

Article Effects of Drying-Rewetting Cycles on Ferrous Iron-Involved Denitrification in Paddy Soils

Yushuai Zhang¹, Baokun Xu^{1,2}, Jiangpei Han¹ and Liangsheng Shi^{1,*}

- ¹ State Key Laboratory of Water Resources and Hydropower Engineering Science, Wuhan University, Wuhan 430072, China; whuzys@whu.edu.cn (Y.Z.); xbk@whu.edu.cn (B.X.); hanjiangpei@163.com (J.H.)
- ² Agricultural Water Conservancy Department, Changjiang River Scientific Research Institute, Wuhan 430010, China
- * Correspondence: liangshs@whu.edu.cn; Tel.: +86-159-0278-1510

Abstract: Soil moisture status has an important effect on the process of denitrification in paddy soils. However, it is unclear how it affects the ferrous iron-involved denitrification. Here, the influence of drying-rewetting cycles on ferrous iron-involved denitrification in paddy soil were studied with batch experiments. The dynamics of nitrate, ammonia, Fe^{2+} , Fe^{3+} and total organic carbon (TOC), as well as nitrous oxide (N₂O) were investigated using the iron-rich paddy soil in Jiangxi province, South China. Results demonstrated that the denitrification rate dropped while ammonia nitrogen content (NH₄⁺-N) showed a rapid accumulation in the drying period. In the rewetting period, organic carbon played two-side roles. Organic carbon and ferrous iron together provided electron donors to denitrification, and organic carbon simultaneously reduced ferric iron under anaerobic environment. There were complex interactions among organic carbon, nitrate and Fe^{2+}/Fe^{3+} under drying-rewetting cycles. Soil rewetting led to denitrification flush, especially after a moderately long drying period, while excessively frequent drying-rewetting alternation was not favorable to nitrate denitrification.

Keywords: denitrification; autotrophic denitrification; iron; drying and rewetting cycle; rewetting frequency

1. Introduction

Intensive use of fertilizers to agricultural land can lead to nitrate contamination in both surface water and groundwater ecosystems [1]. China has been experiencing serious nitrogen pollution threats [2]. As the largest chemical fertilizer consumption nation worldwide, the consumed fertilizer in China has reached 60.22 million tons in 2015, of which the nitrogen fertilizer accounted for 40% [3]. In contrast to large plot size and mechanized farming in many countries, applying fertilizer by hand to millions of small plots is still the most popular farming method in China [4], often resulting in over-application and environmental pollution.

Denitrification, which is proved to be mainly a biological process, is defined as a dissimilatory reduction process of nitrate, nitrite, or other ionic nitrogen oxides to the gaseous oxides by aerobic bacteria under anoxic conditions [5,6]. There is denitrification in natural habitats such as lake sediments, river banks and farmland [7–9]. Usually, denitrification contains two forms depending on the source of reducing energy: heterotrophic denitrification and autotrophic denitrification. The former one is a process that needs organic substrates as the electron donor to reduce nitrate, while the latter one is an autotrophic process that the nitrate is degraded by sulfate, ferrous iron, or other inorganic matters [10]. Autotrophic denitrification seems more autonomous than heterotrophic process because there is no need of an external organic carbon source [11,12]. In a number of autotrophic denitrification reactants, ferrous iron is an important electron donor in the paddy soil.



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Iron, as the fourth most abundant element in the Earth's crust, is a transition metal that can contribute or accept electron during valence conversion [13–15]. In the drying soil, the iron is mainly ferric iron which can accept electrons from reductive substances (e.g., organic matter and hydrogen). When switching from drying status to flooding condition, there would be more ferrous iron which can devote electron ion to oxidizing substances (e.g., nitrate). Since the nitrate-dependent iron-oxidizing bacteria was detected in a lagoon, various denitrifying bacteria which can oxidize ferrous iron with nitrate have been found [16,17]. Melton et al. [18] systematically described the process of Fe^{2+} oxidation by nitrate with the denitrifying bacteria in the sedimentary and terrestrial environments. Mejia et al. [19] found that nitrate reduction driven by ferrous iron is an important process in the biogeochemical cycles of nitrogen, not only in anoxic environment but also in aerobic subsurface environment. Han et al. [12] proved a positive relationship between the denitrification process and ferrous iron oxidation in the paddy soil of rich iron content. However, ferrous iron, as a donation of electron, does not always promote denitrification. Baeseman et al. [20] found that the addition of ferrous and ferric iron could decrease the denitrification rate because the iron may complex strongly with organic carbon and the complexation may inhibit the nitrate reacting with ferrous iron. Overall, the denitrification involved with ferrous iron requires further understanding in the paddy soil of rich iron content.

Almost one-third of the land cover on earth is the arid and semi-arid area, where drastic soil moisture change is common [21]. In China, intermittent irrigation for paddy rice fields is popular, which also generates the drying and rewetting cycle [22]. In soils that are undergoing drying, the loss of unbound free-water leads to the substrate diffusion and enzyme activity decreasing [23–25]. The microbial metabolic activity is forced to reduce with decreasing soil moisture content [26,27]. On the other hand, rewetting of dry soils changes the physical properties and available substance of the soil. When the drying soil is rewetted, the rapid entry of water would break the aggregates, resulting in the release of organic carbon held within aggregates [28]. Above all, drying and rewetting events not only lead to the change of oxygen level and the soil microbial activity [26,29], but also affect the geochemical reactions and microbiological processes [6,30,31]. It is expected that drying-rewetting cycles complicate the interactions among organic carbon, nitrate and ferrous iron.

The objective of this study is to investigate the effect of drying-rewetting cycles on ferrous iron-involved denitrification in paddy soils. In this study, we designed incubation experiments with several different drying-rewetting cycles using the iron-rich soil. The goals are: (1) to reveal the interactions among organic carbon, nitrate and Fe^{2+}/Fe^{3+} under drying-rewetting conditions; (2) to assess the effect of drying-rewetting cycles on soil denitrification in paddy soils.

2. Materials and Methods

2.1. Soil Samples

The paddy soil was collected after the rice harvest in De'an, Jiangxi province of China $(29^{\circ}16'34'' \text{ N}, 115^{\circ}43'38'' \text{ E})$. A two-crop paddy was maintained, and nitrogen fertilizer was applied at a rate of about 400 kg N (ha year)⁻¹. Soil samples were randomly collected from five sampling sites. Surface soil (0–20 cm) samples were collected with taking care to avoid stock camps, roots and other such artefacts. The five soil samples were then air-dried and were passed through a 2 mm sieve to remove stones and plant residues. Then all the soil samples were mixed thoroughly to form a composite sample. Some physical properties of the soil are shown in Table 1.

2.2. Design of Batch Experiments

The experiments were designed following a method described by Xu and Cai [32] with some modifications. A total of 138 Erlenmeyer flasks (250 mL) were prepared and 40 g of dry soil with 30 mL of deionized water were placed into each flask for pre-incubation. All of the flasks were kept at 30 °C in the dark for 12 days. In the absence of oxygen, the

microbial activity of the soil could be activated [33], and the reduction of ferric iron in soils could be accelerated. As a result of pre-incubation, there were more available ferrous iron for denitrification in the next experiments [34]. Before the experiment begins, the field water-holding capacity was measured by the cutting ring method for determination of field water-holding capacity in soil [35]. The water content of the soil in the wetting period was determined as the gravimetric water contents of saturated soils (gravimetric moisture content: 52%). The water content of the soil in the drying period was 23% gravimetric moisture content. The two water contents were chosen because they were the standard of irrigation content in the intermittent irrigation for paddy rice fields of Southern China.

Table 1. The chemical and physical properties of the soil used.

Soil Site	pН	TN	TOC	NO_3^N	NH_4^+-N	Fe ³⁺	Fe ²⁺	Sand	Silt	Clay
Son She		g/kg	g/kg	mg/kg	mg/kg	g/kg	g/kg	%	%	%
De'an	5.70 ± 0.01	2.23 ± 0.39	12.47 ± 0.20	1.15 ± 0.03	4.61 ± 0.26	2.03 ± 0.16	0.23 ± 0.01	26.74 ± 0.10	44.76 ± 0.36	28.50 ± 0.10

TN total nitrogen, TOC total organic carbon. Values are the mean \pm standard deviation (n = 3). Fe³⁺, Fe²⁺ represents extractable Fe³⁺ and Fe²⁺ by 0.5 mol/L HCl extraction, respectively.

At the start of the treatment phase, 11 mL solution of KNO₃ was added into the flasks so as to the original content is the same (490 mg N kg⁻¹ soil). Three random replicates of pre-incubated flasks were chosen to obtain the physiochemical properties of the soil in the next stage of the experiment (Table 1). The changes of the soil moisture content in 5 different treatments were schematically presented in Figure 1. The soil water contents of continuously wet/dry treatments were maintained at saturated content and 80% field waterholding capacity, respectively. Three drying-rewetting treatments were designed with the same numbers of wet/dry days (both are 12 days). Thus, the treatments varied only in the drying-rewetting frequency. Above all, the experiment was composed of the following treatments: (i) continuously dry/wet treatment (CD/CW); (ii) 3-day drying-rewetting treatment (T₃); (iii) 6-day drying-rewetting treatment (T₆) and (iv) 12-day drying-rewetting treatment (T₁₂).



Figure 1. Moisture content design of different drying-rewetting treatments: (**a**) Continuously wet (solid line), continuously dry (dotted line); (**b**) Drying-rewetting alternation at an interval of 3 days; (**c**) 6 days and (**d**) 12 days.

To ensure rapid soil drying, 30 g silica gel was packaged and hung carefully in the flasks to avoid attaching to the surface of soil, in order to absorb water quickly. When the soil reached the designed content, the packaged silica gel was taken out after the gas in the flasks was measured. The silica gel could be regenerated at 105 °C using an air-blowing thermostatic oven. All of the flasks were sealed by 704 silicone sealants. At the start of the wetting period, the soils were wet to saturated content by adding reverse osmosis water with an automatic pipette, and then the flask was sealed again immediately. Acetylene (C_2H_2) at 10 kPa was applied into the flasks in order to inhibit nitrification and potential N₂O reduction after the flasks were sealed every time. The soil water moisture was monitored by weighing the flask every day during the experiment.

 N_2O was sampled by using a gas-tight syringe at first and determined by gas chromatography method immediately to avoid the samples being contaminated with air. After that, the sealed flasks were destroyed and sampled for testing soil chemical properties once every three days (e.g., soil pH, NO_3^- , NO_2^- , NH_4^+ , TOC, Fe^{2+} and Fe^{3+} contents). All soils were destructively sampled in this experiment. Three random Erlenmeyer flasks for each treatment were tested and then abandoned after sampling.

2.3. Measurements

Each gas sample was collected by using a gas-tight syringe at the beginning of every specified time point. Before collecting the gas sample, the gas-tight syringe piston would be pulled and pushed three times in order to make the gas in the flasks homogenized. Then, each gas sample was tested immediately using gas chromatograph (GC-2014C, Shimadzu, Japan). The electron detector for the N₂O content of the gas chromatograph was electron capture detector (GC-ECD). The carrier gases were N₂ (99.999%) and H₂ (99.999%). The detector temperature for analyzing N₂O was 300 °C.

Soil particle size distribution was measured by the hydrometer method. Soil pH was measured by pH-electrode method at soil-water ratio of 1:2.5 using a pH portable analyzer (PQ-40D; Hach, USA) [36]. Total nitrogen was measured by the automatic Kjeldahl analyzer. Total organic carbon (TOC) was determined by organic matter-ignition loss method using a TOC analyzer (TOC-L CPN, Shimadzu, Japan) which was equipped with solid sample combustion unit (SSM-5000A, Shimadzu, Japan) [37]. Inorganic N was extracted by adding 1M KCl solution and the concentrations of NO₃⁻, NH₄⁺ were measured using the extraction with potassium chloride solution-spectrophotometric method [38]. The Fe²⁺ and Fe³⁺ ions were extracted by adding 0.5 mol·L⁻¹ HCl solution in which the aqueous solution should be degassed. The Fe²⁺ content was determined by using phenanthroline-spectrophotometric method without phenanthroline solution [39]. Specially, the Fe³⁺ content was calculated by the difference value between the Fe²⁺ value of the extracted liquid which was added with the same deionized water.

2.4. Statistical Analyses

Statistical analyses were calculated by using SPSS 24 for Windows 10. Two-way analysis of variance (ANOVA) was carried out to determine the significant differences of Fe²⁺ and TOC. Multiple comparison was used to assess the relationships between the NO_3^{-} -N content and other properties such as Fe²⁺ and TOC content.

3. Results

3.1. Dynamics of the Soil Nitrate Nitrogen

The temporal variations of soil nitrate nitrogen (NO₃⁻-N) content under different treatments are shown in Figure 2. The nitrate contents were reduced continuously for all the treatments. A minimum reduction was observed in the continuously dry (CD) treatment, as the nitrate content dropped from 490 mg/kg to 411 mg/kg at the end of the experiment. The amount of the nitrate reduction in the continuously wet treatment was about 73 mg/kg, which was the maximum reduction in the five treatments. The final NO₃⁻-N content from treatment T₃ was lower than that from the CD treatment while higher than that from all other treatments (393 mg/kg and 411 mg/kg in treatment T₃ and CD, respectively). Treatment T₆ and T₁₂ had similar NO₃⁻-N loss in terms of the

final NO₃⁻-N content (369 mg/kg and 363 mg/kg in treatment T₆ and T₁₂, respectively). Comparing the dynamics of the nitrate in the different treatments, it is seen that more frequent drying and rewetting cycles lead to less of a drop in NO₃⁻-N content.



Figure 2. The dynamic of NO_3^- -N content in different treatments. The solid and dotted lines represent the rewetting and drying period, respectively. The vertical bars refer to one standard deviation (n = 3).

The instantaneous reduction rate (*IRR*) of NO₃⁻-N was defined as:

$$IRR_{NO_3^- - N} = \frac{mC_t - mC_{t - \Delta t}}{m\Delta t} \tag{1}$$

where C_t is the content of nitrate (NO₃⁻-N) at the day t, $C_{t-\Delta t}$ is the content of nitrate at the day $t-\Delta t$, m is the mass of the dry soil in the flask, Δt is the measured interval that is 3 days.

Figure 3a presents the NO₃⁻-N instantaneous reduction rates (*IRR*) under different treatments. *IRR* declined from 10.8 mg/kg/d to 7.1 mg/kg/d in the first 9 days and remained stable (around 4 mg/kg/d) in the following process under the CW treatment. Drying-rewetting cycles imposed an impact on the reduction rate. For all the drying and rewetting treatments, *IRR* dropped in the dry period regardless of the dry-rewetting frequency. For the treatment T₃, a pulse of *IRR* was observed when the soil was rewetted. Such pulse of *IRR* was particularly obvious in the treatment T₆ when the soil was rewetted at day 12, and a significant rise of *IRR* was found during day 12 and 15 and remained high during day 15 and 18.

The cumulative NO_3^- -N reduction under different treatments in the dry and wet period, as well as the overall NO_3^- -N reduction, are shown in Figure 3b. The soils subject to drying-rewetting alternation generated similar cumulative NO_3^- -N reduction in the 12 drying days regardless of the treatments. However, it is seen that cumulative NO_3^- -N reduction in treatment T_6 during 12 wetting days contributed most to the overall reduction. Although treatments of T_3 , T_6 and T_{12} had the same number of drying and wetting days, the cumulative reduction of NO_3^- -N within the wetting period, as well as the contribution to the overall reduction, was different for each treatment.



Figure 3. Instantaneous reduction rate of NO₃⁻-N in different treatments. (**a**) The solid and hollow boxes represent the soil in the rewetting and drying period, respectively; (**b**) Cumulative reduction of NO₃⁻-N in different treatments. The vertical bars refer to one standard deviation (n = 3).

The temporal variations of the soil NH₄⁺-N content under different treatments are shown in Figure 4. Treatments of CW and CD produced the least and second least NH₄⁺-N accumulation, respectively. Treatments subject to drying-rewetting alternation generated more final NH₄⁺-N than continuously dry or wet treatments. There was a pulse of NH₄⁺-N during the drying period in treatment T₆ (from 7.6 mg/kg at day 6 to 11.8 mg/kg at day 12). Similarly, the treatment T₁₂ produced stable NH₄⁺-N during the first 12 wet days as in the treatment CW, while a much faster increase of NH₄⁺-N was observed during the following 12 drying days. The final NH₄⁺-N content was twice that of the initial NH₄⁺-N content in the treatment T₁₂. Comparing T₃ and T₆ with T₁₂, it is seen that more frequent dryrewetting alternation led to more NH₄⁺-N accumulation. Although treatments of T₆ and T₁₂ had similar NO₃⁻-N loss, their NH₄⁺-N accumulations were different and treatment T₆ produced more NH₄⁺-N than T₁₂. It seemed that the drying process after wetting period facilitated the production of NH₄⁺-N.



Figure 4. The dynamic of NH_4^+ -N content in different treatments. The solid and dotted lines represent the rewetting and drying period, respectively. The vertical bars refer to one standard deviation (n = 3).

3.2. Dynamic of the Soil Ferrous Iron Content

The temporal variations of ferrous iron (Fe²⁺) under different treatments are presented in Figure 5. In the CD treatment, the Fe²⁺ content was decreasing due to the oxidation of Fe²⁺ driven by oxygen. In the CW treatment, although the soils were in anaerobic condition, the Fe²⁺ content was declining continuously. Stronger oxidation of Fe²⁺ was observed in the CD than in the CW. The Fe²⁺ content in the treatment T₃ and T₆ showed a dropping trend during the drying periods and a rising trend during the rewetting periods. The treatment T₁₂ presented a similar Fe²⁺ changing trend as in CW during the first 12 wetting days, while a faster decrease of Fe²⁺ content occurred during the last 12 drying days for T₁₂. The results also demonstrated that dry-rewetting frequency exerted an influence on the final ferrous iron content. Lower dry-rewetting frequency led to less final ferrous iron.

The Fe³⁺ content increased in the first few days and decreased in the late period for all treatments (Figure 6). In the CW treatment, as well as in the treatment T_{12} , the Fe³⁺ content reached the peak at t = 6 days and then decreased continuously. In the CD treatment, the Fe³⁺ content was stable from t = 3 days to t = 15 days and started to drop after t = 15 days. In the treatment T_3 , the Fe³⁺ content kept rising in the first 9 days and continuously dropped after that. The Fe³⁺ accumulation pattern in the treatment T_6 was similar to treatments of CW and T_{12} , while there was a rise during t = 15 days and t = 18 days. Unlike the changing pattern of Fe²⁺, drying-rewetting cycles did not cause the fluctuation of Fe³⁺ content.

3.3. Dynamics of Total Organic Carbon in the Soil

The temporal variations of the total organic carbon (TOC) content under different treatments are shown in Figure 7. It is seen that the overall trend of the TOC contents was decreasing with time for all treatments. In the continuously wet treatment, the TOC content decreased quickly in the early period and declined slowly in the late period. In the continuously dry treatment, the TOC content decreased stably while at a slower rate than other treatments during the whole period. Comparing to CW and CD, the soils subject to drying-rewetting cycles had lower TOC content. However, the final TOC contents of three different drying-rewetting treatments (T_3 , T_6 and T_{12}) were close.

Due to the application of acetylene inhibition in the experiment, nitrous oxide (N_2O) was the main gas emission (Figure 8). The accumulative N_2O emission is overall consis-

tent with NO₃⁻-N reduction in all treatments. For instance, the nitrate loss was about 104 mg/kg (NO₃⁻-N) in the T₃ treatment. At the same time, the cumulative emission of the N₂O was about 97 mg/kg (N₂O-N) in this treatment. There was 7.9% mismatch in it.



Figure 5. The dynamic of Fe²⁺ content in different treatments. The solid and dotted lines represent the rewetting and drying period, respectively. The vertical bars refer to one standard deviation (n = 3).



Figure 6. The dynamic of Fe³⁺ content in different treatments. The solid and dotted lines represent the rewetting and drying period, respectively. The vertical bars refer to one standard deviation (n = 3).



Figure 7. The dynamic of TOC content in different treatments. The solid and dotted lines represent the rewetting and drying period, respectively. The vertical bars refer to one standard deviation (n = 3).



Figure 8. The accumulated N₂O emission in different treatments. The solid and dotted lines represent the rewetting and drying period, respectively. The vertical bars refer to one standard deviation (n = 3).

4. Discussion

4.1. Nitrogen Dynamics

Drying and rewetting events affect the nitrogen transformation process from several aspects. Firstly, it could lead to the change of soil moisture content and oxygen level in soil, which eventually influences N dynamics. Secondly, the drying and rewetting process breaks the soil aggregates, resulting in the release of organic carbon held within aggregates. Thirdly, microorganism richness and community composition are affected by drying and rewetting process. Similar to respiration flush induced by rewetting dry

soil [29,31], denitrification flush was also observed for treatments of T_3 and T_6 when the soil was rewetted (Figure 3a). The rewetting denitrification pulse may be attributed to the increase of easily available soil organic matter. The microbial mineralization of cytoplasmic solutes and the release of soil organic matter protected within microaggregates are two major sources of mineralized carbon [40]. Rewetting after a short drying period (3 days in T_3) leads to slightly enhanced denitrification. However, rewetting after a moderately long drying period (6 days in T_6) significantly stimulates denitrification intensity. The spike of denitrification during 12 and 18 d in T_6 may be attributed to stronger mineralization of organic carbon (especially cytoplasmic solutes) after longer drying days. Thus, a moderately frequent drying-rewetting alternation may be not favorable to the population of denitrification bacteria. A decrease in microbial biomass with increased frequency of drying-rewetting cycles has been observed by previous studies [41,42].

In this study, more NH_4^+-N was produced under more frequent drying-rewetting treatments, which is consistent with previous studies [42]. The enhanced NH_4^+-N is likely due to the microbial mortality caused by water stress under drying conditions. Such NH_4^+-N pulse is particularly obvious after a longer wetting period. For example, there is a rise in NH_4^+-N during 6~12 d, 12~18 d for Treatment T₆, and the NH_4^+-N pulse during 12~24 d for Treatment T₁₂. For Treatment T₆, it is interesting to observe that a faster NH_4^+-N accumulation coincides with a slower NO_3^--N reduction during the drying-rewetting cycles. A stronger denitrification corresponds to a weaker NH_4^+-N production. For Treatment T₁₂, the phenomenon is similar. It is likely that anaerobic bacteria (part of them are denitrification bacteria) were killed during the drying period.

There are many factors which can affect the denitrification rate for soil, including the moisture content, temperature, nitrate content, total organic carbon and so on [10]. Our results indicated that denitrification rates under different drying and rewetting cycles vary markedly. The NO_3^- -N content significantly correlated with the TOC content (Table 2), which agreed with the results of previous studies [11,18].

	NO ₃ N	Fe ²⁺	Fe ³⁺	TOC	NH4 ⁺ -N	N_2O
NO ₃ ⁻ -N	1					
Fe ²⁺	0.661 **	1				
Fe ³⁺	-0.533 **	-0.517 *	1			
TOC	0.943 **	0.721 **	-0.555 **	1		
NH4 ⁺ -N	-0.709 **	-0.864 **	-0.609 **	-0.833 **	1	
N ₂ O	-0.967 **	-0.550 **	-0.385 **	-0.900 **	0.611 **	1

Table 2. Correlation matrix of the chemical properties in all soil samples.

Sample size: n = 135. ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

The nitrate reduction in the drying and rewetting cycles resulted in the production of N₂O gases. A higher NO₃⁻-N reduction corresponds to higher N₂O emission. Correlation analysis showed that nitrate content was significantly correlated with the emission of N₂O (r = -0.967, p < 0.01). Previous reported results showed that N₂O was higher in the drying and rewetting treatment compared to the continuously wet treatment [14]. More extensive soil drying and more extensive rewetting stimulated N₂O emission [27]. Our results seemed opposite to previous studies since the CW treatment produced the most N₂O emission in this study. It is likely that with no acetylene inhibition, more N₂ can be produced in the CW treatment [43]. It is reasonable since the denitrification process is a multi-step process and N₂ is the end production without acetylene inhibition.

4.2. Fe Dynamics

There existed Fe^{2+} driven denitrification in this study since the content of Fe^{2+} declined continuously in the CW treatment. It is interesting to observe that the denitrification pulse

was accompanied by Fe²⁺ content increase, which can be found in T₃ (during 12~15 d, 18~21 d) and T₆ (during 12~18 d). The increment of ferrous iron may be due to the reaction of ferric iron and organic carbon. Lots of archaebacteria were found in wetland and Fe³⁺ reduction occurred when microorganisms generated chemical substances which would promote the nonenzymatic conversion of Fe³⁺ to Fe²⁺. The reaction between iron and organic carbon can be described by the following chemical equations [44,45]. However, we observed that the increase in Fe²⁺ did not match with the decline in Fe³⁺, which was possibly attributed to the formation of poorly soluble Fe³⁺ products [19]:

$$CH_2O + 2Fe^{3+} + 2OH^- \rightarrow 2Fe^{2+} + H_2O + 2CO_2$$
 (2)

Thus, the ferrous iron is one of the electron donors for denitrification although the ferrous iron itself was the reductive production of ferric iron with organic carbon in the anaerobic environment [19,45]. As the experiment went by, the available ferrous iron was consumed by ferrous iron-involved denitrification. However, rewetting restored the available ferrous iron at the expense of consuming more organic carbon.

Fe²⁺ content had been reported to be significantly with nitrate content, suggesting that Fe²⁺ participates in the denitrification [10,16]. The NO₃⁻-N content significantly correlated with the Fe²⁺ content. The value of correlation coefficient for the relationship between nitrate content and Fe²⁺ content (r = 0.661, *p* < 0.01; Table 2) was lower than the value of correlation coefficient for the relationship between nitrate content and TOC content (r = 0.943, *p* < 0.01; Table 2). Overall, from the results, C, N and Fe exist as complex interactions in the experimental soils. Drying-rewetting cycles further complicate the interaction, and C simultaneously oxidizes Fe³⁺. The soil organic carbon consumption under drying-rewetting cycles is much higher than that under continuously dry or wet conditions. However, the higher TOC consumption is not due to stronger denitrification, because the reduced nitrate was lower under drying and rewetting condition than under the continuously wet condition. It is attributed to stronger soil respiration and additional consumption for Fe²⁺ oxidization.

5. Conclusions

This paper investigated the effect of drying-rewetting cycles on ferrous iron-involved denitrification in paddy soils. The results revealed the complex interactions among organic carbon, nitrate and Fe^{2+}/Fe^{3+} under drying-rewetting condition. Soil rewetting led to denitrification flush, especially after a moderately long drying period, while excessively frequent drying-rewetting alternation was not favorable to nitrate denitrification. NH_4^+ -N accumulated fast during the drying period. Fe^{2+} contributed to the denitrification process. Drying-rewetting cycles led to additional consumption of organic carbon and increased the available Fe^{2+} for autotrophic denitrification.

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