

Gross nitrogen transformations and N₂O emission sources in sandy loam and silt loam soils postprint

Authors: LANG Man, LI Ping, WEI Wei, LI Ping

Date: 2021-06-04T00:00:00+00:00

Abstract

The soil type is a key factor influencing N (nitrogen) cycling in soil; however, gross N transformations and N₂O emission sources are still poorly understood. In this study, a laboratory ¹⁵N tracing experiment was carried out at 60% WHC (water holding capacity) and 25°C to evaluate the gross N transformation rates and N₂O emission pathways in sandy loam and silt loam soils in a semi-arid region of Heilongjiang Province, China. The results showed that the gross rates of N mineralization, immobilization, and nitrification were 3.60, 1.90, and 5.63 mg N/(kg • d) in silt loam soil, respectively, which were 3.62, 4.26, and 3.13 times those in sandy loam soil, respectively. The ratios of the gross nitrification rate to the ammonium immobilization rate (n/ia) in sandy loam soil and silt loam soil were all higher than 1.00, whereas the n/ia in sandy loam soil (4.36) was significantly higher than that in silt loam soil (3.08). This result indicated that the ability of sandy loam soil to release and conserve the available N was relatively poor in comparison with silt loam soil, and the relatively strong nitrification rate compared to the immobilization rate may lead to N loss through NO₃⁻ leaching. Under aerobic conditions, both nitrification and denitrification made contributions to N₂O emissions. Nitrification was the dominant pathway leading to N₂O production in soils and was responsible for 82.0% of the total emitted N₂O in sandy loam soil, which was significantly higher than that in silt loam soil (71.7%). However, the average contribution of denitrification to total N₂O production in sandy loam soil was 17.9%, which was significantly lower than that in silt loam soil (28.3%). These results are valuable for developing reasonable fertilization management and proposing effective greenhouse gas mitigation strategies in different soil types in semiarid regions.

Full Text

Preamble

Gross Nitrogen Transformations and N O Emission Sources in Sandy Loam and Silt Loam Soils

LANG Man^{1,2}, LI Ping^{1,2*}, WEI Wei^{1,2}

¹ Jiangsu Key Laboratory of Agricultural Meteorology, Nanjing University of Information Science & Technology, Nanjing 210044, China

² Department of Agricultural Resources and Environment, Nanjing University of Information Science & Technology, Nanjing 210044, China

Abstract: Soil type is a key factor influencing nitrogen (N) cycling in soil; however, gross N transformations and N O emission sources remain poorly understood. In this study, a laboratory ¹⁵N tracing experiment was conducted at 60% WHC (water holding capacity) and 25°C to evaluate gross N transformation rates and N O emission pathways in sandy loam and silt loam soils from a semi-arid region of Heilongjiang Province, China. The results showed that the gross rates of N mineralization, immobilization, and nitrification were 3.60, 1.90, and 5.63 mg N/(kg · d) in silt loam soil, respectively, which were 3.62, 4.26, and 3.13 times those in sandy loam soil. The ratios of gross nitrification rate to ammonium immobilization rate (n/i_a) in sandy loam and silt loam soils were both higher than 1.00, whereas the n/i_a in sandy loam soil (4.36) was significantly higher than that in silt loam soil (3.08). This indicated that sandy loam soil had a relatively poor capacity to release and conserve available N compared with silt loam soil, and the relatively strong nitrification rate relative to the immobilization rate may lead to N loss through nitrate leaching. Under aerobic conditions, both nitrification and denitrification contributed to N O emissions. Nitrification was the dominant pathway leading to N O production in soils and was responsible for 82.0% of total emitted N O in sandy loam soil, which was significantly higher than that in silt loam soil (71.7%). However, the average contribution of denitrification to total N O production in sandy loam soil was 17.9%, which was significantly lower than that in silt loam soil (28.3%). These results are valuable for developing reasonable fertilization management practices and proposing effective greenhouse gas mitigation strategies for different soil types in semi-arid regions.

Keywords: gross N transformation rates; ¹⁵N tracing; N O emission sources; sandy loam; silt loam; semi-arid region

1 Introduction

Soil N transformations are microbially mediated processes affected by a range of factors, including moisture content, temperature, cultivation practices, soil type, and land use type (Hoyle et al., 2006; Lan et al., 2013; Zhu et al., 2019). Among these factors, soil type is considered one of the most important regu-

lating factors. Numerous studies have investigated the effect of soil type on N transformations by examining net rates (Grünzweig et al., 2003; Tripathi and Singh, 2009; Yang et al., 2010). However, net mineralization or nitrification rates are only valuable for indicating N availability to plants and cannot provide detailed information on the production and consumption processes of soil inorganic N (Murphy et al., 2003).

Alternatively, gross N transformation rates quantified by the ^{15}N isotope-labeling technique can estimate the gross rates of N mineralization, nitrification, and immobilization, respectively, providing deeper and more fundamental understanding of the inherent N cycle in soil (Müller et al., 2007).

N_2O is an important greenhouse gas involved in global warming and stratospheric ozone layer destruction (Byrnes, 1990). Agricultural soil is a major source of N_2O emissions, responsible for 70% of anthropogenic emissions (Kroeze et al., 1999). N_2O is mainly produced through two biological processes in soil: nitrification and denitrification (Stevens et al., 1997). These two processes are considered to occur simultaneously due to soil heterogeneity, which allows aerobic and anaerobic zones to coexist within soil aggregates (Steven et al., 1997). However, the magnitude of N_2O emissions derived from nitrification and denitrification depends on many factors such as temperature, moisture content, fertilizer application, and soil type (Dobbie and Smith, 2001; Khalil et al., 2004; Pihlatie et al., 2004; Lan et al., 2013; Zhang et al., 2019; Castellano-Hinojosa et al., 2020; Li et al., 2020), with soil type regarded as one of the most important controllers. Although some studies have estimated the specific processes leading to N_2O emissions from different soils, results have been variable. For example, most studies showed that N_2O was mainly released via nitrification at 30% to 60% water-filled pore space (WFPS) in agricultural soils (Steven et al., 1997; Pihlatie et al., 2004; Bateman and Baggs, 2005; Well et al., 2008; Lan et al., 2014). However, Zhu et al. (2011) reported that denitrification was the dominant source of N_2O emissions from agricultural soils at 50% water holding capacity (WHC), and Li et al. (2016) indicated that the contribution of denitrification to N_2O emissions exceeded 50% at 40% to 60% WFPS in a sandy loam soil. These discrepancies could result from differences in soil texture, pH, and the availability of C and N; however, the underlying mechanisms and regulatory processes remain poorly understood.

The semi-arid region of Heilongjiang Province is an important agricultural area in Northeast China, where sandy loam and silt loam soils are two typical agricultural soil types and maize is grown annually. Large amounts of N fertilizer have been applied each year to increase crop yield (Wang et al., 2013), and excessive N inputs are an environmental concern as they can cause water pollution and greenhouse gas emissions. It is especially crucial to better understand N transformations in these two typical soil types to optimize fertilizer management and increase N use efficiency. However, few studies have examined the characteristics of gross N transformations in agricultural soils of semi-arid regions. In addition, since soil N_2O emissions are mainly controlled by nitrification and den-

itrification, the contributions of these two processes to N₂O emissions in soils need to be studied to better understand N₂O emission sources for different soil textures in semi-arid regions, to accurately estimate N₂O inventories and plan mitigation strategies.

The aims of this study were (1) to elucidate the gross N transformation rates in sandy loam and silt loam soils, and (2) to assess the relative importance of nitrification and denitrification to N₂O emissions under aerobic conditions. To meet these objectives, the ¹⁵N tracing technique was used under laboratory incubation conditions to identify N₂O emission sources in the two agricultural soils with substantially different properties, and gross N transformations were calculated using the FLUAZ model.

2.1 Study Site and Soil Sampling

The study sites were located in Qiqihar City (45°53′–48°56′ N, 122°24′–126°41′ E) in southwest Heilongjiang Province, China, which has a typical temperate continental semi-arid monsoon climate. Precipitation is rare and strong winds occur frequently in spring. The annual mean temperature is 2.3°C, and mean annual precipitation is 454.0 mm, with approximately 70% falling between June and September (Jiang et al., 2014). Two types of soil samples were collected from agricultural fields and classified as sandy loam and silt loam according to the soil texture classification system issued by the International Society of Soil Science. The conventional cropping practice is continuous maize cultivation from May to October each year, with a mean annual N fertilizer application of approximately 750 kg N/hm².

The soil types used in this study represent typical cultivated soils, as sandy loam and silt loam cover approximately 6.68% and 5.29%, respectively, of the local agricultural land area (Du et al., 1994).

Soil sampling was conducted after crop harvest in October 2018. For each soil type, three sites of 100 m × 100 m were randomly selected as spatial replicates. At each site, three plots (4 m × 4 m) were randomly established, and four soil cores (5 cm diameter) were collected from the 0–20 cm depth in each of four sampling quadrats (2 m × 2 m) per plot. A total of 12 soil cores from each replicated site were pooled to form a composite sample. Roots and plant litter were removed by hand, and all soils were passed through a 2-mm sieve and split into two subsamples. One subsample was stored at 4°C for incubation within one week, and the other was air-dried to measure basic physical and chemical properties.

2.2 ¹⁵N Tracing Experiment

The ¹⁵N tracing experiment was conducted using the method of Lang et al. (2010) with modifications. Briefly, two ¹⁵N-labeled treatments (each with three replicates) were established: ¹⁵N-labeled ammonium (¹⁵NH₄⁺) and ¹⁵N-labeled nitrate (NO₃⁻) with 5-atom% ¹⁵N isotopic excess. For each

soil, 30 g (dry weight basis) of fresh soil was weighed into 30 Erlenmeyer flasks (250 mL) (2 ^{15}N -labeled treatments \times 3 replicates \times 5 sampling times = 30 flasks).

Each ^{15}N -labeled NH_4NO_3 solution was added to 15 flasks at 1 mL volume to reach a final applied N concentration of 40 g $\text{NH}_4\text{NO}_3\text{-N/g}$ soil, and soil water content was adjusted to 60% WHC. Flasks were sealed with silicone rubber stoppers and incubated for 7 days at 20°C in darkness. During incubation, stoppers were removed for 30 minutes daily to maintain aerobic conditions, and water lost through evaporation was compensated by adding deionized water via pipette as needed.

At days 0, 1, 3, 5, and 7 after NH_4NO_3 solution addition, gas samples were collected from inside the flasks. For each sampling time, three replicate flasks from each ^{15}N -labeled treatment were randomly selected and flushed three times with room air using a multiport vacuum manifold (4 minutes each time), then immediately sealed with silicone rubber stoppers (with gas collection ports) for 24 hours. When sampling, headspace gas was thoroughly mixed by repeated extraction and injection five times with a gas-tight syringe. Subsequently, 20 mL of headspace gas was collected and injected into 12.5 mL pre-evacuated vials to determine $\text{N}_2\text{O-N}$ concentration and ^{15}N isotopic excess using an Agilent 7890 gas chromatograph (Agilent Technologies, USA) and a Finnigan MAT 253 mass spectrometer (Thermo Electron Corporation, USA), respectively.

After gas sampling, soil samples were extracted with 2 mol/L KCl solution (soil:water ratio 1.0:2.5) by shaking for 1 hour at 250 r/min with a mechanical rotary shaker. The solution was filtered into 100 mL plastic bottles and stored at 4°C for analysis of NH_4NO_3 concentrations and ^{15}N isotopic excess within one week. To analyze ^{15}N isotopic excess in the labeled soil, we used the steam distillation method to separate NH_4NO_3 . Specifically, 10 mL of KCl extract was first steam-distilled with magnesium oxide to separate NH_4NO_3 , then distilled again with Devarda's alloy to separate NO_3^- . NH_4NO_3 and NO_3^- were liberated as NH_3 and absorbed in 5 mL of H_2SO_4 solution (0.010 mol/L). To prevent cross-contamination of ^{15}N isotopic excess between samples, 20 mL of ethyl alcohol was distilled after each sample to clean the distillation system. Concentrations of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were determined by titrating excess H_2SO_4 with 0.010 mol/L NaOH. The titrated solution was then acidified with 0.005 mol/L H_2SO_4 to pH 3 to avoid microbial contamination and dried at 65°C in an oven to constant weight. The dried material was packed into tin capsules for ^{15}N isotopic excess analysis using a stable isotope mass spectrometer (Finnigan MAT 253, Thermo Electron Corporation, USA). The extracted soil was washed three times with 150 mL deionized water to remove residual KCl, then dried at 65°C and finely ground with a ball grinder. A portion of the soil was packed into tin capsules to measure organic N concentration and ^{15}N isotopic excess using a CN analyzer (NA Series 2, Capillary Electrophoresis Instruments, Italy) linked to the stable isotope ratio mass spectrometer.

2.3 Soil Property Analysis

Soil samples were stirred quickly with a glass rod for 1 minute after adding deionized water (soil:water ratio 1.0:2.5), then left to stand for 30 minutes before measuring pH with a DMP-2 mV/pH detector (Quark Ltd., China). Soil WHC was determined after waterlogging air-dried soil for 2 hours and natural draining for 12 hours (Fierer and Schimel, 2002). Particle size distribution was measured using the hydrometer method after dispersing soil with pyrophosphate for 12 hours. Sand, silt, and clay contents are expressed as percentage by mass of dispersed soil. Soil organic carbon (SOC) was analyzed by titration with FeSO after H SO -K Cr O digestion, and total N (TN) was analyzed via semi-micro Kjeldahl digestion with K SO , CuSO , and Se powder mixture as catalysts.

Water-soluble organic carbon (WSOC) and water-soluble organic N (WSON) were determined according to Burford and Bremner (1975). Inorganic N (NH - N and NO -N) in air-dried soil was extracted with 2 mol/L KCl (soil:water ratio 1.0:2.5) on a mechanical shaker for 60 minutes, then filtered. Concentrations of NH -N and NO -N in the filtrate were determined using a continuous-flow analyzer (Skalar Analytical Corporation, Netherlands).

2.4 Calculation and Statistical Analysis

Gross N transformation rates were calculated using the FLUAZ model (Mary et al., 1998). This model's calculations are based on isotopic dilution and enrichment principles. It uses a numerical fourth-order Runge-Kutta algorithm with variable time-step to solve the differential system, and a non-linear fitting program (based on Marquardt's algorithm) to calculate unknown N transformation rates between NH , NO , and organic N pools. Minimizing the mean weighted error (MWE) is used as a criterion instead of sum of squares for optimal fitting of experimental data, which accounts for measurement variance and normalizes various variables (Mary et al., 1998). In this study, five gross N transformation rates were estimated by the FLUAZ model: gross N mineralization rate (m , mg N/(kg · d)), gross nitrification rate (n , mg N/(kg · d)), gross N immobilization rate (i , mg N/(kg · d)), NO immobilization rate (i_n , mg N/(kg · d)), and NH immobilization rate (i_a , mg N/(kg · d)) from measurements of amounts and ¹⁵N isotopic excesses of NH , NO , and organic N. Time-weighted average rates for the whole incubation period were calculated in addition to individual time interval rates. N O flux was calculated according to Lang et al. (2011). N O emission fractions derived from nitrification and denitrification were calculated as described by Steven et al. (1997). Within the two emission pools in their model, the contribution of denitrification (d) and nitrification ($1 - d$) to N O emissions could be calculated from the NH ¹⁵NO labeled treatment using the following equation:

$$d = \frac{a_m - a_n}{a_d - a_n}$$

where a_m is the ^{15}N isotopic excess of the N O mixture (atom%); a_n is the ^{15}N isotopic excess of NH_3 (atom%); and a_d is the ^{15}N isotopic excess of NO_3^- (atom%).

Differences in measured soil properties and time-weighted average gross N transformation rates between sandy loam and silt loam soils were assessed by paired sample t-tests. All statistical analyses were performed using SPSS 13.0 (IBM, USA).

3.1 Soil Properties

As shown in Table 1, significant differences in soil properties existed between sandy loam and silt loam soils. Sandy loam soil had a sand content of 80.5%, significantly higher than silt loam soil (23.4%), while silt and clay contents in sandy loam soil were 11.1% and 8.40%, respectively, significantly lower than those in silt loam soil. WHC, SOC, TN, WSOC, WSON, NH_3 , and NO_3^- contents in sandy loam soil were all significantly lower compared to silt loam soil, whereas no significant difference in pH was found between the two soils.

Table 1 Basic physical and chemical properties of the tested soils

Property	Sandy loam soil	Silt loam soil
pH	$6.3 \pm 0.5a$	$5.7 \pm 0.4a$
WHC (%)	$39.3 \pm 3.2b$	$55.5 \pm 3.9a$
SOC (g/kg)	$14.6 \pm 2.6b$	$21.4 \pm 2.1a$
TN (g/kg)	$1.1 \pm 0.1b$	$2.1 \pm 0.2a$
C/N ratio	$12.9 \pm 2.0a$	$10.3 \pm 1.0a$
WSOC (mg C/kg)	$154.1 \pm 13.0b$	$257.2 \pm 11.3a$
WSON (mg N/kg)	$16.4 \pm 2.1b$	$28.9 \pm 2.3a$
Sand (>50 μm) (%)	$80.5 \pm 4.1a$	$23.4 \pm 1.9b$
Silt (2–50 μm) (%)	$11.1 \pm 0.7b$	$62.3 \pm 2.1a$
Clay (<2 μm) (%)	$8.4 \pm 0.4b$	$14.3 \pm 2.1a$
NH_3 -N (mg/kg)	$1.8 \pm 0.1b$	$5.1 \pm 0.3a$
NO_3^- -N (mg/kg)	$9.7 \pm 1.5b$	$14.8 \pm 1.4a$

Note: WHC, water holding capacity; SOC, soil organic carbon; C, carbon; N, nitrogen; TN, total N; WSOC, water soluble organic C; WSON, water soluble organic N. Different lowercase letters within the same row indicate significant differences at $P < 0.05$ level. Mean \pm SD; $n = 3$.

3.2 Concentrations and ^{15}N Isotopic Excess of Inorganic N

For both soils, an obvious decrease in NH_3 concentration and increase in NO_3^- concentration over time indicated that net nitrification occurred (Fig. 1 [Figure 1: see original paper]). The decrease in NH_3 concentration and increase

in NO_3^- concentration were larger in silt loam soil than in sandy loam soil. In the $^{15}\text{NH}_4^+$ NO_3^- treatments for both soils, the ^{15}N isotopic excess of NH_4^+ resulting from mineralization of native organic N was continuously input into the NH_4^+ pool. In the same ^{15}N -labeled treatment, the ^{15}N isotopic excess of NO_3^- increased gradually during the whole incubation period due to input from nitrified NH_4^+ . In the NH_4^+ $^{15}\text{NO}_3^-$ labeled treatments, the ^{15}N isotopic excess of NH_4^+ was very low and showed no significant change between sampling times (Fig. 2c and d), suggesting that remineralization of immobilized NO_3^- could be neglected. Conversely, in the ^{15}N -labeled NO_3^- treatments, the ^{15}N isotopic excess of the NO_3^- pool decreased over time, caused by nitrification of naturally abundant ^{15}N -containing NH_4^+ pool (Fig. 2b and d). The ^{15}N isotopic excess of NO_3^- in silt loam soil increased much faster than in sandy loam soil in the $^{15}\text{NH}_4^+$ spiked treatment (Fig. 2a and b), consistent with the faster decrease of ^{15}N isotopic excess of NO_3^- in silt loam soil than in sandy loam soil in the $^{15}\text{NO}_3^-$ -spiked treatment (Fig. 2c and d), indicating that nitrification was stronger in silt loam soil.

Fig. 1 Concentrations of NH_4^+ and NO_3^- in sandy loam and silt loam soils over the 7-d incubation time. Since values of N were equivalent for the ^{15}N -labeled NH_4^+ and NO_3^- treatments, data were pooled. Vertical bars indicate standard deviations ($n = 6$).

Fig. 2 ^{15}N isotopic excess of NH_4^+ , NO_3^- , and N_2O in the studied soils over the 7-d incubation time. (a) ^{15}N isotopic excess of sandy loam soil in the $^{15}\text{NH}_4^+$ NO_3^- treatment; (b) ^{15}N isotopic excess of silt loam soil in the $^{15}\text{NH}_4^+$ NO_3^- treatment; (c) ^{15}N isotopic excess of sandy loam soil in the NH_4^+ $^{15}\text{NO}_3^-$ treatment; (d) ^{15}N isotopic excess of silt loam soil in the NH_4^+ $^{15}\text{NO}_3^-$ treatment. Vertical bars indicate standard deviations ($n = 3$).

3.3 Gross Nitrogen Transformations

For silt loam soil, the gross N mineralization rate reached its highest value during the first time interval and then decreased gradually until the end (Table 2). However, the gross N mineralization rate in sandy loam soil showed an initial increase until peaking during the day 3–5 interval, then declined thereafter. The average gross N mineralization rate in silt loam soil was 3.595 mg N/(kg · d), 3.62 times that in sandy loam soil (0.993 mg N/(kg · d)) (Fig. 3 [Figure 3: see original paper]). Similar temporal patterns in gross N immobilization rates were observed in both soils, generally characterized by highest rates during the first time interval and subsequent decrease toward the end of incubation (Table 2). The average gross N immobilization rate in silt loam soil was 1.897 mg N/(kg · d), 4.26 times that in sandy loam soil (0.445 mg N/(kg · d)) (Fig. 3). The gross nitrification rate in sandy loam soil remained nearly constant over the first 5 days, then dropped sharply, whereas in silt loam soil it peaked during the day 1–3 interval and then decreased (Table 2). The average gross nitrification rate in silt loam soil was 5.632 mg N/(kg · d), 3.13 times that in sandy loam soil (1.796 mg N/(kg · d)) (Fig. 3).

Results from the FLUAZ model showed that both NH_4^+ and NO_3^- were immobilized in both soils throughout the incubation period (Table 2). However, NH_4^+ immobilization rates were much higher than NO_3^- immobilization rates for each time interval in both soils (Table 2). The amount of NH_4^+ immobilized accounted for 70.2% to 98.0% of all immobilized N, demonstrating that microbes preferentially utilize NH_4^+ rather than NO_3^- .

Table 2 Gross N transformation rates in sandy loam and silt loam soils at different time intervals

Gross N transformation rate (mg N/(kg · d))	Soil type	Day 0–1	Day 1–3	Day 3–5	Day 5–7
m (gross N mineralization)	Sandy loam	0.133± 0.071	0.289± 0.051	2.831± 0.427	0.289± 0.061
	Silt loam	6.169± 0.776	5.119± 0.864	3.340± 0.520	1.041± 0.433
i (gross N immobilization)	Sandy loam	0.980± 0.104	0.377± 0.107	0.280± 0.051	0.412± 0.024
	Silt loam	5.025± 0.863	1.951± 0.233	1.985± 0.325	0.191± 0.062
ia (NH_4^+ immobilization)	Sandy loam	0.917± 0.090	0.350± 0.124	0.258± 0.063	0.377± 0.068
	Silt loam	4.927± 0.792	1.881± 0.290	1.923± 0.144	0.134± 0.110
in (NO_3^- immobilization)	Sandy loam	0.064± 0.014	0.027± 0.004	0.023± 0.014	0.035± 0.024
	Silt loam	0.098± 0.068	0.070± 0.014	0.062± 0.011	0.057± 0.022
n (gross nitrification)	Sandy loam	2.198± 0.331	2.089± 0.411	2.273± 0.313	0.826± 0.091
	Silt loam	5.093± 0.663	6.854± 0.950	6.043± 0.740	4.270± 0.225

Note: *m*, gross N mineralization rate; *i*, gross N immobilization rate; *i_a*, NH_4^+ immobilization rate; *i_n*, NO_3^- immobilization rate; *n*, gross nitrification rate. Mean±SD; *n* = 3.

Fig. 3 Time-weighted average gross N transformation rates during day 0–7 in sandy loam and silt loam soils. *m*, gross N mineralization rate; *i*, gross N immobilization rate; *i_a*, NH_4^+ immobilization rate; *n*, gross nitrification rate. Vertical bars indicate standard deviations (*n* = 3). Different lowercase letters on a pair of columns represent significant differences between the two soils at *P* < 0.05 level.

The ratio of gross nitrification rate to NH_4^+ immobilization rate (n/i_a) can indicate the dominant consumption pathway of NH_4^+ in soil. The n/i_a ratio was

4.36 in sandy loam soil, significantly higher than in silt loam soil (3.08).

3.4 N O Flux and Sources

In silt loam soil, N O flux increased sharply from day 1 to 3 then declined quickly over the remaining incubation time. In sandy loam soil, N O flux showed a slow increase from day 1 to 5, followed by gradual decrease until the end of incubation (Fig. 4 [Figure 4: see original paper]). At each sampling time, N O flux in silt loam soil was significantly higher than in sandy loam soil ($P < 0.01$). The average time-weighted N O flux over the incubation period was 490 ng N O/(kg·h) in silt loam soil, significantly higher than in sandy loam soil (174 ng N O/(kg·h)).

For both soils, the ^{15}N isotopic excess of the N O pool was always between that of the NH_4^+ and NO_3^- pools for both $^{15}\text{NH}_4^+$ and $\text{NH}_4^+^{15}\text{NO}_3^-$ treatments (Fig. 2), and the ^{15}N isotopic excess of N O was closer to that of the NH_4^+ pool. This demonstrated that N O was derived from both nitrification and denitrification, but nitrification contributed more to N O emissions. The N O emission fractions derived from nitrification and denitrification varied with sampling time (Fig. 5 [Figure 5: see original paper]). The relative contribution of denitrification to N O emissions tended to first increase then decrease with time in silt loam soil, whereas it showed a decrease-increase-decrease pattern in sandy loam soil. The opposite trend was observed for nitrification's relative contribution. For both soils, nitrification was the dominant source contributing to N O emissions, as the N O emission fraction from nitrification was significantly higher than from denitrification over the whole incubation period (Fig. 5). In sandy loam soil, the average contribution of nitrification to total N O production (82.1%) was significantly higher than in silt loam soil (71.7%), while the average contribution of denitrification was 17.9%, significantly lower than in silt loam soil (28.3%).

Fig. 4 N O flux from sandy loam and silt loam soils over the 7-d incubation time. Since N O flux values were equivalent for the ^{15}N -labeled NH_4^+ and NO_3^- treatments, data were pooled. Vertical bars indicate standard deviations ($n = 6$).

Fig. 5 N O emission fraction derived from nitrification and denitrification processes in sandy loam and silt loam soils over the 7-d incubation time.

4.1 Nitrogen Transformation Rates

Soil N transformations are microbially regulated processes influenced by various factors, among which soil type is regarded as one of the most important controllers (Cookson et al., 2007; Lang et al., 2010; Zhang et al., 2013; Zhu et al., 2019). Our research supports this general assertion.

This study showed that gross N mineralization and immobilization rates were significantly lower in sandy loam than in silt loam soil. The reason for lower gross N turnover rates may be the lower carbon (C) content in sandy loam

soil (Table 1). Soil C provides substrate and energy for microbial growth, and positive correlations between SOC or WSOC and gross N turnover rates have been reported in many studies (Cookson et al., 2007; Lang et al., 2010, 2016; Zhang et al., 2013). Therefore, higher SOC and WSOC contents in silt loam soil may promote microbial growth and propagation, resulting in higher N mineralization and immobilization rates. However, some studies showed that gross N mineralization rate was more influenced by SOC content and not affected by substrate quality, while immobilization was mainly affected by soluble organic C availability (Hoyle et al., 2006), since microbes involved in mineralization can use various complex organic substrates while those dominating immobilization can only utilize simple carbohydrates (Gibbs and Barraclough, 1998). Thus, higher gross N mineralization and immobilization rates in silt loam soil could be attributed to higher SOC and WSOC contents, respectively.

Consistent with gross N mineralization and immobilization rates, gross nitrification rate in sandy loam soil was also lower than in silt loam soil. Given similar pH in both soils, other factors such as NH_4^+ availability and SOC may regulate nitrification (Hart et al., 1994). Kuroiwa et al. (2011) reported strong correlations between gross nitrification and mineralization rates and soil C concentrations, consistent with our results, suggesting that SOC quantity controls gross nitrification rate by regulating gross mineralization rate, which generates available NH_4^+ for nitrifiers. The relatively lower SOC content and gross N mineralization rate in sandy loam soil may therefore contribute to its lower gross nitrification rate. In addition to SOC, soil texture may be another crucial parameter influencing gross nitrification (Pereira e Silva et al., 2012). Gross nitrification rates correlate positively with clay content and negatively with sand content (Elrys et al., 2020), agreeing with our findings. The lower gross nitrification rate in sandy loam soil may be attributed to: (1) little available NH_4^+ produced via exchange equilibrium in sandy soil (Aulakh et al., 1996); (2) higher abundance and diversity of nitrifying bacteria in soils with larger clay content (Hosny, 1979; Elrys et al., 2020); and (3) strong cation exchange capacity in clay-rich soil that can reduce NH_4^+ concentration in soil solution to nontoxic levels for microorganisms (Lam et al., 2018). Our results highlight that soil texture and SOC content may be the dominant factors controlling gross nitrification rates in these soils.

In soils under laboratory incubation, nitrification and immobilization are the two main consumption fates for NH_4^+ . The ratio of gross nitrification rate to ammonium immobilization rate (n/i_a) reflects the dominant NH_4^+ consumption pathway and can be used as an effective indicator for assessing nitrate leaching potential (Stockdale et al., 2002; Murphy et al., 2003). In this study, n/i_a ratios of sandy loam soil (4.36) and silt loam soil (3.08) were both higher than 1.00, suggesting strong risk of nitrate leaching in both soils. However, the significantly low rates of gross N mineralization and immobilization combined with high n/i_a ratio in sandy loam soil indicated that it has relatively weak capacity for production and conservation of available N and is more vulnerable to nitrate leaching compared with silt loam soil.

4.2 N O Emission Pathways

Our study showed that N O emissions from silt loam soil were significantly higher than from sandy loam soil, agreeing with previous findings of Pihlatie et al. (2004) and Syväsalo et al. (2004), who indicated larger N O emissions in clay than loamy sand soils. High N O emission in silt loam soil could be attributed to high C and N contents providing sufficient substrates for nitrification and denitrification. Furthermore, the significantly high clay content in silt loam soil may favor development of anaerobic microsites, creating conditions conducive to denitrification and greater N O emissions (Smith, 1980).

Under aerobic incubation conditions, nitrification was the dominant process regulating N O production, though denitrification also played an important role. For example, Bateman and Baggs (2005) showed that in agricultural soils at 60.0% and 50.0% WFPS, 18.5% and 23.9% of N O was emitted from denitrification, respectively. Pihlatie et al. (2004) found that when water content was adjusted to 60% WFPS in peat, clay, and loamy sand soils, denitrification contributed 22.0% to 33.0% of N O production. Lan et al. (2013) demonstrated that denitrification contributed 16.3% and 23.9% of total N O emission in two paddy soils at 65.0% WHC. In this study, average denitrification contributions to total N O emission were 18.0% and 28.3% in sandy loam and silt loam soils, respectively, consistent with these findings. The substantial denitrification contribution suggests it occurred under aerobic conditions, even though gross denitrification rates calculated by the FLUAZ model were near zero in both soils (data not shown). Large N O emissions might result from anaerobic microsite formation. Renault and Stengel (1994) noted that rapid microbial growth consumes substantial oxygen in soil aggregates, leading to anoxic microenvironments. Additionally, anaerobic microsites may form in aerobic soils with high clay content and high nitrification rates, since rapid nitrification limits O₂ supply and high clay content prevents O₂ diffusion (Smith, 1980), creating anaerobic environments where denitrification can occur. Moreover, when N solution was added via pipettes, it could not distribute uniformly in soil aggregates, potentially saturating some aggregates and making them conducive to denitrification. Garrido et al. (2002) also reported aerobic denitrification in soil, but underlying mechanisms remain poorly understood.

As discussed above, the low clay content and low gross nitrification rate in sandy loam soil compared with silt loam soil (Table 1) may explain the lower denitrification contribution to N O emissions in sandy loam soil (17.9%) than in silt loam soil (28.3%). Our result agrees with Pihlatie et al. (2004), who found lower denitrification contribution to N O emissions in loamy sand (22.5%) than in clay soil (33.5%) under aerobic conditions. Among mineral soil physical properties, texture, macro- and micro-porosity, and soil aeration are important factors regulating N O emission pathways (Simojoki and Jaakkola, 2000). Sandy loam soil would have larger macro-porosity, allowing O₂ transport and supply, which would be unfavorable for denitrification.

Our results are supported by Pihlatie et al. (2004), who reported that nitrification remained the dominant N O emission process even at 100% WFPS for loamy sand soil that was visibly waterlogged. Castellano-Hinojosa et al. (2020) also showed that in sandy loam soil incubated at 80% WFPS, N O was emitted mainly from nitrification rather than denitrification, indicating the good aerobic properties of sandy loam soil that favor nitrification.

Our results are consistent with most published findings showing nitrification as the main N O production pathway under aerobic conditions (Steven et al., 1997; Pihlatie et al., 2004; Well et al., 2008; Lan et al., 2014). In contrast, some studies showed denitrification as the dominant pathway under aerobic conditions. For example, Zhang et al. (2011) indicated that denitrification accounted for 53.5% to 56.1% of total N O production in four acidic forest soils incubated under aerobic conditions (40% to 52% WFPS). Li et al. (2016) reported that denitrification contributed more than 50% of N O emissions in loamy sand soil at 40% to 60% WFPS when crop residues were added. The different result of Zhang et al. (2011) may be because fungi prevailed in acidic forest soils, and fungal-mediated denitrification was an important N O source due to potential lack of N O reductase in fungal biomass (Laughlin and Stevens, 2002). In Li et al. (2016), crop residue incorporation caused large oxygen consumption, leading to denitrification predominance as an N O source. Thus, the relative dominance of nitrification and denitrification in N O release under aerobic conditions is influenced by soil type and substrate supply.

Our results indicated that N O emission fractions from nitrification and denitrification fluctuated during the 7-day incubation in both soils, consistent with Khalil et al. (2004), Li and Lang (2014), and Li et al. (2016), who found similar phenomena in unsaturated soils. However, Stevens et al. (1997) found that denitrification contribution to N O emissions decreased after two days of incubation regardless of soil water content. In some cases, the N O emission fraction from denitrification increased with time under aerobic conditions (Wolf and Russow, 2000; Lan et al., 2013). These conflicting results may relate to soil type, incubation time, or substrate availability, and underlying mechanisms require further research.

4.3 Implications of Gross N Transformation Rates and N O Emission Pathways

The sandy loam and silt loam soils selected for this study are typical of local agriculture in western Heilongjiang Province, China. Significant differences in soil properties led to different gross N transformation rates and N O emissions. Sandy loam soil had lower gross N mineralization and immobilization rates than silt loam soil due to relatively low C content, but the ratio of gross nitrification to ammonium immobilization rate was relatively high. This suggests that mineral N produced from mineralization in sandy loam soil was low, requiring large amounts of N fertilizer to meet crop nutrient requirements. However, it is better to apply N fertilizer at low intensity and high frequency to avoid nitrate

leaching, considering the soil's low capacity to preserve available N. Additionally, this study showed that nitrification was the dominant N₂O emission process in both soils under aerobic conditions, with higher nitrification contribution in sandy loam soil than in silt loam soil. These differences provide improved understanding of the relative importance of the two N₂O production processes in sandy loam and silt loam soils, which is valuable for improving predictability of soil N₂O emissions, proposing mitigation strategies, and establishing greenhouse gas inventory models. However, caution is needed when extrapolating these laboratory results to field conditions.

5 Conclusions

Our study demonstrated that soil type is an important factor influencing gross N transformations and N₂O emissions from agricultural soil. Gross N mineralization, immobilization, and nitrification rates were significantly lower in sandy loam than in silt loam soil, but the ratio of gross nitrification rate to NH₄⁺ immobilization rate was much larger in sandy loam soil, though both ratios exceeded 1.00. This suggests that sandy loam soil has relatively poor ability to supply and conserve available N and is more vulnerable to nitrate leaching compared with silt loam soil. Nitrification and denitrification occurred simultaneously at 60% WHC in both soils, with nitrification being the dominant N₂O production source and its contribution to N₂O emissions higher in sandy loam soil than in silt loam soil. These differences in gross N transformation rates imply that soil type must be considered when managing fertilizer applications, and data on N₂O production pathways from different agricultural soils are valuable for proposing effective mitigation strategies and developing process-based models to reduce uncertainties in N₂O emission estimates. However, caution is needed because this study was conducted under laboratory conditions, and further field-scale research is required to consider additional factors.

Acknowledgements: This study was financed by the National Natural Science Foundation of China (41301345, 41101284).

References

- Aulakh M S, Kuldip S, Bijay S. 1996. Kinetics of nitrification under upland and flooded soils of varying texture. *Communications in Soil Science and Plant Analysis*, 27(9–10): 2079–2089.
- Bateman E J, Baggs E M. 2005. Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils*, 41(6): 379–388.
- Burford J R, Bremner J M. 1975. Relationships between the denitrification capacities of soils and total water soluble and readily decomposable soil organic matter. *Soil Biology and Biochemistry*, 7(6): 389–394.

- Byrnes B H. 1990. Environmental effects of N fertilizer use—an overview. *Fertilizer Research*, 26: 209–215.
- Castellano-Hinojosa A, Charteris A F, Müller C, et al. 2020. Occurrence and ^{15}N -quantification of simultaneous nitrification and denitrification in N-fertilized soils incubated under oxygen-limiting conditions. *Soil Biology and Biochemistry*, 143: 107757, doi: 10.1016/j.soilbio.2020.107757.
- Cookson W R, Osman M, Marschner P, et al. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. *Soil Biology and Biochemistry*, 39(3): 744–756.
- Dobbie K E, Smith K A. 2001. The effects of temperature, water-filled pore space and land use on N O emissions from an imperfectly drained gleysol. *European Journal of Soil Science*, 52(4): 667–673.
- Du M G, Li Y F, Qiu Z Y, et al. 1994. The improvement approaches and utilization of sand-drift soil in semiarid area in the west part of Heilongjiang Province. *Agricultural Research in the Arid Areas*, 12(1): 68–73. (in Chinese)
- Elyrs A S, Raza S, Elnahal A S M, et al. 2020. Do soil property variations affect dicyandiamide efficiency in inhibiting nitrification and minimizing carbon dioxide emissions? *Ecotoxicology and Environmental Safety*, 202: 110875, doi: 10.1016/j.ecoenv.2020.110875.
- Fierer N, Schimel J P. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, 34(6): 777–787.
- Garrido F, Hénault C, Gaillard H, et al. 2002. N O and NO emissions by agricultural soils with low hydraulic potentials. *Soil Biology and Biochemistry*, 34(5): 559–575.
- Gibbs P, Barraclough D. 1998. Gross mineralisation of nitrogen during the decomposition of leaf protein I (ribulose 1,5-diphosphate carboxylase) in the presence or absence of sucrose. *Soil Biology and Biochemistry*, 30(13): 1821–1827.
- Grünzweig J M, Sparrow S D, Chapin F S. 2003. Impact of forest conversion to agriculture on carbon and nitrogen mineralization in subarctic Alaska. *Biogeochemistry*, 64: 271–296.
- Hart S C, Nason G E, Myrold D D, et al. 1994. Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. *Ecology*, 75(4): 880–891.
- Hosny L. 1979. Biological oxidation of ammoniacal fertilizers as affected by the physical properties of soil. *Zentralbl Bakteriol Naturwiss*, 134(6): 513–527.
- Hoyle F C, Murphy D V, Fillery I R P. 2006. Temperature and stubble management influence microbial CO₂-C evolution and gross N transformation rates. *Soil Biology and Biochemistry*, 38(1): 71–80.

- Jiang H T, Liu W H, Fang Y F, et al. 2014. Study on soil types and utilization in Qiqihar City. *Protection Forest Science and Technology*, 5: 95–96. (in Chinese)
- Khalil K, Mary B, Renault P. 2004. Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O₂ concentration. *Soil Biology and Biochemistry*, 36(4): 687–699.
- Kroeze C, Mosier A, Bouwman L. 1999. Closing the global N O budget: A retrospective analysis 1500–1994. *Global Biogeochemical Cycles*, 13(1): 1–8.
- Kuroiwa M, Koba K, Isobe K, et al. 2011. Gross nitrification rates in four Japanese forest soils: heterotrophic versus autotrophic and the regulation factors for the nitrification. *Journal of Forest Research*, 16(5): 363–373.
- Lam S K, Sutera H, Daviesb R, et al. 2018. Direct and indirect greenhouse gas emissions from two intensive vegetable farms applied with a nitrification inhibitor. *Soil Biology and Biochemistry*, 116: 48–51.
- Lan T, Han Y, Roelcke M, et al. 2013. Processes leading to N O and NO emissions from two different Chinese soils under different soil moisture contents. *Plant and Soil*, 371(1–2): 611–627.
- Lan T, Han Y, Roelcke M, et al. 2014. Sources of nitrous and nitric oxides in paddy soils: Nitrification and denitrification. *Journal of Environmental Sciences*, 26(3): 581–592.
- Lang M, Cai Z C, Mary B, et al. 2010. Land-use type and temperature affect gross nitrogen transformation rates in Chinese and Canadian soils. *Plant and Soil*, 334: 377–389.
- Lang M, Cai Z C, Chang S X. 2011. Effects of land use type and incubation temperature on greenhouse gas emissions from Chinese and Canadian soils. *Journal of Soils and Sediments*, 11(1): 15–24.
- Laughlin R J, Stevens R J. 2002. Evidence for fungal dominance of denitrification and co-denitrification in grassland soil. *Soil Science Society of America Journal*, 66(5): 1540–1548.
- Li P, Lang M. 2014. Gross nitrogen transformations and related N O emissions in uncultivated and cultivated black soil. *Biology and Fertility of Soils*, 50(2): 197–206.
- Li X X, Sørensen P, Olesen J E, et al. 2016. Evidence for denitrification as main source of N O emission from residue-amended soil. *Soil Biology and Biochemistry*, 92: 153–160.
- Li Z G, Xia S J, Zhang R H, et al. 2020. N O emissions and product ratios of nitrification and denitrification are altered by K fertilizer in acidic agricultural soils. *Environmental Pollution*, 265: 115065, doi: 10.1016/j.envpol.2020.115065.
- Mary B, Recous S, Robin D. 1998. A model for calculating nitrogen fluxes in soil using ¹⁵N tracing. *Soil Biology and Biochemistry*, 30(14): 1963–1979.

- Müller C, Rütting T, Kattge J, et al. 2007. Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling. *Soil Biology and Biochemistry*, 39(3): 715–726.
- Murphy D V, Recous S, Stockdale E A, et al. 2003. Gross nitrogen fluxes in soil: theory, measurement and application of ^{15}N pool dilution techniques. *Advances in Agronomy*, 79: 69–118.
- Pereira e Silva M C, Poly F, Guillaumaud N, et al. 2012. Fluctuations in ammonia oxidizing communities across agricultural soils are driven by soil structure and pH. *Frontiers in Microbiology*, 3: 1–22.
- Pihlatie M, Syväsalo E, Simojoki A, et al. 2004. Contribution of nitrification and denitrification to N_2O production in peat, clay and loamy sand soils under different soil moisture conditions. *Nutrient Cycling in Agroecosystems*, 70: 135–141.
- Renault P, Stengel P. 1994. Modeling oxygen diffusion in aggregated soils: I. Anaerobiosis inside the aggregates. *Soil Science Society of America Journal*, 58(4): 1017–1023.
- Simojoki A, Jaakkola A. 2000. Effect of nitrogen fertilization, cropping and irrigation on soil air composition and nitrous oxide emission in a loamy clay. *European Journal of Soil Science*, 51(3): 413–424.
- Smith K A. 1980. A model of the extent of anaerobic zones in aggregated soils, and its potential application to estimates of denitrification. *Journal of Soil Science*, 31(2): 263–277.
- Stevens R J, Laughlin R J, Burns L C, et al. 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry*, 29(2): 139–151.
- Stockdale E A, Hatch D J, Murphy D V, et al. 2002. Verifying the nitrification to immobilisation ratio (N/I) as a key determinant of potential nitrate loss in grassland and arable soils. *Agronomie*, 22: 831–838.
- Syväsalo E, Regina K, Pihlatie M, et al. 2004. Emissions of nitrous oxide from boreal agricultural clay and sandy loam soils. *Nutrient Cycling in Agroecosystems*, 69: 155–165.
- Tripathi N, Singh R S. 2009. Influence of different land uses on soil nitrogen transformations after conversion from an Indian dry tropical forest. *Catena*, 77(3): 216–223.
- Wang M X, Zhang Y X. 2013. Effects of different tillage measures on maize growth and yield in aeolian sandy soil area in Heilongjiang province. *Bulletin of Soil and Water Conservation*, 33: 59–63. (in Chinese)
- Well R, Flessa H, Lu X, et al. 2008. Isotopologue ratios of N_2O emitted from microcosms with NH_4^+ fertilized arable soils under conditions favoring nitrification. *Soil Biology and Biochemistry*, 40(9): 2416–2426.

Wolf I, Russow R. 2000. Different pathways of formation of N₂O, N₂ and NO in black earth soil. *Soil Biology and Biochemistry*, 32(2): 229–239.

Yang L L, Zhang F S, Gao Q, et al. 2010. Impact of land-use types on soil nitrogen net mineralization in the sandstorm and water source area of Beijing, China. *CATENA*, 82(1): 15–22.

Zhang J B, Cai Z C, Zhu T B. 2011. N₂O production pathways in the subtropical acid forest soils in China. *Environmental Research*, 111(5): 643–649.

Zhang J B, Zhu T B, Meng T Z, et al. 2013. Agricultural land use affects nitrate production and conservation in humid subtropical soils in China. *Soil Biology and Biochemistry*, 62: 107–114.

Zhang Y, Wang J, Dai S Y, et al. 2019. Temperature effects on N₂O production pathways in temperate forest soils. *Science of the Total Environment*, 691: 1127–1136.

Zhu G D, Song X T, Ju X T, et al. 2019. Gross N transformation rates and related N₂O emissions in Chinese and UK agricultural soils. *Science of the Total Environment*, 666: 176–186.

Zhu T B, Zhang J B, Cai Z C. 2011. The contribution of nitrogen transformation processes to total N₂O emissions from soils used for intensive vegetable cultivation. *Plant and Soil*, 343: 313–327.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.