

# Soil pH controls N<sub>2</sub>O and N<sub>2</sub> emissions from upland agricultural soils across China

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## Abstract

Soil N<sub>2</sub> emission is an important pathway of N losses which is difficult to quantify in terms of both the magnitude and the contributions of the processes involved, yielding uncertainty in closing the N budget for agricultural systems. In this study, by adopting <sup>15</sup>N labelling and <sup>15</sup>N pairing technique under *in vitro* anaerobic conditions, the potential production rates of N<sub>2</sub>O and N<sub>2</sub>, the N<sub>2</sub>:N<sub>2</sub>O, and factors controlling denitrification, combined co-denitrification and anaerobic ammonium oxidation (anammox) were investigated for soils from 8 maize-growing regions across China from 26° N to 46° N latitude. The measured potential rates of N<sub>2</sub>O and N<sub>2</sub> productions were 0.1 to 6.5 nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup> and 8.1 to 41.5 nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup>, respectively. The dominance of N<sub>2</sub> over N<sub>2</sub>O production resulted in N<sub>2</sub>:N<sub>2</sub>O ratios from 4 to 372 in these soils. N<sub>2</sub>O production was high at low pH soils, suggesting possible inhibition on N<sub>2</sub>O reduction. Denitrification dominated both N<sub>2</sub>O and N<sub>2</sub> production, contributing to 85 ~ 99% of N<sub>2</sub>O, and 65 ~ 100% of N<sub>2</sub> productions, respectively. Correlation analysis suggested that soil pH explained 30% and 46% of the variations in <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> production, respectively. Soil pH was the most important factors controlling the ratio of N<sub>2</sub> to N<sub>2</sub>O emissions through denitrification in the studied upland agricultural soils across China, which implies that we may be able to estimate soil N<sub>2</sub> losses from upland soils at a regional scale using N<sub>2</sub>O emission rates and soil pH.

**Keywords:** greenhouse gases, denitrification, co-denitrification, anammox

## Introduction

Denitrification produces N<sub>2</sub>O and N<sub>2</sub> during a stepwise anaerobic reduction of nitrate (NO<sub>3</sub><sup>-</sup>) via nitrite (NO<sub>2</sub><sup>-</sup>), and NO to N<sub>2</sub>O and finally to N<sub>2</sub> (Zumft 1997). As the main products of denitrification, N<sub>2</sub>O and N<sub>2</sub> released are often variable. Environmental factors known to influence the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) are pH, organic carbon, NO<sub>3</sub><sup>-</sup> availability, water content, and O<sub>2</sub> partial pressure (Cuhel et al., 2010). Among these factors, soil pH might be the most important factor influencing both denitrification and N<sub>2</sub>O production (Simek and Cooper 2002). In general, the denitrification rate increases with increasing pH values (up to the optimum pH), while, the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) ratio decreases (Simek and Cooper 2002). This relationship has been characterized both in laboratory experiments (Cuhel and Simek 2011; Simek and Cooper 2002) and in the field (Cuhel et al., 2010) by artificially modifying soil pH to achieve gradients from acid to alkaline conditions. However, such studies were considered to capture the responses of targeted system to sudden and short-term changes in soil pH, by which the adaptation of the microbial community were ignored and not taken into account in interpretation of results (Baggs et al., 2010). Indeed, both ammonia oxidation and denitrification are generally considered to be the results of long-term adaptation of each microbial community to *in situ* differences in environmental characteristics (Nicol et al., 2008; Simek and Cooper 2002). "Historical pH" was demonstrated to play the more important role in influencing denitrifying enzyme activity (DEA), with current pH (immediate pH adjustment) more affecting the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) ratio (Cuhel and Simek 2011). However, it remains largely unclear if the same relationships exist between pH and the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) ratio in natural soil pH gradients over relatively large regional scales.

In addition, co-denitrification was shown to also generate N<sub>2</sub>O and/or N<sub>2</sub> two decades ago, but in a different

manner compared to the traditional denitrification (Shoun et al., 1992; Tanimoto et al., 1992). Co-denitrification produces hybrid N-N species ( $N_2O$  or  $N_2$ ), with one N atom from  $NO_2^-$  and the other from a co-metabolized N compound, such as azide, amines, imines within the substrate (Tanimoto et al., 1992). In spite of its unique characteristics, the co-denitrification process had been less intensively investigated for microbial N-N gas formation in soils. Studies focusing on this process had been carried out in grassland soils from northern Ireland (Laughlin and Stevens 2002), soil suspension from an agricultural research site in Germany (Spott and Stange 2011), several agricultural soils from USA (Long et al., 2013), and soils from permanent grazed grassland soil in southeast Ireland subjected to a large input of livestock urine (Selbie et al., 2015). Among these studies, co-denitrification was shown to contribute to 32 to 78% (Long et al., 2013), 92% (Laughlin and Stevens, 2002) and 98.1% (Selbie et al., 2015) of  $N_2$  production as compared with denitrification. In contrast,  $N_2O$  formations were entirely attributed to denitrification in the two studies by Laughlin and Stevens (2002) and Selbie et al. (2015), although the magnitude of  $N_2O$  production differed greatly. As pointed out by Spott et al. (2011), we know rather little about the contribution of co-denitrification to soil  $N_2O$  and  $N_2$  release and environmental factors that influence the process. Anammox, which refers to the anaerobic ammonium oxidation with nitrite reduction to  $N_2$ , is another process that can produce hybrid  $N_2$  species by combining one N atom from ammonium and the other from nitrite (deGraaf et al., 1996). This reaction has been reported in marine ecosystems and land-freshwater interfaces, like anoxic water column (Dalsgaard et al., 2003) oxygen-minimum zones (Kuypers et al., 2005), estuary sediments (Hou et al., 2013; Risgaard-Petersen et al., 2004), river sediments (Zhao et al., 2013; Zhou et al., 2014) and paddy soils (Sato et al., 2012; Yang et al., 2015; Zhu et al., 2011) and its contribution to  $N_2$  loss was estimated to be 50% in some marine environments (Devol 2015) and 1 to 37% for some paddy soils (Sato et al., 2012; Zhu et al., 2011). Co-denitrification and anammox both contribute to soil  $N_2$  emissions but their respective contributions are still hard to separate (Xi et al., 2016).

In this study, we investigate the influence of environmental factors, especially pH, on  $N_2O$  and  $N_2$  production, as well as on the  $N_2O/(N_2O + N_2)$  ratios in eight areas from three representative agricultural regions across China (northeast, central and southern China). These soils are expected to vary in textures, physicochemical properties, covering a relatively wide range of soil pH (from 4.3 to 8.7). In combination with  $^{15}N$  tracer technique (addition of  $^{15}N$  enriched denitrification substrates), we adopted the recently improved isotope ratio mass spectrometry technique by Yang *et al.* (2014) to track subsequent  $^{15}N_2O$  and  $^{15}N_2$  productions. We also partitioned the sources of  $N_2O$  and  $N_2$  to denitrification or co-denitrification plus anammox based on the different  $^{15}N$  isotope pairing between these processes (Nielsen, 1992). The overall objective was to improve our understanding of potential losses of  $N_2O$  and  $N_2$ , as well as the  $N_2O/(N_2O + N_2)$  ratio and controlling factors, and responsible processes (denitrification vs. co-denitrification plus anammox) from major Chinese upland agricultural soils. We hypothesized that ambient soil pH is the major component influencing the end products of denitrification ( $N_2O$  vs.  $N_2$ ) and thus the  $N_2O/(N_2O + N_2)$  ratio. If so, it implies that we may be able to map annual soil  $N_2$  losses using annual  $N_2O$  emission rate and soil pH at a large regional scale.

## Methods

In the growing season of 2014 (from the middle July to early August), surface layer samples (0-15 cm) were collected in triplicate from 84 maize fields across 8 maize cultivation regions of China, spanning latitude from  $26^\circ N$  to  $46^\circ N$ . These regions included Harbin (HEB), Changchun (CC), and Gongzhuling (GZL) from northeast China; Luancheng (LC) and Fengqiu (FQ) from central China; and Taoyuan (TY), Qiyang (QY), and Yingtian (YT) from southern China. These sampling areas were expected to exhibit broad-scale variations in soil physicochemical properties with respect to the parent material, soil texture, soil pH and organic matter. All fields were grown with maize following typical regional management practices and had been treated with inorganic fertilizers for years. Soil samples from each core were homogenized and passed through 2-mm sieves before storage in a  $4^\circ C$  refrigerator. The sieved soil samples were divided into three sets of subsamples. One set was stored at  $4^\circ C$  for ammonium ( $NH_4^+$ ) and nitrate ( $NO_3^-$ ) concentration analysis. The second part was used for isotope tracer incubation, and the remaining sample was air dried for soil texture, C and N content, and pH analyses. Laboratory incubations were conducted to measure  $^{15}N_2O$  and  $^{15}N_2$  production rates with the addition of enriched  $NH_4^{15}NO_3$ .

## Results

We calculated  $^{15}N_2O$  fluxes from measured net  $N_2O$  fluxes and the  $^{15}N$  enrichment of  $N_2O$ . The  $^{15}N_2$  production rates were calculated using the mass of  $N_2$  in the vial headspace (nmol) and the  $^{15}N$  mole fractions of sampled

N<sub>2</sub> at the end of incubation. In the soils from all eight areas, N<sub>2</sub> was the dominant end product of denitrification over N<sub>2</sub>O under our experimental conditions, especially in FQ and LC (central China). Large variations in both <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> production were observed among these areas. For <sup>15</sup>N<sub>2</sub>O, the lowest flux of 0.1 nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup> was recorded in LC, central China, while the highest was recorded in YT, southern China (6.5 nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup>). The <sup>15</sup>N<sub>2</sub>O fluxes from soils in TY, QY, and YT in southern China were significantly higher than those from FQ and LC in central China ( $P = 0.022$ ). For <sup>15</sup>N<sub>2</sub>, the fluxes ranged from 8.7 nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup> in CC to 41.5 nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup> in LC. Significantly higher <sup>15</sup>N<sub>2</sub> was recorded in FQ and LC in central China than other areas in southern and northeast China ( $P < 0.001$ ).

The dominance of <sup>15</sup>N<sub>2</sub> potential production resulted in large <sup>15</sup>N<sub>2</sub>:<sup>15</sup>N<sub>2</sub>O ratios, with the production of <sup>15</sup>N<sub>2</sub> exceeding those of <sup>15</sup>N<sub>2</sub>O by a factor of 3 (YT, southern China) to 371 (LC, central China). A significantly higher <sup>15</sup>N<sub>2</sub>:<sup>15</sup>N<sub>2</sub>O ratio occurred in LC, central China compared with all other areas ( $P < 0.001$ ). The relative share of the <sup>15</sup>N<sub>2</sub>O emission from total emissions (<sup>15</sup>N<sub>2</sub>O + <sup>15</sup>N<sub>2</sub>) was 51%, 38% and 52% in TY, QY, and YT from southern China, respectively; 43%, 26%, 16% in CC, GZL, and HEB from northeast China, respectively; and only 4% and 0.003% in FQ and LC from central China, respectively, with those relative shares from TY and YT (southern China) being significantly higher than those from FQ and LC (central China) ( $P = 0.022$ ).

A stepwise multiple linear regression model identified soil pH as the most important factor influencing both <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> production (data not shown). Pearson's correlation analyses indicated that soil pH alone explained 30% and 46% of the variation in <sup>15</sup>N<sub>2</sub>O production and <sup>15</sup>N<sub>2</sub> production, respectively ( $P < 0.001$  for both), although the direction of the pH influence was different. <sup>15</sup>N<sub>2</sub>O emissions were high from the soil with low pH and decreased with increasing pH ( $y = -1.97x + 16.43$ ,  $R^2 = 0.30$ ,  $P < 0.001$ ); in contrast, <sup>15</sup>N<sub>2</sub> production increased steadily as soil pH increased ( $y = 8.52x - 34.4$ ,  $R^2 = 0.46$ ,  $P < 0.001$ ). Consequently, the <sup>15</sup>N<sub>2</sub>O proportion (<sup>15</sup>N<sub>2</sub>O/(<sup>15</sup>N<sub>2</sub>O + <sup>15</sup>N<sub>2</sub>)) decreased sharply as the soil pH increased, with 39% of the variation explained by pH ( $y = -0.0628 + 0.775e^{-(x-4.24)/2.11}$ ,  $R^2 = 0.39$ ;  $P < 0.001$ ). <sup>15</sup>N<sub>2</sub>:<sup>15</sup>N<sub>2</sub>O ratios, on the other hand, stayed low for low pH soils and increased exponentially as the soil pH increased, peaking at a soil pH of approximately 8 ( $y = e^{(x-2.97)}$ ,  $R^2 = 0.28$ ,  $P < 0.001$ ). The factors other than soil pH affecting <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> production included the soil texture (clay content for <sup>15</sup>N<sub>2</sub>O,  $R^2 = 0.1$ ,  $P = 0.002$ , data not shown) and soil background nitrate concentrations (for <sup>15</sup>N<sub>2</sub>O,  $R^2 = 0.08$ ,  $P < 0.001$ , for <sup>15</sup>N<sub>2</sub>,  $R^2 = 0.07$ ,  $P = 0.02$ , data not shown). Pearson's correlation analysis also showed an explanatory power of 44% for CO<sub>2</sub> respiration on (<sup>15</sup>N<sub>2</sub>O + <sup>15</sup>N<sub>2</sub>) production. (<sup>15</sup>N<sub>2</sub>O + <sup>15</sup>N<sub>2</sub>) production increased as more CO<sub>2</sub> was respired but started to drop when CO<sub>2</sub> respiration went above 1200 nmol g<sup>-1</sup> h<sup>-1</sup>.

Based on the <sup>45</sup>N<sub>2</sub>O, <sup>46</sup>N<sub>2</sub>O, and <sup>29</sup>N<sub>2</sub>, <sup>30</sup>N<sub>2</sub> productions from the <sup>15</sup>NO<sub>3</sub><sup>-</sup> incubation, the production rates of N<sub>2</sub>O and N<sub>2</sub> from denitrification and combined anammox and co-denitrification were calculated respectively (Table 3). The relative contribution of each process to N<sub>2</sub>O and N<sub>2</sub> productions were also given in Table 3. For both N<sub>2</sub>O and N<sub>2</sub>, denitrification was the dominant producing process. N<sub>2</sub>O produced by denitrification ranged from 0.1 in LC, central China to 6.7 nmol N g<sup>-1</sup> h<sup>-1</sup> in YT, southern China, accounting for 85 to 99% of total N<sub>2</sub>O production. Co-denitrification-contributed N<sub>2</sub>O productions ranged from 0.02 nmol N g<sup>-1</sup> h<sup>-1</sup> to 0.41 nmol N g<sup>-1</sup> h<sup>-1</sup>, accounting for 0.5 to 15% of total N<sub>2</sub>O production. Comparison between areas indicated that denitrification contribution to N<sub>2</sub>O production was significantly higher in HEB, northern China than that from LC, central China ( $P < 0.05$ ), while co-denitrification-contributed N<sub>2</sub>O fraction were highest in LC, central China, and lowest in HEB, northern China ( $P < 0.05$ ). For N<sub>2</sub>, denitrification-derived rates were from 8.6 nmol N g<sup>-1</sup> h<sup>-1</sup> to 43.6 nmol N g<sup>-1</sup> h<sup>-1</sup>, accounting for 65 to 100% of N<sub>2</sub> rates. Co-denitrification plus anammox contributed 0 to 0.55 nmol N g<sup>-1</sup> h<sup>-1</sup> of N<sub>2</sub>, representing 0 to 35% of N<sub>2</sub> rates. In FQ, LC in central China and HEB, northern China, N<sub>2</sub> were produced solely by denitrification. Correlation analyses showed that denitrification positively correlated with co-denitrification to produce N<sub>2</sub>O ( $R^2 = 0.22$ ,  $P < 0.001$ ), but correlated negatively to produce N<sub>2</sub> ( $R^2 = 0.19$ ,  $P < 0.001$ ).

## Conclusions

In summary, our results indicate that soil pH was the most important factor influencing the potential productions of both N<sub>2</sub> and N<sub>2</sub>O, as well as N<sub>2</sub>:N<sub>2</sub>O ratios under anaerobic conditions in maize soils across China. As the soil pH increased, the N<sub>2</sub>O production decreased, the N<sub>2</sub> emissions and total denitrification rates increased, resulting in elevated N<sub>2</sub>:N<sub>2</sub>O ratios. Denitrification, as compared with co-denitrification (for N<sub>2</sub>O) or co-denitrification plus anammox (for N<sub>2</sub>), dominated both N<sub>2</sub>O and N<sub>2</sub> production, contributing to 85.2~99.5% of N<sub>2</sub>O, and 65.3~100% of N<sub>2</sub> potential productions, respectively. Soil pH appeared to be one of the most

important controllers of  $\text{N}_2\text{O}$  and  $\text{N}_2$  emissions through denitrification in upland agricultural soils across China, which may supply us with the possibility of estimating soil  $\text{N}_2$  losses using *in situ*  $\text{N}_2\text{O}$  fluxes and soil pH at a large regional scale.

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