Effect of carbon source on dissimilatory nitrate reduction to ammonium in coastal wetland sediments

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Abstract

¹⁵N tracing technique was applied to investigate the effects of various organic carbon (OC) sources on dissimilatory nitrate (NO₃⁻) reduction to ammonium (NH₄⁺) (DNRA) rates in the coastal wetland sediments. Soils collected from the Chongming Dongtan wetland were incubated at 25 °C in the dark for 24 h following the additions of OC sources (glucose, acetate, malate, citrate and oxalate (500 µg C g⁻¹ dry soil)) and ¹⁵N-labeled NH₄⁺¹⁵NO₃⁻ (initial ¹⁵N atom% of NO₃⁻-N is 20%). The results showed that soil DNRA rates varied from 0.018-0.497 mg N kg⁻¹ dry soil d⁻¹ during the whole incubation, and the rates differed significantly among treatments following the order: oxalate > citrate > glucose > acetate > malate > no exogenous C addition over the first 12-h incubation. This was possibly caused by the different decomposition rates of various OC sources, which further influenced the available energy provided for DNRA microorganisms. Soils with no addition of exogenous C showed low soil DNRA rates, presumably because of the low C/NO₃⁻ ratio as well as energy availability. The relative lower soil DNRA rates over the 24-h incubation indicated that DNRA is a fast process. Our results suggest that DNRA could be controlled by OC sources, especially organic acids, demonstrating that the widespread use of glucose in soil laboratory studies might limit our understanding on the effects of OC on soil DNRA process.

Keywords: Dissimilatory nitrate reduction to ammonium, carbon source, ¹⁵N tracing technique, organic acid, coastal wetland

1. Introduction

Nitrogen (N) is an essential nutrient affecting biological production in terrestrial and aquatic ecosystems, hence it is important to understand how this element is cycled in a specific region or ecosystem

(Francis *et al.*, 2007; Zhao *et al.*, 2013). Coastal wetlands reside at a critical interface between terrestrial land and ocean, which plays a crucial role in balancing and sequestering N as well as protecting

biodiversity of coastal ecosystems (Bai *et al.*, 2012). N has been believed to be a major limiting nutrient for plant growth in pristine coastal wetlands. However, an increase in human disturbance along the coast over recent decades has accelerated N loading into coastal areas via nutrient-rich rivers, groundwater as well as atmospheric N deposition (Koop-Jakobsen and Giblin, 2010). As a result, coastal ecosystems have always experienced eutrophication, which caused severe environmental problems, such as increased frequencies and magnitudes of phytoplankton blooms and loss of habitats for submerged vascular plants (Rabalais and Nixon, 2002). An improved understanding of N dynamics in coastal wetlands is needed to maintain regional and global ecological security (Bai *et al.*, 2012).

For a multitude of coastal wetlands, the dominant form of anthropogenic N inputs is nitrate (NO_3^-) (Burgin and Hamilton, 2007). There are two important NO_3^- removal pathways in wetland sediments: one is denitrification (DNF), which sequentially reduces NO_3^- to dinitrogen (N_2) via nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O), and the other is dissimilatory NO_3^- reduction to ammonium (NH_4^+) (DNRA), by which NO_3^- is reduced to bioavailable NH_4^+ (Zumft, 1997; Rütting *et al.*, 2011). Because of the different productions, DNF has been not only considered as a major pathway of N loss in soil-plant systems (Gong *et al.*, 2013), but also the major biological process responsible for global increases in atmospheric N_2O (Baggs, 2008; Morley *et al.*, 2014). In contrast, DNRA conserves N in soils as NH_4^+ , thus retaining N levels of soils (Yin *et al.*, 2002; Koop-Jakobsen and Giblin, 2010). For a long time, DNF is considered as the predominant pathway to remove NO_3^- (Burgin and Hamilton, 2007). However, over the last few decades, applications of ^{15}N tracer technique and microbial molecular methods have generated growing evidence that DNRA is also an important N reduction process

that cannot be ignored (Giblin *et al.*, 2013). As an example, Giblin *et al.*, (2013) compared the importance of DNF versus DNRA at 55 coastal sites and found that DNRA accounted for more than 30% of the NO_3^- reduction at 26 sites. Consequently, understanding what controls the DNRA process is vital to regulate N loadings in coastal wetland sediments.

Among various factors influencing DNRA, carbon (C)/ NO_3^- ratio has been confirmed to be one of the most important drivers regulate DNRA. For example, Tiedje (1988) argued that DNRA was favored when the ratio of labile C/ NO_3^- was higher, and later studies further verified his hypothesis (Silver *et al.*, 2001; Yin *et al.*, 2001). In addition, as an essential electron donor of DNRA, organic C (OC) modifies the rate of DNRA by supporting respiration or fermentation that influences the population of DNRA bacteria. However, despite this requirement for OC, the regulation of DNRA by OC is poorly understood compared to other soil parameters such as pH, temperature, moisture and redox status (Rütting *et al.*, 2011), and most existing publications only took glucose into consideration (Henderson *et al.*, 2010; Lu *et al.*, 2013). Nevertheless, plant-derived low molecular C compounds in soils, which are key factors affecting the rhizosphere microbial community, are always ignored (Morley and Baggs, 2010; Morley *et al.*, 2014). Different OC substrates may also have different effects on the activity of enzyme (Morley and Baggs, 2010). Morley and Baggs (2010) proposed that OC may alter the fate of NO_3^- in soils on the basis of these hypotheses. In addition, Bonin *et al.* (1999) pointed out that the theoretical energy of various C substrates was quite different, which probably also affect the DNRA process. Consequently, application of glucose alone may not provide enough oversight of the regulatory factors controlling DNRA. In order to advance understanding with a more realistic perspective, other C sources, especially the organic acids generated from root exudates and

the decomposition of organic matter in plant residues, need to be considered to elucidate their unknown influences on DNRA. Under the background of rapid development of coastal economy, understanding the effects of OC on soil DNRA will help formulating appropriate strategies for the reduction of high N loadings in soils of coastal ecosystems.

In this study, ^{15}N -labelled NO_3^- -N tracer technique was applied to determine the effects of individual additions of different C sources (glucose, acetate, malate, citrate and oxalate) and no exogenous C addition (here termed as NC) on DNRA process in sediments of Chongming Dongtan wetland in Changjiang Estuary. The organic acid selected could be released in soils by rhizosphere fungi, root exudates and the decomposition of organic matter in plant residues.

2. Materials and Methods

2.1. Study area

Chongming Island ($31^\circ25'\text{N}$ - $31^\circ38'\text{N}$, $121^\circ50'\text{E}$ - $122^\circ05'\text{E}$) is the third largest island in China and one of the largest alluvial islands in the world, which is formed by alluvial silt and soil from the upper reaches of Yangtze River (Li *et al.*, 2010). On the eastern end of the island is the Chongming Dongtan wetland, which is the largest and youngest natural tidal flat in the Changjiang Estuary (Zhang *et al.*, 2011). It has a northern subtropical ocean climate, with an average annual temperature of 15.3°C , while mean monthly temperature ranges from 2.7°C in January to 27.6°C in August. The mean annual precipitation is 1022 mm, with more than 70 % of the annual rainfall occurring between April and September (Li *et al.*, 2010). The vegetation area of the tidal flat covers 27.51 km^2 and the vegetations primarily consists of *Phragmites communis* and *Scirpus mariqueter*. However, with its recent rapid spread and growth, the

invasive *Spartina alterniflora* has occupied 33.1% of the vegetation area, and mainly distributed in the northeast of Chongming Dongtan wetland (Gan *et al.*, 2009).

2.2. Soil sampling

Soil samples (0-15 cm depth) were collected from a site covered with *Phragmites communis* ($31^\circ37'\text{N}$, $121^\circ42'\text{E}$) in Chongming Dongtan wetland in October 2013, and they were transported to the laboratory within 12 h. Once returned to the laboratory the soils were air-dried in the shade and sieved ($\leq 2\text{ mm}$). The visible plant materials in sieved soils were then removed. It was a silty clay loam (sand 44.18%, silt 55.56%, clay 0.26%) with a pH (H_2O) of 8.65, soil organic carbon (SOC) of 8.39 g C kg^{-1} and total nitrogen (TN), NH_4^+ -N and NO_3^- -N of 1000.00, 6.20 and 3.40 mg kg^{-1} , respectively.

2.3. Experimental set-up

Soil samples (30 g at 5% gravimetric water content) were packed into 250 mL Erlenmeyer flasks, and then 60 mL of deionized water were added into the flasks. The flasks were then capped with breathable films. Afterwards, the flasks were pre-incubated at 25°C in the dark for 7 days. As soon as the pre-incubation finished, different OC substrates, including glucose (GC), acetate (AT), malate (ML), citrate (CT) and oxalate (OL), were applied into the flasks and each treatment had 6 flasks. In addition, 6 flasks with no exogenous C addition (NC) were also included as a control treatment. In order to make all samples the same liquid volume, 10 mL of OC solutions (19.79 mM GC, 59.38 mM sodium acetate, 29.69 mM sodium malate, 19.79 mM sodium citrate or 59.38 mM sodium oxalate) or 10 mL deionized water (NC) was added to each treatment. The final C added in soils

was equivalent to 500 $\mu\text{g C g}^{-1}$. After additions of C, 1 mL ^{15}N -labeled ammonium nitrate ($\text{NH}_4^{15}\text{NO}_3$) (15.2 mM at 30.15 atom % excess ^{15}N) was applied into each flask which resulted in a target ^{15}N enrichment of 20% of soil NO_3^- -N pool. All amendments were added to flasks by syringes through the breathable films. Moreover, 3 additional flasks without addition of C and ^{15}N -labeled were also prepared to measure the contents of organic N (ON), NH_4^+ -N and NO_3^- -N after pre-incubation, which represented the initial N contents before the ^{15}N tracer experiment. Therefore, there were total 39 flasks used in this study.

After all amendments were added, the flasks were immediately incubated in the dark at 25 °C. It is generally believed that DNRA is a fast process (Yin *et al.*, 2002; Lu *et al.*, 2013). In order to make our investigation comparable to previous ones, 12 h and 24 h were chosen as the incubation time, thus 3 flasks of each treatment were destructively sampled after 12 and 24 h incubations, respectively. To extract soil inorganic N, 80 mL of 1.88 M KCl was added to each flask. Because 60 mL deionized water and 10 mL C solutions had been added into each flask at the start of the pre-incubation, the final KCl concentration was about 1 M. The soil extracts were analyzed for the concentrations as well as ^{15}N atom% of inorganic N (NH_4^+ -N and NO_3^- -N). The extracted soils were then washed with deionized water and oven-dried at 60°C for the determination of concentrations and ^{15}N atom% of TN in the residuals, which was considered to be ON.

2.4. Soil measurements

Soil NH_4^+ -N and NO_3^- -N concentrations in the extracts were determined colorimetrically by ultraviolet spectrophotometer (UV-2550, Shimadzu Co., Ltd., Kyoto, Japan). Soil TN and ON were measured by semimicro Kjeldahl digestion selenium (Se), copper sulfate (CuSO_4) and potassium sulfate (K_2SO_4) as catalysts. SOC was determined by concentrated sulfuric acid (H_2SO_4) potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) oxidation and subsequent titration. Soil pH was determined by a combination electrode in the 1:5 soils to water suspension (w:v). Soil water content was determined gravimetrically after subsamples were dried at 105°C to a constant weight. The diffusion method (Brooks *et al.*, 1989) was used for extracting soil $^{15}\text{NH}_4^+$ -N and $^{15}\text{NO}_3^-$ -N while oven-dried soil was directly used for ON- ^{15}N atom% determination. An automated C and N analyzer interfaced with an isotope ratio mass spectrometer (Flash EA-Delta V Advantage, Thermo-Fisher Scientific Co., Ltd., Massachusetts, USA) was used for determining N isotope ratios.

2.5. Calculations and statistical analyses

According to the procedures of Buresh *et al.*, (1978), Chen *et al.*, (1995) and Yin *et al.*, (2002), DNRA rate (d , mg N kg^{-1} dry soil d^{-1}) and relative potential of DNRA (d' , %) were calculated using Equation 1 and Equation 2, respectively:

$$d = \frac{([\text{NH}_4]_{t_2} \times f_{t_2} - [\text{NH}_4]_{t_1} \times f_{t_1}) + ([\text{ON}]_{t_2} \times f_{t_2}' - [\text{ON}]_{t_1} \times f_{t_1}')}{100 \times (t_2 - t_1)} \quad (1)$$

where $[\text{NH}_4]_{t_1}$ and $[\text{NH}_4]_{t_2}$ are the soil NH_4^+ -N concentrations at time t_1 and t_2 (mg N kg^{-1} dry soil), respectively; f_{t_1} and f_{t_2} are the soil NH_4^+ - ^{15}N atom% at time t_1 and t_2 (%), respectively; $[\text{ON}]_{t_1}$ and $[\text{ON}]_{t_2}$ are soil

ON concentration at time t_1 and t_2 (mg N kg^{-1} dry soil), respectively; f_{t_1}' and f_{t_2}' are the soil ON- ^{15}N atom% at time t_1 and t_2 (%), respectively; t_1 is the beginning time of incubation and t_2 is the end time of incubation.

$$d' = \frac{([NH_4]_{t_2} \times f_{t_2} - [NH_4]_{t_1} \times f_{t_1}) + ([ON]_{t_2} \times f_{t_2}' - [ON]_{t_1} \times f_{t_1}')}{[NO_3]_{t_1} \times f_{t_1}'' - [NO_3]_{t_2} \times f_{t_2}''} \times 100 \quad (2)$$

where $[NH_4]_{t_1}$, $[NH_4]_{t_2}$, $[ON]_{t_1}$, $[ON]_{t_2}$, f_{t_1} , f_{t_2} , f_{t_1}' , f_{t_2}' , t_1 and t_2 are the same as the variables in Equation (1); $[NO_3]_{t_1}$ and $[NO_3]_{t_2}$ are the soil NO_3^- -N concentrations at time t_1 and t_2 (mg N kg⁻¹ dry soil), respectively; f_{t_1}'' and f_{t_2}'' are the soil NO_3^- -¹⁵N atom% at time t_1 and t_2 (%), respectively. It should be noted that at the beginning of the first incubation stage, natural abundance of ¹⁵N (0.366%) was applied to stand for the value of NH_4^+ -¹⁵N atom% and ON -¹⁵N atom%, while the value of NO_3^- -¹⁵N atom% was 20% which resulted from the additions of $NH_4^{15}NO_3$ and calculated using the Equation (3) as following:

$$atom\% = (S \times 0.366\% + A \times E) / (S + A) \quad (3)$$

where atom% is the value of ¹⁵N enrichment, S is the concentration of soil nitrate, 0.366% is the natural abundance of ¹⁵N, A is the concentration of nitrate added after OC additions, E is the abundance of ¹⁵N of added NO_3^- -N (30.15%).

To test whether there were differences among treatments in concentrations and ¹⁵N atom% of various N fractions, soil DNRA rate as well as relative potential of soil DNRA, one-way analysis of variance (ANOVA) followed by LSD post hoc test was performed using SPSS 16.0 (SPSS Inc., Chicago, USA). The t test was used to examine the differences in soil indexes mentioned-above between 12-h and 24-h incubation. OriginPro 8.0 was used for performing figures (OriginLab, USA).

3. Results

3.1. Concentrations and ¹⁵N atom% of soil N pools

After pre-incubation, soil NH_4^+ -N, NO_3^- -N and ON were 10.50 ± 0.42 , 4.13 ± 0.26 and 942.00 ± 9.00 mg N

kg⁻¹ dry soil, respectively, and their kinetics over the 24-h tracer experiment were illustrated in Figure 1. Compared with NC, additions of OC significantly decreased soil NH_4^+ -N during both stages ($P < 0.05$) (Figure 1-a). Different soil NH_4^+ -N decrements were also detected among five OC treatments and the order was $OL > ML > GC > AT > CT$. In addition, soil NH_4^+ -N concentrations were significantly lower over the 24-h incubation than that over the 12-h incubation in all treatments ($P < 0.05$). OC treatments also had negative effects on soil NO_3^- -N concentrations, and the lowest value occurred in treatment OL (Figure 1-b). As the incubation went on, soil NO_3^- -N concentrations decreased in spite of the different treatments. Soil ON also decreased under different OC treatments, and showed the same response with inorganic N (Figure 1-c). Over the first 12-h incubation, soil ON concentrations decreased following the order: $NC > AT > OL > GC > ML > CT$, while the order changed to $OL > ML > CT > AT > NC > GC$ over the second 12-h incubation. For treatments GC, AT, CT and NC, soil ON concentrations over the 24-h incubation were significantly lower in comparison with that of over the 12-h incubation ($P < 0.05$). Nevertheless, significant differences were not observed in treatments ML and OL ($P > 0.05$).

As shown in Table 1, soil NH_4^+ -¹⁵N atom% increased to 0.470-0.654% after 12-h incubation, and the highest and lowest NH_4^+ -¹⁵N atom% were found in treatment OL and NC, respectively. The second 12-h incubation further increased NH_4^+ -¹⁵N atom% in all treatments, although the values did not differ significantly in OC treatments between the two incubation stages ($P > 0.05$). Soil NH_4^+ -¹⁵N atom% of OL was significantly higher than those in other treatments ($P < 0.05$). Soil ON -¹⁵N atom% also showed an increasing trend,

although the increments were lower compared with the natural abundance of ON-¹⁵N atom%. During the entire incubation, soil ON-¹⁵N atom% ranged between 0.373% and 0.407%. On the contrary, significant reductions of ¹⁵N atom% were detected in soil NO₃⁻-N pool. After 24-h incubation, soil NO₃⁻-¹⁵N atom% de-

creased to just 0.570-1.270% in treatments with OC additions, which were lower compared with the initial NO₃⁻-¹⁵N atom%. However, these huge reductions did not occur in treatment NC. Furthermore, the NO₃⁻-¹⁵N atom% also differed significantly between the two incubation stages (*P*<0.05), indicating soil NO₃⁻-¹⁵N pool was continuously diluted over time.

Figure 1. The concentrations of soil NH₄⁺-N (a), NO₃⁻-N (b) and ON (c) in sediments of Chongming Dongtan wetland supplied with different forms of OC substrates or none (NC) after 12 and 24-h of incubations. The same uppercase letter indicates values are not significantly different among treatments (*P*<0.05) within a same incubation period. The same lowercase letter indicates values are not significantly different between two periods (*P*<0.05) with the same treatment. The error bars stand for standard deviations of the means (*n*=3).

Table 1. ^{15}N atom% of various N fractions in the sediments of Chongming Dongtan wetland supplied with different forms of OC substrates or none (NC) after 12 and 24 h of incubations.

Treatment	$\text{NH}_4^+ - ^{15}\text{N}$ atom% (%)		$\text{NO}_3^- - ^{15}\text{N}$ atom% (%)		$\text{ON} - ^{15}\text{N}$ atom% (%)	
	12 h	24 h	12 h	24 h	12 h	24 h
GC	0.537(0.022) ^a Ca ^b	0.597 (0.035) Ba	8.253 (0.136) Ca	0.873 (0.175) Bb	0.386 (0.002) Bb	0.403 (0.001) Ba
AT	0.563 (0.012) BCa	0.562 (0.022) Ba	9.735 (0.170) Ba	1.047 (0.144) Bb	0.383 (0.001) Cb	0.395 (0.001) Ca
ML	0.568 (0.013) Ba	0.581 (0.010) Ba	9.103 (0.917) BCa	0.639 (0.078) Bb	0.384 (0) Cb	0.390 (0.001) Da
CT	0.587 (0.004) Ba	0.588 (0.022) Ba	7.171 (0.075) Da	0.606 (0.028) Bb	0.400 (0.001) Ab	0.407 (0.002) Aa
OL	0.654 (0.019) Aa	0.706 (0.028) Aa	5.142 (0.271) Ea	1.065 (0.177) Bb	0.401 (0) Ab	0.404 (0.001) Ba
NC	0.470 (0.017) Db	0.515 (0.020) Ca	14.406(0.920) Aa	11.061(1.326) Ab	0.373 (0) Db	0.383 (0.001) Ea

^a Values are means (SD) (n=3).

^b The same uppercase letter indicates values are not significantly different among treatments ($P < 0.05$) within a same incubation time. The same lowercase letter indicates values are not significantly different between two periods ($P < 0.05$) with the same treatment.

3.2. Soil DNRA rate

Different OC sources significantly affected soil DNRA rates in both incubation stages (Figure 2). Soil DNRA rates varied from 0.018–0.497 mg N kg⁻¹ dry soil d⁻¹, and the maximum and minimum were measured in OL over the 12-h incubation and in AT over the 24-h incubation, respectively. For the first 12-h incubation, the order of DNRA rates followed: OL>CT>GC>AT>ML>NC, while soil DNRA rates of GC and AT did not differ significantly ($P > 0.05$). Notably, the mean DNRA rate

in soils of NC was only 0.033 mg N kg⁻¹ dry soil d⁻¹, which was significantly lower than that in soils with OC additions ($P < 0.05$). Incubation time also influenced soil DNRA rates: soil DNRA rates over the second 12-h incubation were significantly lower than that over the 12-h incubation in most of the treatments except NC ($P < 0.05$). During the second incubation stage, soil DNRA rates in treatment OL were not the highest due to their rapid decreases. Instead, the maximum of soil DNRA rates was detected in treatment AT, although its mean value was only 0.074 mg N kg⁻¹ dry soil d⁻¹.

Figure 2. DNRA rates in sediments of Chongming Dongtan wetland supplied with different forms of OC sources or none (NC) over 12 and 24 h of incubations. The same uppercase letter indicates values are not significantly different among treatments ($P < 0.05$) within a same incubation period. The same lowercase letter indicates values are not significantly different between two periods ($P < 0.05$) with the same treatment. The error bars stand for standard deviations of the means ($n=3$).

3.3. Relative potential of soil DNRA

Relative potential of DNRA, which also refers to the contribution of DNRA to total dissimilatory NO_3^- reduction, was estimated by the recovery of ^{15}N in NH_4^+-N and ON divided by the changes of ^{15}N in NO_3^--N . As shown in Figure 3, different OC treatments influenced the relative potential of soil DNRA during both incubation stages. For

the first 12-h incubation, the relative potential of soil DNRA varied from 4.50–27.57%, and values of soils amended with CT were significantly higher than those of ($P < 0.05$), although its soil DNRA rate was not the highest (Figures 2 and 3).

Moreover, similar to the DNRA rate, relative potential of soil DNRA also showed decreasing tendencies in all treatments during the second 12-h incubation.

Figure 3. Relative potential of soil DNRA in sediments of Chongming Dongtan wetland supplied with different forms of OC substrates or none (NC) after 12 and 24 h of incubations. The same uppercase letter indicates values are not significantly different among treatments ($P < 0.05$) within a same incubation period. The same lowercase letter indicates values are not significantly different between two periods ($P < 0.05$) with the same treatment. The error bars stand for standard deviations of the means ($n=3$).

4. Discussion

In this study, inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) and ON concentrations as well as the ^{15}N atom% of $\text{NO}_3^-\text{-N}$ decreased over the course of the incubation, while ^{15}N atom% of $\text{NH}_4^+\text{-N}$ and ON increased with time. The characteristics of soil N concentrations along time were in line with some previous investigations (Yin *et al.*, 2002; Scott *et al.*, 2008; Lu *et al.*, 2015). It is widely believed that $\text{NH}_4^+\text{-N}$ is an important product of DNRA (Rütting *et al.*, 2011; Giblin *et al.*, 2013), and the $\text{NH}_4^+\text{-N}$ generated from DNRA can be converted into other N pools through other processes,

one of which is $\text{NH}_4^+\text{-N}$ immobilization (Tobias *et al.*, 2001; Yin *et al.*, 2002; Zhang *et al.*, 2014). For instance, Tobias *et al.*, (2001) and Yin *et al.*, (2002) claimed that $\text{NH}_4^+\text{-N}$ generated from DNRA was mainly immobilized in the soil ON pool, while Scott *et al.*, (2008) pointed out that DNRA provided little $\text{NH}_4^+\text{-N}$ to nitrification. As the microorganisms were stimulated by exogenous C sources, $\text{NH}_4^+\text{-N}$ immobilization might be enhanced, which would accelerate the consumption of $\text{NH}_4^+\text{-N}$ (Yin *et al.*, 2002). Therefore, $\text{NH}_4^+\text{-N}$ in soils amended with OC decreased rapidly in contrast with that in NC. In addition, as a major reactant of DNRA as well as other NO_3^- reduction

processes, NO_3^- -N was continuously consumed and transformed into other N pools such as NH_4^+ -N, ON, N_2 and N_2O . Different OC sources also affected NO_3^- -N consumption rate, possibly through influencing activities of enzyme and/or microorganisms (Bonin *et al.*, 1999). However, the NO_3^- -N concentrations did not decrease drastically while the atom% of NO_3^- - ^{15}N did, indicating that nitrification was occurring during incubations, which generated NO_3^- - ^{14}N and diluted the NO_3^- - ^{15}N pool. Furthermore, Rice and Tiedje (1989) pointed out that NO_3^- assimilation could be inhibited by 60% and 80% under low (0.1 mg N kg^{-1} dry soil) and higher soil NH_4^+ -N concentrations (10 mg N kg^{-1} dry soil), respectively. In this study, NH_4^+ -N concentrations in most treatments were higher than 10 mg N kg^{-1} dry soil over the 24-h incubations, hence NO_3^- assimilation was probably inhibited and the formed ON- ^{15}N might come from NH_4^+ -N immobilization. The low NH_4^+ - ^{15}N atom% also indicated that NH_4^+ - ^{15}N generated through DNRA probably converted into the ON pool.

Different OC additions significantly increased soil DNRA rates during the first 12-h incubation in the present study. As a common exogenous OC source, glucose has been widely selected to estimate the effect of C on soil DNRA process, whereas the existing results of its effect on DNRA are contradictory. Some studies demonstrated that the addition of glucose could support respiration as well as fermentation, thus stimulating DNRA (Buresh and Patrick, 1978; Yin *et al.*, 2002). However, DeCatanaro *et al.*, (1987) and Chen *et al.*, (1995) found that the addition of glucose did not influence soil DNRA. Some other publications also indicated that other C sources such as straw, glycerol, methanol and succinate had little effects on soil DNRA process (Buresh and Patrick, 1978; Yin *et al.*, 1998). Buresh and Patrick (1978) and Yin *et al.*, (1998) attributed these findings to the fact that the mentioned C sources were poor substrates for fer-

mentation. However, there are three distinct pathways of microbial DNRA when available reductants such as sulfide and reduced iron are present, fermentative DNRA, respiratory DNRA and Chemoautotrophic DNRA (Roberts *et al.*, 2014). Hence, it is possible that the above mentioned C sources did not favor the three DNRA pathways controlled by the species of soil bacteria. In the present study, additions of glucose significantly increased soil DNRA rates compared with the NC treatment, indicating that bacteria involving in the DNRA process exist in the collected sediments of Chongming Dongtan wetland. Nevertheless, what kind of DNRA bacteria dominated the process are still unclear and deserve further investigations.

Low molecular organic acids are commonly present and important factors in affecting transformation and transfer of heavy metals in terrestrial ecosystems (Schwab *et al.*, 2008), but their effects on simulating soil DNRA are still unknown. Results of this study indicate that the four organic acid treatments have positive effects on soil DNRA, and the effects on enhancing soil DNRA rate follows the order: OL>CT>AT>ML. The differences are presumably caused by the different decomposition rates of organic acids. In general, organic acids are decomposed by microorganisms through two pathways, oxidation and decarboxylation (McCollom *et al.*, 2003). Some studies indicated that the decomposition rates of citrate and oxalate were faster than acetate (Jones *et al.*, 2001; Hees *et al.*, 2002). The fast degradations could rapidly provide energy for soil DNRA (Morley and Baggs, 2010). When the organic acids were almost entirely decomposed by soil microorganisms, the decomposition rates would be stable (Jones, 1998). Consequently, the soil DNRA rates in OL and CT were significantly higher than that in AT over the 12-h incubation, while they were higher in AT rather than OL and CT over the second 12-h incubation. Nevertheless, whether soil DNRA rate is associated to the

decomposition rate of OC is still unclear and need further study. In the Chongming Dongtan wetland, the proportion of vegetation coverage area to total area is high (Gan *et al.*, 2009). Large root exudates and plant residues may lead to high concentrations of soil organic acids. Consequently, glucose may not be the most appropriate C source for understanding the regulation of soil DNRA capacity *in situ*.

In treatment NC, the relative potential of DNRA was about 5% which was in agreement with some previous studies (Tobias *et al.*, 2001; Yin *et al.*, 2002), implying that the SOC was limiting for soil DNRA, possibly due to lack of energy. Moreover, additions of ^{15}N increased concentrations of soil NO_3^- -N as well, which created low C/NO_3^- ratios in NC soils. It is widely accepted that the low C/NO_3^- ratio is unfavorable for DNRA (Rütting *et al.*, 2011), thus the DNRA process must be weak in NC treatment. However, the effects of C/NO_3^- with different OC sources on soil DNRA are still unclear and need to be investigated in the future. In addition, soil NH_4^+ - ^{15}N and ON- ^{15}N atom% showed little increase over the second 12-h incubation, thus induced low soil DNRA rates in all-treatments. This result demonstrated that DNRA was a fast process in soils, which was in consistent with some previous studies (Yin *et al.*, 2002; Lu *et al.*, 2013).

5. Conclusion

In this study, the effects of various C sources on soil DNRA rate were estimated using the ^{15}N tracer technique. After the 24 h incubation, the results showed that concentrations of inorganic N, ON as well as the ^{15}N atom% of NO_3^- -N were lower compared with the initial values, while ^{15}N atom% of NH_4^+ -N and ON showed increasing tendencies, indicating that DNRA, DNF, inorganic N immobilization and ON mineralization occurred during the incubation periods. Over the

first 12-h incubation, soils with OL and CT exhibited greater soil DNRA rates as well as the relative potential of soil DNRA than that with GC, demonstrating that organic acids have different impacts on soil DNRA from glucose. The reason might be the different decomposition rates of various OC compounds influencing the available energy provided for DNRA microorganisms. In contrast, soils with no addition of exogenous C showed lower soil DNRA rates, presumably due to the lack of available energy and the low C/NO_3^- ratio. In addition, soil DNRA rates over the 12-h incubation were significantly higher than those over the 24-h incubation, demonstrating again that DNRA is a fast process. These findings indicate that soil DNRA can be regulated by different OC sources, suggesting that only taking glucose as C source may not reflect the soil DNRA capacity well in the field. In the future, more attention should be paid to investigate how soil pH, different quantities of various OC types as well as C/NO_3^- ratio with different OC types affect soil DNRA process.

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