Short communication

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

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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 (ζ_{lip}). With increasing SWP, net nitrification rates increased from 2.3 to 9.8 mg N kg⁻¹ d⁻¹, while ζ_{lip} increased from 0.25 to 0.31 at field-capacity (SWP of –33 kPa) but decreased with increasing SWP above field capacity. The increased ζ_{lip} at field-capacity indicated that intracellular NH₄⁺ concentration increased as a result of NH₄⁺ supply exceeding NH₃ oxidation, while NH₃ oxidation exceeding NH₄⁺ supply above field-capacity resulted in both decreased intracellular NH₄⁺ concentration and ζ_{lip}. Our results suggest that NH₄⁺ diffusion contributes more sensitively to increasing SWP than NH₃ 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 NH₄⁺/NH₃ into NO₂⁻ and then into NO₃⁻. Measurement of the concentrations or isotope compositions (¹⁵N/¹⁴N) of soil inorganic N pools is a good metric of nitrification kinetics (Stark and Firestone, 1995; Yun et al., 2011). Natural abundance of ¹⁵N/¹⁴N 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 δ¹⁵N of soil inorganic N during N transformation occur as a consequence of isotope fractionation that discriminates against the heavier isotope (¹⁵N) (Kerley and Jarvis, 1996). Apparent isotope fractionation factor (ζ_{lip}) for ammonia oxidation ranged from 1.015 to 1.035, was 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 NH₄⁺ level (Casciotti et al., 2003; Yun et al., 2011). Obviously, soil moisture can regulate intracellular NH₄⁺ concentration, because it affects extracellular NH₄⁺ transport by changing diffusivity of the soil and enzymatic NH₃ oxidation by changing intracellular enzyme activity (Stark and Firestone, 1995). Therefore, ζ_{lip} 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 δ¹⁵N of soil NH₄⁺ and NO₃⁻ during aerobic incubation to examine the effect of soil moisture on apparent N isotope fractionation (ζ_{lip}).

A loam soil (fine, silty, mixed, thermic family of Fluventic Dystropepts, 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 22.9 and 7.6 g kg⁻¹, respectively. Concentrations and δ¹⁵N 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 NH₄⁺-N and 70.5 mg N kg⁻¹ and +29.0% for NO₃⁻-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 (NH₄)₂SO₄ 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.
The SWP for each treatment was adjusted by adding distilled water to maintain their initial weights during incubation. Three bottles for each treatment were 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$_2$SO$_4$ solution and titrated with a standard NaOH solution. After the titration, the solutions were adjusted to pH 2–3 using diluted H$_2$SO$_4$ and then evaporated to dryness. The dried samples were analyzed for $^{15}$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 $^{15}$N of samples were expressed in parts per thousand deviations from the atmospheric N$_2$ as described by the following equation: $^{15}$N ($\%$) = $(R_{sample}/R_{standard}) - 1) \times 1000$, 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 $^{15}$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:

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

where $f$ is the unreacted fraction of NH$_4^+$ – N at time t, and $\delta_s$ and $\delta_t$ are the $^{15}$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$_4$)$_2$SO$_4$ 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 $^{15}$N of NH$_4^+$ was close to $0_{\text{atm}}$ after the addition of (NH$_4$)$_2$SO$_4$ solution, peaked within 25 DOI for soils treated at SWP greater or equal to $-300$ kPa, and thereafter decreased abruptly below $+5.0_{\text{atm}}$ (Fig. 3a). In contrast, the $^{15}$N of NH$_4^+$ at SWP of $-1100$ kPa increased gradually to $+33.2_{\text{atm}}$. On the other hand, the patterns of temporal variations in $^{15}$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 SWP...
soil temperature on $\alpha_{\text{NH}_4}$ associated with denitrification (Mariotti et al., 1981) and nitrification (Yun et al., 2011). In both studies, $\alpha_{\text{NH}_4}$ p decreased with increasing reaction rates as soil temperature increased. In this study, however, $\alpha_{\text{NH}_4}$ p showed an inverted V-shaped pattern, while nitrification rate increased with increasing SWP, and this two-phase change pattern of $\alpha_{\text{NH}_4}$ p indicated a possibility that at least one more factor in addition to soil moisture is involved in the discrimination against $^{15}$N during nitrification. A possible mechanism to explain such variation in $\alpha_{\text{NH}_4}$ p under different SWP would be the balance between the intracellular NH$_4^+$ transport (supply) and NH$_3$ oxidation (consumption). Stark and Firestone (1995) observed that nitrification was inhibited mostly via substrate-limitation (supply) at SWP higher than $-0.6$ MPa, but otherwise by cell dehydration that can suppress enzyme activity (consumption). A disturbance in equilibrium between NH$_4^+$ supply and NH$_3$ consumption can alter intracellular NH$_4^+$ concentration, and this change may in turn lead to unique isotope fractionation. The changes in intracellular NH$_4^+$ concentration and $\alpha_{\text{NH}_4}$ can be explained by the ratio of final (C$_f$) to initial (C$_i$) intracellular NH$_4^+$ concentration (C$_f$/C$_i$) as postulated by Yun et al. (2011). Both NH$_4^+$ supply and NH$_3$ oxidation in microbial cells may increase with increasing SWP, resulting in the increase in NNR (Fig. 3). However, a greater increase in NH$_4^+$ supply than in NH$_3$ oxidation may increase C$_i$/C$_f$ ratio, leading to a greater apparent isotope fractionation; otherwise, $\alpha_{\text{NH}_4}$ p decreased by lowering C$_i$/C$_f$ ratio (approach of $\delta^{15}$N of accumulated NO$_3^-$ to that of substrate NH$_4^+$). In this study, the increased $\alpha_{\text{NH}_4}$ p with increasing SWP to field-capacity suggests that a greater increase in NH$_4^+$ supply than the increase in NH$_3$ oxidation would cause a reduction of intracellular NH$_4^+$ level, resulting in a decrease in intracellular C$_i$/C$_f$ ratio. In contrast, the reason for the decreased $\alpha_{\text{NH}_4}$ p with increasing SWP above field-capacity would be a greater increase in NH$_3$ oxidation than an increase in NH$_4^+$ supply, causing a decrease in intracellular NH$_4^+$ level. A possible explanation for the decreased $\alpha_{\text{NH}_4}$ p observed in this moisture domain below saturation would be faster oxidation of intracellular NH$_4^+$, leading to a decrease in intracellular C$_i$/C$_f$ ratio. N mineralization may also affect $\alpha_{\text{NH}_4}$ p by changing NH$_4^+$ pool size and $\delta^{15}$N, but its effect may be negligible because net N mineralization was low and $\alpha_{\text{NH}_4}$ p was assessed before the abrupt decrease in $\delta^{15}$N of NH$_4^+$ (Yun et al., 2011). Furthermore, $\delta^{15}$N of inorganic N, which was obtained from concentration and $\delta^{15}$N of NH$_4^+$ and NO$_3^-$ using mass balance equation (Karamanos and Rennie, 1981), varied little during the assessment, less than $1\%$, (data not shown), and so the effect of mineralization on $\alpha_{\text{NH}_4}$ p would be little.

Our results showed that NNR increased with increasing SWP, while $\alpha_{\text{NH}_4}$ p increased up to field-capacity but decreased above this moisture level. This finding indicates the sensitivity of microbial NH$_3$ oxidation and the availability of NH$_4^+$ to changing soil moisture regimes, affecting intracellular NH$_4^+$ concentrations that vary depending on the balance between NH$_4^+$ transport and enzymatic oxidation. Despite the lack of direct information supporting the response mechanisms suggested in this study, the differences in $\alpha_{\text{NH}_4}$ p under different SWP indicate that $\alpha_{\text{NH}_4}$ p might be a useful tool to qualitatively assess how changing soil moisture regimes affect nitrification at cellular level. However, it should be noted that further investigations or formulation on the intracellular NH$_4^+$ and the associated $\alpha_{\text{NH}_4}$ p at the cellular level are required to explain how the relationship between NH$_4^+$ transport and enzymatic oxidation determines intracellular NH$_4^+$ concentrations. In addition, our findings should be extrapolated to a wide range of soil types to better understand the mechanism and to get more general notions.

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