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Blank experimental study on the determination of nitrogen and oxygen isotopes by chemical conversion method

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The reduction of nitrate to nitrous oxide by chemical conversion has the characteristics of simple operation and high sensitivity. Therefore, it is widely used in the pretreatment of nitrogen and oxygen isotopes. In this paper, a series of blank experiments were performed to determine various factors influencing the determination process for the chemical conversion method. To determine blank background values for reagents such as deionized water, NaCl and cadmium powder used in the experiment, the optimal experimental conditions of NaN_3, NaCl and cadmium powder were determined to maximize the nitrate reduction rate. The results showed that NaCl was burned at 450 °C for 48 hours, and the placement time was not more than one day, which could minimize the pollution introduced by NaCl. When 0.3 g of cadmium powder and 600 nmol of NaN_3 were added, the overall reduction efficiency reached 90%. After measuring actual standard samples, the method demonstrated a good accuracy and applicability.

Introduction

Nitrogen is an important component for all living things, the primary limiting factor for the productivity of most terrestrial ecosystems and affects the global nitrogen cycle. Nitrate is an important nitrogen source in terrestrial and aquatic ecosystems. With the increase in fossil fuel combustion and agricultural activities, the importance of nitrate in atmospheric deposition, plants, soil and aquatic ecosystems has become the focus of environmental science and ecological research.[1–4] The isotopic composition of nitrogen in nitrate ($\delta^{15}N$ and $\delta^{18}O$) can provide valuable information to characterize the dominant sources of nitrate contamination.[5] The nitrate stable isotope technique, with its tracer, indicator and integrative functions, has been widely used to study the dynamics and sources of nitrate in atmospheric deposition, aquatic ecosystems, soil and plants.[6–10]

With the development of testing technology, the latest methods for the determination of nitrogen and oxygen isotopes in nitrate are the chemical conversion method and biological conversion method. The biological conversion method has more requirements in the laboratory; the bacterial survival

conditions are strict and the survival rate is lower.[11] However, chemical conversion is easy to operate and the mechanism is simple where nitrate is reduced to nitrous oxide in only two steps.

Lachouani *et al.* used vanadium chloride (VCl_3) as a reducing agent in the first step to convert NO_3^- into NO_2^- and sodium azide was used in the second step under acidic conditions to convert NO_2^- into N_2O.[12] However, this method could not determine the value of $\delta^{18}O$ and the nitrate pollution in the second step of the reductant, sodium azide, led to a larger blank value for the entire method. McIlvin *et al.* reduced the nitrate to nitrite with sponge cadmium or active cadmium in the first step of the reduction process.[13] The greatest advantage of this method is that it could simultaneously determine the nitrogen and oxygen isotope composition in nitrate. However, the preparation of sponge cadmium or active cadmium is complicated. Therefore, Tu *et al.* improved the azide method for the analysis of nitrate isotopes.[14] In other words, NO_3^- was reduced to NO_2^- using cadmium powder instead of sponge cadmium or active cadmium under an ultrasonic wave. In addition, the amount of the toxic azoic acid acetate buffer in the second reduction reaction was reduced to 1/100th the original trial amount. The method is simple in operation, short in preparation time and easy to establish.[15]

The results from Bell *et al.* showed that the background nitrate contamination introduced by different brands of KCl were different.[16] However, in the chemical denitrification process, more chemical reagents such as NaCl, NaN_3, Cd power, *etc.* are used in the reagent preparation process. Different levels of nitrogen pollution are inevitable. Therefore, the background

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value of nitrogen introduced by the reagents during the chemical denitrification process could not be ignored. At present, there are few studies on reducing the influence of the blank background value on a nitrogen-oxygen stable isotope measurement by controlling the pretreatment method and chemical reagent process.[17]

Based on the above analysis, the blank value and reduction rate were studied using the modified azide method. More specifically, the blank value of each reagent was first measured. The experimental blank value was reduced by changing the NaCl placement time after firing and the purge time for the sodium azide acetate buffer. The amount of cadmium added, the addition of sodium azide and the reaction time in the nitrous oxide experimental conditions were optimized to ensure a high yield.[18–21]

In this paper, we examined the blank background values for NaCl, Cd power and deionized water with the chemical denitrification method, determined the optimal experimental conditions for the method and established a good experimental basis for the subsequent detection of sample isotope values. By measuring the isotope value of the natural sample, it showed that the above method has good applicability.[22]

Experimental

Sample analysis

There were two main steps performed before determining the nitrogen and oxygen isotopes in the sample.[14] First, nitrate was reduced to nitrite by cadmium powder. Specifically, a sample containing 25 nmol of nitrate was added to a 10 mL centrifuge tube, followed by dilution with deionized water to 5 mL. NaCl was added to provide a final Cl^- concentration of 5 M. Then, 0.3 g of Cd powder without pretreatment was added, followed by 0.1 mL of a 1 M imidazole solution. The centrifuge tube was sealed, mixed, placed in an ultrasonic environment, sonicated at 40 °C for 2 h and allowed to stand at room temperature overnight after sonication. Second, sodium azide reduced nitrite to nitrous oxide. Specifically, 0.65 g of NaN_3 in a 20 mL headspace bottle was added to 5 mL of deionized water in a fume hood and the mixture was shaken. Then, 5 mL of a 20% acetic acid solution was added for acidification. The solution was purified with the reagent at 70 mL min^{-1} N_2 for 1.5 h (long needle air intake, short needle gas outlet) to remove the N_2O gas in the solution. The supernatant was removed from the centrifuge tube in the 4 mL to 20 mL headspace bottle. The rubber plug and aluminum cover were installed. The above reaction was added to each headspace bottle with 0.2 mL of NaN_3 buffer. The mixture was shaken for 30 min and the reaction was terminated by the addition of 0.4 mL of 10 M NaOH. Lastly, a certain amount of gas in the upper part of the headspace bottle was injected into an isotope ratio mass spectrometer with an automatic preconcentration device to determine the nitrogen and oxygen isotope ratio of N_2O in the gas sample.

Experiment 1: effect of NaCl on its background N_2O value

To evaluate the background N_2O value for NaCl caused by the firing temperature for NaCl and the time after firing, NaCl was

pre-combusted at 450 °C and 550 °C for 48 hours in the muffle furnace. Then, NaCl was placed in a glass vacuum dryer for different days (1 to 6 days). The purpose of the pre-combusted NaCl was to remove any moisture and other impurities from the crystal.

Experiment 2: effect of whether or not the supernatant was filtered on the second step of the reduction

Since the experiment was performed two steps, the solution containing the cadmium powder obtained in the first step of the reduction process may have affected the second step reaction. The solution obtained in the first step was filtered to see if filtration had an effect on the experimental results. We chose two different types of filters, 0.2 μm and 0.45 μm hydrophilic filters.

Experiment 3: effect of the Cd powder addition on the reduction efficiency of NO_3^- to NO_2^-

In order to maximize the NO_3^- reduction yield, we checked the effect of the Cd powder addition on the reduction efficiency. In this experiment, except for the amount of Cd powder, all reaction conditions were the same as those in the previous experiment. Before this experiment, we compared the reduction rate of nitrate during the first step of the reduction process with several of the same experiments.

Experiment 4: effect of the NaN_3 purge time on its background N_2O value and NaN_3 on the reduction efficiency of NO_2^- to N_2O

In the experiment, the sample was purged with a nitrogen gas flow to remove the nitrogen impurities in NaN_3 and eliminate the negative pressure in the headspace bottle. To determine if the background N_2O value for NaN_3 was affected by its purge time, we tested four different times (0.5 hours, 1 hour, 1.5 hours and 2 hours). In the second reduction reaction step, NO_2 was converted into N_2O using the azide buffer. Because NaN_3 is extremely toxic and volatile, reducing the amount of NaN_3 is important for the experiment. Therefore, we implemented a program to test its minimum usage without affecting its results.

Results and discussion

The whole blank composition of chemical conversion method

Based on the above experimental methods, we first determined the blank composition of some major substances. These substances included deionized water (DIW), NaCl, Cd and a total blank composition. The m/z 44 peak amplitude detected by the mass spectrometer indicated the N_2O content in the substance (Fig. 1). Then, the value of the m/z 44 peak represented the ion signal strength and was based on the response value for N_2O. We analyzed the composition of the total blank during the experiment by measuring the m/z 44 peak.[16] As shown in Fig. 1, a lack of attention to detail could result in different blank errors, even with the same operation. Therefore, we needed to refine the details of each step. If NO_3^- was not
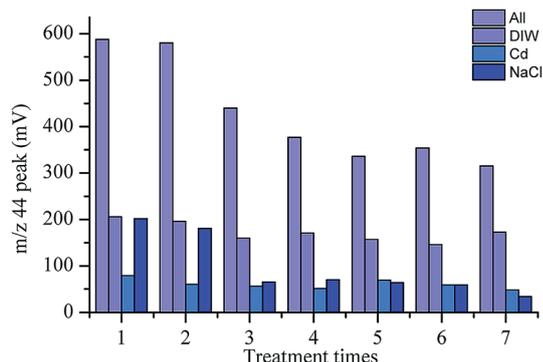
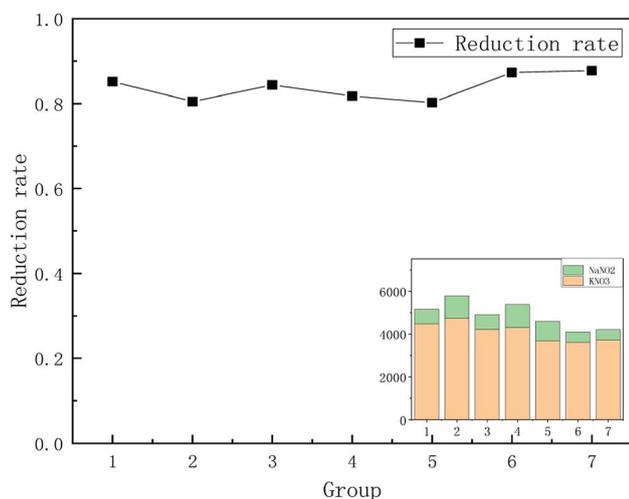Fig. 1 Variation in the peak amplitude range (m/z 44) for three main substances and the total blank composition.

Fig. 2 Reduction rate for NO_3^- to NO_2^-.

completely converted to N_2O, isotopic fractionation could be caused, resulting in measurement errors. Therefore, we needed to determine the conversion rate of nitrate. We calculated the reduction rate of nitrate by measuring the m/z 44 peak for KNO_3 and $NaNO_2$. After the calculation, we determined that the reduction rate of nitrate during the first step of the reduction was between 80% and 90% (Fig. 2).

The exploration of N_2O background value in NaCl

The N_2O produced by the NO_3^- reduction was introduced into an isotope-ratio mass spectrometer. The N and O isotopic composition was measured for N_2O. The value for N_2O was closely related to the measured isotope value. It was necessary to explore the N_2O background value in the reagents used. We found that the blank value for deionized water in the first two experiments was about 200, which was higher than the other experimental values (150–170). This was because the blank value for deionized water in the first two experiments was relatively large after being added for a period of time. Therefore, deionized water was used immediately after receival. Then, the value for the m/z 44 peak in Cd ranged from 30 to 80. In the previous experiment, we determined that NaCl had a greater impact on the background value (Fig. 1). Therefore, it made sense to explore the impact of NaCl on the blank value. In this experiment, the variation in the m/z 44 peak for NaCl was larger and the difference was obvious. The minimum value was 33 and the maximum value was 202. This indicated that although the blank for deionized water was relatively large, the influence of NaCl was the largest.[18] This effect was minimized by making the standards and blanks in the same NaCl solution.

NaCl was analyzed by X-ray diffraction. As shown in Fig. 3, the content for the other impurities in the NaCl reagent was all below the normal range, which indicated that the higher N_2O background value in NaCl was caused by operation and pretreatment during the experimental process.
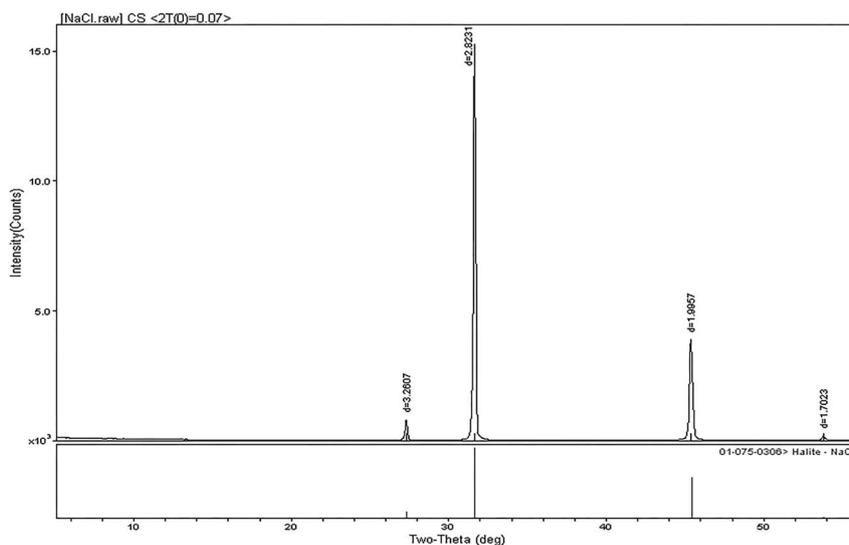

Fig. 3 The X-ray diffraction pattern for NaCl.

The NaCl added in the experiment required pre-combustion. By comparing the combustion at two temperatures (450 °C and 550 °C), we saw that there was no distinct difference between the two temperatures (Fig. 4). The results of our study suggest that a pre-combustion of NaCl at 450 °C ensured that the contaminant content was minimized. We did not find a significant effect of the firing temperature on the m/z 44 peak amplitude for the NaCl blanks. However, we did find that the NaCl placement days was correlated with its blank value. As shown in Fig. 5, the longer the placement time, the larger the N_2O background value for NaCl. We speculated that this phenomenon might have been due to increased reagent purity reducing other background molecules that interfered with the bacterial conversion of NO_3^- into N_2O. Therefore, NaCl was used immediately on the day of pre-combustion.

NO_3^- conversion efficiency

Cadmium powder plays a vital role in the first step of the reduction process as it has the ability to reduce nitrate to nitrite. By changing the amount of Cd powder added, we found that the m/z 44 peak for NO_3^- and NO_2^- changed significantly. As the added amount of cadmium powder increased, the peak decreased. More specifically, when the added amount of cadmium powder was less than 0.3 g, the m/z 44 peak value for NO_3^- showed no significant changes. However, when it was larger than 0.3 g, the m/z 44 peak value began to decrease. As the cadmium powder content increased to 1.5 g, the m/z 44 peak value for NO_3^- was reduced to zero. The reason, however, for this situation was not that the reduction capacity of the cadmium powder was insufficient. The reduction was too strong due to the excessive cadmium powder content, which further reduced N_2O to N_2, making it impossible to detect in an isotope mass spectrometer. The results showed that 0.3 g of Cd powder efficiently reduced the 5 mL samples from NO_3^- to NO_2^-. In addition, the m/z 44 peak for NO_2^- also decreased with the addition of Cd powder. These results indicated that the optimal amount of Cd powder was 0.3 g (Fig. 6).

Based on previous research, in order to eliminate the influence of cadmium powder on the second step in the reduction process, we further explored whether filtration of the supernatant obtained in the first step of the reduction process for cadmium powder would affect the second step of the reduction (Fig. 7). The m/z 44 peak values for each substance measured before and after filtration were compared. Then, we found that the results were consistent. Therefore, the supernatant could be used without filtration.

Influence of NaN$_3$

NaN$_3$ was a key reagent throughout the experiment as it formed an azide buffer with acetic acid and reduced NO_3^- to NO_2^-. With the increase in the NaN$_3$ consumption, we found that the m/z 44 peak value for NaNO$_2$ increased. However, when the added amount of NaN$_3$ was more than 600 nm, the growth
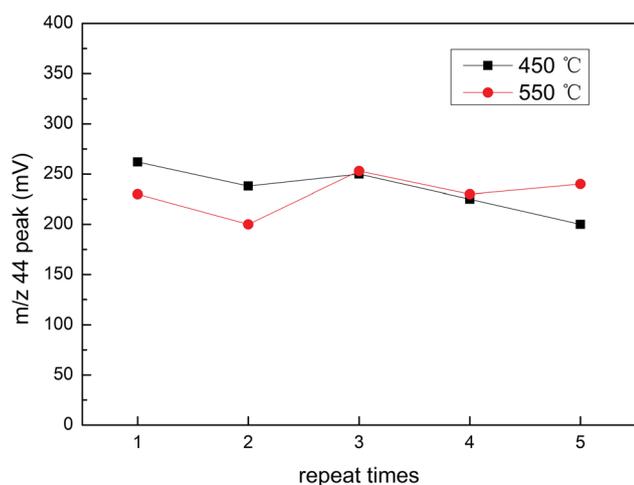

Fig. 4 Correlation between the firing temperature of NaCl and its m/z 44 peak amplitude.

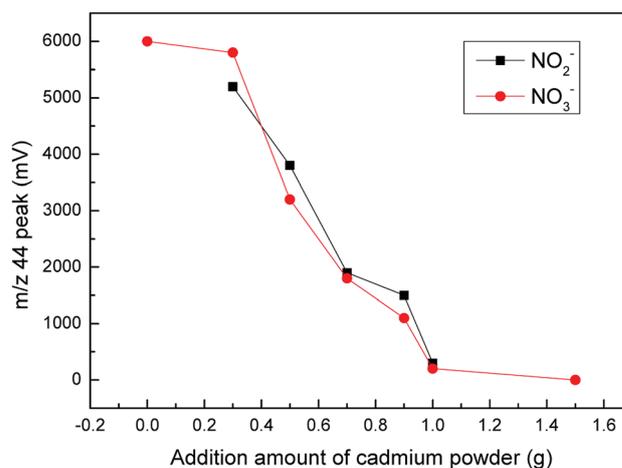Fig. 5 Correlation between the NaCl placement days and its m/z 44 peak amplitude.

Fig. 6 Relationship between the m/z 44 peak amplitudes for the NO_3^-, NO_2^- and Cd powder addition.

Fig. 7 Effect of the supernatant filtration on the reduction.

Fig. 9 Effect of the purge time on the m/z 44 peak amplitude for NaN$_3$.

range decreased, which meant that the reduction efficiency was not significantly improved (Fig. 8). We could also conclude that 200 nmol of NaN$_3$ could not completely reduce 25 nmol of NO$_3^-$. If too much NaN$_3$ is added, it will increase the pollution during the experiment, which may cause unnecessary harm. Therefore, 600 nmol was selected as the best added amount of NaN$_3$. The purge time for NaN$_3$ had an effect on the m/z 44 peak value of NaN$_3$ (Fig. 9). The results showed that the measured m/z 44 peak value for NaN$_3$ was 261 when the purge time was 0.5 hours. As the purge time increased, the m/z 44 peak value was lower. However, when the purge time was increased from 1.5 hours to 2 hours, the change in the m/z 44 peak was very small. The numerical difference was only 8. Therefore, we think that 1.5 hours already had a good purge effect. Taking into account the time and cost, we chose 1.5 hours as the best purge time.

Calibration of measurement results

It can be seen from the above studies that in the process of chemical denitrification, the nitrogen and oxygen isotope
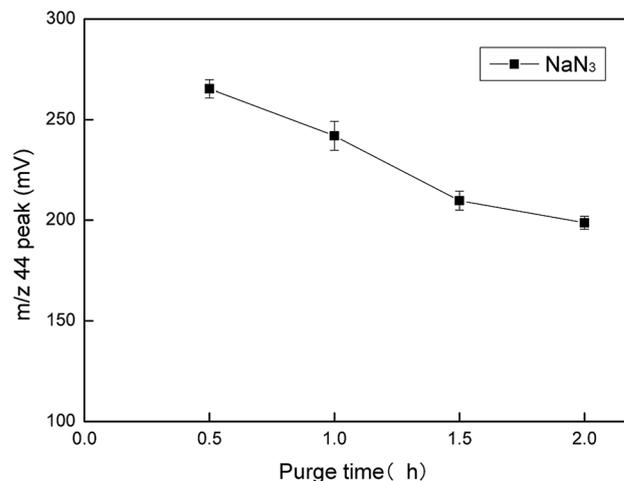
determination will inevitably produce blank pollution, so we used the standard material solution to prepare the standard curve to correct the measurement results.[23] Commonly used international standard substances that participate in the calibration curve are USGS-32(KNO$_3$), USGS-34(KNO$_3$), USGS-35(NaNO$_3$) and IAEA-N3(KNO$_3$). The calibration equation for the δ^{15}N value is:[14]

$$\delta^{15}N_{NO_3^- \text{ sample}} = \left(\delta^{15}N_{N_2O \text{ sample}} - \text{intercept}\right)/\text{slope}$$

where the intercept and slope were obtained from the linear regression, which was established by the measured δ^{15}N values of the four NO$_3^-$ standards against the assigned δ^{15}N values.

The standard curve shown in Fig. 10 indicates a good correlation between the measured δ^{15}N value and the true value of δ^{15}N. In Table 1, we list the true values and corrected values that passed the standard curve of the four international standard samples. We found that the blank contamination could be calculated and calibrated using the calibration curve. Therefore,
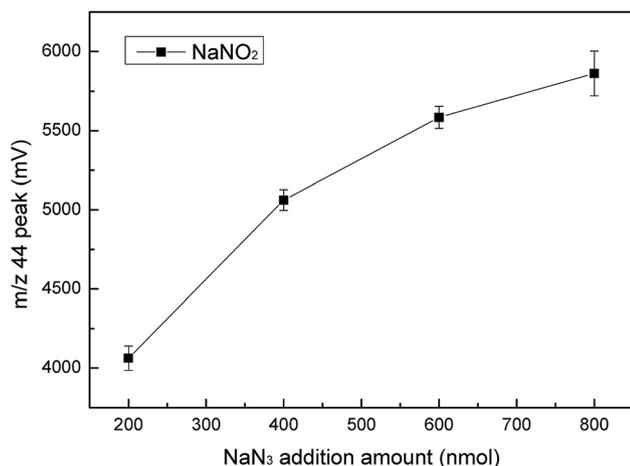

Fig. 8 The m/z 44 peak amplitude for NaNO$_2$ changed as the NaN$_3$ usage increased.

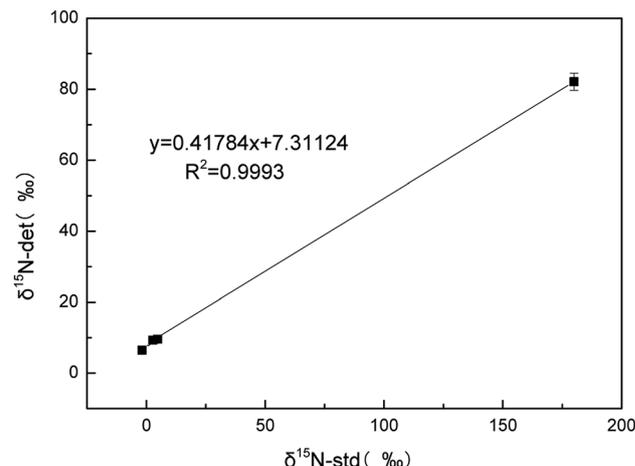

Fig. 10 Linear regression analysis of the measured values and true values for δ^{15}N in four standard substance solutions.

Table 1 Results from the nitrogen isotope determination of the international standard samples

Standard sample	Standard value (‰)	Calculated value (‰)	RE (%)
USGS-32	180	185	2.7
USGS-34	−1.8	−2.0	11.1
USGS-35	2.7	3.3	22.2
IAEA-N3	4.7	5.3	12.7

during the chemical experiment process, as long as we strictly followed the above measurement conditions, the blank pollution in the experiment could be corrected using the standard curve.

We believe that the chemical conversion method for the determination of nitrogen and oxygen isotopes can more accurately determine the sample.

Conclusion

This paper presented a relatively simple, highly accurate chemical conversion method (azide method) for the determination of nitrogen and oxygen isotope values in nitrate. Our study revealed the influencing factors for the blank background value in this method and determined the best experimental conditions to improve the reduction conversion efficiency.

By testing the blank background values in the experiment, it was found that NaCl was the root cause of the fluctuation in the blank value. Then, we explored the cause of the change in the blank value by changing the burning time of NaCl and the time of placement. We found that a temperature of 450 °C was sufficient to remove nitrogen impurities. However, NaCl should be used immediately after burning and should not be added for a long time. After adding 0.3 g of Cd powder and 600 nmol of NaN_3, NO_3^- was completely reduced and the reduction effect was better.

Afterwards, we plan to apply this method to practical measurements, which can be used to measure nitrogen sources in soil and rivers.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Notes and references

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