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Key Factors, Soil Nitrogen Processes, and Nitrite Accumulation Affecting Nitrous Oxide Emissions

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A better understanding of the factors and processes affecting N_2O emissions is essential for developing mitigation strategies. This research aimed to examine the factors and processes affecting N_2O emissions and N dynamics. Laboratory incubation experiments examined the effects of N (urea) application rate (0–150 mg N kg^{-1}); soil water content (5–30%, w/w); temperature (10–40°C); and incorporation of biochar (1%, w/w), a urease inhibitor (Agrotain Ultra), and a nitrification inhibitor (N-Serve 24) on N_2O emissions and N transformation dynamics in a Hanford sandy loam soil. Nitrous oxide emissions, soil pH, and mineral N species were monitored for 35 d. Peak emission rates and cumulative losses of N_2O increased more than the N application rate increased. Soil water content at 20 and 30% [above the water holding capacity (WHC) of 12%] resulted in much higher total N_2O emissions (9.3 and 8.0% of total soil inorganic N, respectively) than that from 5 and 10% water content (0.2 and 0.3%, respectively). Increasing soil water content above WHC caused higher N_2O emissions than increasing the soil temperature, which demonstrates that soil moisture is more significant in affecting the process. Nitrite concentration was highly correlated with N_2O emissions within two water content ranges (above or below WHC). Amendment with biochar, Agrotain Ultra, and N-Serve 24 reduced N_2O emissions by 74, 78, and 74%, respectively. This research provide further understanding of the processes affecting N_2O emissions from soil, which can assist in developing management practices.

Abbreviations: DI, deionized; GC, gas chromatograph; TIN, total inorganic N; WHC, water holding capacity

Core Ideas

- **Nitrite concentration was highly correlated with N_2O emissions within two distinct water content ranges.**
- **Soil moisture was the most important environmental factor affecting N_2O emissions.**
- **Nitrous oxide emissions increased exponentially as the N application rate increased.**
- **Biochar and N transformation inhibitors showed great potential to reduce N_2O emissions.**

Nitrous oxide has a global warming potential 310 times that of CO_2 (on a per molecule basis over a 100-yr period) and also plays an important role in atmospheric photochemical reactions that contribute to stratospheric ozone destruction (Prather et al., 2001; Portmann et al., 2012; Intergovernmental Panel on Climate Change, 2014). Because of the importance and widespread use of synthetic N fertilizers, agriculture accounts for 60% or more of the global N_2O emissions (Intergovernmental Panel on Climate Change, 2014; US Environmental Protection Agency, 2016). Thus reducing N_2O emissions from fertilizer applications is necessary to combat climate change.

Nitrous oxide is produced primarily via microbial nitrification and denitrification processes (Khalil et al., 2004; Wrage et al., 2005; Gillam et al., 2008). Nitrogen in the soil undergoes highly complex transformations. Synthetic N fertilizers are in the form of urea, ammonium, and nitrate (Glibert et al., 2006). Under oxygenated conditions, urea hydrolyzes first to NH_3 or NH_4^+ (Eq. [1]) and then oxidizes to NO_3^- (Eq. [2]). The process results in a net production of two protons (H^+) per molecule of urea although hydrolysis produces two hydroxyls first (Barak et al., 1997; Bolan and Hedley, 2003):

$$CO(NH_2)_2 + 3H_2O = 2NH_4^+ + 2OH^- + CO_2 \text{ (hydrolysis)}; \quad [1]$$

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$$NH_4^+ + 2O_2 = NO_3^- + 2H^+ + H_2O \text{ (nitrification).} \quad [2]$$

The nitrification (oxidation of NH_4^+ to NO_3^-) process includes two stages. The first stage is the oxidation of NH_4^+ or NH_3 to NO_2^- (Eq. [3]), mediated by NH_3 oxidizers; the second stage is the oxidation of NO_2^- to NO_3^- (Eq. [4]), performed by NO_2^- oxidizers (Wrage et al., 2001):

$$2NH_4^+ + 3O_2 = 2NO_2^- + 4H^+ + 2H_2O \text{ (ammonium oxidation);} \quad [3]$$

$$2NO_2^- + O_2 = 2NO_3^- \text{ (nitrite oxidation).} \quad [4]$$

There are four proposed biological pathways for N_2O formation associated with the N transformation processes (Fig. 1). In the first stage of nitrification and under oxygenated conditions, NH_3 is oxidized by ammonia monooxygenase to NO_2^- via NH_2OH, from which N_2O can be formed through chemical decomposition (chemodenitrification). This was referred to as the nitrifier nitrification pathway by Kool et al. (2011) (Pathway A). Under O_2–limited conditions, N_2O can be produced through denitrification by nitrifiers, which is referred to as nitrifier denitrification or as nitrification-coupled denitrification by both nitrifiers and denitrifiers (Pathways B and C, respectively) (Wrage et al., 2001; Maharjan and Venterea, 2013; Zhu et al., 2013). Under highly anaerobic conditions, N_2O production is dominated by denitrification (Pathway D) (Kool et al., 2011).

Although N_2O production is directly associated with N transformation, no correlations have been reported between N_2O emissions and soil NH_4^+ or NO_3^- species (Venterea et al., 2010; McGeough et al., 2012; Zebarth et al., 2012) but a correlation was found between cumulative N_2O emissions and NO_2^- intensity (Maharjan and Venterea, 2013). A linear response of N_2O production rate to NO_2^- was observed when soil was amended with NO_2^- (Venterea, 2007). Nitrite metabolism in the soil is reported to be extremely rapid (Burns et al., 1995) but can accumulate under certain environmental stress such as NH_3 toxicity to NO_2^-–oxidizing bacteria at elevated soil pH and high NH_4^+ concentrations (Burns et al., 1995; Huang et al., 2014). Little information is available on how environmental factors directly affect NO_2^- changes in soil and its correlation with N_2O emissions.

Developing mitigation strategies for N_2O emissions depends on better understanding of the environmental factors, mechanisms, and soil processes involved. Soil moisture and temperature are two main factors affecting N_2O production following N fertilization (Barnard et al., 2005; Gillam et al., 2008). Soil moisture regulates O_2 concentration in the soil and affects nitrifier activity, denitrifier activity, and the pathways for N_2O production (Fig. 1) (Bateman and Baggs, 2005). Soil temperature affects all aspects of respiration processes (Parkin and Tiedje, 1984). In searching for strategies to reduce N_2O emissions, soil amendment with biochar or the use of N transformation inhibitors have been investigated (e.g., Cayuela et al., 2014; Li et al., 2014). Biochar has been shown to slow nitrification by adsorption and retention of the NH_4^+ that may have influenced the production of N_2O (Singh et al., 2010; Ahmad et al., 2014). However, in some cases, biochar is not always effective in reducing N_2O emissions (e.g., Xiang et al., 2015). A urease inhibitor, *N*-(n-butyl)thiophosphoric triamide [the active ingredient in the commercial product Agrotain (Koch Agronomic Services LLC, Wichita, KS), was found to effectively reduce N_2O emissions by inhibiting the reaction in Eq. [1] (Zaman et al., 2009). A nitrification inhibitor, dicyandiamide, was found to delay the oxidation of NH_3 to NH_2OH and further oxidation to NO_3^- or N_2O formation (McGeough et al., 2012). The effectiveness of these inhibitors to reduce N_2O emissions depends on their impact on N transformation processes; however, these have not been well examined.

There is a significant knowledge gap on how N_2O emissions are linked to N transformation processes or if the N dynamics are affected by environmental condition changes. The objective of this research was to examine the important factors and major processes that affect N_2O emissions and N dynamics. The effect of N application rate, soil water content, temperature, biochar amendment, and N transformation inhibitors on N_2O emission, N transformation, soil pH, and their correlations were determined under controlled laboratory conditions.

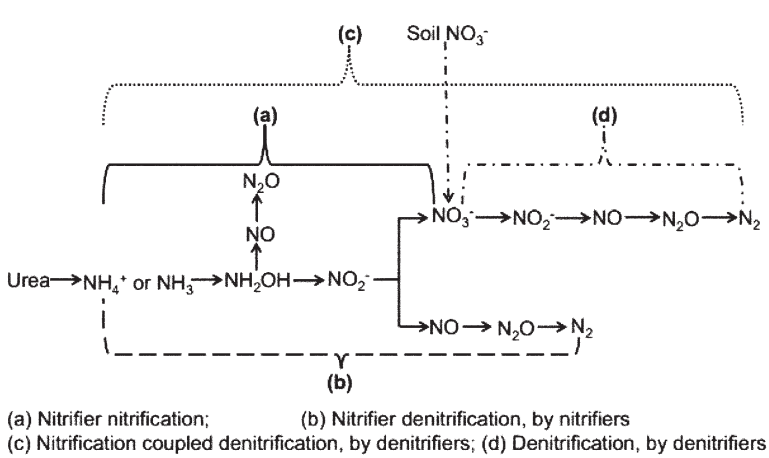

Fig. 1. Proposed pathways for N_2O production.

MATERIAL AND METHODS

Soil, Biochar, and Chemicals

The soil used in this study was collected from a pomegranate (*Punica granatum* L.) orchard at the University of California Kearney Agricultural Research and Extension Center, Parlier, CA, USA (36°36′42″ N, 119°31′34″ W). The soil was a Hanford sandy loam (coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthents). Surface soil (0–20 cm) was collected from areas of the orchard with a history of low N application rates, air-dried to ~3% water content (w/w), sieved to pass through a 4-mm sieve, thoroughly mixed using a portable concrete mixer, and stored in sealed plastic bags for analysis and incubation experiments. The soil contained 0.4% total organic C, 0.04% total N determined by the combustion

method (AOAC International, 2006), a cation exchange capacity of 5.9 meq 100 g^{-1} determined via the barium replacement method (Rible and Quick, 1960), a water content at -33.4 kPa or a holding capacity of 12% (w/w), and 24% water content in a saturated paste following the procedures outlined by Rhoades (1982). The electric conductivity (saturation extract), pH (saturation extract), and NO_3^-–N content extracted with 2M KCl solutions (Knepel, 2003) were 171 μS cm^{-1}, 7.3, and 8.4 mg kg^{-1} soil, respectively. The particle size analysis method of Sheldrick and Wang (1993) indicated a distribution of 59% sand, 32% silt, and 9% clay.

Biochar (CoolTerra, Camarillo, CA) was sieved to pass through a 2-mm sieve and thoroughly mixed. The biochar was derived 100% from coconut shell feedstock, pyrolyzed at 550°C in controlled kiln-based operations, then underwent a proprietary post-production treatment to neutralize the pH and remove residual elements like volatile organic compounds, dioxins or furans. The properties of the biochar were 78.5% total C, 5.5% total O, 0.5% total H, 0.5% total N, a pH of 7.60 (1:10 biochar/water extract), and a specific surface area of 348.8 m^2 g^{-1}. The elemental composition and surface area were determined using a CHN element analyzer (Leco Corp., St. Joseph, MI), and a V-Sorb 2800P surface area and pore distribution analyzer (Gold APP Instruments Corporation, Beijing, China), respectively. Agrotain Ultra [a urease inhibitor containing 15–40% *N*-(n-butyl)thiophosphoric triamide] and N-Serve 24 [a nitrification inhibitor containing 22.2% 2-chloro-6-(trichloromethyl)pyridine] were obtained from Koch Agronomic Services and Dow AgroSciences LLC (Indianapolis, IN, USA), respectively. Urea (46% N) was purchased from Fisher Scientific (Pittsburgh, PA).

Incubation Experiments

Air-dried soil (3.0 kg oven-dried basis) was weighed into 4-L high density polyethylene containers (20 by 20 by 10 cm) with lids (Rubbermaid Commercial Products Inc., Winchester, VA. A urea stock solution (32.16 g L^{-1}) was prepared with deionized (DI) water. A known volume of the solution was diluted with DI water before spraying onto the soil to simultaneously achieve the target N application rate and the desired soil water content. The procedures for each experiment are described below:

1. Nitrogen application rate. Different volumes of the urea stock solution [0 (no urea addition or control), 10, 20, or 30 mL] were mixed with different amounts of DI water, sprayed onto the air-dried soil (3.0 kg oven-dried basis) and mixed thoroughly to achieve a final 10% water content (w/w) and N application rates at 0, 50, 100, and 150 mg N kg^{-1}.
2. Soil water content. Twenty mL of the urea stock solution was diluted with different amounts of DI water added to the air-dried soil for a container to achieve the target water contents of 5, 10, 20, and 30% (w/w). The water contents that were well above WHC were included to represent the conditions that can occur near surface drip irrigation or fertigation lines (Skaggs et al., 2010).
3. Temperature. Soils treated with 150 mg N kg^{-1} and 10% water content were incubated at 10, 25, and 40°C.
4. Biochar amendment. Thirty g of biochar was mixed with the air-dried soil for each container of soil treated with 150 mg N kg^{-1}. The biochar was added before the soil was adjusted to 10% water content (w/w) using DI water.
5. Nitrogen transformation inhibitors. Agrotain Ultra (Agrotain) (Koch Agronomic Services) and N-Serve 24 (N-Serve) (Dow AgroSciences) were tested in soil treated with 150 mg N kg^{-1}. The recommended rates were used for the products (3 U.S. quarts per ton urea, equivalent to 6.7 mL kg^{-1} N for Agrotain, and 1 U.S. quarts per 100 lb N, equivalent to 20.9 mL kg^{-1} N for N-Serve).

For each treatment, the soil and all the materials required were initially mixed in a 10-L plastic bucket. To ensure the uniformity of a treatment, mixing of the soil with a solution was done manually by spraying incremental small amounts of the solution while the soil was continuously mixed. The well-mixed soil was then transferred and placed into the 4-L polyethylene containers without packing to a specified bulk density. All containers were covered with Al foil perforated with evenly spaced holes. The containers were weighed every 3 or 4 d and DI water was sprayed on the soil surface to compensate for water lost through evaporation and mixed again. All containers were incubated in a room with a controlled temperature (25 ± 1°C) except those for the 10 and 40°C treatments, which were kept in incubators set at the target temperature. All treatments were tested in triplicate.

Soil mineral N species and N_2O emissions as well as soil pH were measured during a 35-d incubation period. Treatments for N application rate and soil water content were conducted in the first batch of experiments (21 containers in total). Treatments for temperature, biochar, and N transformation inhibitors were tested in a second batch (18 containers in total) using similar sampling protocols. Sampling for N_2O emissions and soil mineral N species as well as pH were conducted as early as 6 h after urea application and treatment, daily within the first week, and at longer time intervals thereafter.

Nitrous Oxide Emission Measurement and Analysis

For N_2O emission measurements, the perforated Al foil covering the incubation container was replaced with air-tight lids fitted with a sampling port and rubber septa. For each sampling, four gas samples were withdrawn at 0 (immediately after the lid was placed), 0.5, 1, and 1.5 h using a 20-mL plastic syringe. The gas samples were immediately transferred to 10-mL glass headspace vials sealed with Teflon-faced butyl rubber septa secured with Al crimp seals (Agilent Technologies, Santa Clara, CA). The vials were previously flushed with ultra-zero grade air (Praxair, Fresno, CA) to reduce the background N_2O level. Before analysis, the pressure of each vial was released by quickly inserting and withdrawing a syringe needle (0.7 mm by 25 mm). Releasing the vial pressure resulted in more reproducible results than analyzing these results directly from the pressurized vials. Standards of N_2O were prepared in the same manner as the test samples. The N_2O concentration was analyzed on a gas

chromatograph (GC) (6890N, Agilent Technologies) equipped with a split–splitless inlet, a micro-electron capture detector, a headspace sampler (G1888) functioning as a gas autosampler connected to the 6890N GC using an electronic pneumatics control, and a capillary column (HP-PLOT Q, 0.53 mm i.d. by 30 m length by 40 mm film thickness). Helium (Praxair) was the carrier gas and P-5 (a mixture of 5% methane and 95% argon; Praxair) was the make-up gas for the detector. The GC inlet temperature was set at 35°C. Using the split mode, the GC inlet pressure, split ratio, split flow, and total flow were 34.5 kPa, 6:1, 24.3 mL min^{-1}, and 31.4 mL min^{-1}, respectively. The GC oven temperature was maintained at 30°C with a total run time of 5 min. For the micro-electron capture detector, the temperature, mode, and combined flow were 350°C, constant column + makeup flow, and 20.0 mL min^{-1}, respectively. The following parameters were used for the headspace sampler: sample loop size, 3 mL; sample loop temperature, 45°C; vial headspace pressure, 86.2 kPa; vial oven temperature, off; transfer line temperature, off; vial equilibrium time, 1 min; pressurization time, 0.5 min; loop fill time, 0.5 min; loop equilibrium time, 0.5 min; injection time, 1 min; shake setting, low. Under these conditions, the retention time for N_2O was 3.7 min and the detection limit (3× the background noise level) was 0.03 μL N_2O L^{-1} or 0.034 ng N_2O-N cm^{-3}. The analytical N_2O standards (0, 0.101, 0.303, 1.01 and 10.1 mL L^{-1}, v/v) were prepared in 1-L Tedlar bags from a dilution of 10.1 mL L^{-1} (v/v) N_2O (Air Liquide America Specialty Gases LLC., Santa Fe Springs, CA, USA).

Soil Sampling and Analysis

Soil samples were collected from the same incubation container to monitor the N transformation processes immediately after N_2O emissions were sampled. Soil in the container was mixed with a spatula before sampling five different spots inside the container for one composite sample. After sampling, the soil in the container was leveled again by gentle shaking, weighed, and covered to continue incubation. Duplicate soil samples (20 g each) were weighed into 120-mL plastic bottles and either processed immediately or stored in a freezer at −4°C until analysis. The freezing effect on the stability of NH_4^+, NO_2^-, and NO_3^- was tested by spiking the soil with a known concentration of each species. Next, three of the samples was shaken immediately with 2 M KCl for 1 h to determine the initial concentration at freezing time zero and others were frozen. After freezing soil samples for 1 wk, the recovery rates for NH_4^+, NO_2^-, and NO_3^- were 101, 95, and 97%, respectively, but after 40 d, they were 101, 88, and 103%, respectively, indicating significant oxidation of NO_2^-. Most of the samples in this study were extracted within 1 wk after sampling. One sample was extracted with 50 mL of 2 M KCl for NH_4^+, NO_2^-, and NO_3^- determination. The bottle was shaken for 1 h on the reciprocating shaker, the suspension was vacuum-filtered through Whatman #1 filter paper (Whatman International Ltd, Maidstone, UK), and the filtrate was analyzed using the colorimetric methods described in Mulvaney (1996) with modifications using an Astoria 2 Analyzer (Astoria-Pacific Inc., Clackamas, OR). The analysis was done for NH_4^+, NO_3^- + NO_2^-, and NO_2^- separately; NO_3^- was obtained by difference. The second duplicate sample was used for pH measurement at 1:1 (w/v) soil/DI water ratio using a digital 420A pH meter with a combined glass electrode (Orion Research Inc., Boston, MA). By the end of the incubation experiment, repeated soil sampling had removed 21 to 26% (depending on the number of sampling times and the soil water content) of the initial soil mass in the container. At the end of the incubation experiment, 0.84, 0.80, 0.73, and 0.68 kg soil (oven-dried basis) in total was removed from the containers for the 5, 10, 20, and 30% water content treatments, respectively, in the first batch of experiments; 0.62 kg soil was removed in the second batch of experiments, including temperature, biochar, and inhibitor treatments.

DATA ANALYSIS

The N_2O emission rate (μg N kg^{-1} h^{-1}) for each measurement was calculated from linear regression of the N_2O concentration increase inside the incubation container with time, gas-phase volume (which was adjusted for soil removal) and the soil mass remaining in each container at the time of sampling (Eq. [5]).

$$N_2O \text{ emission rate} = \frac{bV_a}{m_s}, \quad [5]$$

where b is the change in N_2O concentration with time (μg N L^{-1} h^{-1}), V_a is the air volume (L) (corrected for soil, water, and biochar materials with average density of 2.65, 1.0, and 0.5 g cm^{-3}, respectively), and m_s *is* the soil mass (kg) remaining in the container at the sampling time. The density of biochar materials was based on the range reported by Brewer et al. (2014). Conversion of N_2O from volume to mass concentrations (i.e., from v/v to w/v) was done using a correction factor of 1.148 assuming normal temperature (25°C) and pressure (100 kPa). Cumulative emissions (mg N kg^{-1}) for each container were estimated by summing the products of the emission rates (μg N kg^{-1} h^{-1}) and the sampling intervals during the incubation period.

The N mass balance at the end of incubation was calculated. The percent of N_2O emission and soil residual N was calculated. The unaccounted N loss, which was assumed mainly to be NH_3 and N_2 and other minor gases, was estimated via Eq. [6]:

$$N_{\text{unaccounted}}(\%) = \frac{\text{TIN-}\ NH_4^+\text{-N-}NO_3^-\text{-N-}NO_2^-\text{-N-}N_2O\text{-N}}{\text{TIN}} \times 100\%, \quad [6]$$

where the total inorganic N (TIN, mg N kg^{-1}) in each treatment was estimated as the amount of urea-N applied plus any N in the soil as well as the N mineralized during incubation, which was determined from the control; NH_4^+–N, NO_3^-–N, and NO_2^-–N were the concentrations (mg N kg^{-1}) in the soils; and N_2O-N was the cumulative emissions (mg N kg^{-1}) during the 35-d incubation period.

SPSS for Windows, version 16.0 (SPSS Inc. Chicago, IL) was used for statistical analysis of the data. The data distribution normality of the N_2O emission rate and of soil NH_4^+–N,

NO_3^-–N + NO_2^-–N, NO_2^-–N, and pH was verified using the Kolmogorov–Smirnov test. Differences in peak emission rates, N species, and the cumulative emissions were analyzed using one-way ANOVA ($P < 0.05$). Mean separation was conducted using Tukey's adjustment for all possible comparisons. Linear regression analyses ($P < 0.05$) were performed to determine correlations between the N_2O-N emission rate and soil NO_2^-–N.

RESULTS

Nitrogen Application Rate

Nitrous oxide emissions were affected significantly by N application rate (Fig. 2a). The N_2O emission rate increased markedly following urea addition and reached its peak values of 0.8, 1.9, and 4.2 μg N kg^{-1} h^{-1} by Days 3, 5, and 11 for the 50, 100, and 150 mg N kg^{-1} treatments, respectively. The emission rate decreased significantly thereafter until no difference from the control was seen on Days 7, 13, and 23 for the 50, 100, and 150 mg N kg^{-1} treatments, respectively. Furthermore, the increase in the peak was at a higher rate than the N application rate increase. At 150 mg N kg^{-1}, the peak emission rate was 2.2 times that at 100 mg N kg^{-1}. At 100 mg N kg^{-1}, the peak was 2.5 times that at 50 mg N kg^{-1}. The cumulative (total) emission loss during the 35-d incubation period (1.3 mg N_2O-N kg^{-1}) from the 150 mg N kg^{-1} treatment was about 21, 13, and 4 times those from the 0, 50, and 100 mg N kg^{-1} treatments (<0.1, 0.1, and 0.3 mg N_2O-N kg^{-1}, respectively) (Table 1). The cumulative N_2O emissions were found to increase exponentially with N application rate ($y = 0.0465e^{0.0208x}$, $r^2 = =0.97$)where both y and x are in mg N kg^{-1}).

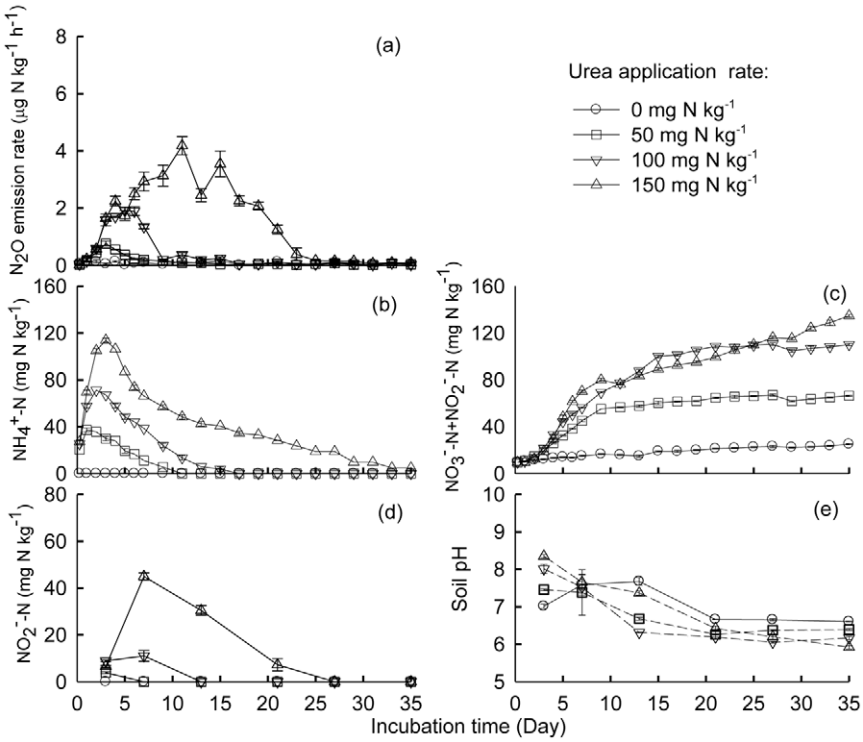

Fig. 2. Effects of N application rates on (a) the NO_2 emission rate and changes in soil (b) NH_4^+–N concentration, (c) NO_3^-–N + NO_2^-–N concentration, (d) NO_2^-–N concentration, and (e) pH. The treated soil with a 10% water content was incubated at 25°C for 35 d. Error bars show the standard error of the mean (n = 3).

Nitrogen transformations after urea application to soil are reflected in the concentration changes of NH_4^+–N, NO_2^-–N, and NO_3^-–N + NO_2^-–N (Fig. 2b–d). Urea hydrolysis (Eq. [1]) resulted in increased NH_4^+ concentration, with the highest observed on Days 1, 2, and 3 for the 50, 100, and 150 mg N kg^{-1} treatments, respectively, suggesting that urea hydrolysis occurred within a couple of days in soil. The peak NH_4^+ concentration (37.3, 71.2, and 113.8 mg N kg^{-1} for the 50, 100, and 150 mg N kg^{-1} treatments, respectively) increased linearly with the N application rate ($P < 0.01$). After the peak, the NH_4^+–N concentration began to decrease until no difference from the control (no urea addition) was seen by Days 11 and 17 for the 50 and 100 mg N kg^{-1}, respectively; for the 150 mg N kg^{-1} treatment, the NH_4^+–N concentration decreased to 4.8 mg N kg^{-1} by Day 35, which was still significantly higher than that of all other N rate treatments.

Nitrification resulted in a decrease in NH_4^+–N concentration, accompanied by a increase in NO_2^- and NO_3^- concentration (Fig. 2b,c). A marked increase in NO_2^- + NO_3^- was observed in 2 to 3 d for all N treatments. As an intermediate product during nitrification, peak NO_2^- concentration was found to increase exponentially with N application rates, similar to the N_2O emissions. The peak concentrations of 3.7, 11.0, and 44.9 mg N kg^{-1} were observed on Days 3, 7, and 7 for the 50, 100, and 150 mg N kg^{-1} treatments, respectively (Fig. 2d). By Day 35, NO_3^- was the dominant form of soil N in all treatments. Changes in NO_2^- concentration followed a similar trend as the N_2O emission rate (Fig. 2a,e).

Changes in soil pH followed the N transformation processes because urea hydrolysis consumes protons (causing pH increase) and nitrification produces protons (causing pH decrease) (Eq. [1] and Eq. [2]). For this experiment, the first soil sample was collected on Day 3 when all soils had a higher pH than the control (Fig. 2e), indicating the dominance of hydrolysis. On Day 7, soil pH measurements from all N treatments were similar and then decreased to below that of the control indicating dominance of the nitrification process. By Day 35, the soil pH was 6.39, 6.17 and 5.92 (or a pH drop of 0.21, 0.43, and 0.68 from the control soil) for the 50, 100, and 150 mg N kg^{-1} treatments, respectively.

Soil Moisture

Soil water content had profound effects on N_2O emissions, depending on whether it was below or above the WHC of 12% (w/w) (Fig. 3a). The 30% soil water content was near saturation. With the 100 mg N kg^{-1} application rate, nearly dry soil (5% water content, air-dried) resulted in the lowest peak emission rate (1.1 μg N kg^{-1} h^{-1}) by Day 11, which occurred later

Table 1. Estimates of N mass balance components for treatments with urea application and various conditions after 35 d of incubation†

Treatments and incubation conditions		N_2O-N		NH_4^+–N		NO_3^-–N		NO_2^-–N		Unaccounted N§
		Total emissions	% of TIN‡	Content	% of TIN	Content	% of TIN	Content	% of TIN	
		mg N kg^{-1}		mg N kg^{-1}		mg N kg^{-1}		mg N kg^{-1}		%
Urea-N application rate (mg kg^{-1}) (10% soil water content; 25°C)	0	<0.1(c)	<0.4	0.0 ± 0.0(b)	0.0 ± 0.0	25.0 ± 0.5(d)	100.0 ± 0.0	0.0 ± 0.0(a)	0.0 ± 0.0	–
	50	0.1 ± 0.0 (c)	0.1 ± 0.0	0.0 ± 0.0(b)	0.0 ± 0.0	66.5 ± 0.5(c)	88.6 ± 0.6	0.0 ± 0.0(a)	0.0 ± 0.0	11.3 ± 0.6(b)
	100	0.3 ± 0.0(b)	0.3 ± 0.0	0.0 ± 0.0(b)	0.0 ± 0.0	110.2 ± 0.9(b)	88.1 ± 0.7	0.0 ± 0.0(a)	0.0 ± 0.0	11.6 ± 0.7(b)
	150	1.3 ± 0.1(a)	0.7 ± 0.0	4.8 ± 0.0(a)	2.8 ± 0.0	134.7 ± 1.2(a)	77.0 ± 0.7	0.0 ± 0.0(a)	0.0 ± 0.0	19.5 ± 0.7(a)
Soil moisture (w/w, %) (100 mg urea-N kg^{-1} soil application rate, 25°C)	5	0.3 ± 0.0(b)	0.2 ± 0.0	7.6 ± 5.5(c)	6.0 ± 4.4	83.9 ± 6.8(b)	67.1 ± 5.4	0.0 ± 0.0(b)	0.0 ± 0.0	26.7 ± 1.3(c)
	10	0.3 ± 0.0(b)	0.3 ± 0.0	0.0 ± 0.0(d)	0.0 ± 0.0	110.2 ± 0.9(a)	88.1 ± 0.7	0.0 ± 0.0(b)	0.0 ± 0.0	11.6 ± 0.7(d)
	20	11.6 ± 1.5(a)	9.3 ± 1.2	19.9 ± 1.8(b)	15.9 ± 1.4	2.6 ± 0.0(c)	2.1 ± 0.0	2.3 ± 0.0(a)	1.8 ± 0.0	70.9 ± 2.2(a)
	30	10.0 ± 1.1(a)	8.0 ± 0.9	38.1 ± 2.0(a)	30.5 ± 1.6	0.4 ± 0.6(c)	0.3 ± 0.5	0.0 ± 0.0(b)	0.0 ± 0.0	61.1 ± 0.5(b)
Temperature (°C) (150 mg urea-N kg^{-1} soil application rate; 10% soil water content)	10	<0.1(b)	0.0 ± 0.0	119.4 ± 1.9(a)	68.2 ± 0.0	15.9 ± 1.1(b)	9.1 ± 0.8	0.0 ± 0.0(a)	0.0 ± 0.0	22.7 ± 0.8(a)
	25	1.0 ± 0.1(a)	0.6 ± 0.1	1.9 ± 1.9(c)	1.1 ± 0.0	130.3 ± 4.2(a)	74.4 ± 1.8	0.0 ± 0.0(a)	0.0 ± 0.0	23.9 ± 1.9(a)
	40	1.1 ± 0.3(a)	0.6 ± 0.1	16.8 ± 11.2(b)	9.6 ± 2.1	123.4 ± 19.3(a)	70.5 ± 9.7	0.0 ± 0.0(a)	0.0 ± 0.0	19.3 ± 7.6(a)
Biochar amendment and N transformation inhibitor)¶ (150 mg urea-N kg^{-1} soil application rate, 25°C)	No amendment	1.0 ± 0.1(a)	0.6 ± 0.1	1.9 ± 1.9(a)	1.1 ± 0.0	130.3 ± 4.2(bc)	74.4 ± 1.8	0.0 ± 0.0(a)	0.0 ± 0.0	23.9 ± 1.9(a)
	Biochar	0.3 ± 0.0(b)	0.2 ± 0.0	0.0 ± 0.0(a)	0.0 ± 0.0	133.8 ± 0.9(c)	76.4 ± 1.6	0.0 ± 0.0(a)	0.0 ± 0.0	23.4 ± 1.6(a)
	Agrotain Ultra	0.2 ± 0.0(b)	0.1 ± 0.0	0.0 ± 0.0(a)	0.0 ± 0.0	155.3 ± 0.9(a)	88.7 ± 0.8	0.0 ± 0.0(a)	0.0 ± 0.0	11.2 ± 0.8(c)
	N-Serve 24	0.3 ± 0.0(b)	0.2 ± 0.0	0.0 ± 0.0(a)	0.0 ± 0.0	141.5 ± 2.4(b)	80.8 ± 1.2	0.0 ± 0.0(a)	0.0 ± 0.0	19.0 ± 1.2(b)

† Values in the table are presented as means ± SE (n = 3). Different letters in parentheses indicate significant (P < 0.05) differences among the treatments in the same column within the single experiment.

‡ Total inorganic N (TIN) in each treatment was estimated by the amount of urea-N applied plus N mineralized from the no N application control;

$$\%\ \text{of TIN} = \frac{NH_4^+\text{-N or } NO_3^-\text{-N , or } NO_2^-\text{-N at the end of incubation or cumulative } N_2O\text{-N emissions}}{\text{TIN}} \times 100$$

§ See Eq. [6].

¶ Biochar was applied at the rate of 1% (dry mass basis, w/w); the final inhibitor application rates (recommended rates from the manufacturers) were equivalent to 6.7 mL kg^{-1} N for Agrotain Ultra and 20.9 mL kg^{-1} N for N-Serve 24.

than that for 10% water content (Day 5, 1.9 μg N kg^{-1} h^{-1}) (Fig. 2b). At 20 and 30% water content, the N_2O emission rate spiked quickly to 25.7 and 38.7 μg N kg^{-1} h^{-1}, respectively on Day 1 and then dropped to undetectable levels. After a lag period of about 1 wk, the N_2O emission rate increased again, continued for about 3 wk at high rates, and eventually dropped by the end of incubation period. The second-highest peak emission rates, 41.1 and 36.1 μg N kg^{-1} h^{-1}, were observed on Days 19 and 29 for the 20 and 30% water content treatments, respectively. The cumulative N_2O emissions were much higher from the 20 and 30% water content (11.6 and 10.0 mg N_2O-N kg^{-1}, respectively) than that from the 5 and 10% treatments (0.3 and 0.3 mg N_2O-N kg^{-1}, respectively) (Table 1).

The initial hydrolysis of urea did not appear to be affected by soil water content, as the peak NH_4^+ concentration was observed between 2 and 3 d in all treatments (Fig. 3b). Ammonium concentration began to decrease thereafter, which was accompanied by increasing NO_3^- + NO_2^- concentrations (Fig. 3c) at 5 and 10% water content but little change at 20 and 30% water content. The peak NH_4^+ concentrations followed the order of soil water content: 30% > 20% > 5% > 10%. By Day 35, NH_4^+ was detectable in soils at 5, 20, and 30% water content but NO_3^- + NO_2^- was only detectable at 5 and 10% water content. The NO_2^- concentration appeared to be high when the N_2O emission rates were high but not proportionally when compared among the different water treatments (Fig. 3d). Soil pH dropped the most at 10% water content among all the treatments, indicating that the most nitrification occurred (Fig. 3e). The highest and most stable soil pH was observed at 20 and 30% water content (pH 7.47–8.97) throughout the incubation period, which indicated that minimal nitrification occurred.

Temperature

The temperature effect on N_2O emission is shown in Fig. 4a. A low N_2O emission rate (<0.17 μg N kg^{-1} h^{-1}) was observed at 10°C. At 25°C, the N_2O emission rate increased markedly between 3 and 5 d and then fluctuated between 1.9 and 2.8 μg N kg^{-1} h^{-1} until 22 d before decreasing to a level comparable to that at 10°C. At 40°C, the N_2O emission rate was low during the first 5 d, increased abruptly

to above that at 25°C from 0.7 μg N kg^{-1} h^{-1} on Day 6 to about 5.0 μg N kg^{-1} h^{-1} on Days 7 to 9, and then quickly decreased to a comparable level to all other temperatures by Day 25. The cumulative N_2O emissions at 25 and 40°C were similar (1.0 and 1.1 mg N_2O-N kg^{-1}, respectively) and significantly higher than that of the 10°C incubation (<0.1 mg N_2O-N kg^{-1}).

Ammonium concentration increased markedly following urea addition and peaked by Days 5, 2, and 2 at 10, 25, and 40°C, respectively (Fig. 4b). Thereafter, NH_4^+ concentration did not change at 10°C but had a remarkable decrease on Days 3 and 6 at 25 and 40°C, respectively. The concentration of NO_2^-+NO_3^- also did not change at 10°C during the incubation period (Fig. 4c) but increased significantly after Days 3 or 6 when the soil was incubated at 25 and 40°C, respectively. The highest temperature resulted in a lower peak NO_2^- concentration as compared with 25°C (Fig. 4d). Soil pH at 10°C increased from 7.9 to 9.0 by Day 5 and stayed above 8.6 thereafter, indicating minimal nitrification (Fig. 4e). At the end of the incubation period, soil pH was 8.61, 5.72 and 6.07 for the 10, 25, and 40°C treatments, respectively. The largest pH drop seen at 25°C suggests that the strongest soil acidification was caused by nitrification.

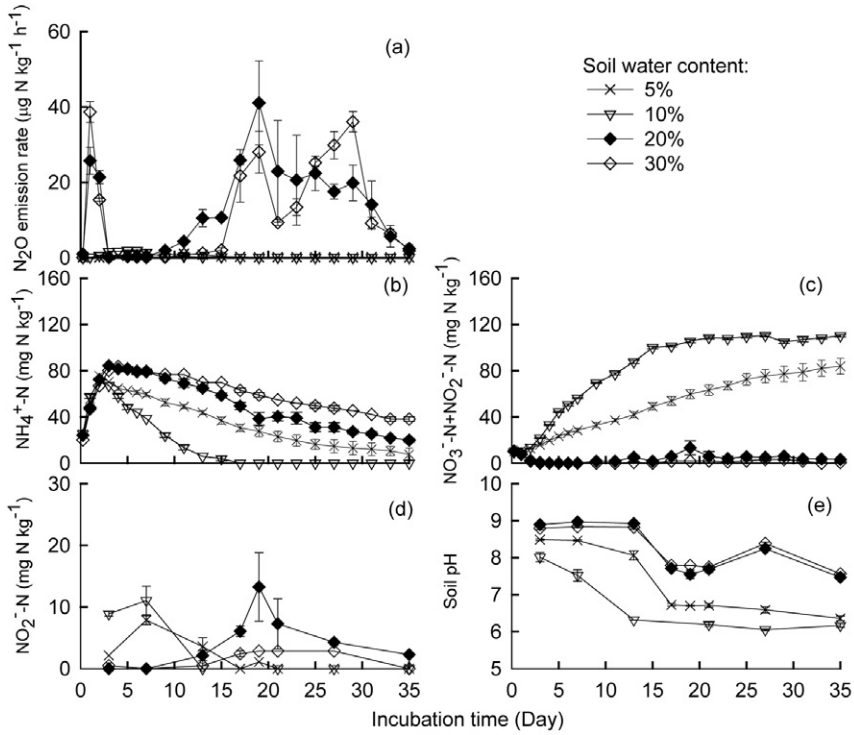

Fig. 3. Effects of soil water content on (a) the NO_2 emission rate and changes in soil (b) NH_4^+–N concentration, (c) NO_3^-–N + NO_2^-–N concentration, (d) NO_2^-–N concentration, and (e) pH. The soil was treated with 100 mg N kg^{-1} and incubated at 25°C for 35 d. Error bars show the standard error of the mean (*n* = 3).

Urea hydrolysis and nitrification were significantly affected by the N transformation inhibitors (Fig. 5b–d). Under the no amendment and biochar treatments, NH_4^+ concentration

Biochar and N Transformation Inhibitors

Amendment with biochar, Agrotain, and N-Serve all reduced N_2O emissions (Fig. 5a). With no amendment, N_2O emission rates increased markedly following urea application, peaked (2.7 μg N kg^{-1} h^{-1}) on Day 6, fluctuated between 2.0 and 2.7 μg N kg^{-1} h^{-1} until Day 19, and then decreased to below 0.1 mg N kg^{-1} by Day 26. Amendment with 1% biochar initially resulted in a similar N_2O emission rate increase as the no-amendment control but plateaued around 1.0 μg N kg^{-1} h^{-1} between 5 and 12 d before decreasing. Both Agrotain and N-Serve delayed N_2O emissions, with increases observed after Day 6 and peaks of 2.4 and 1.6 μg N kg^{-1} h^{-1} on Days 9 and 11 for the two inhibitors, respectively. The emission rate dropped quickly thereafter to below 0.3 μg N kg^{-1} h^{-1} by Day 16 compared to 2.4 μg N kg^{-1} h^{-1} for no amendment. Biochar, Agrotain, and N-Serve reduced the cumulative N_2O emission loss by 74, 78, and 74% (0.3, 0.2, and 0.3 mg N kg^{-1}, respectively) compared with no amendment (1.0 mg N kg^{-1}).

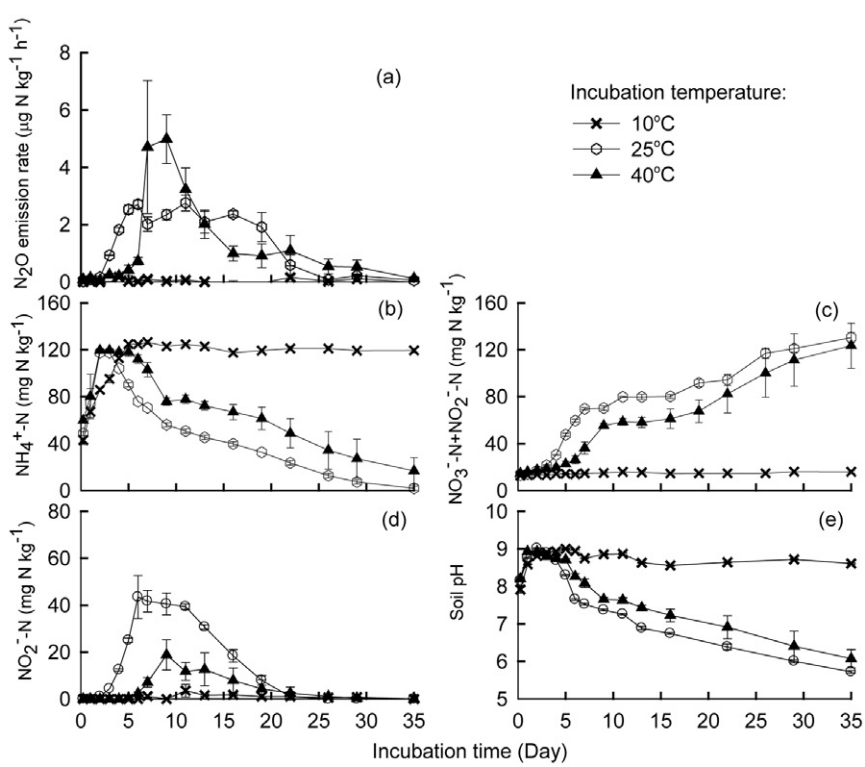

Fig. 4. Effects of temperature on (a) the NO_2 emission rate and changes in soil (b) NH_4^+–N concentration, (c) NO_3^-–N + NO_2^-–N concentration, (d) NO_2^-–N concentration, and (e) pH. The soil was treated with 150 mg N kg^{-1} as urea and 10% water content (w/w). Error bars show the standard error of the mean (*n* = 3).

changes followed exactly the same pattern, i.e., increased quickly following urea addition and peaked (115.7–117.5 mg N kg^{-1}) on Day 3 before decreasing, except with lower concentrations under the biochar treatment after Day 9. The N transformation inhibitors, however, significantly affected urea transformation. Agrotain (a urease inhibitor) delayed NH_4^+ formation, with a reduced concentration and peak (79.5 mg N kg^{-1}), which was observed on Day 9. The nitrification inhibitor, N-Serve, had no impact on NH_4^+ formation and extended the high concentration level around the peak (121.3 mg N kg^{-1}) until Day 7 before decreasing. By the end of the experiment, there were no significant differences in NH_4^+ concentration among the treatments.

Nitrite concentration changes resulting from the biochar amendment and N transformation inhibitors are shown in Fig. 5d. The no-amendment control had a peak NO_2^- concentration of 43.6 mg N kg^{-1} observed on Day 6, which lasted for several days before a significant decrease. Biochar amendment resulted in a similar initial increase but after reaching a peak of 31.7 mg N kg^{-1} on Day 6, NO_2^- concentration began to decrease. Agrotain or N-Serve delayed the NO_2^- peak (which occurred on Days 9 and 11, respectively) and also reduced the peak concentration to 7.6 and 16.4 mg N kg^{-1}, respectively. All NO_2^- concentration changes followed a similar pattern to the N_2O emission rates. Changes in soil pH and soil NH_4^+ followed a generally similar trend (Fig. 5b,e). For the no-amendment, biochar, and N-Serve treatments, soil pH increased significantly within the first 3 d following urea addition and then decreased, although with a 2-d delay for the N-Serve. Agrotain slowed urea hydrolysis, thereby reducing pH elevation during the early stage of incubation.

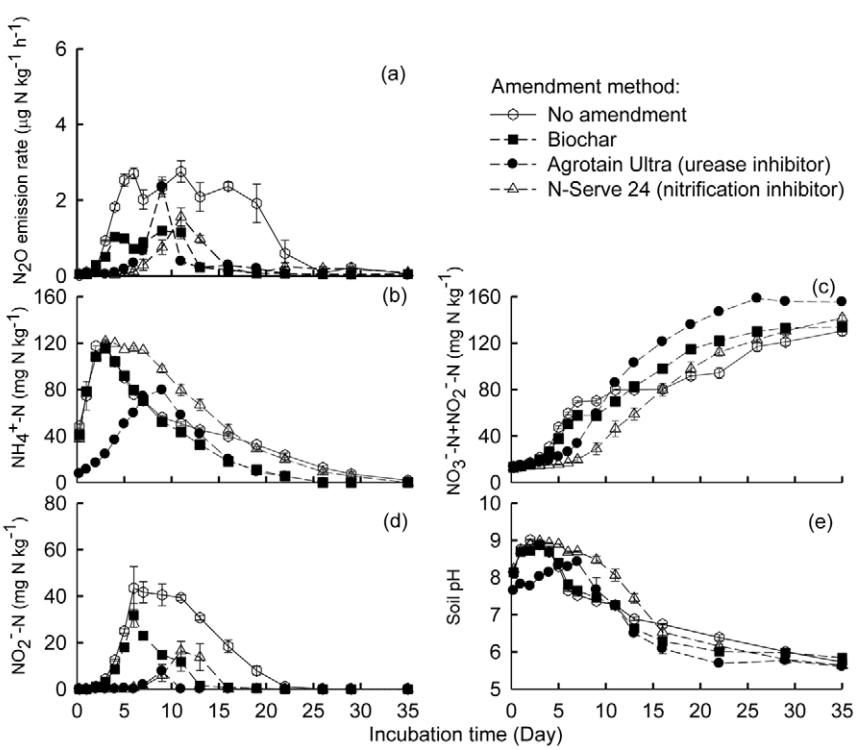

Fig. 5. Effects of soil amendment with biochar, Agrotain (a urease inhibitor), and N-Serve (a nitrification inhibitor) on (a) the NO_2 emission rate and changes in soil (b) NH_4^+–N concentration, (c) NO_3^-–N + NO_2^-–N concentration, (d) NO_2^-–N concentration, and (e) pH. The soil was treated with 150 mg N kg^{-1} as urea and 10% water content (w/w). Error bars show the standard error of the mean (n = 3).

Correlation between Soil N Species and N_2O Emissions

Neither the NH_4^+ nor the NO_3^- concentration was found to correlate with the N_2O emission rate in any of the experiments in this study. As the peak N_2O emission rate and NO_2^- concentration occurred simultaneously in most of the individual experiments (except the soil moisture experiment), positive correlations were found between the two parameters. For example, in the N application rate experiment, the positive linear correlation was , y=0.0712x+0.2309, r^2=0.85, where y was the N_2O emission rate in μg N kg^{-1} h^{-1} and x was the NO_2^- concentration in mg N kg^{-1}. However, when we plotted the data from all experiments throughout the 35-d incubation period, there was no significant correlation between the two parameters. We then divided the data into two groups [i.e., treatments with 5 and 10% water content (below WHC of 12%), including N application rate, temperature, and amendments with biochar and N transformation inhibitors and treatments with 20 and 30% water content (above WHC)] and found significant correlations between the N_2O emission rate and NO_2^- concentration within each group (Fig. 6). The slope for the higher water content group (3.181) was significantly greater than that for the lower water content (0.066).

DISCUSSION

A High Urea Application Rate Induced Much Higher N_2O Emissions

Peak emission rate and a cumulative emission loss of N_2O as well as the peak NO_2^- concentration in the soil increased exponentially instead of linearly as the N application rate increased (Fig. 2a,d; Table 1). Under the study conditions, N_2O production probably included nitrifier nitrification and nitrifier denitrification (Fig. 1). Both ammonia toxicity and O_2 limitation have been reported to cause NO_2^- accumulation as a result of urea application (Maharjan and Venterea, 2013). In our case, O_2 was unlikely to have been limited at 10% soil water content but the higher N application rate would have resulted in higher NH_4^+ concentration and elevated soil pH at the early stage of incubation as a result of urea hydrolysis (Fig. 3a, Fig. 5a), which would shift the NH_4^+/NH_3 equilibrium toward NH_3. The high NH_3 toxicity to NO_2^- oxidizers would have caused NO_2^- accumulation (Eq. [4]) (Venterea, 2007; Huang et al., 2014), leading to high N_2O emissions via nitrifier denitrification (Pathway B, Fig. 1). Using the isotope technique, Poth and Focht (1985) concluded that *Nitrosomonas europaea*, which is involved in the oxidation of NH_4^+ to NO_2^- (Eq. [3]), is also a denitrifier

which, under oxygen stress, uses NO_2^- as a terminal electron acceptor and produces N_2O. On the other hand, the oxidation of NH_4^+ could consume enough O_2 (Eq. [3]) to result in anaerobic microsites slowing down oxidation of NO_2^- and promoting N_2O production via nitrifier denitrification, as observed by Zhu et al. (2013) and Huang et al. (2014).

The nonlinear response of N_2O emissions to the N application rate was also observed by others under field conditions. McSwiney and Robertson (2005) found that N_2O fluxes were moderately low at levels of N addition to 101 kg N ha^{-1}, where maize (*Zea mays* L.) grain yields were maximized, beyond which, fluxes more than doubled at 134 kg N ha^{-1} N addition. Hoben et al. (2011) further determined that N_2O emission flux increased exponentially with N rate (0–225 kg N ha^{-1}) across five fields for 2 yr of data. At 180 and 225 kg N ha^{-1}, which were above the recommended level for maximum economic return (135 kg N ha^{-1}), the N_2O fluxes were 43% and 115% higher. These studies illustrated the potential to lower agricultural N_2O flux within the range of N fertilization that does not affect economic return from grain yield. Our laboratory data show that the exponential increase in N_2O emission rates could expand to any N application rate when no plant uptake is excluded. Based on a meta-analysis, Shcherbak et al. (2014) suggested a general trend of exponentially increasing N_2O emission rates as N inputs exceed crop needs.

The High Impact of Increasing Soil Moisture on N_2O Emissions

Much higher N_2O emissions were found at 20 and 30% water content than at 5 to 10% (Fig. 3a; Table 1). The data on soil mineral N species and pH (Fig. 2b-e) suggest that nitrification and nitrifier denitrification were the dominant pathways in N_2O production at 5 and 10% water content. Nitrifier denitrification can occur under fully to moderately oxic conditions (Venterea, 2007; Maharjan and Venterea, 2013; Huang et al., 2014). The lower N_2O emissions at 5% water content were caused by decreased activity of the NH_3–oxidizers that control the first step of nitrification (Eq. [3]), which resulted in lower concentrations of substrates (NH_2OH or NO_2^-) for N_2O production through nitrifier nitrification (Conrad, 1996).

The cumulative N_2O emissions at 20 and 30% soil water content (11.6 and 10.0 mg N_2O-N kg^{-1}, respectively) were 45 and 30 times that at 5 and 10% water content, respectively (Table 1). The two emission peaks during the 35-d incubation period reflect different mechanisms or pathways for N_2O production. Denitrification was clearly responsible for the first peak in the N_2O emission rate under high moisture conditions, as the $NO_3^- + NO_2^-$ concentration decreased markedly within the first 3 d (Fig. 3a,c). After that, NH_4^+ concentration under higher moisture decreased much more slowly than at 5 and 10% water content (Fig. 3c). However, there was an increase in soil NO_2^-, with little NO_3^- corresponding to the second peak N_2O emission rate at 20 and 30% water content. Nitrous oxide emissions from soil at high moisture are generally thought to be a result

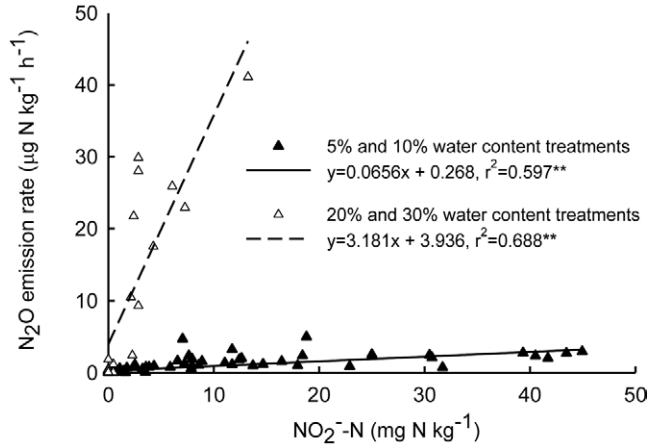

Fig. 6. Relationship between N_2O emission rates and soil NO_2^- concentration. **, correlation is significant at the α = 0.01 level.

of denitrification performed by denitrifiers (Pathway d) (Wrage et al., 2001; Bateman and Baggs, 2005). In our study, the complete denitrification process was uncertain for the second peak because of the absence of NO_3^- in the soil. The data suggest that nitrifier denitrification, nitrification coupled with denitrification, and denitrification may have all contributed to the second peak in N_2O emissions. Nitrifier denitrification becomes more important in N_2O production as soil water content increases (Kool et al., 2011). Nitrous oxide production via nitrifier denitrification increased 81-fold as O_2 concentration decreased from 21 to 0.5% but heterotrophic denitrification was responsible for all N_2O production at 0% O_2 (Zhu et al., 2013). Hu et al. (2015) found that an increase in soil moisture led to a higher denitrification rate and a higher conversion rate of N_2O to N_2. The lag period between the two N_2O emission peaks suggest changes in the microbial community. Di et al. (2014) also reported that the abundance of denitrifier genes increased as soil moisture increased from 60% field capacity to 100% and 130% field capacity. Most denitrifiers were facultative aerobes but could use various forms of NO_2 as an electron acceptor under increasingly anaerobic conditions (Wrage et al., 2001; van Spanning et al., 2007). In our study, the soil at 20 and 30% water content was unlikely to be totally anaerobic, as the second peak of NO_2^- was observed (Fig. 5d) but the lack of NO_3^- suggests increased denitrification performed by both nitrifiers and denitrifiers.

Relatively low and stable N_2O emission rates were observed in soil incubated at 10°C, probably because no nitrification occurred and most N remained as NH_4^+ throughout the incubation period (Fig. 4a,b). Increasing the temperature to 25 and 40°C resulted in 10 times higher cumulative N_2O emissions (1.0 and 1.1 mg N_2O-N kg^{-1}, respectively). Nitrogen transformation and pH change data show a relatively higher nitrification rate at 25°C than at 40°C (Fig. 4b–e). The nitrification response to temperature is bell-shaped, with an optimum between 20 and 35°C (Hadas et al., 1986). The reduced nitrification rate at 40°C was probably caused by increased biological O_2 consumption (Smith et al., 2003) and the temperature might become too high to support the growth of chemoautotrophic bacteria for a long enough

period of time for nitrification (Williams et al., 1992). This could explain the much higher N_2O emission rates between Days 7 and 9, which had much lower NO_2^- concentrations for 40°C than for 25°C. At 40°C, increased respiration could have led to anaerobic microsites for N_2O production through nitrifier denitrificaion (Smith et al., 2003). The heterotrophic microorganisms that are responsible for both nitrification and denitrification have greater tolerance to high temperature; thus denitrification might be a source of N_2O under high temperatures (Williams et al., 1992).

Overall, our results showed that increasing the temperature from 10 to 25 or 40°C only increased cumulative N_2O emission up to 0.6% of TIN but up to 9.3% when soil water content was increased from <10% to ≥20%, indicating that soil moisture is a more important factor affecting N_2O emissions. The 20 and 30% water content (above a WHC of 12%) are not uncommon, as soil moisture can exceed field capacity easily under surface drip irrigation and fertigation events. In areas close to the irrigation tubing, high soil water content resulted in much higher N_2O emissions than subsurface drip irrigation (with a dry surface) in a pomegranate orchard (Gao et al., 2013). In any case, it is unlikely that surface soil can retain such high moisture across the field and for a long period of time, especially with evapotranspiration and plant uptake; thus the laboratory results are probably somewhat higher than what would be expected under field conditions. However, these results indicate that reducing N applications in soils with higher surface moisture can reduce N_2O emissions.

Nitrous Oxide Emission Reduction by Biochar and N Transformation Inhibitors

Amending soil with biochar, Agrotain, or N-Serve reduced cumulative N_2O emissions by over 70% (Table 1). Different hypotheses have been postulated to explain why biochar can decrease N_2O emissions from soils, including increasing N immobilization and modifying enzymatic activities, as well as potentially inducing a toxic effect on nitrifier and denitrifier communities (Cayuela et al., 2014). Our data indicate that biochar did not have an effect on the urea transformation rate, as indicated by similar NH_4^+ concentration increases (Fig. 5b) and $NO_3^- + NO_2^-$ concentration increases (Fig. 5c) for both biochar and no amendment within the first week. Although the initial NO_2^- concentration in biochar-amended soil increased similarly to no amendment, it dropped more quickly (Fig. 5b). The >50% reduction in the N_2O emission rate from biochar-amended soil on or after Day 5 may indicate the reduced availability of substrate for N_2O reduction. Biochar has been reported to have strong adsorption for NH_4^+ because of its high surface area (Singh et al., 2010; Ahmad et al., 2014). The biochar materials used in our study were estimated for their NH_4^+ adsorption capacity, which increased linearly with NH_4^+ concentration in soil; thus adsorption could be the most important mechanism affecting N_2O emissions (Gao and Cai, 2015).

For the N transformation inhibitors, Agrotain (an urease inhibitor product) inhibited urea hydrolysis by reducing NH_4^+ formation and pH elevation (Fig. 5b,e), both of which could reduce NH_3 toxicity, leading to low NO_2^- accumulation in the soil (Fig. 5d). Once applied to soil, the active ingredient in the product, *N*-(n-butyl)thiophosphoric triamide can quickly convert to its more effective O analog, *N*-(n-butyl)phosphoric triamide, which then forms a tridentate ligand with the urease enzyme to slow down urea hydrolysis (Zaman et al., 2009), resulting in a reduced NH_4^+ concentration (Fig. 5b) and lower pH (Fig. 5e) following urea addition than in treatments without the inhibitor. The nitrification inhibitor, N-Serve, contains the active ingredient dicyandiamide, which is reported to depress the activity of the NH_3 monooxygenase enzyme in soil and delay the oxidation of NH_3 to NH_2OH and further oxidation to NO_3^- or N_2O formation (McGeough et al., 2012). Our data indicate that N-Serve slows the first stage of nitrification (Eq. [3]) by delaying NH_4^+ oxidation to NO_2^- (Fig. 5d) and reduces $NO_3^- + NO_2^-$ production (Fig. 5c). Both inhibitors effectively decreased NO_2^- accumulation in the soil, leading to significantly lower total N_2O emissions (Table 1).

The data indicate the potential of using biochar and the transformation inhibitors to reduce N_2O emissions. However, their effects are short-term because, following application, most N transformation processes, including N_2O emissions, were completed within 3 wk (Fig. 5 a–e). In the field, with plant uptake and other N losses from the soil, N_2O emissions might be reduced further. The effectiveness of these remedies to reduce N_2O emissions should be evaluated further under field conditions.

Nitrite as an Indicator of N_2O Emissions for Two Grouping Mechanisms

A linear correlation between NO_2^- concentration and N_2O emissions was identified but a third variable, soil water, clearly divided the linear correlation into two separate regions (i.e., below or above WHC). We speculate that different mechanisms or processes were involved in N_2O formation and emissions depending on the soil water content or the dominance of nitrification or denitrification processes (Fig. 1), as discussed in previous sections. Nitrification was dominant under low soil moisture conditions. Denitrification, nitrifier denitrification or nitrification-coupled denitrification were expected to intensify at high water contents (20 and 30%), causing extremely high N_2O emissions. Maharjan and Venterea (2013) reported that cumulative N_2O emissions were correlated with NO_2^- intensity under field conditions. Our research revealed that this correlation is highly affected by soil water content and depending on whether the conditions have sufficient or limited O_2. The positive correlation between the N_2O emission rate and NO_2^- concentration may imply that when conditions favor NO_2^- accumulation, potentially high N_2O emissions are expected and NO_2^- concentration in soil could be used to predict N_2O emissions.

Unaccounted N Loss from Urea Application under Various Incubation Conditions

The unaccounted N losses (Table 1) mainly include the unmeasured gases and those immobilized in the soil that cannot be recovered by the 2 M KCl extraction procedure. The unmea-

sured gas loss includes N_2 and NH_3, which are potentially significant under anoxic and high soil pH conditions, respectively, and other minor gases (NO, NO_2). The immobilized N would include microbial immobilization depending on organic C and N status in the soil and mineral NH_4^+ fixation, which depends on soil mineralogy (Robertson and Groffman. 2007; Nieder et al., 2011). As the same amount of soil was used in each incubation container, mineral fixation for NH_4^+ was expected to be similar among the treatments. Microbial immobilization could vary depending on microbial activity and the availability of N, but since no additional energy source was added and because of the unknown effects of biochar, microbial immobilization was not expected to contribute to the large differences in the unaccounted N loss among the different treatments. Our discussion here focuses on N transformation processes and conditions that may have significantly affected NH_3 and N_2 volatilization losses.

The unaccounted N loss was large from this study, ranging from 11.2% in Agrotain treated soil to 70.9% in the soil at 20% water content (Table 1). At the highest N application rate (150 mg N kg^{-1}), the unaccounted loss was 19.5% of TIN or ~ 70% higher than that under the 50 and 100 mg N kg^{-1} treatments. This could have been partially attributed to the elevated soil pH (Fig. 5a) and higher NH_4^+ concentration from urea hydrolysis at the beginning of incubation shifting the NH_4^+–NH_3 equilibrium toward NH_3 for volatilization. Higher soil moisture conditions have resulted in exceptionally high unaccounted N loss (70.9% and 61.1% of TIN at 20 and 30% water content, respectively) compared to <27% at lower water contents. The high unaccounted loss could be either NH_3 because of the high pH and NH_4^+ concentration in soil (Fig. 3b,e) or N_2 from denitrification, as evidenced by the small increase in NO_2^-+NO_3^- concentration. All other treatments, including temperature change, biochar, and N transformation inhibitors, did not show extremely low or high unaccounted N loss. The data again illustrated that soil moisture becomes a crucial factor for reducing N loss to the environment. Nitrogen fertilizer application into surface soil with a high water content should be avoided.

CONCLUSIONS

This study examined the key factors and processes involved in N_2O production under various controlled laboratory conditions. Peak emission rates and cumulative N_2O emissions increased exponentially as the N application rate increased from 0 to 150 mg N kg^{-1}, probably caused by increased NH_3 toxicity to NO_2^- oxidizers, NO_2^- accumulation, and subsequent nitrifier denitrification to N_2O. Cumulative N_2O emissions increased 30 to 45 times as the soil water content increased from 5 to 10% to 20 to 30%, when nitrifier denitrification or nitrification-coupled denitrification and denitrification began to play a major role in N_2O production. Amending the soil with biochar, Agrotain Ultra, or N-Serve 24 reduced N_2O emissions by >70%. The research revealed that the positive correlation between N_2O emissions and soil NO_2^- accumulation is strongly affected by soil moisture conditions and was clearly defined within two soil water content ranges, depending on whether water content was below or above WHC, or O-sufficient or -limited conditions. High soil moisture conditions (20–30% in this study) also resulted in exceptionally high unaccounted N losses up to 71%. The results imply that soil moisture and N application rate are the most significant factors that can be managed to minimize N_2O emissions and total N losses by supplying N with the amount needed for maximum crop yield or economic return and avoiding high soil water content and high N concentration at the same time in surface soils. Although the measured N loss data from this study cannot be used directly to represent field conditions because of a lack of plants and other processes, these findings can be used to direct further evaluations of effective strategies for reducing N_2O emissions and other N losses under field conditions.

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