Soil Biology & Biochemistry xxx (2014) 1–4

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short communication

Can nitrogen isotope fractionation reveal ammonia oxidation responses to varying soil moisture?

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ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form
11 April 2014
Accepted 21 April 2014
Available online xxx

Keywords:
Ammonia oxidation
Isotope fractionation factor
Nitrification
Soil moisture
Substrate diffusion

ABSTRACT

To interpret the response of ammonia oxidation to changing soil moisture, we conducted a batch aerobic incubation with a loam soil at different soil water potentials (SWP) from −1100 to −11 kPa, and calculated net nitrification rates and apparent isotope fractionation factors ($\alpha_{s/p}$). With increasing SWP, net nitrification rates increased from 2.3 to 9.8 mg N kg^{-1} d^{-1}, while $\alpha_{s/p}$ increased from 1.025 to 1.031 at field-capacity (SWP of −33 kPa) but decreased with increasing SWP above field capacity. The increased $\alpha_{s/p}$ at field-capacity indicated that intracellular NH_4^+ concentration increased as a result of NH_4^+ supply exceeding NH_3 oxidation, while NH_3 oxidation exceeding NH_4^+ supply above field-capacity resulted in both decreased intracellular NH_4^+ concentration and $\alpha_{s/p}$. Our results suggest that NH_4^+ diffusion contributes more sensitively to increasing SWP than NH_3 oxidation below field-capacity, while the reverse is the case above field-capacity.

Many attempts have been made to investigate the effect of environmental changes on the kinetics of the nitrification process, the biological oxidation of NH_4^+/NH_3 into NO_2^- and then into NO_3^-. Measurement of the concentrations or isotope compositions ($^{15}N/^{14}N$) of soil inorganic N pools is a good metric of nitrification kinetics (Stark and Firestone, 1995; Yun et al., 2011). Natural abundance of $^{15}N/^{14}N$ ($\delta^{15}N$) of soil inorganic N pool can provide integrated insight and/or specific evidence for naturally-occurring N transformation processes, because N isotope fluxes during N transformation imprint specific N isotope signals on soil N pools. Therefore, variations in $\delta^{15}N$ of soil inorganic N during N transformation occur as a consequence of isotope fractionation that discriminates against the heavier isotope (^{15}N) (Kerley and Jarvis, 1996). Apparent isotope fractionation factor ($\alpha_{s/p}$) for ammonia oxidation ranged from 1.015 to 1.035, similar between ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Högberg, 1997; Santoro et al., 2011), and can vary with extracellular abiotic conditions through influencing intracellular NH_4^+ level (Casciotti et al., 2003; Yun et al., 2011). Obviously, soil moisture can regulate intracellular NH_4^+ concentration, because it affects extracellular NH_4^+ transport by changing diffusivity of the soil and enzymatic NH_3 oxidation by changing intracellular enzyme activity (Stark and Firestone, 1995). Therefore, $\alpha_{s/p}$ obtained during ammonia oxidation may reflect the integrated microbial response to changing soil moisture regime. Here, we periodically analyzed the time-course patterns of concentrations and $\delta^{15}N$ of soil NH_4^+ and NO_3^- during aerobic incubation to examine the effect of soil moisture on apparent N isotope fractionation ($\alpha_{s/p}$).

A loam soil (fine silty, mixed, thermic family of Fluventic Dystrudepts, USDA classification) was collected from an experimental field of the university farm of Seoul National University, Korea, air-dried, passed through a 2-mm sieve, and used for incubation experiment. Soil pH in distilled water was 6.1, and clay and carbon content was 229 and 7.6 g kg^{-1}, respectively. Concentrations and $\delta^{15}N$ of soil N were 0.9 g N kg^{-1} and +7.9‰ for total N, 2.7 mg N kg^{-1} and +0.4‰ for NH_4^+–N and 70.5 mg N kg^{-1} and +29.0‰ for NO_3^-–N, respectively. The soil had a moisture content of 0.24 kg kg^{-1} at soil matric potential of −33 kPa. Six soil moisture treatments were set up in triplicate, and their respective soil water potentials (SWPs) were −1100, −300, −100, −33, −16, and −11 kPa. Each 25 g of soil was individually transferred into 100-ml polyethylene bottles, covered with a perforated cap, and pre-incubated at 27 ± 1 °C in the dark for two weeks. After pre-incubation, 1 ml of 3.00 g N l^{-1} $(NH_4)_2SO_4$ solution was added to each bottle, mixed homogeneously, packed to give a soil bulk density of 1.2 Mg m^{-3}, and then incubated at 27 ± 1 °C in the dark.

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http://dx.doi.org/10.1016/j.soilbio.2014.04.032

Please cite this article in press as: Yun, S.-I., Ro, H.-M., Can nitrogen isotope fractionation reveal ammonia oxidation responses to varying soil moisture?, Soil Biology & Biochemistry (2014), http://dx.doi.org/10.1016/j.soilbio.2014.04.032

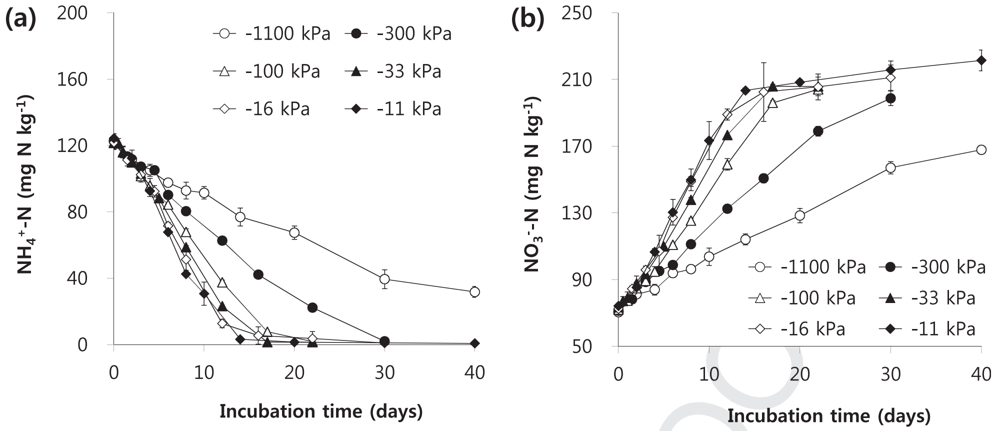

Fig. 1. Temporal changes in concentrations of 2 M KCl-extractable (a) NH_4^+–N and (b) NO_3^-–N in soils incubated at different soil water potentials: −1100, −300, −100, −33, −16, and −11 kPa. Values are means of triplicates. Vertical bars mean standard error ($n = 3$).

The SWP for each treatment was adjusted by adding distilled water to maintain their initial weights during incubation. Three bottles for each treatment were destructively sampled periodically throughout the incubation. At sampling, NH_4^+ and NO_3^- from soil were extracted with 100 ml of 2 M KCl solution. A 40-ml aliquot of each extract was added to a distillation flask and steam-distilled with MgO and Devarda's alloy to determine NH_4^+ and NO_3^- (Bremner and Keeney, 1966; Keeney and Nelson, 1982). The ammonia liberated during each steam-distillation was collected into a H_2SO_4 solution and titrated with a standard NaOH solution. After the titration, the solutions were adjusted to pH 2–3 using diluted H_2SO_4 and then evaporated to dryness. The dried samples was analyzed for $\delta^{15}N$ using an IsoPrime-EA stable isotope ratio mass spectrometer (Micromass, UK). The accuracy and reproducibility checked with reference materials (IAEA-N2, ammonium sulfate, +20.3‰) were better than 0.2‰. The $\delta^{15}N$ of samples were expressed in parts per thousand deviations from the atmospheric N_2 as defined by the following equation: $\delta^{15}N$ (‰) = [(R_{sample}/$R_{standard}$) − 1] × 1000, where R_{sample} and $R_{standard}$ are the ^{15}N/(^{14}N+^{15}N) ratios of samples and the atmospheric N_2.

Net nitrification rates (NNR) and $\alpha_{s/p}$ were calculated using concentration and $\delta^{15}N$ of NH_4^+ observed at 40, 16, 12, 8, 8, and 10 days after incubation for soils at SWP of −1100, −300, −100, −33, −16, and −11 kPa, respectively. Rate constants (k, NNR) for a decrease in NH_4^+ concentration were obtained by using zero-order kinetics described as follows: $C]C_0-kt$, where C_0 and C are the concentrations of substrate at time 0 and t, respectively. The $\alpha_{s/p}$ was calculated according to Mariotti et al. (1981) using the following equation:

$$\ln\left(\frac{10^{-3}\delta_s+1}{10^{-3}\delta_{s,\,0}+1}\right)=\left(\frac{1}{\alpha_{s/p}}-1\right)\ln f \qquad (1)$$

where f is the unreacted fraction of NH_4^+–N at time t, and $\delta_{s,0}$ and δ_s are the $\delta^{15}N$ of NH_4^+–N at time 0 and t, respectively. The $\alpha_{s/p}$ was calculated from the slope ($1/\alpha_{s/p}-1$) of the straight line on a natural logarithmic scale. Data were analyzed using the SAS software package (SAS Institute Inc., Cary, USA). NNR and $\alpha_{s/p}$ were compared among soil moisture treatments using the least significance differences test after a one-way ANOVA for the completely randomized design to assess the significance of any differences among the treatments at the significance level of $\alpha = 0.05$.

While NH_4^+ concentrations after adding $(NH_4)_2SO_4$ decreased rapidly from 120 to a level below 10 mg N kg^{-1} within 30 days of incubation (DOI) for soils treated at SWP greater than or equal to −300 kPa, those of soils at SWP of −1100 kPa decreased gradually to 32 mg N kg^{-1} during 40 DOI (Fig. 1a). On the other hand, NO_3^- concentration rose rapidly from 70 to 200 mg N kg^{-1} except for SWP of −1100 kPa, which increased gradually to 168 mg N kg^{-1} during 40 DOI (Fig. 1b). The quasi-linearly decreasing patterns of NH_4^+–N concentrations with time (Fig. 1a) were fitted well with zero-order kinetics; the determination coefficient mean is 0.981 (range 0.964–0.995, data not shown). The estimated NNR increased with increasing SWP, which was consistent with previous studies (Malhi and McGill, 1982; Stark and Firestone, 1995; Bateman and Baggs, 2005). Although field-capacity condition (SWP of −33 kPa) is optimal for nitrification, NNR increased steadily even above field-capacity (Fig. 2).

The $\delta^{15}N$ of NH_4^+ was close to 0‰ after the addition of $(NH_4)_2SO_4$ solution, peaked within 25 DOI for soils treated at SWP greater or equal to −300 kPa, and thereafter decreased abruptly below +5.0‰ (Fig. 3a). In contrast, the $\delta^{15}N$ of NH_4^+ at SWP −1100 kPa increased gradually to +33.2‰. On the other hand, the patterns of temporal variations in $\delta^{15}N$ of NO_3^- were opposite to those of NH_4^+ (Fig. 3b). Isotope fractionation factors ($\alpha_{s/p}$) increased from 1.025 to 1.031 with increasing SWP up to field-capacity, and decreased to 1.027 above field-capacity (Fig. 4). To our knowledge, there is no systematic investigation that addresses the question of how changing SWP affects $\alpha_{s/p}$ under unsaturated soil moisture condition. Meanwhile, a few studies have investigated the effect of changing

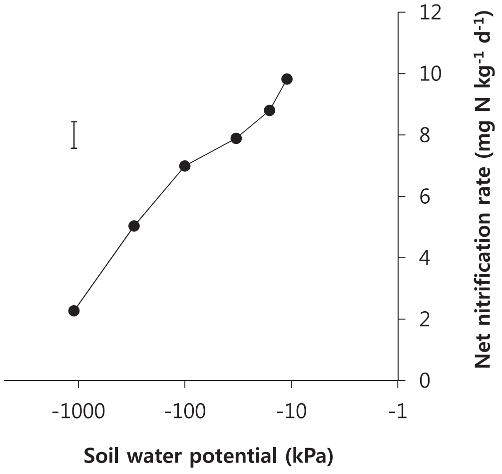

Fig. 2. Net nitrification rates in soils incubated at different soil water potential: −1100, −300, −100, −33, −16, and −11 kPa. Values are means of triplicates. Vertical bar presents LSD ($P = 0.05$).

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