Fertilizer nitrogen loss via N$_2$ emission from calcareous soil following basal urea application of winter wheat

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**ABSTRACT**

The ratio of nitrous oxide (N$_2$O) to N$_2$O plus nitrogen gas (N$_2$) emitted from soils (N$_2$O/(N$_2$O+N$_2$)) is regarded as a key parameter for estimating fertilizer nitrogen (N) loss via N$_2$ emission at local, regional or global scales. However, reliable measurement of soil N$_2$ emissions is still difficult in fertilized soil-crop systems. In this study, the N loss via N$_2$ emission following basal urea application (with a dose of 150 kg N ha$^{-1}$) to a calcareous soil cultivated with winter wheat was quantified using the helium-based gas-flow-soil-core technique. Emissions of N$_2$ and N$_2$O from sampled fresh soils were measured under simulated field soil temperature and oxygen conditions. Our observation performed on the first day after irrigation and rainfall events showed the highest N$_2$ and N$_2$O emissions, which amounted to approximately 11.8 and 3.8 μg N h$^{-1}$ kg$^{-1}$ dry soil, corresponding to 3304 and 1064 μg N m$^{-2}$ h$^{-1}$, respectively. The N$_2$O/(N$_2$O+N$_2$) molar ratios within about 10 days following fertilization ranged from 0.07 to 0.25, which were much larger than those at the other time. During the one-month experimental period, the urea-N loss via emissions of N$_2$, N$_2$O, and N$_2$+N$_2$O was 1.6%, 0.6%, and 2.2%, respectively. Our study confirms that the widely applied acetylene-inhibition method substantially underestimates fertilizer N losses via N$_2$ emissions from calcareous soils cultivated with winter wheat.

1. Introduction

Anthropogenic activities have dramatically increased the amount of reactive nitrogen (N$_r$) circulating in the biosphere and atmosphere, creating a series of environmental consequences, such as eutrophication, acidification, and air pollution (Fowler et al. 2013). Terrestrial denitrification converts 30%–60% of the N$_r$ back into nitrogen gas (N$_2$) (Ciais et al. 2013), and thus contributes to close the global nitrogen (N) cycle (Gruber and Galloway 2008). Moreover, denitrification is an important process of fertilizer N loss from agricultural systems (Bouwman et al. 2013), as it converts nitrate and nitrite into nitric oxide (NO), nitrous oxide (N$_2$O), and N$_2$.

Among these gases, the N loss through N$_2$ emission is especially most uncertain in terrestrial N budgets because the high background atmospheric N$_2$ concentration makes soil N$_2$ emissions difficult to measure (Davidson and Seitzinger 2006). This situation is greatly hampering accurate quantification of terrestrial N budgets using experimental or modeling approaches. Previous studies have mainly quantified the N loss via denitrification in rice paddy fields or wetlands (Burgin and Groffman 2012; Wang et al. 2017). By contrast, research on upland soils has been much less common. Most previous studies on N loss through denitrification in upland soils have been based on the acetylene inhibition (AI) method.
(e.g. Cheng et al. 2004; Ju et al. 2009). Meanwhile, only a few researchers have adopted other methods that can specifically determine N₂ emission, such as the gas-flow-soil-core (GFSC) technique (e.g. Scheer et al. 2009) or the ^15^N gas flux method (e.g. Buchen et al. 2016). However, many studies have reported that the AI method, which usually determines the N loss in the form of N₂+NO, can severely bias the results owing to its shortcomings (Groppman et al. 2006). Because of its sensitivity and high costs, the ^15^N gas flux method is rarely applied in croplands, even though it directly measures N₂ emissions in-situ (e.g. Buchen et al. 2016). The helium-based GFSC technique (Butterbach-Bahl, Willibald, and Papen 2002; Molstad, Dorsch, and Bakken 2007; Wang et al. 2011) allows for direct measurements of the emissions of N₂, N₂O, and NO from soils under given conditions. Using the GFSC technique, in this study we attempted to quantify the fertilizer N loss via N₂ emission in an upland soil. In the North China Plain (NCP), typically with calcareous soils and widely cultivated with irrigated winter wheat and rainfed summer maize, excessive fertilizer-N is usually used as part of an intensive cultivation of both crops. For instance, Zhao et al. (2006) reported that N fertilizers were conventionally applied in the NCP at a rate of 550–600 kg N ha⁻¹ y⁻¹, which more than doubled the optimum demand, 286 kg N ha⁻¹ y⁻¹, for both crops (Ju et al. 2009). However, fertilizer-N losses via N₂ emission due to denitrification in intensive agricultural soils have been poorly quantified. In particular, existing studies use different methods that feature very large uncertainties. For example, the reported fertilizer-N loss in the form of N₂+NO via soil denitrification in wheat-maize rotation fields of the NCP ranges from 0.1%–3.3% to 13%–29%. The former rates of loss, which were measured using the AI method (Ju et al. 2009), are obviously much lower than the later ones determined via the mass balance approach (Cai and Yang 1998). In fact, neither method separately quantifies the losses of individual denitrification gases, i.e. NO, N₂O, and N₂.

In this study, soil samples were collected after basal urea application to a winter wheat field in the NCP. Using the GFSC technique, we directly measured the emissions of N₂, N₂O, and NO from the fresh samples under the given conditions of soil temperature, moisture, and oxygen, which were the same as those in-situ at soil sampling. The objectives were to (1) identify the temporal variations in N₂ emissions and N₂O/(N₂O+N₂) ratios following the basal N fertilization, and (2) quantify the rate of urea-N loss via N₂ emission.

2. Materials and methods

2.1. Description of experimental field site

The investigated experimental fields, which were located in the northern suburbs of Beijing and belonged to the Shangzhuang Agricultural Experimental Station (39°48′N, 116°28′E) of China Agricultural University, were long-term rotationally cultivated with winter wheat and summer maize. There is a typical calcareous fluvo-aquic soil, which on average contains 28% clay (<0.002 mm), 32% silt (0.05–2 mm), 0.71% organic carbon, and 0.08% total N in the 0–20 cm layer, showing an average pH (H₂O) value of 8.1 and a bulk density (BD) of 1.4 ± 0.1 g cm⁻³ in the cultivation horizon.

The conventional fertilization treatment (Nᵣᵣᵣ) of a long-term experiment initiated 12 years ago for the typical winter wheat-summer maize rotation system was selected for this study. It has three replicated square field plots (64 m² for each), which were designed by completely randomized blocks with those of other field treatments. The location of each field plot was fixed since establishment of the experiment. The winter wheat was sown in early October and harvested in late May or early June. The preceding crop straw was fully removed from the field. Urea was applied as the basal N fertilizer for the winter wheat, at a rate of 150 kg N ha⁻¹, prior to rotary plough tillage (to 10 cm in depth) followed by sowing. Both phosphorus and potassium fertilizers were basally applied together with the urea at a rate of 100 kg ha⁻¹ in phosphorus pentoxide and potassium oxide. During the observation period (1–26 October 2017) of this study, 28 mm of sprinkling irrigation was supplied on 7 October to promote seed germination (Figure 1(a)). For more detailed information about the experimental site and field management practices, refer to Huang et al. (2013) and Huang, Ju, and Yang (2017).

2.2. Field sampling and soil-core preparation

To determine the soil emissions of N₂, as well as N₂O and NO, topsoil (0–20 cm) samples were collected from the winter wheat field of Nᵣᵣᵣ after basal fertilization. Using the GFSC technique, we directly measured the emissions of these three gases from the soil samples incubated under soil conditions of temperature, moisture, and oxygen that were the same as those in-situ when the soil samples were collected.

In this study, the soils were sampled seven times in the one-month period following basal fertilization. The soil sampling was carried out once every two days during the first two weeks following fertilization and/or following an irrigation/rainfall event (but with exceptions on 8 and 10 October due to excessively wet soil for field sampling.
operation). During the remaining period, the soil sampling was conducted weekly. About 0.8 kg of soil was randomly collected each time for each replicated plot. When soil samples were collected, soil environmental variables (temperature, moisture, and oxygen concentration of the soil air) were recorded (see subsection 2.4 for details). The collected soil samples were stored under cool conditions and transported to the laboratory within 2 h after sampling. Prior to measurement, the soil sample from each plot was filled into four stainless-steel rings (5.6 cm in diameter, 4 cm in height) and repacked at a BD of 1.4 g cm\(^{-3}\), which was the same as the value measured in-situ. The four repacked soil cores were enclosed into one of the three incubation vessels of the applied GFSC system for simultaneous dynamical measurements of N gas emissions from the three field replicates.

### 2.3. Laboratory measurement of N gas emissions from soil cores

The GFSC technique adopted in this study for detection of \(\text{N}_2\), \(\text{N}_2\text{O}\), and NO emissions from incubated soil cores has been described in detail in previous publications (Liao et al. 2013; Wang et al. 2011). Immediately after gas-tight sample installation, the air in soil pores and the enclosure headspace was replaced with an \(\text{N}_2\)-free artificial atmosphere (20% oxygen in helium) at a temperature above 0°C but below 4°C by thoroughly flushing for 20–25 h. Then, the enclosure headspaces were continuously flushed under the ‘field soil condition’ for 4 h to re-establish a ‘natural’ gradient of gases within each soil core. Thereafter, four emission rates (6 h for each) were measured for each of the three nitrogenous gases during a 24-h period with headspace flushing at the ‘field soil condition’. The temperature and oxygen concentrations measured in-situ at the time of field sampling were set for the incubated soil cores to establish the ‘field soil conditions’. The moisture of soil cores was not adjusted since it could represent the field water condition at sampling. This was because (1) the fresh soils were immediately incubated after sampling and (2) the soil air replacement and the following headspace flushing did not significantly reduce the soil moisture (Wang et al. 2013).

The emission rate of each gas was determined with five headspace gas concentrations measured over a 4-h period at 1-h intervals. The detection limits of the applied GFSC system for the \(\text{N}_2\), \(\text{N}_2\text{O}\), and NO emission rates were 0.65, 0.002, and 0.01 \(\mu\text{g N h}^{-1}\text{ kg}^{-1}\text{ d.s.}\), respectively. The measured emission rate of each gas for a replicated field plot on the sampling day was reported as the average of the four measurements of the corresponding vessel. The gas emission rates in \(\mu\text{g N h}^{-1}\text{ kg}^{-1}\text{ d.s.}\) were also converted to units of \(\mu\text{g N m}^{-2}\text{ h}^{-1}\) for the 0–20 cm field soil layer (with an average BD of 1.4 g cm\(^{-3}\)).

### 2.4. Auxiliary field measurements

Daily precipitation was automatically recorded using a meteorological station located adjacent to the experimental field. The moisture, temperature, and oxygen
concentration of soil air at the 10-cm depth in each field plot were automatically recorded at 30-min intervals using a combined moisture and temperature sensor (STM, Decagon Devices Inc., USA) and an oxygen sensor (SO-100, Apogee Instruments, Logan, UT, USA). The data acquisition was interrupted during the soil plough tillage stage (3–6 October) before the wheat was sown. According to the soil BD (g cm\(^{-3}\)) and a theoretical soil particle density (\(\rho\), g cm\(^{-3}\)), the recorded soil water content (\(\theta_v\), cm\(^3\) cm\(^{-3}\)) was converted to water-filled pore space (WFPS, %) following WFPS = \(\theta_v/(1 – BD/\rho)\).

3. Results

3.1. Environmental variables

The soil temperature ranged from 9.1 to 20.9°C (mean: 13.9°C) during the one-month experimental period (Figure 1(a)). The irrigation and subsequent precipitation supplied 80 mm of water to the field within four days (Figure 1(a)), and thus increased the soil moisture from 29% to 70% WFPS, while simultaneously decreasing the oxygen concentrations in soil air ([O\(_2\)]) from 21% to 18% (Figure 1(b)). These two variables showed a significant negative correlation, which could be described by \([O_2] = -0.077WFPS + 24.36\) (\(R^2 = 0.60, p < 0.05\)).

3.2. Temporal variations of N gas emissions and their molar ratios

Before basal fertilization, as Figure 2(a) shows, the emission rates of the three nitrogenous gases were all low enough to be close to their detection limits. Fertilization greatly promoted N gas emissions from soil. For instance, the magnitudes of N\(_2\), N\(_2\)O, and NO emission rates on average reached 1.8, 0.3, and 0.5 μg N h\(^{-1}\) kg\(^{-1}\) d.s. corresponding to 504, 84, and 140 μg N m\(^{-2}\) h\(^{-1}\), respectively, on the first day after fertilization, which were approximately 2, 96, and 108 times higher than those prior to urea application. Subsequent irrigation, occurring on the fourth day after urea incorporation into the soil, and heavy rain falling immediately after, substantially stimulated the emissions of N\(_2\) and N\(_2\)O. On the first day after three rainy days, both gases showed maximum emissions, of 11.8 ± 6.6 and 3.8 ± 2.1 μg N h\(^{-1}\) kg\(^{-1}\) d.s. (Figures 1(a) and 2(a)), corresponding to 3304 ± 1848 and 1064 ± 588 μg N m\(^{-2}\) h\(^{-1}\) for N\(_2\) and N\(_2\)O, respectively. On this day, however, the NO emission level dropped to

![Figure 2](image-url)
a value (around 0.02 µg N h⁻¹ kg⁻¹ d.s.) very close to its detection limit. Afterwards, the emission rates of both N₂ and N₂O also quickly decreased, to levels of approximately 1.1 and 0.005 µg N h⁻¹ kg⁻¹ d.s., respectively (Figure 2(a)).

The N fertilization, as well as the subsequent irrigation/rainfall, resulted in significant variations in molar ratios of the N gas product. As Figure 2(b) illustrates, N₂O/(N₂O+N₂) ratios increased from a level close to zero before fertilization, to 0.07–0.25. Afterwards, the N₂O/(N₂O+N₂) ratios gradually dropped back to the near-zero level. The ratios of NO/N₂O were approximately 3.5, but exhibited no significant difference between before and after fertilization under conditions of low soil moisture (< 30% WFPS). However, they decreased significantly, to 0.03–0.21, when the soil moisture increased to over 60% WFPS. As the soil moisture dropped below 55%, however, the NO/N₂O ratios rose again to the initial level or even higher (Figure 2(c)).

### 3.3. Fertilizer-N loss via emissions of gases

The soil N₂, N₂O, and NO emissions cumulated to 1456.9 ± 628.1, 309.5 ± 161.3, and 40.1 ± 7.2 µg N kg⁻¹ d.s., corresponding to 4.1 ± 1.8, 0.9 ± 0.5, and 0.11 ± 0.01 kg N ha⁻¹, respectively, during the one-month period following fertilization. Assuming that the emission rate prior to N addition could represent the background gas emissions under unfertilized conditions, the rates of fertilizer-N loss via N₂, N₂O, and NO emissions during the observation period following basal fertilization were estimated at 1.6% ± 1.2%, 0.6% ± 0.3%, and 0.07% ± 0.01%, respectively.

### 4. Discussion

#### 4.1. Fertilizer-N loss via N₂ emission

In this study, the soil emissions of N₂ and N₂O following basal fertilization of winter wheat on average accounted for 1.6% and 0.6% of the applied urea-N, respectively. This fertilizer-N loss rate via emission of N₂+N₂O (2.2% on average) was higher than the range (0%–0.4%) measured by the AI method for wheat fields in China (adapted from Wang and Yan (2016)). In particular, our loss rate was 21 times higher compared to the average value (0.1%) measured using the AI method in a winter wheat field with similar soil, climate, and management practices (Ju et al. 2009). On the contrary, this study resulted in a much lower fertilizer-N loss via N₂ emission as compared to some other previous results, e.g. 10.5% (corn field) measured by the ¹⁵N gas flux method (Li et al. 2002), 13%–29% (wheat field) determined by the ¹⁵N mass balance method (Cai and Yang 1998), and 10%–70% (irrigated cotton field) detected by the GFSC technique (Scheer et al. 2009). The higher soil moisture and temperature than this study may account for the much higher losses observed in corn and cotton fields by Li et al. (2002) and Scheer et al. (2009).

It should be noted that this study might to some extent have underestimated the fertilizer-N losses via emissions of N₂, N₂O, and/or NO. Two reasons likely account for the possible underestimation. One is that abrupt changes and especially the true emission peaks might have been unfortunately missed due to the limitations of instrument capacity as well as inconvenience of field sampling operation (Figure 2(a)). Because of these limitations, low observation frequencies of at most once every two days following fertilization, irrigation and heavy rainfall events had to be adopted for soil sampling and subsequent gas measurements. The other is the lack of a true control treatment without N application. Deduction of the background emissions estimated as pre-fertilization levels likely biased the results due to the unavoidable ‘memory’ effect of preceding fertilizer amendments. Therefore, further studies are still needed to assess the effects of improving measurement schedules on the accuracy of fertilizer-N loss via N₂ emission measured using the method adopted in this study. The assessment also needs to involve the use of other techniques suitable for undisturbed measurements in-situ, such as the ¹⁵N gas flux method (Stevens et al. 1993).

#### 4.2. Molar ratios of N gas emissions

This study confirmed substantial N₂ emissions occurring simultaneously with N₂O at high soil moisture resulting from heavy rainfall/irrigation events following N fertilization. Using the ¹⁵N gas flux method to measure N₂ losses in corn fields receiving the same N dose as this study, Li et al. (2002) observed a peak emission of 2666 µg N m⁻² h⁻¹ following heavy rainfall after fertilizer addition, which is comparable with our maximum. Using a similar GFSC system, Scheer et al. (2009) observed a lower maximum N₂ emission (361 µg N m⁻² h⁻¹ on average) from soils of irrigated cotton fields than this study. The low N₂ emission was probably due to the lower N application compared to this study (75 versus 150 kg N ha⁻¹). However, much higher N₂ emissions from the denitrification of calcareous soils in the NCP than those of this study (133–500 versus 11.8 µg N h⁻¹ kg⁻¹ d.s.) were observed under anaerobiosis, even though the soil was treated with lower (67%–81%) N doses (Wang et al. 2013; Yuan et al. 2017). These differences in N₂ emissions can be attributed to the oxygen availability determined by precipitation, irrigation, or experimental manipulation. This is because
oxygen is the most important regulator for denitrification (Bouwman et al. 2013), in which the activity of N$_2$O reductase encoded by the nos gene is generally thought to increase with decreasing oxygen availability (Chapuis-Lardy et al. 2007). In this study, the observed lowest oxygen concentrations in soil pores were approximately 18%, which is still high enough to induce a less efficient expression of the nos gene. Thereafter, the reduction of N$_2$O to N$_2$ was limited and thus led to much lower N$_2$ emissions (Figure 2(a)) than those under anaerobiosis (Wang et al. 2013). The measurements under anaerobiosis resulted in N$_2$O/(N$_2$O+N$_2$) ratios of approximately 0.37 for a calcareous soil (adapted from Wang et al. 2013), which is higher than in our study (Figure 2(b)). This suggests that limitation by oxygen does not induce higher N$_2$O/(N$_2$O+N$_2$) ratios than denitrification under anaerobic conditions.

Our investigations showed that fertilization increased N$_2$O/(N$_2$O+N$_2$) ratios, making them much higher than prior to N amendment (Figure 2(a,b)). However, our observed N$_2$O/(N$_2$O+N$_2$) ratios (0.07–0.25) following urea application were lower than previously reported values (−0.51–0.69) for fertilized intact soil cores of a winter wheat field in France (Mathieu et al. 2006) and fertilized upland soils in China (Wang and Yan 2016). The aforementioned possible underestimation of N$_2$ and N$_2$O emissions due to the adopted insufficient measurement frequencies might have contributed to the lower ratio values of this study. However, the truth remains to be revealed by further studies.

The N$_2$O/(N$_2$O+N$_2$) ratio ($r$) can be used as a key parameter, together with given N$_2$O emissions ($F_{N_2O}$), to estimate soil N$_2$ emissions ($F_{N_2}$) at local, regional and/or global scales, following $F_{N_2} = (1/r - 1) F_{N_2O}$ (Bouwman et al. 2013; Schlesinger 2009). Accordingly, underestimated N$_2$O/(N$_2$O+N$_2$) ratios would lead to overestimated N$_2$ emissions, and vice versa. This emphasizes the need for further research to elucidate the regulating factors that influence the accuracy and precision of measured N$_2$O/(N$_2$O+N$_2$) ratios for soils under different conditions.

5. Conclusion

Using the GFSC technique, emissions of N$_2$, N$_2$O, and NO from a calcareous soil following basal urea fertilization of winter wheat were measured under simulated field conditions of temperature, and pore oxygen concentrations and fresh soil moisture. Our study reveals that irrigation/heavy rainfall following N fertilization not only substantially promotes the emission of both N$_2$ and N$_2$O, but also greatly enhances the N$_2$O/(N$_2$O+N$_2$) ratio. Approximately 1.6% and 2.2% of the basally applied urea-N was estimated to have been lost via emissions of N$_2$ and N$_2$+N$_2$O, respectively. However, these percentages were possibly underestimations of the true losses owing to instrumental limitations and the inconvenience of field sampling operations. These limitations might also have led to underestimated N$_2$O/(N$_2$O+N$_2$) ratios, which is a potentially key parameter for estimating fertilizer-N losses via N$_2$ emissions under different conditions. Further studies are still needed to clarify this issue.

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Disclosure statement

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