ANALYSIS OF CHARACTERISTICS OF NITROGEN MIGRATION IN THE PROCESS OF VEGETATION CONCRETE DEGRADING AMMONIA NITROGEN BASED ON THE ^{15}N ISOTOPE TRACER TECHNIQUE

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Abstract. Aiming at analyzing the contribution of every part of vegetation concrete system degrading ammonia nitrogen and exploring the nitrogen migration characteristics during denitrification process, ^{15}NH$_4$Cl tracer was used and added to six airtight glass containers in this study. Samples of concrete aggregate, Bahia grass (Paspalum notatum Flüggé), water and air were acquired on the 1std and 8thd. The results showed that the ^{15}N Atom % of air samples had no obvious change. ^{15}N tracer in each kind of samples taking up the percent of the initial dosing quantity were respectively, on the 1std: Bahia grass 3.8%~6.6%, aggregate 11.9%~17.2%, water 2.9%~80.5%, loss 3.3%~3.8%; on the 8thd: Bahia grass 65.3%~68.7%, aggregate 25.6%~26.2%, water 4.6%~4.8%, loss 0.8%~3.9%. Variance of the ^{15}N abundance of each kind of samples indicate that ^{15}NH$_4$Cl tracer added in the culture solution was mainly absorbed by aggregate at early phase (1 d), while by plant assimilation at later phase (8 d).
Keywords: *nitrogen migration, concrete aggregate, Bahia grass, ^{15}N tracer, plant assimilation*

Introduction

Nitrogen is a necessary element for organisms and is also one of the main elements of the water eutrophication. With the expansion of the population, and the rapid advance of industrialization, agricultural modernization and urbanization, eutrophication has become a global water pollution problem. To this, a lot of studies (Hopfensperger et al., 2014; Winter et al., 2015; Zhou et al., 2022) aimed at the occurrence and development mechanism and control technology of eutrophication have resulted in considerable progress. Such as wetlands, ecological floating bed *et al.*, technologies in view of the eutrophication, are widely used in water environment restoration. Among them, the vegetation concrete embankment technique (Faiz et al., 2022; Xiong et al., 2023) has drawn more and more concern as it benefits for water purification, environmental greening, improving the ecological landscape and slope protection. Vegetation concrete refers to a kind of ecological slope protection material applied to the eutrophication of rivers and lakes. It is based on a certain aperture, a certain porosity of special concrete for the skeleton. In concrete pore filling material needed for plant growth, root grows in pores or penetrates concrete matrix in the soil beneath the porous concrete skeleton.

As a non-radioactive, non-destructive tracer, stable isotope ^{15}N is widely used in tracing inorganic nitrogen source, migration, and transformation on all kinds of water environmental research studies (Panno et al., 2008; Chang et al., 2009; Liao and Inglett, 2014; Ding et al., 2015). Stable isotope technique is a kind of application technology developed in the 1970s. It has multiple functions such as tracing, integration, and instruction, and has the characteristics of rapid detection, accurate results, and noninterference.

Previously reported works on vegetation concrete skeleton (porous concrete), concrete composition and optimization, screening adaptive plants, water permeability and denitrification & phosphorus removal effect, have achieved fruitful results (Zhang et al., 2012; Yue et al., 2012; Chen et al., 2013). However, the research on the pollutant removal mechanism of vegetation concrete is relative insufficient. To analyze the ability of various parts of the vegetation concrete system in the process of ammonia nitrogen removal and explore the characteristics of nitrogen migration, experiments presented in this paper were conducted in a sealing good transparent glass container for denitrification. Mass spectrometry method was used to test each component of vegetation concrete quantitatively, and the contribution to the denitrification and nitrogen migration characteristics of each vegetation concrete's component with the help of ^{15}N isotope tracer technique with ^{15}NH$_4$Cl as the tracer was also explored.

Experimental section

Materials

Vegetation concrete skeletons (porous concrete) were made with zeolite, steel slag and pumice. After procedures of reducing alkali, perfusing raw materials (main ingredients are pond sludge, organic fertilizer, slow-release fertilizer), Bahia (Paspalum notatum flugge) seeds (about 15 g/m^2) were spread on the porous concrete. After a week the seeds were germinated. After 60 days, overground part of Bahia grass grew well that roots vegetating through the concrete specimen. Vegetation concrete skeleton and Bahia grass fit into experimental vegetation concrete pot (here in after refers to potted plants). After four months of cultivation (calculated from seed germination), six pots well-growing vegetation concrete were selected for the experimental study.

Potted plants type C, mass ratio of zeolite to steel slag is 1:3; Potted plants type F, mass ratio of zeolite to pumice is 1:3; Ammonium chloride (^{15}NH$_4$Cl, ^{15}N, 99%, Cambridge Isotope Laboratories, Inc.).

The weight of the vegetation concrete is 3.5 kg, among which the aggregate quality is 2.0 kg. The basic structure was shown in *Fig. 1*. From *Fig. 1*, it can be seen that the porous concrete cylindrical part is 10 cm high (diameter 20 cm) including porous concrete and nutrient soil, and upper is well-growing Bahia grass (plant height 30 ~ 60 cm). Plant roots penetrate porous concrete specimen.

Experimental design

Pots for test were harvested from cultivating greenhouses, then they were placed in a shallow pool with water immerge 14 days to elute root soil and adapting to the hydroponic conditions. The size of the six transparent and good sealing glass incubators was: length × width × height = 350 × 350 × 800 mm. A water inlet tap is equipped at 100 mm height from the bottom of the device, and an air sample tap is set at the top of the device.

15 L of water was added to the incubator first, and then the Bahia grass vegetation concrete was laid on the steel mash support in the center of the incubator bottom. Incubator device was shown in *Fig. 2*. All incubators were put in well-lit place to incubate 7 d with adding water timely. Test was begun to conduct after the vegetation concrete was stabilized. The processing methods for each device were shown in *Table 1*. Before the test, 2 pots of Bahia grass vegetation concrete (KB_1, KB_2) were broken for sampling, then samples of concrete, plants (Bahia grass), water and air were made to determinate indicators such as TN、 ^{15}N、 NO_3^--N、 NH_4^+-N and NO_2^--N as the background value.
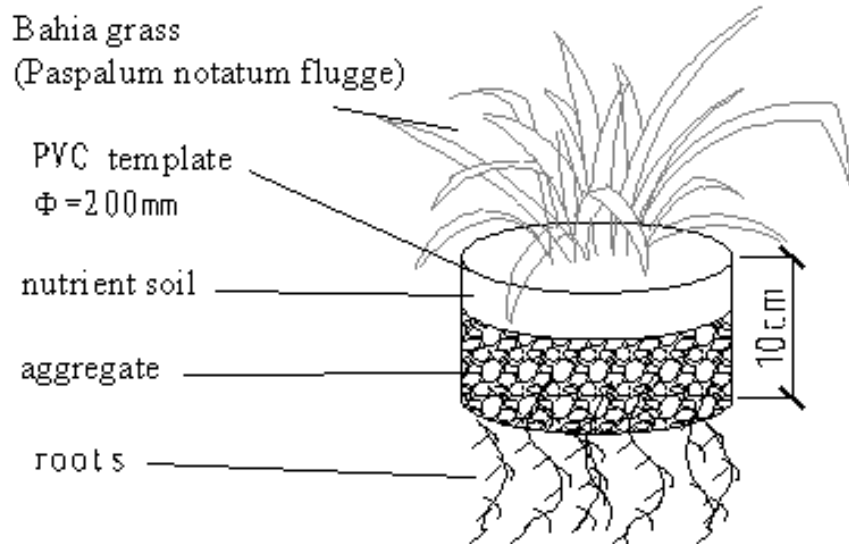

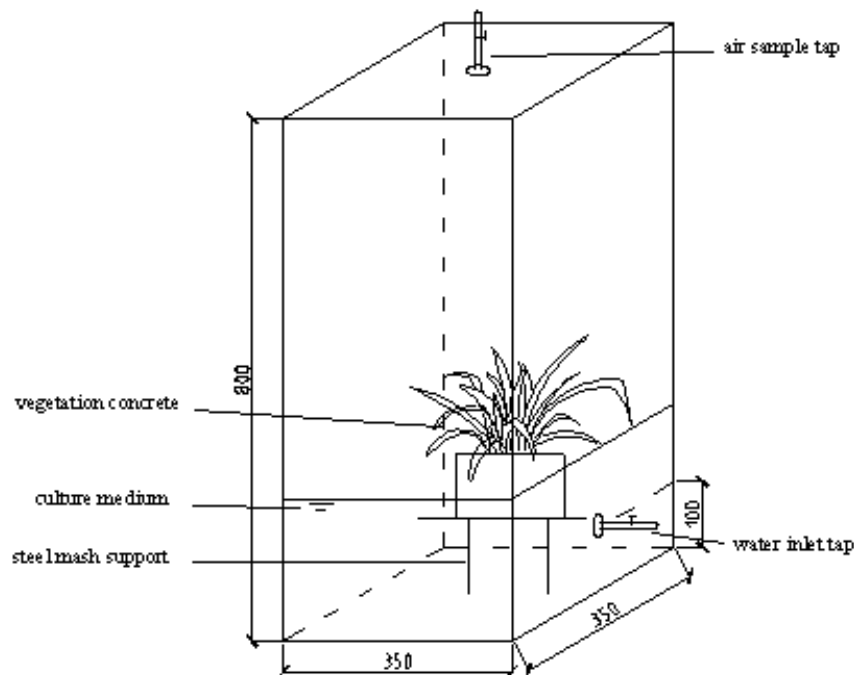*Figure 1. Basic structure of vegetation concrete for experiment*

Figure 2. Incubator device (unit of size: mm)

Table 1. The processing method of each sample

Test number	Test time	Types of vegetation concrete
KB1	0d	type C
KB2	0d	type F
1	1d	type C
2	8d	type F
3	1d	type C
4	8d	type F

Device 1&2 were sampled on the 1st day after the trial began, and device 3&4 were sampled on the 8th day. Type C pots were place in singular number devices and type F pots were place in dual number devices. 15 L culture medium (preparation of NH_4Cl and KH_2PO_4; ^{15}N tracers, ammonia nitrogen concentration for 2 mg·L^{-1}) were added to devices 1 to 4. All devices were sealed. Sustained observation was kept for eight days, and on the 8th day specimens of 100 mL air, plant, concrete and water were prepared for TN, ^{15}N, NO_3^{-}-N, NH_4^{+}-N and NO_2^{-}-N indicators determination.

Sample preparation and analysis

Collection and pre-processing method of sample

(1) Concrete sample: each group of vegetation concrete aggregate (composite aggregate) was crushed, and then dried under 40 °C for 24 h, then it was smashed and sieved through 100 mesh sieve (sieve pore diameter is 0.150 mm). 10.0 g sample was extracted by four points method, and it was sent into the elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) to measure nitrogen content and ^{15}N Atom %.

(2) Bahia grass sample: after the experiment, the pot was taken out, and the concrete block was broken up. The whole plant (including fracture in the block) was picked out, then was dried in the oven under temperature of 80~90°C for 15~30min after washed out. Then the Bahia grass was cooled to 65 °C in order to dehumidify for 12 ~ 24 h, and it was smashed and sieved through 60 mesh sieve (sieve pore diameter is 0.250 mm). 5.0 g sample was extracted by four points method, and it was sent into the EA-IRMS to measure nitrogen content and ^{15}N Atom %.

(3) Water sample: the water sample was filtered by 0.45 μm *Whatman* filter membrane, and 500 ml of it was extracted, then was kept under -18 °C for cryopreservation.

(4) Air sample: air sample was collected from incubator with a syringe. It was kept in 100 ml air sample bag at room temperature.

Analytical method

The related indexes were measured by China national standard method (Editorial Board of Water and Wastewater Monitoring and Analysis Method of the State Environmental Protection Administration, 2002). Total nitrogen (TN): alkaline potassium persulfate digestion UV spectrophotometric method. Nitrate nitrogen (NO_3^{-}-N): ultraviolet spectrophotometric method. Ammonia nitrogen (NH_4^{+}-N): Nessler's reagent spectrophotometric method. Nitrite nitrogen (NO_2^{-}-N): N-(1-Naphthalene)-Ethylenediamine dihydrochloride reagent spectrophotometric method.

The analytical instrument used to determine TN and NO_3^--N is 752N ultraviolet spectrophotometer, *Shanghai sincere dedication of science and technology innovation, Co., Ltd.* The analytical instrument used to determine NH_4^+-N and NO_2^--N are 721G visible spectrophotometers, *Shanghai sincere dedication of science and technology innovation, Co., Ltd.*

The analytical instrument used to determine pH is HACH HQ30d portable, USA.

Data analysis

^{15}N samples analysis using the difference value method, i.e., the sample's ^{15}N value minus the blank group (background value) ^{15}N value. All analyses were conducted using SPSS software (version 19.0) and Origin (version 8.0).

The value of δ^{15}N was calculated according to the following formula Eq. (1):

$$\delta^{15}N(‰)=\{[\ R(^{15}N/^{14}N)\ _{sample}\ /R(^{15}N/^{14}N)_{standard}]-1\}\times1000 \qquad (Eq.1)$$

In the above formula, R $(^{15}N/^{14}N)$ $_{sample}$ stands for the abundance ratio of heavy isotope nitrogen atoms (^{15}N) in the sample with light isotope atoms (^{14}N) in the sample. R $(^{15}N/^{14}N)$ $_{standard}$ stands for the abundance ratio of heavy isotope nitrogen atoms (^{15}N) in the sample with light isotope atoms (^{14}N) in the international standard atmosphere (IST). If the value of δ^{15}N(‰) is positive, the sample enriches ^{15}N reference to the standard sample; while dilutes, on the contrary.

Results

Water quality indexes of tap water used for preparation of culture medium and ^{15}N culture medium (initial solution) were listed on *Table 2*.

Table 2. Water quality indexes of tap water used for preparation of culture medium and ^{15}N culture medium (initial solution)

Water sample	TN (mg N L^{-1})	NH^{4+} (mg N L^{-1})	NO^{3-} (mg N L^{-1})	NO^{2-} (mg N L^{-1})	pH
tap water	0.29	0.13	-	-	6.9
^{15}N culture medium	2.38	2.02	-	-	7.0

"-": Not detected

Content variation of NH^{4+}-N, NO^{3-}-N, NO^{2-}-N and TN in culture medium

Content variation of NH_4^+-N, NO_3^--N, NO_2^--N and TN in culture medium was listed on *Table 3*. Ammonia nitrogen (NH_4^+-N) content in the aqueous solution decreased with time, from the initial 2.02 mg·L^{-1}, reduced to 1.54 ~ 1.61 mg·L^{-1} (1 d), and then dropped to 0.09 mg·L^{-1} (8 d). 8d's NH_4^+-N concentration had been lower than that of the blank group 0.13 mg·L^{-1}.

Total nitrogen (TN) concentration decreased with time, from the initial 2.38 mg·L^{-1}, reduced to 1.73 ~ 2.00 mg·L^{-1} (1 d), and then dropped to 0.23 mg·L^{-1} (8 d). 8 d's TN concentration had been lower than that of the blank group 0.29 mg·L^{-1}.

Nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) were not detected in all samples.

Table 3. *Content variation of NH^{4+}-N, NO^{3-}-N, NO^{2-}-N and TN in culture medium (mg N L^{-1})*

Test number	NH^{4+}-N	NO^{3-}-N	NO^{2-}-N	TN
KB1 (type C)	0.14	-	-	0.29
KB2 (type F)	0.13	-	-	0.30
1 (type C)	1.61	-	-	2.00
2 (type F)	1.54	-	-	1.73
3 (type C)	0.09	-	-	0.23
4 (type F)	0.09	-	-	0.23

"-": Not detected

The value of $\delta^{15}N$ and ^{15}N Atom% of air samples

Compared with the IST (the natural abundance of ^{15}N atom is 0.366 %), the value of δ^{15}N changed tiny between - 0.164 ‰ and + 0.164 ‰, and the value of ^{15}N Atom % had no obvious change (*Table 4*). This indicates that during the test, ^{15}NH$_4$Cl tracers are not transformed into ^{15}N$_2$ through the physical, chemical, and biological action.

Table 4. *The value of $\delta^{15}N$ and ^{15}N atomic percentage (Atom %) of the air samples in the test devices*

Test number	δ^{15}N (‰)	^{15}N Atom % (%)
KB1 (type C)	+0.167	0.366533
KB2 (type F)	+0.145	0.366525
1 (type C)	-0.155	0.366415
2 (type F)	-0.164	0.366412
3 (type C)	-0.139	0.366421
4 (type F)	+0.180	0.366538

The value of $\delta^{15}N$ and ^{15}N atomic percentage (atom %) of Bahia grass samples and stone samples

The value of δ^{15}N of both the Bahia grass and stone samples were positive, in addition, 1's was less than 2's while 3's was less than 4's (*Table 5*). This indicates that the absorption effect of ^{15}N in each group of vegetation concrete is obvious. From the δ^{15}N value in each group of Bahia grass and stone samples, we can conclude that vegetation concrete type C was superior to F in terms of the adsorption capacity for ammonia nitrogen.

Table 5. *The $\delta^{15}N$ value in each group of Bahia grass and stone samples*

Test number	Bahia grass samples	Stone samples
KB1 (type C)	25.314	17.800
KB2 (type F)	38.633	21.226
1 (type C)	182.311	53.296
2 (type F)	453.127	94.199
3 (type C)	1137.181	89.441
4 (type F)	1370.970	121.244

Statistics of ^{15}N content in each part of vegetation concrete

Statistics of ^{15}N content in each part of vegetation concrete were listed in *Table 6*. The ^{15}N initial dosing quantity in each device was 4.3092 mg. The ^{15}N content of Bahia grass samples and stone samples increases, while that of water samples decreases when incubation time was prolonged. Accounting for the same ^{15}N initial dosing quantity of tracers, the percentage of the tracers in all kinds of samples was shown in *Figure 3*. Tracers distribution were: on the 1st day, 3.8% ~ 6.6% in Bahia grass, 11.9% ~ 17.2% in stone, 72.9% ~ 72.9% in water, and 3.3% ~ 3.3% lost; on the 8th day, 65.3%~68.7% Bahia grass, 25.6%~26.2% in stone, 4.6%~4.8% in water, and 0.8%~3.9% lost. The ^{15}N abundance variation in all kinds of samples showed that the ^{15}NH$_4$Cl markers added to the culture medium were removed relying mainly on the adsorption action of aggregate in the early (1 d), while giving priority to the assimilation action of Bahia grass in the late (8 d).

Table 6. *Statistics of ^{15}N content in each part of vegetation concrete (mg)i*

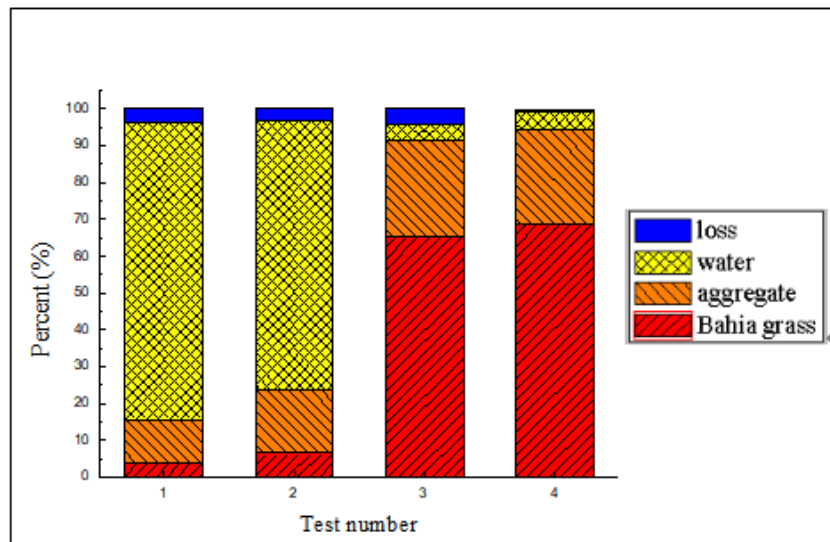| Test number	Bahia grass samples	Stone samples	Water samples	Loss
1 (type C)	0.1649	0.5106	3.4702	0.1635
2 (type F)	0.2830	0.7419	3.1403	0.1440
3 (type C)	2.8158	1.1291	0.1971	0.1672
4 (type F)	2.9604	1.1041	0.2090	0.0357

Figure 3. *The percentage of the 15N tracers in all kinds of samples compared to the initial dosing quantity*

Discussions

Through analyzing the ^{15}N content variation in the samples of aggregate, Bahia grass, culture medium and air, we found that the adsorption action of aggregate played a dominant role in the early stage, and the assimilation action of Bahia grass played a dominant role in the late stage, while the contribution of microbial action was almost none. In addition, statistics of δ^{15}N in samples indicated that the adsorption capacity for

ammonia nitrogen of vegetation concrete type C was superior to F. In this test we made quantitative analysis on the migration characteristics of ammonia nitrogen and calculated the contribution to ammonia nitrogen removal of the vegetative concrete matrix (aggregate) and plants (Bahia grass) in the early and late test stage, which is consistent with previous report (Chen et al., 2013).

Vegetation concrete systems are similar to tiny artificial wetland treatment systems, it contains the nutrient soil, microbes, plants, and porous concrete skeleton which act as a filler. Whether its denitrification process is similar to artificial wetland system or not, it's worth further discussing.

Artificial wetland degrading nitrogen combines physical, chemical, and biological function. After artificial wetland operates mature and stable, the packing surface and the plant roots were covered with bacteria biofilm formed by microorganisms. Benefiting from plant roots transferring and releasing oxygen, the roots surrounding environment brings out the aerobic, anoxic, and anaerobic condition in turn, which ensures the nitrogen in the wastewater can not only be absorbed by plants and microorganisms as nutrients directly, but also removed from wastewater by nitrification and denitrification process. Basically, nitrogen in wastewater exists in the form of organic nitrogen and inorganic nitrogen. In general, organic nitrogen is degraded into ammonia nitrogen by microbes, while inorganic nitrogen (NH_4^+-N, NO_3^--N) can be directly ingested by plants as an indispensable substance in the process of plant growth to synthesize plant proteins and other organic nitrogen. Inorganic nitrogen is removed from wastewater and wetland system through the harvest of the plant.

The nitrogen removal mechanism of the wetland includes volatile, substrate adsorption, microbiological nitrification-denitrification, and plant uptake. Ammonia volatilization is a physical and chemical process. The dissociation balance equation of ammonia nitrogen in water is:

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O \qquad (Eq.2)$$

(1) When pH = 9.3, the proportion of NH_3 and NH_4^+ is 1:1, ammonia volatilization is significant; (2) When pH = 7.5 ∼ 8.0, ammonia volatilization is not significant; (3) When pH < 7.5, ammonia volatilization is negligible. During the trial, pH was around 7.0, so ammonia volatilization had little effect on denitrification in system.

The plant's denitrification contribution in this experiment has large difference to the reported study results. Plenty of research results showed that microbial nitrification-denitrification played a main role in the artificial wetland denitrification process. Research results of Matheson concluded that the plants uptake could contribute only 11 ∼ 15% to the whole denitrification process (Matheson et al., 2002). Many studies had shown that the main mechanism of nitrogen removal in the wetlands was microbiological nitrification-denitrification (Stottmeister et al., 2003). Martin et al. (2013) found that the contribution of microbial nitrification-denitrification to TN removal was 83.3%. Nevertheless, in this study the plant (Bahia grass) played a main role in nitrogen removal. Analyzing ^{15}N of the plant samples showed that on the 8th day, ^{15}N tracers in plants samples took up 65.3% ∼ 65.3% percent of the initiate dose. However, $^{15}N_2$ produced by nitrification-denitrification action was hardly detected, which could ascribe to two aspects. One was that potted plants soaked in often replaceable water repeatedly in the early stage of the test, leading the microbial breeding dormancy; the other was that at the

start of the trial, microorganism in the system was still in the stage of a growth and adaptation, and had not yet blooms. Therefore, substrate adsorption and plant uptake predominates.

The contribution of microbial nitrification-denitrification removing nitrogen was not reflected in this test, which was the deficiency of this study. In the future study, it is recommended that first, cultivate of vegetation concrete for long enough, making the microorganisms breed massively to form biofilm, and then adding tracer began to test. To study the microbial denitrification, expand vegetation concrete denitrification mechanisms of the system.

Matheson et al. (2013) found in the experiment that undetected ^{15}N tracers were 36%. But our experiment was conducted in good sealing glass container, undetected ^{15}N accounted for only 0.8% ~ 3.9% percent of total dosed tracers. There existing tiny loss of ^{15}N in its recycling checking, many reasons accounted for this. For instance, ^{15}N$_2$ produced by microbes was sandwiched and wrapped in soil and plant aerenchyma (Lindau et al., 1988). Drying and sieving in the process of sample preparation were the possible reasons of the ^{15}N loss. ^{15}N loss factors also included the error of the sample testing.

Conclusions

This study provided a deep insight into the contribution of every part of vegetation concrete system degrading ammonia nitrogen. Tracers distribution were:1st day, 3.8% ~ 6.6% in Bahia grass, 11.9% ~ 17.2% in stone, 72.9% ~ 72.9% in water, and 3.3% ~ 3.3% lost; 8th day, 65.3%~68.7% Bahia grass, 25.6%~26.2% in stone, 4.6%~4.8% in water, and 0.8%~3.9% lost. ^{15}NH$_4$Cl markers added to the culture medium was removed relying mainly on the adsorption action of aggregate in the early (1 d), while given priority to the assimilation action of Bahia grass in the late (8 d).

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