Dissimilatory nitrate reduction to ammonium, denitrification and anaerobic ammonium oxidation in paddy soil

Arjun Pandey[1], Helen Suter[1], Jizheng He[1], Deli Chen[1]

[1] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Burnley Campus, 500 Yarra Boulevard, Richmond, Victoria 3121, http://fvas.unimelb.edu.au/. Email: arjunp@student.unimelb.edu.au

Abstract

Nitrogen (N) is the most important yield-limiting nutrient for rice production. Flooding of rice paddies for an extended period of time creates anoxic conditions in soil which can favour a simultaneous occurrence of several microbial N transformation processes, such as dissimilatory nitrate (NO_3^-) reduction to ammonium (NH_4^+) (DNRA), denitrification and anaerobic NH_4^+ oxidation (anammox). Little is known about the role of DNRA and anammox in N cycling in paddy soils, and of the simultaneous occurrence of these N transformations. This study utilized a ^{15}N isotopic approach to determine the rates of DNRA, denitrification and anammox processes simultaneously in a paddy soil. The paddy soil was collected from the Riverina region in New South Wales, Australia and studied under laboratory conditions. The rates of the processes were investigated after a week of flooding of paddy soil after a basal dose of N application at the rate of 1.6 g N m^{-2} (farmers practice in the region). Results showed that DNRA contributed to the formation of 0.34 µmole NH_4^+-N hr^{-1} kg^{-1} soil. Denitrification and anammox produced 3.35 µmole N_2 and 0.65 µmole N_2 hr^{-1} kg^{-1} soil, respectively. Denitrification was the major pathway contributing to N_2 production which accounted for 83% of total N_2 produced. Anammox contributed to 17% of total N_2 production. Considering the bulk density of soil (1.3 g cm^{-3}), it can be estimated that DNRA can retain 0.03 g N m^{-2} day^{-1}, whereas denitrification and anammox can contribute to a loss of 0.58 and 0.11 g N m^{-2} day^{-1}, respectively, after the first week of flooding of paddy soil.

Key Words

Paddy soil, DNRA, denitrification, anammox

Introduction

Paddy soils are fundamentally different from other arable soils due to the extended period of flooding. Oxygen in the flooded soil is rapidly consumed and the anaerobic condition (Eh < 0 mV) is developed within a few days of flooding (Das et al. 2016). However, a few millimetres of the water surface are oxic due to the diffusion of atmospheric oxygen. The anaerobic condition with oxic interfaces ensures the continuous availability of different forms of mineral N such as ammonium (NH_4^+) and nitrate (NO_3^-) (Aulakh et al. 2001).

Nitrogen (N) is often the most important yield-limiting nutrient for rice. Rice culture is highly N intensive in the majority of the world's top rice producing countries but the N use efficiency (NUE) of rice production is below 40% due to the rapid loss of N through several pathways (volatilization, denitrification, leaching, runoff) (Fageria et al. 2010). Urea is commonly used as a N source in rice paddies (Palanivell et al. 2016). Hydrolysis of urea as well as mineralization of organic N in soil produce NH_4^+, and the nitrification of produced NH_4^+ (in the presence of O_2) produces NO_2^- and NO_3^- (Simek 2000). Nitrate is highly mobile and is subject to loss through different pathways (denitrification and leaching) while the NH_4^+ is less mobile and is retained in soil (Huygens et al. 2008).

Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) may be an important N retention mechanism prevailing in paddy soils, however there is currently limited information available. As the name suggests, DNRA transforms the highly mobile NO_3^- to less mobile NH_4^+ thus reducing the loss of NO_3^- from denitrification and leaching. Anaerobic microsites with readily available C are thought to be hotspots for the DNRA process in forest soils (Silver et al. 2001). A recent study suggested that DNRA could also be an important N retention mechanism in paddy soils (Zhang et al. 2015). Denitrification is the major microbial N transformation pathway in paddy soils which alone can contribute to the loss of around 33% of the N applied in rice paddies (Aulakh et al. 2001). Denitrification is the process of microbial reduction of NO_3^- to N_2O and/or N_2 (Aulakh et al. 2001). The anaerobic conditions along with readily available C favour rapid reduction of NO_3^- to N_2, thus, loss of N from paddy soils. Use of N fertilizer significantly increases denitrification loss of N in rice paddies (Aulakh et al. 2001). However, recent studies have indicated that denitrification is not the only process producing N_2 in anaerobic systems (Thamdrup & Dalsgaard 2002; Dalsgaard et al. 2003). The process of anaerobic NH_4^+ oxidation (anammox) carried out by strictly anaerobic

bacteria has been found to be responsible for around 60% of the total N_2 produced in anaerobic sea sediments (Thamdrup & Dalsgaard 2002). During the anammox process, NH_4^+ is oxidised with NO_3^-/NO_2^- (nitrite) to produce N_2 (Dalsgaard et al. 2005). The first known study of anammox in rice paddies indicated that more than 41% of the total N_2 produced can be due to anammox (Zhu et al. 2011). The finding has challenged our previous view on strategies to reduce N loss from the rice paddies. However, there is still a lack of knowledge on how N input in rice paddies affects anammox rates.

Dissimilatory NO_3^- reduction to NH_4^+, denitrification and annamox have a complex relationship in terms of the use of the N substrates. Therefore, a better understanding of the contribution of each of these processes in paddy soil N cycling can only be achieved by a simultaneous study of these processes. However, there are no reported studies that have looked into all these processes simultaneously in terrestrial ecosystems including paddy soils. Therefore, this study utilized a ^{15}N isotopic approach to determine the rates of DNRA, denitrification and anammox simultaneously during flooding of a paddy soil. The objective of this study was to develop an understanding on the significance of the DNRA, denitrification and anammox processes in N cycling in paddy soils during the first week of flooding.

Methods
Soil sampling
Soil samples (0-20 cm) were collected from a rice paddy in the Riverina region of New South Wales (NSW), Australia; the biggest rice growing area in Australia (Kinoshita et al. 2015). Soil samples were collected from five random positions in the rice paddy just before the sowing of rice, and were gently crushed and homogenised after collection. Soil pH was 5.18 and EC was 131.56 μS cm^{-1}. Total soil organic C was 19.8 g kg^{-1} soil and total soil N was 1.77 g kg^{-1}. Soil $NO_3^- + NO_2^-$ was 17.83 mg kg^{-1} dry soil and NH_4^+ was 72.96 mg kg^{-1} dry soil. Approximately 4.5 kilograms of soil was then placed into four replicate PVC pots (15 cm diameter and 25 cm height) and incubated for one month in the glasshouse. The soil was irrigated occasionally to prevent soil from drying out. After one month of incubation, pre-germinated rice seeds were sown and 64.64 mg urea (equal to 16 kg N ha^{-1} basal application which is equal to farmers' practice) was applied to each pot. The PVC pots were flooded with 3-5 cm water 24 hour after urea application. After one week of flooding, the soils from the replicate pots were sampled, composited and homogenised.

Chemical analysis
Chemical analysis was performed in four replicates. Soil pH and electrical conductivity (EC) were determined in soil-water (1:5) suspension. Total C and total N was determined using Dumas method. Soil $NO_3^- + NO_2^-$ and NH_4^+ were determined after extraction with 2M KCl (1:1, soil:KCl solution).

Measurement of DNRA, denitrification and anammox
The DNRA rates were measured using the method described by Trimmer and Nicholls (2009) and denitrification and anammox was measured using the methods described by Thamdrup and Dalsgaard (2002). Briefly, ~3.5 g of the homogenised soil was transferred to 12.5 ml vials (Exetainer, Labco, UK). The vials were then degassed and purged with Helium (He) gas. The vials were filled with He purged water and then pre-incubated for 48 h to deplete soil NO_3^- and O_2. After the pre-incubation 12 sets of vials were added with 100 μL of He purged either $^{15}NH_4Cl$ (> 99 % ^{15}N, 12 mM N), $^{15}NH_4Cl + K^{14}NO_3$ (> 99 % ^{15}N in $^{15}NH_4Cl$, 12 mM N), or $K^{15}NO_3$ (>99% ^{15}N, 12 mM N). Thereafter, microbial activities in the triplicate vials with each of the tracers were stopped by injecting 200 μL of saturated $ZnCl_2$ solution at 0, 6, 12 and 24 h to measure denitrification and anammox. Exactly 4 ml of water in the vials was replaced with He. The vials were vigorously shaken and the headspace gas was analysed for $^{29}N_2$ and $^{30}N_2$ content using GC-IRMS. In exactly the same way, 12 sets of vials were prepared to measure DNRA, but only with $K^{15}NO_3$ (>99% ^{15}N, 12 mM N) tracer. The vials prepared for DNRA were frozen immediately after $ZnCl_2$ injection at each time point. Thereafter, content of the vials was extracted with 2M KCl (1:1 soil:KCl solution) and the $^{15}NH_4^+$ in the samples was determined using alkaline-hypobromite method (Trimmer & Nicholls 2009). Reference vials without ^{15}N tracer amendment were also prepared for both the methods to calculate natural $^{29}N_2$ and $^{30}N_2$ and $^{15}NH_4^+$ production. The DNRA, denitrification and anammox were calculated using the procedure described by Thamdrup and Dalsgaard (2002).

Results and discussion
Measurement of DNRA, denitrification and anammox
As shown in Figure 1, the NO_3^- level in soil was under the detection limit already after 12 h of pre-incubation. There was no excess $^{29}N_2$, $^{30}N_2$ and $^{15}NH_4^+$ production in the reference vials. There was also no

$^{15}N_2$ ($^{29}N_2$ and $^{30}N_2$) production in the vials with only $^{15}NH_4Cl$ tracer, whereas $^{29}N_2$ was produced in the vials added with $^{15}NH_4Cl + K^{14}NO_3$ tracer, which indicates that there is anammox activity in the soil. After the confirmation of anammox activity, DNRA, denitrification and anammox were calculated from the rate of production of $^{15}NH_4^+$ and accumulation of $^{29}N_2$ and $^{30}N_2$ in the vials with added $^{15}NO_3^-$ (Figure 2). Results showed that the DNRA has the potential for production of 0.34 µmole NH_4^+-N hr^{-1} from the reduction of NO_3^- in per kilogram of paddy soils flooded for a week. Denitrification, however, was a dominant N transformation pathway in the paddy soil which contributed to 3.35 µmole N_2 hr^{-1} kg^{-1} soil, whereas anammox rate was 0.65 µmole N_2 hr^{-1} kg^{-1} paddy soil. Considering the soil bulk density (1.3 g cm^{-3}) of the top 20 cm of the collected soil, DNRA can retain 0.03 g N m^{-2} day^{-1} after the first week of flooding, whereas denitrification and anammox can contribute to a loss of 0.58 and 0.11 g N m^{-2} day^{-1} after the time period. Our study showed that favourable conditions for DNRA, denitrification and anammox are created within a week of flooding of rice paddies. It should be noted that, farmers in the region apply more than 70 kg and 50 kg N ha^{-1} during the second and the third topdressing, respectively. As the application of higher dose of N (in the form of urea) changes the level of NH_4^+ and NO_3^- availability of in the soil it can also change the rate of the processes. As the growth of rice plant is almost negligible, it has little effect on N transformations during the first week of flooding. But during the later stages, rice plants supply significant amount of dissolve organic carbon through the root exudates and decaying plant parts, which further strengthen the soil anaerobic conditions (Aulakh et al. 2001). The availability of labile organic carbon can enhance DNRA and denitrification processes (Aulakh et al. 2001; Silver et al. 2001) but its direct effect on anammox is little known. In addition, the rice plants supply O_2 to its roots through aerenchyma. The supplied O_2 also gets leaked to the adjacent soil which creates aerobic environment around the rice roots. The aerobic environment can influence the nitrification and enhance NO_3^- availability (Li et al. 2008). Therefore the rice plants can have a significant effect on the process rates. Nie et al. (2015) observed a significant effect of rice rhizosphere on the rates of denitrification and anammox where denitrification and anammox were higher in nonrhizosphere than in rhizosphere. However, there are no studies conducted so far that looked at rhizospheric effect on DNRA rates.
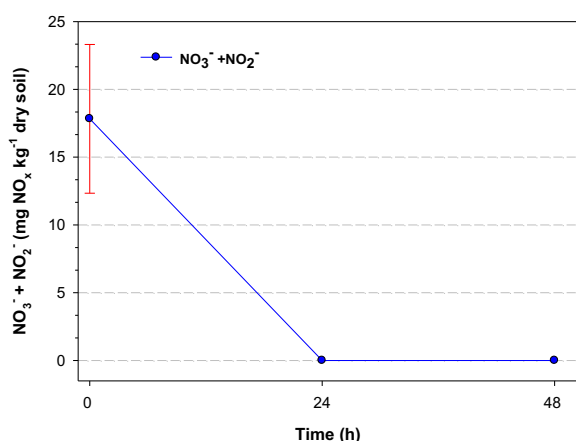

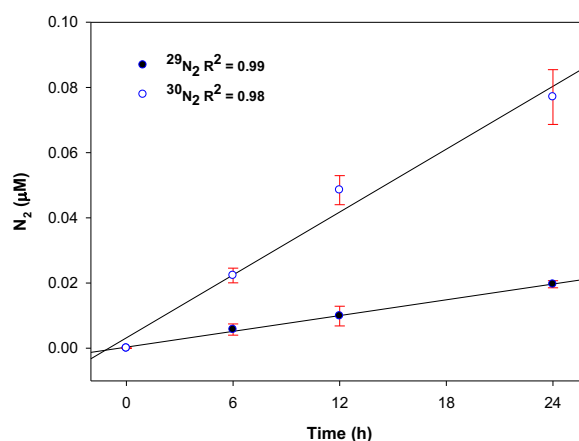Figure 1. Concentration of $NO_3^- + NO_2^-$ during the pre-incubation. The detection limit for $NO_3^- + NO_2^-$ was 0.5 ppm.

Figure 2. Accumulation of $^{29}N_2$ and $^{30}N_2$ in $^{15}NO_3^-$ added vials at different time period.
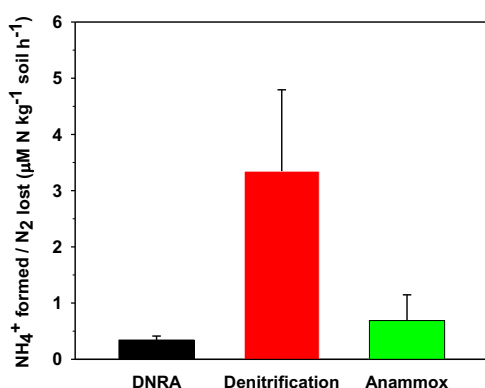
Figure 3. Rates of DNRA, denitrification and anammox. NH_4^+ produced refers to DNRA rate and N_2 produced refers to denitrification and anammox rates.

Conclusion

Results showed that DNRA, denitrification and anammox are significant N transformation pathways in rice paddies. These processes can start within a week of flooding of rice paddies. This study only investigated the processes rates during the first week of flooding which does not represent the whole rice growing period. The process rates can be affected by the rice roots growth and the higher dose of N that is top-dressed during the later stage of rice growth. Therefore there is a need of study looking at these processes throughout the rice growing period.

Acknowledgement

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