Ammonium fertilization causes a decoupling of ammonium cycling in a boreal forest

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ABSTRACT

The forest-floor organic layer of the boreal coniferous forest is generally characterized by large mineral-N pools (especially ammonium), high rates of gross N mineralization, and low rates of autotrophic nitrification and nitrate immobilization. As atmospheric N deposition increases in boreal regions, it is expected to increase N losses from the forest-floor organic layer, which could affect the N status and microbial N cycling of the underlying mineral soil. To test this possibility, we conducted a long-term experiment, starting in 2010, consisting of three N addition levels (0, 20, and 40 kg NH₄Cl–N ha^{−1} yr^{−1}) in a boreal *Larix gmelinii* forest in the Great Xing'an Mountain, China. We measured mineral N concentrations (2012–2014), the *in-situ* net N-cycling rates (2012 and 2013), the gross N transformation rates (2014), and microbial abundance (2014) in mineral soil (0–10 cm) in the peak growing season. The gross rates of N transformations were quantified via a laboratory, ¹⁵N tracing experiment with a process-based ¹⁵N tracing model. NO₃[−] concentration, *in-situ* net nitrification, heterotrophic nitrification, gross nitrification, NO₃[−] immobilization, and dissimilatory NO₃[−] reduction to NH₄⁺ (DNRA) neither increased nor decreased, suggesting that NO₃[−] loss, production and retention were not affected by continual NH₄⁺ additions. However, the NH₄⁺ concentration and *in-situ* net ammonification rates increased under continued high NH₄⁺ additions, reflecting a change in soil NH₄⁺ status. As a result, microbial NH₄⁺ cycling was in uncoupled state in the high N addition plots (NH₄⁺ immobilization rates were incomparable to gross N mineralization rates), but this was not the case for the control and low N addition plots. Interestingly, the NH₄⁺ oxidation rates decreased rather than increased with decreased NH₄⁺ immobilization rates in the high N addition plots. However, the decreased NH₄⁺ oxidation rates were paralleled by a reduction in ammonia-oxidizing archaea (AOA) abundance. Our results indicate that for this boreal coniferous forest, enhanced NH₄⁺ deposition could alter mineral soil NH₄⁺ status and NH₄⁺ consumption. We show that NH₄⁺ fertilization could inhibit NH₄⁺ oxidation in forest soils.

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1. Introduction

Atmospheric deposition of reactive nitrogen (N), has substantially increased over the past century, and is expected to increase further in the coming decades (Galloway et al., 2003, 2008). Nitrogen deposition could shift an ecosystem from previously N-

limited into N saturated, driving N loss, soil acidification and productivity reduction (Aber et al., 1998; Fenn et al., 1998). The adverse effects of N deposition might be a particular concern in boreal regions, as ecosystems with short growing seasons and shallow soils have lower capacities to sequester N (Williams et al., 1996; Curtis et al., 2005; Bowman et al., 2008).

Key factors influencing N losses and the functional stability of boreal ecosystems under anthropogenic N inputs could be the changes in soil N cycling and retention (Aber et al., 1998; Fenn et al., 1998; Gundersen et al., 1998). However, information on how boreal

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soil N cycling and retention processes respond to N deposition is currently lacking (Aber et al., 1998; Allison et al., 2008). It has become clear to us that increased N availability and changes in microbial N cycling under N deposition could negatively affect the main root system of trees that are generally distributed in mineral soil, resulting in forest decline (Aber et al., 1998; Janssens et al., 2010). Previous studies have shown that enhanced deposition of N to forest ecosystems can alter soil N cycling and retention not only in the organic layer (if present), but also in the mineral layer, especially when the organic layer is rich in N availability, and is not thick (Corre et al., 2003, 2007, 2010; Zak et al., 2006; Baldos et al., 2015).

Boreal forests are generally dominated by coniferous trees, which produce litter with high contents of lignin and phenolic compounds that are resistant to microbial decay (Aerts, 1995). In coniferous forests, decomposition of litter and humus could be the rate-limiting step for microbial N cycling in the organic layer (Aber et al., 1998; Prescott et al., 1999), while microbial N cycling in the underlying mineral soil might be greatly influenced by mobilized N (Bengtsson and Bergwall, 2000). As needle inputs take a considerable time to decompose (Prescott et al., 1999, 2000), microbial N cycling in the coniferous organic layer is typically unresponsive to N fertilization (Bengtsson and Bergwall, 2000; Allison et al., 2008). However, the coniferous forest organic layer generally has a large mineral-N pool, a high rate of gross N mineralization, and low rates of autotrophic nitrification and NO_3^- immobilization (Gundersen et al., 1998; Bengtsson and Bergwall, 2000; Gao et al., 2013), which creates a potential for high N-leaching under N deposition conditions (Gundersen et al., 1998; Meiwes et al., 1998; Corre et al., 2003). By contrast, the mineral soil of the coniferous forest is often characterized by small mineral-N pools and low rates of net N mineralization (Gundersen et al., 1998; Gao et al., 2013, 2015a,b). Previous studies have suggested that N-cycling microbes in low-N soils are highly sensitive to changes in soil N availability (Boyle et al., 2008; Allison et al., 2009). Thus, it was expected that N deposited to the boreal coniferous forest-floor would induce a change in microbial N cycling in the underlying mineral soil.

Net N-cycling rates (net N mineralization and net nitrification) provide an index of plant-available N and N leaching (Booth et al., 2005; J.B. Zhang et al., 2012). However, they overshadow the mechanisms and dynamics of the soil internal N cycle (Gao et al., 2015a). Determination of soil gross N transformation rates can provide additional insights into microbially mediated N processes (Booth et al., 2005), as well as the N status of the soil (Corre et al., 2003; Venterea et al., 2004). Thus, an assay of both net and gross N transformations could increase our understanding of the relationship between soil N status and microbial N cycling, and microbial control of plant-available N and N leaching.

The respective gross N-cycling rates of organic and mineral soils are generally estimated via a separation of organic and mineral layers after ^{15}N injection into intact soil cores and incubation (Huygens et al., 2008; Corre et al., 2010; Baldos et al., 2015). The intact-core incubation technique avoids a major disturbance to the soil physical characteristics (Brenner et al., 2005). To minimize the influence of soil heterogeneity, temperature and moisture, gross N transformations are often assayed under controlled laboratory conditions. Although the N transformation rates obtained from laboratory incubations may differ from those derived in the field (Arnold et al., 2008), differences in N-cycling rates among treatments are likely to be identified from laboratory incubation (Paterson, 2003).

Since May in 2010, we have conducted an NH_4^+ addition experiment in a boreal *Larix gmelinii* forest located in the Great Xing'an Mountain region of China. The soil organic layer has a high N content ($101.9 \pm 10.9 \text{ mg NH}_4^+\text{-N kg soil}^{-1}$ and $1.9 \pm 0.2 \text{ mg NO}_3^-\text{-N}$

kg soil^{-1}) (Gao et al., 2013), and sampling through the growing season showed that experimental NH_4^+ additions have induced a 50% increase in $\text{NH}_4^+\text{-N}$ concentration in the 0–10 cm mineral soil (Gao et al., 2015b). However, it remained unclear if this change in N status would affect soil internal N cycling.

In this study, we examined the impact of experimental NH_4^+ additions on net N cycling (*in-situ* incubations), gross N transformations (laboratory incubations), and microbial abundance in the 0–10 cm mineral soil in the peak growing season (August). To get a better resolution of soil N cycling and N retention processes, we employed both ^{15}N tracing experiments and full process-based N cycling models to quantify N-pool-specific and process-specific gross N transformation rates. The objective of our study was to determine if and how gross N transformations (N mineralization, NH_4^+ immobilization, gross nitrification, autotrophic nitrification, heterotrophic nitrification, NO_3^- immobilization and DNRA) in the mineral soil respond to experimental NH_4^+ additions.

2. Materials and methods

2.1. Site descriptions and N fertilization experiment

The study site is located on the northwest slope of Great Xing'an Mountains in Inner Mongolia, China ($50^\circ 49' - 50^\circ 51' \text{E}$, $121^\circ 30' - 121^\circ 31' \text{N}$, ~826 m above sea level). The study area belongs to the cold-temperate humid climate. The annual precipitation averages from 450 to 550 mm, with 60% falling from July to August. During the snowfall period (from October to April of the following year), the soil is covered by a 20–40 cm snow layer, corresponding to 12% of the annual precipitation. The annual evaporation is approximately 800–1200 mm. The mean annual sunshine duration is 2594 h, with a frost-free period of approximately 80 days. The annual temperature averages -5.4°C , with a mean temperature of $11.4\text{--}19.5^\circ \text{C}$ in growing seasons (May to September). The site is occupied by a 150-year-old *Ledum palustre* L. - *Larix gmelinii* (Rupr.) Kuzen. mixed forest. The *L. gmelinii* forest is the dominant boreal forest type and covers $15.6 \times 10^6 \text{ ha}$ in Northeast China (National Forestry Bureau, 1994). The main canopy layer is dominated by *L. gmelinii* together with the associated tree species mostly *Betula platyphylla* Sukacz. The understory layer is dominated by *Larix palustre*, *Rhododendron simsii* Planch., and *Vaccinium vitis-idaea* L. The forest-floor (average thickness: 10 cm) comprises a thin O_i horizon dominated by leaf litter, and a thicker O_{ea} horizon interpenetrated by a dense root mat. The underlying mineral soil, with an average thickness of 20 cm, is a podzolic soil derived from granite residual. The terrain is flat, with slopes less than 3° .

Background N deposition rates were estimated at $9.87\text{--}14.25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the Great Xing'an Mountain region (Lü and Tian, 2007). To investigate the influence of atmospheric NH_4^+ deposition on microbial N cycling in the boreal coniferous forest soil, we established a NH_4^+ addition experiment in May 2010. We fertilized $20 \text{ m} \times 10 \text{ m}$ plots with 0 (control), 20 and 40 $\text{kg NH}_4\text{Cl-N ha}^{-1} \text{ yr}^{-1}$ in a randomized block design (three replicates per treatment). There was an interval of 20 m between blocks, and 10 m buffer zones between plots within each block. During the growing season (May to September), NH_4^+ solution (each low-N plot: $33.3 \text{ g NH}_4\text{Cl-N} + 20 \text{ L of water}$; each high-N plot: $66.7 \text{ g NH}_4\text{Cl-N} + 20 \text{ L of water}$) was sprayed onto the forest floor using a backpack sprayer at the beginning of each month. During the non-growing season (from October to April of the following year), fieldwork cannot be carried out because of the extremely harsh weather (low temperature and heavy snow). Thus, the NH_4^+ solution (each low-N plot: $233.3 \text{ g NH}_4\text{Cl-N} + 140 \text{ L of water}$; each high-N plot: $466.7 \text{ g NH}_4\text{Cl-N} + 140 \text{ L of water}$) was sprayed

collectively in early October. Control plots received an equal volume of water.

2.2. Measurements of in-situ net N cycling rates

In-situ net ammonification and nitrification were measured in August of 2012 and 2013, in the control and high-N treatments. Besides the N addition rate, factors such as forest-floor organic layer (e.g. thickness and N status) and treatment time also determine the time over which a response occurs in mineral soils. For 2012–2013 to test if the relatively low N addition ($< 40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to the boreal forest floor would induce a response in mineral soils, we only measured high-N addition treatments to observe potential responses.

Net ammonification and nitrification rates ($\text{mg N kg}^{-1} \text{ dry soil d}^{-1}$) were estimated separately from the change in NH_4^+ or NO_3^- concentration, respectively, per incubation period. This was determined using an incubation method, in which three pairs of PVC tubes (inner diameter of 7 cm; length of 20 cm) were installed randomly into the soil in three locations within each plot. PVC tubes covered the O_i and O_{ea} layers and extended into the mineral soil layer (0–10 cm). The perforated PVC tube in each pair remained in the field for the 30-d incubation. The top of the perforated PVC tube was wrapped with Parafilm® during *in-situ* incubation. The perforation allowed roots to enter the soil column. The mineral soil column from the other PVC tube was collected immediately, and shipped in a cold box. Our study site was in a very remote region, and there was no field station and oscillating machine, thus, we transported soil samples to the Key Laboratory of Ecosystem Network Observation and Modeling (Beijing) for mineral N extraction. Soil samples were sieved (2-mm), homogenized, and extracted with KCl after a cold-storage at 4°C for 2 or 3 days. The extracts were analyzed within 1 week of sampling in the field. The extracts were stored at 4°C before analysis.

Soil NH_4^+ and NO_3^- were extracted with 2 mol L^{-1} KCl at a soil/extractant ratio of 1: 5, and were analyzed using a continuous-flow analyzer (Bran Luebbe, Germany). Soil pH from the initial incubation was determined at a soil (air-dry) to water ratio of 1: 2.5 using a pH meter (Mettler Toledo, Switzerland). The soil properties and net N-cycling rates measured in August 2012 and 2013 were presented in Table S1 (see supporting information).

2.3. ^{15}N tracing experiment

In 2014, mineral soil samples (0–10 cm) were collected from all experimental plots (9 plots) to determine gross N transformations in August, a time when soils are characterized by high microbial activity (Gao et al., 2015b). The methods used for soil sampling, transfer and processing followed Reinhart and Rinella (2016) and Hart et al. (1994). Soil samples were collected by plot, and were not mixed for the same treatment. To ensure uniform labeling, organic and mineral horizons were separated before ^{15}N injection for sieving, mixing and pre-incubation (Corre et al., 2003, 2007; Zhang et al., 2013). For each plot, eight subsamples were collected from the top 0–10 cm soil using a PVC tube (inner diameter of 7 cm; length of 20 cm) after removing the O layers, and then were combined into a composite sample (9 samplings in total). The composite sample of each plot was sieved (2-mm), homogenized, and subsequently divided into three subsamples: one for ^{15}N tracing experiment, one for the analysis of soil properties (soil moisture, NH_4^+ , NO_3^- , pH, total C and total N) and the rest one for DNA extraction and qPCR. ^{15}N tracing experiments were carried out within two to three weeks of field sampling, and analyses of soil properties and microbial abundance were carried out within two weeks after field sampling. Soil samples were stored at 4°C before analysis.

For each plot, there were two ^{15}N treatments, of which either ammonium ($^{15}\text{NH}_4\text{NO}_3$) or nitrate ($\text{NH}_4^{15}\text{NO}_3$) were labeled with ^{15}N at 10 atom % excess. For each plot, the sieved soil was placed in four sets of conical flasks (six conical flasks per sets, three of the six conical flasks for $^{15}\text{NH}_4\text{NO}_3$ labeling, and the remaining three for $\text{NH}_4^{15}\text{NO}_3$ labeling; each conical flask containing fresh soil with the equivalent of 20 g of dry soil). The conical flasks were sealed with Parafilm® with five pinholes for gas exchange, and incubated in the dark for 24 h at 20°C before ^{15}N labeling. The incubation temperature was close to the highest average temperature of 19.5°C in the growing season. This incubation temperature was set because N transformation activities (e.g. N mineralization) may be undetectable under low incubation temperature (e.g. 15°C ; Jinbo Zhang, personal communication). Three milliliter of $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$ solution was added to each conical flask at a rate of $2.86 \mu\text{mol N g}^{-1}$ dry soil ($20 \mu\text{g NH}_4^+\text{-N g}^{-1}$ dry soil and $20 \mu\text{g NO}_3^-\text{-N g}^{-1}$ dry soil). The soil were finally adjusted to 60% water holding capacity (WHC), and sealed with parafilm® (with five pinholes for air exchange) and incubated for 144 h at 20°C . Soil extractions were conducted at 0.5, 48, 96, and 144 h after label addition to determine the concentrations and isotopic compositions of NH_4^+ and NO_3^- . A detailed description of the ^{15}N tracing study on each soil sample can be found in Fig. S1 (see supporting information).

Soil NH_4^+ and NO_3^- were extracted using the method described above, but were assayed at Nanjing Normal University (Nanjing) with a different continuous-flow analyzer (Skalar, Breda, the Netherlands). For isotopic analysis, NH_4^+ and NO_3^- were separated by distillation with MgO and Devarda's alloy (Zhang et al., 2009, 2013; L.M. Zhang et al. 2012). The isotopic compositions of NH_4^+ and NO_3^- were measured by an automated C/N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK). Soil moisture was measured at each separate extraction time by drying the soil samples at 105°C for 48 h. Total C and N contents were analyzed by an elemental analyzer (Europa Scientific Integra, UK) using air-dried, finely ground soil (Table S1). Soil pH was determined in an air-dried soil: water ratio of 1:2.5 by a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China).

Fig. 1. Conceptual ^{15}N tracing model (Müller et al., 2007). N_{rec} = recalcitrant organic-N; N_{lab} = labile organic-N; NH_4^+ = ammonium; NO_3^- = nitrate. M_{Nrec} , mineralization of recalcitrant organic-N to NH_4^+ ; M_{Nlab} , mineralization of labile organic-N to NH_4^+ ; $I_{\text{NH}_4\text{-Nrec}}$, immobilization of NH_4^+ to recalcitrant organic-N; $I_{\text{NH}_4\text{-Nlab}}$, immobilization of NH_4^+ to labile organic-N; $R_{\text{NH}_4\text{ads}}$, release of adsorbed NH_4^+ ; A_{NH_4} , adsorption of NH_4^+ on cation exchange sites; O_{NH_4} , oxidation of NH_4^+ to NO_3^- (autotrophic nitrification); O_{Nrec} , oxidation of recalcitrant organic-N to NO_3^- (heterotrophic nitrification); $I_{\text{NO}_3\text{-Nrec}}$, immobilization of NO_3^- to recalcitrant organic-N; and D_{NO_3} , dissimilatory NO_3^- reduction to NH_4^+ .

2.4. ^{15}N tracing model

The 10 simultaneously-occurring gross N transformations were quantified using a process-based ^{15}N tracing model (Fig. 1) (Müller et al., 2007): M_{Nrec} , mineralization of recalcitrant organic-N to NH_4^+ ; M_{Nlab} , mineralization of labile organic-N to NH_4^+ ; I_{NH_4-Nrec} , immobilization of NH_4^+ to recalcitrant organic-N; I_{NH_4-Nlab} , immobilization of NH_4^+ to labile organic-N; R_{NH_4ads} , release of adsorbed NH_4^+ ; A_{NH_4} , adsorption of NH_4^+ on cation exchange sites; O_{NH_4} , oxidation of NH_4^+ to NO_3^- (autotrophic nitrification); O_{Nrec} , oxidation of recalcitrant organic-N to NO_3^- (heterotrophic nitrification); I_{NO_3-Nrec} , immobilization of NO_3^- to recalcitrant organic-N; and D_{NO_3} , dissimilatory NO_3^- reduction to NH_4^+ (DNRA). In this study, we focused on gross N mineralization ($M_{tot} = M_{Nrec} + M_{Nlab}$), gross NH_4^+ immobilization ($I_{NH_4} = I_{NH_4-Nrec} + I_{NH_4-Nlab}$), autotrophic nitrification (O_{NH_4}), heterotrophic nitrification (O_{Nrec}), gross nitrification ($O_{NH_4} + O_{Nrec}$), gross NO_3^- immobilization (I_{NO_3}), and DNRA (D_{NO_3}). They were calculated either by zero (M_{Nrec} and O_{Nrec}) or first-order (M_{Nlab} , I_{NH_4-Nrec} , I_{NH_4-Nlab} , O_{NH_4} , I_{NO_3-Nrec} , and D_{NO_3}) kinetics. The data (Fig. S1) supplied to the model were the concentrations and ^{15}N excess of NH_4^+ and NO_3^- in the form of the mean $\pm$ standard deviations (SD). For each plot, the simulated concentrations and isotopic enrichments were generally within the range of the observed averages $\pm$ SD (see supporting information, Figs. S2–10).

Gross N transformation rates were estimated using the model via simultaneously optimizing the kinetic parameters for each individual process to minimize the misfit between the modeled and observed concentrations of NH_4^+ and NO_3^- and their respective ^{15}N enrichments. To obtain the most suitable model that could simulate the observed data, several model modifications, which vary in the number of N transformations, kinetic settings of individual processes (zero/first-order kinetics/Michaelis–Menten kinetics) and N pools considered, were tested. The final model (Fig. 1) was identified based on Akaike's information criterion (AIC) (Cox et al., 2006), selecting the lowest AIC that indicated the best fit between the observed and modeled data. Initially, all parameters from the conceptual model (Fig. 1) were included in the optimization run and the kinetic settings adjusted to reach an AIC as low as possible. Those parameters approaching zero in the analysis were considered to not significantly improve the model fit and were excluded in the subsequent steps. In general, N transformations (e.g. M_{Nrec} and O_{Nrec}) originating from large pool sizes are likely to follow zero-order kinetics while first-order kinetics are more appropriate to describe N transformations (M_{Nlab} , I_{NH_4-Nrec} , I_{NH_4-Nlab} , I_{NO_3-Nrec} , and D_{NO_3}) originating from small pool sizes (Myrold and Tiedje, 1986). Transformations such as NH_4^+ oxidation are likely to follow Michaelis–Menten kinetics rather than zero- or first-order kinetics, especially when the activity of nitrifying microorganisms undergoes a rapid change during the incubation from non- NH_4^+ limiting conditions (zero-order kinetics) to NH_4^+ limiting conditions (first-order kinetics) (Müller et al., 2007). A detailed description of stepwise modification of parameters and their kinetic settings to find the combination with the lowest resulting AIC can be found in previous studies (Müller et al., 2007; Rütting and Müller, 2007; Inselsbacher et al., 2013). The model parameters were optimized with Markov Chain Monte Carlo Metropolis algorithm (MCMC-MA), which was described in detail by Müller et al. (2007). To get a better resolution of soil processes, and in line with previous studies, the soil organic N pool was conceptually divided into two fractions, a labile (active) and a recalcitrant (slow) pool (Müller et al., 2007; Inselsbacher et al., 2013; Zhang et al., 2013). The initial (i.e. $t = 0$) pool sizes of mineral N (^{14}N and ^{15}N) were estimated based on Müller et al. (2004). In brief, the initial concentrations of NH_4^+ and NO_3^- were obtained by extrapolating the data at $t = 0.5$ h and $t = 24$ h ($t = 48$ h for our study) back to $t = 0$ h. The initial values of

the NH_4^+ were measured as the difference between applied NH_4^+ and the initial concentrations of NH_4^+ . The optimization procedure produced the probability density function (PDF) for each process, allowing the subsequent calculation of the average and standard deviation of each process. If N transformations follow first-order kinetics, the average gross N rates were calculated by integrating the gross N rates over the entire experimental period, divided by the incubation time (Inselsbacher et al., 2013). The gross N transformation rates were expressed in units of mg N kg^{-1} dry soil d^{-1} . The MCMC-MA routine is programmed into the software Matlab (Version 7.2, The Math Works Inc.), which calls models that are set up separately in Simulink (Version 6.4, The Math Works Inc.).

2.5. Measurements of microbial abundance

Due to expensive analysis cost, we only quantified microbial abundance of the control and high N treatments by quantitative real-time PCR (qPCR). DNA was extracted from 0.5-g soil sample using an FASTDNA Spin Kit for soil (MP Biomedicals, USA) according to manufacturer's instructions. The concentration of DNA was quantified photometrically using a NanoDrop ND-1000 UV–visible spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and diluted 10-fold. A brilliant SYBR[®] Premix Ex Taq Kit (Takara, China) and Biorad CFX96 Real-time PCR system (Biorad, USA) were used for qPCR. Each reaction was performed in a 20 μL volume containing 10 μL of $2 \times$ SYBR Premix Ex Taq (TaKaRa, code RR420A), 0.3 μL of each primer and 1 μL of 10-fold diluted DNA template (5.1–17.7 $\text{ng } \mu\text{L}^{-1}$). Bacterial 16S rRNA genes were amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCATCTCTTTRAGTTT-3') (Harris et al., 2004), and fungal internal transcribed spacer (ITS) genes were determined using primers ITS1 (5'-TCCGTAGGTGACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The amplification conditions were as follows: 95 °C for 180 s for predenaturation, followed by 40 cycles at 95 °C for 10 s, at 55 °C (ITS: 53 °C) for 20 s and at 72 °C for 20 s for the denaturation, annealing and elongation steps, respectively. Archaeal *amoA* genes were amplified using primers ArchamoAF (5'-STAATGGTCTGGCTTAGACG-3') and ArchamoAR (5'-GCGGCCATCCATCTGTATGT-3') (Francis et al., 2005) using the following amplification conditions: 95 °C for 180 s; 40 cycles at 95 °C for 15 s, at 55 °C for 20 s and at 72 °C for 25 s, and followed by plate reading at 83 °C. Bacterial *amoA* genes were quantified using the primers amoA1F (5'-GGGGTTTCTACTGGTGGT-3') and amoA2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') (Rotthauwe et al., 1997) with the same amplification conditions for archaeal *amoA* genes except for 10 s for 40 cycles at 95 °C. All PCR amplifications were performed in triplicate. Fluorescence was measured after each extension step. Melting curve and agarose gel electrophoresis were performed for quality verification of PCR products after each qPCR run. Standard curves ($r^2 > 99\%$) were obtained with serial ten-fold plasmid dilutions of a known amount of plasmid DNA containing a fragment of the 16S rRNA (9.43×10^2 to 9.43×10^8 $\text{ng DNA } \mu\text{L}^{-1}$), ITS (1.25×10^2 to 1.25×10^8 $\text{ng DNA } \mu\text{L}^{-1}$), archaeal *amoA* (2.57×10^2 to 2.57×10^8 $\text{ng DNA } \mu\text{L}^{-1}$) and bacterial *amoA* (1.07×10^1 to 1.07×10^7 $\text{ng DNA } \mu\text{L}^{-1}$) genes, respectively. Amplification efficiency (PCR efficiency = $10^{-1/\text{slope}} - 1$) was determined from the slope of the log-linear portion of the calibration curve (Bustin et al., 2009). The amplification efficiency values ranged between 96% and 104%.

2.6. Data analysis

All the data were tested for normality (Shapiro–Wilk test) and homogeneity of variance before analyses (Levene-test). Data that showed normal distribution and homogeneity (In 2012: NH_4^+ and

pH; in 2013: NH_4^+ , pH, and net nitrification; in 2014: M_{tot} , I_{NH_4} , O_{NH_4} , O_{Nrec} , gross nitrification, microbial abundance, total N, soil C: N ratio, pH, and soil moisture) were tested using one-way analysis of variance (ANOVA) with a Least Significant Difference (LSD) test to compare the differences between treatments. If preconditions of ANOVA were not met (In 2012: NO_3^- , net ammonification, net nitrification; in 2013: NO_3^- , net ammonification; in 2014: I_{NO_3} , D_{NO_3} , total C, NH_4^+ and NO_3^-), we employed the Kruskal-Wallis H test with multiple comparison extension to test differences among treatments. In this design, the dose of N addition was set as the main effect with sub-plot measurements considered as treatment replicates. All data used in these analyses were plot means. Microbial abundance was log transformed to meet the preconditions of ANOVA. We tested for significant differences at $\alpha = 0.10$. All analyses were conducted using SPSS version 20.0 (IBM Co., Armonk, NY, USA).

3. Results

3.1. N pools and microbial abundance

In August 2014 (the fifth peak growing season after N additions), the high N-addition plots had a higher concentration of NH_4^+ compared with the control plots ($p = 0.025$; Table 1), which was also the case in the preceding two years (2012 and 2013) (Table S1). In August 2014, the NH_4^+ concentration was also lower in the low N-addition plots than in the high N-addition plots ($p = 0.074$; Table 1). There were no significant differences in NO_3^- concentrations between treatments in any year. Soil total N and C: N ratio were similar across treatments after four-year fertilization (Table 1).

After 4 years of N additions, log number of archaeal *amoA* copies decreased from 7.34 ± 0.01 in the control plots to 6.19 ± 0.25 in the high N-addition plots ($p = 0.023$). Log transformed bacterial 16S rRNA, fungal ITS or bacterial *amoA* gene copies did not differ significantly between treatments (Table 2).

3.2. Net and gross N transformations

In August 2012, the *in-situ* net ammonification rates were similar among treatments (Table S1). However, in August 2013, plots receiving high N-addition had higher net ammonification rates compared with control plots ($p = 0.05$). No significant differences in *in-situ* net nitrification rates were observed between treatments in August of 2012 and 2013 (Table S1).

In the control plots, the major sink for the NH_4^+ produced was NH_4^+ immobilization (80.1%), rather than autotrophic nitrification (Fig. 2). Moreover, gross rates of NH_4^+ immobilization (I_{NH_4} ; $1.09 \pm 0.14 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$) were comparable to gross rates of N mineralization (M_{tot} ; $1.53 \pm 0.09 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$). Compared with the M_{tot} rates, the gross nitrification rates ($O_{\text{NH}_4} + O_{\text{Nrec}}$; $0.43 \pm 0.04 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$) were much lower (less than 30% of M_{tot}). Autotrophic nitrification contributed approximately 63% to

Table 2

Measurements of microbial gene copy numbers (log-transformed, g^{-1} dry soil) in August 2014 in boreal forest soils under simulated N deposition, including control, low N ($20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and high N ($40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) experimental plots.

Treatment	Bacteria	Fungi	AOA	AOB
Control	11.74 ± 0.04	9.28 ± 0.01	7.34 ± 0.01^a	5.84 ± 0.48
Low N	ND	ND	ND	ND
High N	11.64 ± 0.13	9.10 ± 0.19	6.19 ± 0.25^b	5.92 ± 0.41
<i>p</i> -Value	0.57	0.55	0.02	0.87

Values are means with SE ($n = 3$ plots). ND = not detected. Statistical significance for main effects of dose is shown as a *p*-value. Different letters indicate significant differences between treatments at $p < 0.10$.

gross NO_3^- production (Fig. 2). Two NO_3^- consumption processes were quantified in this study, namely NO_3^- immobilization (I_{NO_3}), and dissimilatory NO_3^- reduction to NH_4^+ (DNRA). However, both I_{NO_3} and DNRA showed low potential to retain NO_3^- , with a rate of $0.01 \pm 0.01 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ and $0.06 \pm 0.03 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$, respectively (Fig. 2).

Significant changes in gross N cycling in response to N additions were confined to NH_4^+ immobilization and autotrophic nitrification (Fig. 2). Gross rates of NH_4^+ immobilization in the high N-addition plots ($0.25 \pm 0.09 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$) were lower than in the control ($1.09 \pm 0.14 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$; $p = 0.026$) and the low N-addition plots ($1.03 \pm 0.30 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$; $p = 0.033$). Compared with the control plots ($0.27 \pm 0.01 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$), the high N-addition plots with a higher NH_4^+ concentration and a lower NH_4^+ immobilization rate had a 37% lower rate of autotrophic nitrification ($p = 0.033$; Fig. 2).

4. Discussion

4.1. The effects of NH_4^+ addition on microbial NH_4^+ cycling

The observed increase in the NH_4^+ concentration in August 2012 (Table S1) could be related to high levels of NH_4^+ in the percolate (Gundersen et al., 1998; Meiwes et al., 1998; Corre et al., 2003), rather than increased N release by microbes. This is because relative to the NH_4^+ concentration, the *in-situ* net ammonification rates showed a delayed response (Table S1). This indicates that a change in soil N status is a precursor for subsequent changes in microbial N cycling.

One of the key features of N-limited ecosystems is the tight coupling of microbial N cycling, especially for NH_4^+ (Hodge et al., 2000; Corre et al., 2003, 2007), as observed in the mineral soil of the control plots, where microbial immobilization of NH_4^+ kept pace with the gross rates of N mineralization (Fig. 2). In many temperate and boreal coniferous forests, the forest-floor organic layers are often characterized by large mineral-N pools ($21\text{--}205 \text{ mg NH}_4^+\text{-N kg soil}^{-1}$) and high rates of gross N mineralization ($15.9\text{--}27.5 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$; OM: organic matter, $80.7\text{--}95.0\%$) and net N mineralization ($6.6\text{--}9.5 \text{ mg N kg}^{-1} \text{ OM d}^{-1}$) (Gundersen

Table 1

Measurements of soil characteristics in August 2014 in boreal forest soils under simulated N deposition, including control, low N ($20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and high N ($40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) experimental plots.

Treatment	Soil moisture (%)	WHC (%)	NH_4^+ ($\text{mg N kg}^{-1} \text{ soil}$)	NO_3^- ($\text{mg N kg}^{-1} \text{ soil}$)	pH	Total C ($\text{g C kg}^{-1} \text{ soil}$)	Total N ($\text{g N kg}^{-1} \text{ soil}$)	Soil C: N
Control	24 ± 3	80 ± 10	8.60 ± 0.27^b	7.71 ± 2.03	5.65 ± 0.13	30.71 ± 1.74	1.80 ± 0.07	17.15 ± 1.00
Low N	30 ± 5	90 ± 9	7.78 ± 1.28^b	6.50 ± 0.97	5.55 ± 0.16	41.84 ± 10.74	2.30 ± 0.63	18.37 ± 0.44
High N	27 ± 1	87 ± 3	12.11 ± 0.74^a	10.17 ± 2.38	5.57 ± 0.17	31.67 ± 3.38	2.05 ± 0.32	15.77 ± 1.45
<i>p</i> -Value	0.54	0.50	0.06	0.49	0.85	0.59	0.70	0.29

Values are means $\pm$ standard errors (SE; $n = 3$ plots). Statistical significance for the main effects of each dose is shown as a *p*-value. Different letters indicate significant differences between treatments at $p < 0.10$.

Fig. 2. Gross rates of N cycling in the 0–10 cm mineral soil in response to NH₄⁺ additions. SOM = soil organic matter, NH₄⁺ = ammonium; NO₃⁻ = nitrate. DNRA = dissimilatory NO₃⁻ reduction to NH₄⁺. Rates are means with standard errors (mg N kg⁻¹ dry soil d⁻¹; *n* = 3 plots). There was no difference detected among treatments for N mineralization, heterotrophic nitrification, NO₃⁻ immobilization, and DNRA. For NH₄⁺ immobilization and autotrophic nitrification, means followed by the same letter (upper case for NH₄⁺ immobilization and lower case for autotrophic nitrification) indicate no significance among treatments (one-way ANOVA, least significant difference test at *p* < 0.10).

et al., 1998; Tietema, 1998; Gao et al., 2013), and cannot be considered as N limited. Thus, ecosystem N limitation might occur mainly in the underlying mineral layer and where, in our case, tree roots are mainly distributed. Microbial competition with plant roots for N sustains ecosystem N limitation.

The higher net ammonification rates in the high N-addition plots could be explained by reduced microbial assimilation of NH₄⁺ (Fig. 2). Our measurements for gross N mineralization, and bacterial and fungal abundance (Fig. 2 and Table 2) suggested that, in the high N-addition plots, the reduced NH₄⁺ assimilation by soil microbes could not be attributed to the reductions in NH₄⁺ supplied by N mineralization and microbial biomass, as reported in many studies (Corre et al., 2003, 2007, 2010; Baldos et al., 2015). The observed reductions in microbial assimilation of NH₄⁺ could be related to a declining microbial need for NH₄⁺, inhibitions of enzyme systems for the utilization of alternative N sources, and increased microbial utilization of organic N to meet their C demand (Geisseler et al., 2010).

The absence of a strong response of gross N mineralization to NH₄⁺ additions was in line with the lack of significant changes in its main controls: soil C, soil N, soil C:N ratio, and bacterial and fungal abundance (Fig. 2; Table 1). The insensitivity of N mineralization in the underlying mineral layer to long-term N addition or extremely high N deposition were also reported in temperate (Corre et al., 2003, 2007; Zak et al., 2006) and tropical forests (Corre et al., 2010). These findings highlighted the importance of the forest-floor organic layer in mediating ecosystem-level responses to N deposition, and the changes in the decomposition of humus and

fresh leaf litter, and the soil N status in shaping microbial response (Zak et al., 2006). However, previous studies have suggested that for a temperate or boreal coniferous forest, N additions do not have significant effects on the decomposition rates of needle litter and humus to affect microbial cycling of N in the forest-floor organic layer (Prescott et al., 1999, 2000; Bengtsson and Bergwall, 2000). Moreover, our previous work showed that this boreal forest-floor organic layer had an extremely large NH₄⁺ pool, and that low-dose NH₄⁺ addition (< 40 kg N ha⁻¹ yr⁻¹) could not induce a significant change in the NH₄⁺ pool (Gao et al., 2013). Thus, in the present study, we did not investigate the forest-floor organic layer. Interestingly, although microbial N limitation was alleviated, gross N mineralization did not show an increasing trend in the high N-addition plots. The ratio of gross NH₄⁺ immobilization to gross N mineralization (0.18) was far below 0.5, indicating that the high N-addition plots were N saturated (Aber, 1992). This indicated that ecosystem N retention might decrease with NH₄⁺ deposition, with an apparent threshold deposition rate of 50 kg N ha⁻¹ yr⁻¹ (N addition 40 kg N ha⁻¹ yr⁻¹ + background N deposition 9.9–14.3 kg N ha⁻¹ yr⁻¹).

4.2. The effects of NH₄⁺ addition on microbial NO₃⁻ cycling

The absence of a significant change in mineral soil NO₃⁻ concentration with NH₄⁺ additions (Tables 1 and S1) could be attributed to several causes: low nitrification in forest-floor organic layer, and low autotrophic nitrification and high NO₃⁻ leaching in mineral soil. Two lines of evidence suggest that nitrification activity in this

forest-floor organic layer was low. On the one hand, the mineral N concentration in this boreal forest-floor organic layer was $101.9 \text{ mg N kg soil}^{-1}$ (Gao et al., 2013), which is beyond the threshold for mineral N ($60\text{--}90 \text{ mg N kg soil}^{-1}$), at which nitrification ceases (Vitousek and Reiners, 1982; Bengtsson and Bergwall, 2000). On the other hand, we observed an extremely high ratio of NH_4^+ to NO_3^- (53.6), and low NO_3^- concentration ($1.9 \pm 0.2 \text{ mg N kg soil}^{-1}$) in the forest-floor organic layer (Gao et al., 2013). These values indicated that NO_3^- concentration in the mineral soil was unlikely to be affected by NO_3^- leaching from the forest-floor organic layer. In addition, autotrophic nitrification rates in the mineral soil were too low to have anything but a marginal influence on NO_3^- production (Fig. 2).

The process-specific production (autotrophic nitrification and heterotrophic nitrification) and consumption (NO_3^- immobilization and DNRA) rates of NO_3^- in the mineral soil were comparable to the values observed by Zhang et al. (2013) from the mineral soils of five temperate forests in northern China, with very similar soil properties (e.g. pH) to our study. At our site, the mineral soil had a high ability to produce NO_3^- , but a low ability to retain NO_3^- (Fig. 2). Similarly, the forest-floor organic layers of European temperate coniferous forests showed a lack of NO_3^- retention capacity (Gundersen et al., 1998; Tietema, 1998; Bengtsson and Bergwall, 2000). The uncoupled microbial NO_3^- cycling, together with insignificant *in-situ* net nitrification, suggested a high leaching loss of NO_3^- from this coniferous forest (Table S1; Fig. 2).

Interestingly, autotrophic nitrification rates decreased in the high N-addition plots that exhibited an increase in NH_4^+ concentration and a decrease in NH_4^+ immobilization (Fig. 2). However, this is not surprising considering the lower AOA abundance in the high N addition plots (Table 2). Ammonia oxidation is the first step of autotrophic nitrification, which is mediated by both AOA and AOB (Prosser and Nicol, 2008; Levy-Booth et al., 2014). Similar to our observation (Table 2), in many other forest soils, AOA outnumber AOB based on their *amoA* gene abundances (Isobe et al., 2012; Long et al., 2012; Lu et al., 2015). This indicates the potential for a greater role of archaea in autotrophic ammonia oxidation (L.M. Zhang et al., 2012; Levy-Booth et al., 2014).

Previous studies have demonstrated that the growth or activity of AOA tends to be inhibited by high pH and high NH_3 concentrations (Nicol et al., 2008; Verhamme et al., 2011; Thion and Prosser, 2014). A decrease in NH_4^+ immobilization might result in potential NH_3 accumulation and pH increase in the soil. We have evidence that soil pH increased in the high N addition plots that exhibited a reduction in NH_4^+ immobilization (Table S1; Fig. 2). An increase (0.6 units), not decrease, in soil pH after long-term N application was also observed in Alaskan boreal forest (Allison et al., 2008). Thus, we speculated that the N deposition-induced decrease in NH_4^+ immobilization and increase in $\text{NH}_4^+/\text{NH}_3$ concentrations might inhibit the growth or activity of AOA (Fig. 3).

Heterotrophic nitrification rates are affected by many factors, of which soil acidity, soil C: N ratio and fungal biomass appear to be the most important (De Boer and Kowalchuk, 2001; Zhang et al., 2013, 2015; Zhu et al., 2013). Nitrification tends to be heterotrophic in acidic forest soils, where heterotrophic nitrification rates are positively correlated with both the soil C: N ratio and fungal biomass, (Zhang et al., 2013; Zhu et al., 2013). As mentioned above, soil C: N, soil pH and fungal abundance in the mineral layer were only marginally different with increasing NH_4^+ additions to the forest floor (Tables 1 and 2), which perhaps explains the similar heterotrophic nitrification rates among the treatments.

The lack of response of NO_3^- immobilization is not surprising considering the absence of changes in heterotrophic nitrification and fungal abundance (Table 2 and Fig. 2). Recent studies have suggested that NO_3^- immobilization and heterotrophic nitrification

Fig. 3. Conceptual model for the decline in autotrophic nitrification in the boreal coniferous forest soils in response to NH_4^+ addition. Microbial immobilization of NH_4^+ decreases with increased concentrations of NH_4^+ under enhanced N deposition. A reduction in microbial immobilization of NH_4^+ results in a potential accumulation of NH_3 and rising pH, thereby constraining the activity or growth of ammonia-oxidizing archaea (AOA). As a result, ammonia oxidation is inhibited.

might be functionally linked in forest soils, and heterotrophic nitrification rate and fungal biomass, but not NO_3^- availability, are major controllers of microbial immobilization of NO_3^- (Zhang et al., 2013; Zhu et al., 2013). For example, forest soils, which exhibit high rates of heterotrophic nitrification, are often characterized by high rates of NO_3^- immobilization; however, this is not the case for forest soils with high rates of autotrophic nitrification (Huygens et al., 2007, 2008; Zhang et al., 2013; Zhu et al., 2013). In addition, grassland soils (Rütting et al., 2010; Müller et al., 2011) and agriculture soils (J.B. Zhang et al., 2012) are known to have high NH_4^+ oxidation rates, however, they all show extremely low NO_3^- immobilization capacities.

Several studies have suggested that DNRA is a potential N retention mechanism in N limited forest ecosystems, and its importance in N retention may change with N deposition (Bengtsson and Bergwall, 2000; Huygens et al., 2007; Rütting et al., 2008; Baldos et al., 2015). Our results showed that DNRA retained 7.9–16.3% of the NO_3^- produced in mineral soils (Fig. 2). However, the importance of DNRA in N retention is likely to be underestimated. This is because *in-situ* DNRA rates were probably underestimated by laboratory incubations (Zhang et al., 2013). However, in this study, DNRA rates were similar among treatments (Fig. 2), suggesting that experimental NH_4^+ additions did not affect the rate of N retention via DNRA in mineral soils.

4.3. The effects of Cl^- on microbial N cycling

Ammonium chloride contains 65% of Cl^- relative to 25% of N, which may also produce effects on ecosystem N cycling. The accumulation of Cl^- via the use of NH_4Cl in soils and plants can trigger a series of physiological disorders in plants and microorganisms (White and Broadley, 2001; Megda et al., 2014). However, in our view, microbial N cycling would not be greatly influenced by Cl^- input. First, at our site, the soil had a low risk of Cl^- accumulation. This is because Cl^- shows little adsorption to soil components and is easily leached (White and Broadley, 2001), and the coniferous forest receives high rainfalls during the growing season. Second, the biocide effect of Cl^- accumulation on microbial biomass exhibits a short-term effect, which may diminish over time (Megda et al., 2014). Thus, the impact of Cl^- accumulation on N mineralization and N immobilization could be minor. Third, AOA and AOB

are tolerant to high salinity. For example, archaeal *amoA* genes are detected in coastal and marine waters with high practical salinity units (Venter et al., 2004; Francis et al., 2005). Moreover, community structure of AOB, all of which clustered with *Nitrosospora*-like sequences, remained largely unaffected in NaCl treated soil (Nelson and Mele, 2007). Thus, the influence of Cl^- input on autotrophic nitrification might be minor. Two laboratory studies have shown that Cl^- accumulation can inhibit microbial nitrification in the soil (Golden et al., 1981; Megda et al., 2014). However, it should be noted that in those two studies, Cl^- was added to the soils at a very high dose.

4.4. The effects of soil separation, soil sieving, and cold storage on microbial N cycling

Although gross N transformation rates estimated from laboratory incubations permit the identification of changes in N transformations between treatments, they do not reflect *in-situ* N cycling rates. Because mineral layer was the layer of our interest, a separation of organic and mineral horizons was made before ^{15}N injection and incubation as reported in previous studies (Corre et al., 2003, 2007; Zhang et al., 2013), rather than after ^{15}N injection into intact soil cores (Huygens et al., 2008; Corre et al., 2010; Baldos et al., 2015). However, this might have caused a major disturbance to soil physical characteristics (Brenner et al., 2005), and a reduction in nutrient input into mineral layer (Li et al., 2014), and thus a decline in microbial activity, and N mineralization and NH_4^+ immobilization rates.

To ensure uniform labeling, soil samples were sieved and homogenized. However, soil sieving might have interrupted the established mycelium networks (Johnson et al., 2005; Huygens et al., 2007), negatively affecting the fungi-mediated transformations such as heterotrophic nitrification and NO_3^- immobilization (Huygens et al., 2008; Zhu et al., 2013). Moreover, studies by Schimel et al. (1989) showed that although soil mixing did not affect the rate of NH_4^+ utilization, it significantly increased the rate of NH_4^+ production. More recently, Arnold et al. (2008) found that laboratory measurements from cold-stored tropical forest soils cores (soils were stored at 5 °C for 2–30 days followed by 3-day acclimatization) showed lower gross N mineralization and NH_4^+ consumption rates, and higher gross nitrification and NO_3^- immobilization rates than the *in-situ* measurements. Possible mechanisms are associated with the fact that microorganisms in tropical soils are typically accustomed to relatively high temperatures with small fluctuations, and easily mineralizable organic N decreases during storage (Arnold et al., 2008). However, microbial populations in temperate soils, and probably in boreal soils, could adapt to a large range of temperature, including values below the freezing point (Hart et al., 1994; Arnold et al., 2008). Thus, we consider that the impact of cold storage on microbial N cycling in our soil samples was likely minor.

5. Conclusions

For this boreal coniferous forest, our measurements of mineral N concentration and *in-situ* net N cycling suggested a greater effect of NH_4^+ deposition on mineral soil NH_4^+ status than NO_3^- status. Laboratory ^{15}N tracing experiments showed that the soil N cycling processes affected by NH_4^+ additions were NH_4^+ immobilization and NH_4^+ oxidation, exhibiting a decreasing trend under excessive NH_4^+ input. Our results also revealed that mineral soil NO_3^- production (gross and heterotrophic nitrification) and retention (NO_3^- immobilization and DNRA) were relatively resistant to enhanced NH_4^+ deposition. This study provided new insights on how microbial N cycling in forest soils might respond to N deposition: enhanced

NH_4^+ deposition might increase the NH_4^+ concentration and decrease NH_4^+ immobilization to constrain AOA growth, thereby inhibiting NH_4^+ oxidation. Further investigations on *in-situ* gross N cycling in the organic and mineral layers using intact-core incubation technique should be performed to confirm our findings. In addition, exploration on the fates of N, and export of N (NH_4^+ , NO_3^- , and dissolved organic N) from the organic layer is needed to reveal the factors controlling microbial N cycling in mineral soil under increased NH_4^+ deposition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.07.007>.

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