sterile water. The reaction was performed as follows: initial denaturation (2 min, 95°C), followed by 40 cycles of denaturing (20 s, 95°C), annealing (30 s, 55°C) and elongation (30 s, 72°C). Ten-fold serial dilutions of linearized plasmids containing the 16S rDNA gene were used to create the standard curves for qPCR. The initial copy number of the bacterial gene was determined by comparing the threshold cycle values of each sample with the standard curve.

2.6. Data analysis

After subtracting the average background, the DGGE profiles were digitized. The band (which was determined from the peak in the densitometric curves and indicated the microbial community structure) number and the relative intensity (within a lane) were quantified using QuantityOne software (version 4.5, Bio-Rad, USA), as previously described (Zhang et al., 2011). The Shannon–Weiner diversity index (H) was used to calculate the diversity of the soil bacterial community as follows:

$$H = - \sum \left(\frac{N_i}{N} \right) \ln \left(\frac{N_i}{N} \right),$$

where i is the number of bands in each DGGE profile, N_i is the relative intensity of each band i , and N is the sum of all of the relative intensities in a given DGGE profile (Zeng et al., 2011).

The mean values among E40, E20 and E0 were compared by one way analysis of variance (ANOVA), where the post hoc Tukey's test was used to assess significant differences ($p < 0.05$) in the data. A Pearson correlation analysis was used to test the univariate correlations between the parameters. All of the analyses were conducted using SPSS (version 19).

Canoco (version 4.5, Centre for Biometry, Wageningen, the Netherlands) was used to determine the multivariate relationships between the bacterial community structure and the soil properties. Detrended correspondence analysis (DCA) was implemented to determine whether the data for the bacterial community structure followed a linear or unimodal response model. The length of the first DCA ordination axis was 1.376, which clearly indicated a linear species response. Accordingly, redundancy analysis (RDA) with default settings was performed to ordinate the soil bacterial community structure with the soil properties (Lepš and Šmilauer, 2003). Ordination biplots with scaling focused on inter-species differences displayed bacterial community structure similarities, so that the distances between centroid points for individual samples were easily understood (Wu et al., 2013). In the forward selections, a Monte Carlo permutation test with 499 unrestricted permutations was carried out to determine the parameters that significantly affected the bacterial community structure (Lepš and Šmilauer, 2003).

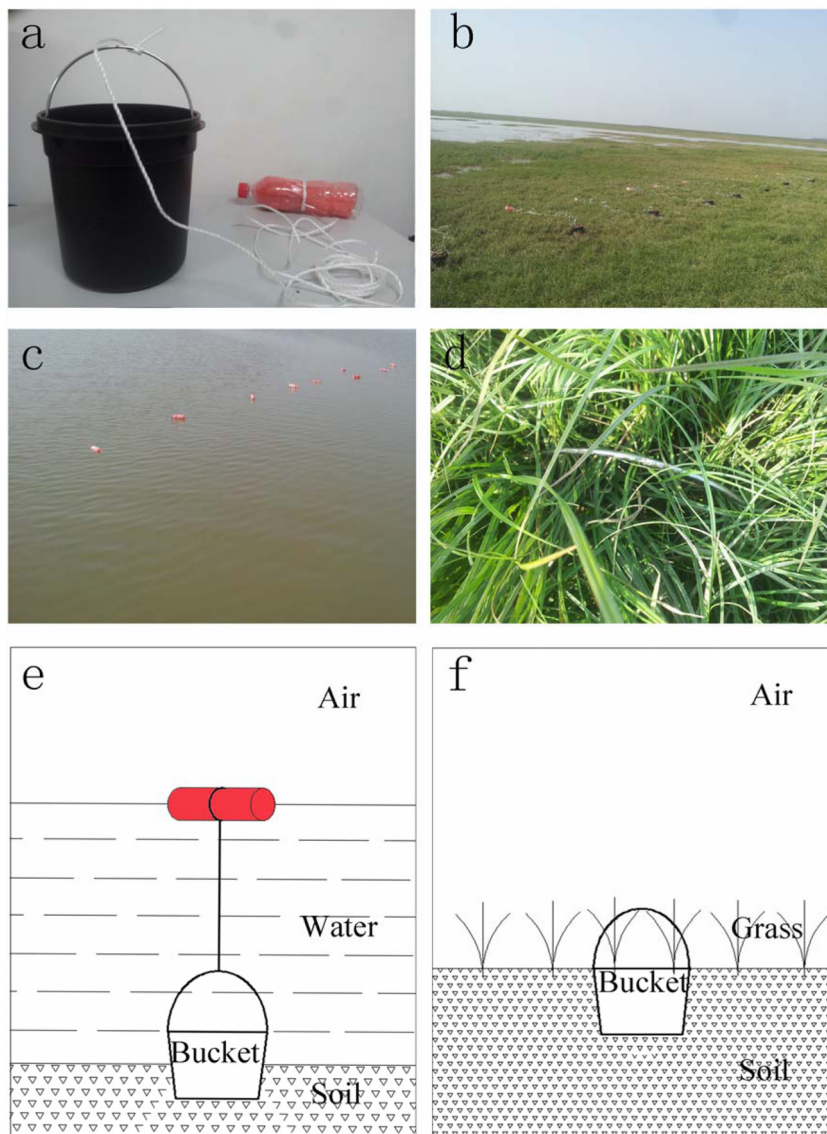


Fig. 2. Images showing status of culturing buckets during the experiments. (a) Photograph of a culturing bucket; (b) photograph of culturing buckets placed on the shallow; (c) photograph of inundated culturing buckets; (d) photograph of culturing bucket placed on the emergence shallow after inundation; (e) sketch of image (c); (f) sketch of image (d).

3. Results

3.1. Responses of soil properties to early dry season

Fig. 3 shows that there were differences in the soil properties among the samples, particularly in terms of the moisture, the SOM and the CEC. The moisture, SOM and CEC of E20 were less than those of E0. However, the moisture, SOM and CEC of E40 exceeded those of E0. With increasingly early start dates, the pH of each soil sample decreased. The soil pH of E40 was significantly lower than that of E0, whereas there was no significant difference between the pH of E20 and those of the other samples. No significant differences were observed between the TNs of the management samples.

The Pearson's correlation coefficients among the considered parameters are shown in Table 1. There was a highly significant ($p < 0.01$) positive correlation between the moisture and the SOM. The correlation analysis also demonstrated a significant ($p < 0.01$) positive correlation between the CEC and the SOM. The soil pH was strongly correlated with the lead time ($p < 0.01$). The moisture, SOM, TN and CEC were not strongly correlated with the lead time.

3.2. Responses of soil microbial biomass to early dry season

There was a significant change in the MBC, which served as a representative for the SMB (Fig. 4). Significant increases in the MBC were observed as the dry season began increasingly earlier. The MBC values for the samples increased in the following order: E0 < E20 < E40. There was a significant ($p < 0.01$) positive correlation between the MBC and the lead time (Table 1).

There was also a change in the quantity of bacteria, which was the primary component of the soil microbes. The initial copy numbers of the bacterial gene of the soil samples are shown in Fig. 4. The change in the initial copy number of the bacterial gene was similar to that of the MBC and increased significantly with the lead time of the dry season.

3.3. Responses of bacterial community structure to early dry season

The differences in the DGGE band numbers and compositions suggested that different bacterial community structures existed in

Table 1

Pearson's correlation coefficients between the parameters.

	Lead time	Moisture	SOM	pH	TN	CEC	MBC	Gene number	Band number	Shannon–Weiner diversity index
Lead time	1.000									
Moisture	0.489 ^{ns}	1.000								
SOM	0.489 ^{ns}	0.945 ^{***}	1.000							
pH	−0.902 ^{***}	−0.379 ^{ns}	−0.435 ^{ns}	1.000						
TN	0.019 ^{ns}	0.065 ^{ns}	0.125 ^{ns}	−0.062 ^{ns}	1.000					
CEC	0.482 ^{ns}	0.926 ^{***}	0.877 ^{**}	−0.378 ^{ns}	0.358 ^{ns}	1.000				
MBC	0.942 ^{***}	0.422 ^{ns}	0.520 ^{ns}	−0.905 ^{***}	−0.055 ^{ns}	0.380 ^{ns}	1.000			
Gene number	0.972 ^{***}	0.477 ^{ns}	0.549 ^{ns}	−0.910 ^{***}	−0.003 ^{ns}	0.448 ^{ns}	0.992 ^{***}	1.000		
Band Number	0.764 [*]	0.576 ^{ns}	0.719 [*]	−0.747 [*]	0.121 ^{ns}	0.586 ^{ns}	0.859 ^{**}	0.845 ^{**}	1.000	
Shannon–Weiner diversity index	0.766 [*]	0.646 ^{ns}	0.707 [*]	−0.637 ^{ns}	−0.061 ^{ns}	0.636 ^{ns}	0.813 ^{**}	0.807 ^{**}	0.930 ^{***}	1.000

SOM: soil organic matter; TN: total nitrogen; CEC: cation exchange capacity; MBC: microbial biomass carbon. Gene number: bacterial 16S rDNA genes copy number. Significance levels are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, while ns indicates no significant correlation ($p > 0.05$).

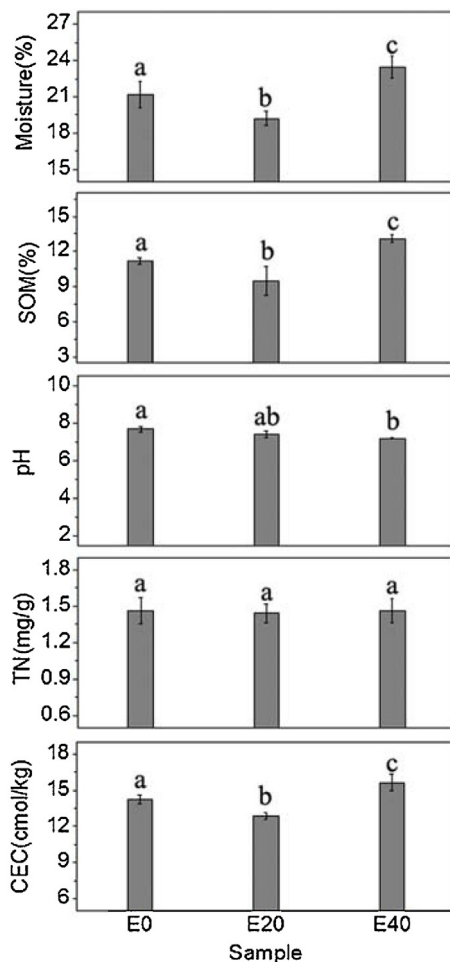


Fig. 3. Soil properties of samples E0, E20 and E40 (the dry season was early by 0, 20 and 40 days, respectively); CEC: cation exchange capacity; TN: total nitrogen; SOM: soil organic matter; bars with different letters correspond to significant differences ($p < 0.05$).

the soil. Figs. 4 and 5 show that the early dry season induced the differences in the soil bacterial community structures. Differences were observed in the DGGE band numbers. The band numbers of E0 and E20 were similar and both smaller than that of E40. Significant differences were also found among the Shannon–Weiner diversity indexes of the samples. There was a significant difference between the Shannon–Weiner diversity index of E40 and those of the other samples, but there was no significant difference between the Shannon–Weiner diversity indexes of E20 and E0, which suggested that there were significant differences in the diversity of

Table 2

The results of Monte Carlo permutation test for the test of influence of the soil properties.

Parameters	% Variation explains	F-value	p-Value
Lead time	46.0	5.961	0.0080
SOM	36.7	4.064	0.0260
Moisture	35.7	3.880	0.0300
CEC	33.9	3.590	0.0320
pH	33.7	3.557	0.0360
TN	4.2	0.309	0.9500
All above together	85.4		

the bacterial community structure between E40 and E0 but not between E20 and E0. Generally, the bacterial community structure of E40 was more diverse and abundant than that of E20, which was similar to that of E0.

The RDA biplot of the soil bacterial community structure and the investigated soil properties are shown in Fig. 6. Table 2 shows that all of the soil properties could explain 85.4% of the variation in the species data. The lead time, the SOM, the moisture, the CEC and the pH exerted highly significant influences on the bacterial community structure. Each soil property explained a different aspect of the variation in the bacterial community structure data. The community variation explained by the soil properties decreased as follows: lead time > SOM > moisture > CEC > pH > TN. The behavior of the lead time could be used to explain 46.0% of the variation in the species data. All of the aforementioned results suggested that lead time of dry season was the dominant influence factor of the soil bacterial community structure.

4. Discussion

4.1. Responses of soil properties to early dry season

The SOM differed significantly among the soil samples and increased in the order, E20 < E0 < E40, where there was a nonlinear relationship between the SOM and the duration of waterlogging. The decrease in the SOM of E20 was attributed to the response of the metabolism and respiration of the aerobic soil microorganisms to the long waterlogging duration and the short recovery period following waterlogging (Anderson and Domsch, 1993; Rinklebe and Langer, 2006). The respiration of the soil microorganisms increased significantly after flooding (Bossio and Scow, 1995). The respiration of the soil microorganisms in E20 lasted longer and was enhanced over that of the soil microorganisms in E0. These differences in the respiratory behavior would have lowered the SOM of E20 below that of E0. In addition, the appearance of plant would have occurred as the emergence time increased. The plants contributed a carbon input to the soil through canopy litterfall and root exudation (Anaya et al., 2007; Hamilton and Frank, 2001). This carbon input may have

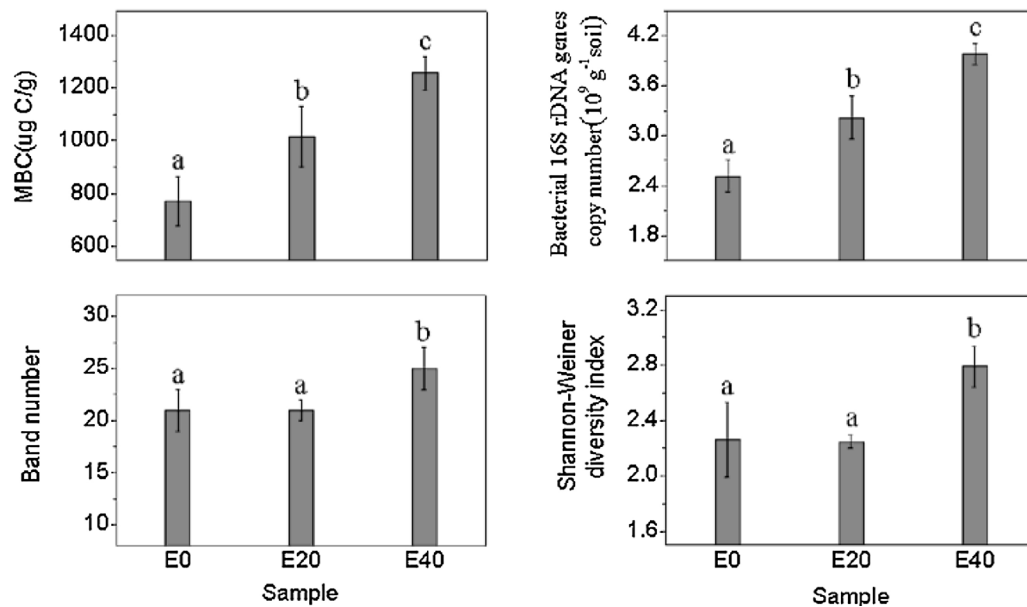


Fig. 4. Soil microbial properties of samples E0, E20 and E40 (the dry season was early by 0, 20 and 40 days, respectively); MBC: microbial biomass carbon; significant differences ($p < 0.05$) are indicated by different letters.

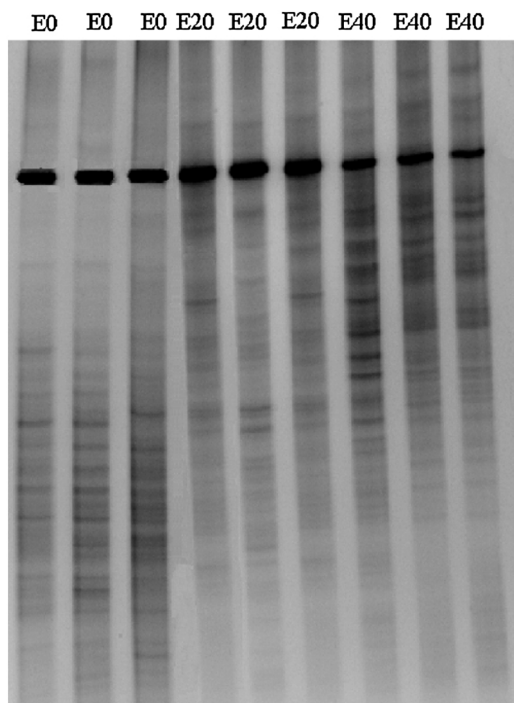


Fig. 5. DGGE photographs of samples E0, E20 and E40 (the dry season was early by 0, 20 and 40 days, respectively).

been the primary reason for why there was a significant increase in the SOM of E40. Therefore, the significant differences in the SOM of the samples (which increased in the order $E20 < E0 < E40$) depended on the balance between the respiration of the aerobic soil microorganisms and the carbon input from the plants.

A positive correlation between the SOM and moisture has been found in several studies, presumably because the SOM enhances the water holding capacity of the soil by increasing the porosity of the soil structure (Douterelo et al., 2009; Dubbin, 2000; Wu et al., 2013). A correlation analysis also showed a significant positive correlation between the CEC and the SOM, which was in agreement with the

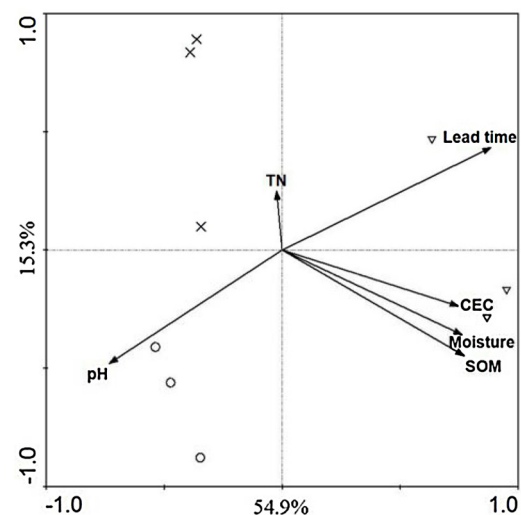


Fig. 6. Redundancy analysis of soil properties and bacterial community structure; soil properties are indicated by solid lines with filled arrows; samples of E0 (the dry season was early by 0 days) are indicated by circles; samples of E20 (the dry season was early by 20 days) are indicated by crosses; samples of E40 (the dry season was early by 40 days) are indicated by triangles; CEC: cation exchange capacity; TN: total nitrogen; SOM: soil organic matter.

results of Wu et al. (2010). This correlation agreed with the theory that the SOM is the most important factor for determining the CEC (Yan et al., 2007).

The moisture and the CEC changed because these parameters were correlated with the SOM. However, the pH of the soil samples exhibited different trends, and no significant change was observed in the TN with changes in the SOM. The soil pH in this study exhibited a decreasing trend as the lead time increased. This result was obtained because waterlogging increased the soil pH. The decrease in pH could be explained by the dissolution of carbonate and bicarbonate during the stages of waterlogging (Wang et al., 2013b). The early dry season meant less dissolution of carbonate and bicarbonate. No significant changes were observed in the TN with the early start date of the dry season, which may have resulted from the

combined effect of the duration of the oxic and anoxic phases, the water holding capacity, the SOM and the C and N that were present in the microbial biomass (De Jager et al., 2012). Rinklebe and Langer (2006) also found that there was no significant difference in the soil TN (0.66 and 0.63%, respectively) from 76 to 175 days after the soils were flooded.

4.2. Responses of soil microbial biomass to early dry season

The results of this study (i.e., the changes in the MBC and the initial copy number of the bacterial gene) implied that the SMB increased with increasingly early starting dates for the dry season. This result was in good accordance with those of Rinklebe and Langer (2006). The authors found that a shorter flooding duration and a longer period after flooding resulted in a higher SMB. There may be two reasons for this behavior: (1) the soil aerobic microorganisms were stressed during flooding because of the absence of oxygen and recovered after flooding (Anderson and Domsch, 1993); and (2) the appearance of plant occurred as the emergence time increased. Plant litterfall and the exudation of the plant roots resulted in a carbon input to the soil, which induced variations in the SMB (Hamilton and Frank, 2001).

Similarly, the change in the SMB that determined the microbial metabolic potential was consistent with the change in the soil CO₂ efflux (Poret-Peterson et al., 2007; Wu et al., 2013). The metabolic products of microorganisms can enhance the effectiveness of soil nutrients and increase the intensity of soil respiration (Li et al., 2010). Lee and Jose (2003) found that the SMB was positively correlated with the soil respiration during agroforestry. However, Wang et al. (2003) obtained different results. These authors argued that the MBC was weakly or insignificantly correlation with the soil respiration and that sufficient substrate input was required for the respiration rates to be proportional to the size of the MBC. The Dongting Lake wetland is likely to meet the conditions for sufficient substrate input, which means that the soil respiration rate of this wetland would increase (Wu et al., 2013). This change in the SMB would affect carbon exchange throughout the ecosystem. Further study is needed to determine how the carbon exchange of the entire ecosystem would be affected. However, in general, such a large increase in the SMB would reduce the carbon storage of the wetland.

4.3. Responses of bacterial community structure to early dry season

In this study, we showed that the lead time of the dry season was the dominant influence factor for variations in the soil bacterial community structure. The hydrologic cycle could affect the duration of the oxic and anoxic phases, the soil moisture and the SOM (De Jager et al., 2012; Ponnamperna, 1972). All of these factors could affect the soil microbial properties (Wu et al., 2013). Bossio and Scow (1998) found that the soil microbial community structure in a paddy rice field was sensitive to flooding. Schimel et al. (1999) argued that the duration of wet and dry periods had a critical effect on the soil microbial community structure. Other studies have also reported that different waterlogging times produce different soil microbial communities (e.g., Tang et al., 2011).

Our results also showed that the SOM, the moisture, the CEC and the pH had significant influences on the bacterial community structure. This is maybe because these soil properties can regulate the nutrient content, the rates of aerobiosis and anaerobiosis and the acidity in the soil, which further affect the soil bacterial community structure (Wu et al., 2013). Tang et al. (2011) also found that soil factors, such as the moisture and the pH, could affect the structure and diversity of the soil community. Both the water regime and the

soil are clearly critical factors in regulating soil microbial diversity in floodplain soils (Rinklebe and Langer, 2006).

In addition, although we took appropriate measures to lower the impacts of air-drying, sieving and mixing to prevent the generation of additional modifications/disturbances and reduced contact with the surrounding soil by the buckets, the aforementioned impacts could not be eradicated. Therefore, there may be differences between the results of this study and the actual impacts of the early dry season. However, considering the function of the necessary measures, this study could basically reflect the actual impacts of the early dry season, and the results appear to be credible.

Previous studies have suggested that the duration of flood inundation is a critical environmental factor that affects the vegetation and soils of floodplains (e.g., Auble et al., 1994; De Jager et al., 2012). Similarly, an early dry season would change the vegetation of lake wetlands of the middle and lower reaches of the Yangtze River (including the two largest freshwater lake wetlands). The changes in the vegetation of these wetlands are important for the wintering habitats of East Asian migratory birds and would affect birds and other animals (Tang et al., 2014; Yuan et al., 2014). The changes in the vegetation would affect the soil microorganisms (Hamilton and Frank, 2001; Rinklebe and Langer, 2006; Tang et al., 2011). The birds and animals would affect the soil microorganisms through their feces, urine and browsing on grass (Sakaran and Augustine, 2004; Xu et al., 2012). All of these factors are important for the ecological service and protection of these wetlands. Therefore, further study is required to determine the effects of an early dry season on the vegetation, birds and animals and the combined impacts of these changes on the soil microorganisms in these wetlands.

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China (51039001, 51479072, 51009063), the State Council Three Gorges Project Construction Committee Projects (SX2010-026), the New Century Excellent Researcher Award Program (NCET-08-0181) from the Ministry of Education of China and the Program for Changjiang Scholars and Innovative Research Team in University (IRT-13R17).

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