



## Short communication

## Interaction between nitrification, denitrification and nitrous oxide production in fumigated soils



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## HIGHLIGHTS

- Fumigation increased soil ammonium content and inhibited nitrification process.
- Denitrification was greatly stimulated by the breakdown products of Pic and DZ.
- N<sub>2</sub>O emissions in Pic and DZ fumigated soils are predominantly due to denitrification.

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## ABSTRACT

Soil fumigation can increase mineral nitrogen due to the mineralization of soil microbial biomass killed during the fumigation, and as a result nitrous oxide (N<sub>2</sub>O) emission would increase. In addition, a fumigant's impact on soil nitrification and denitrification would also alter the dynamics of N<sub>2</sub>O production in fumigated soils. Laboratory incubation studies were conducted to quantify the dynamic changes in N<sub>2</sub>O production following various fumigant treatments, and to determine the interaction between nitrification, denitrification and N<sub>2</sub>O production in fumigated soils. Results showed a substantial increase in NH<sub>4</sub><sup>+</sup>-N and dissolved amino acids (DAA) during 7 days fumigation at 1WAF (week after fumigation). The application of fumigants caused significant inhibition of nitrification. However the results relating to potential denitrification were quite different. The rates of potential denitrification in chloropicrin (Pic) and dazomet (DZ) treatments at 1WAF were 3.5 and 5.6 times higher than the untreated control. Potential denitrification was greatly stimulated after Pic and DZ fumigation. The N<sub>2</sub>O production rates in Pic and DZ fumigated soil were significantly higher than the untreated control at 1WAF in the tested soil type. The cumulative N<sub>2</sub>O emissions in Pic and DZ fumigated soil were also significantly higher than the untreated control, but there were no significant differences among 1,3-dichloropropene (1,3-D), dimethyl disulfide (DMDS) and untreated control. A positive relationship between N<sub>2</sub>O production and potential denitrification (PDN) was observed ( $r = 0.951$ ,  $P < 0.01$ ). Pic and DZ are both nitrogenous compounds. The breakdown products of Pic and DZ would be available for microbial-aided denitrification reactions as nitrogen sources leading to N<sub>2</sub>O production, indicating that Pic and DZ degradation stimulated denitrification activity responsible for soil N<sub>2</sub>O production.

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## 1. Introduction

Soil fumigation – the application of fumigant pesticides to bare soil – is commonly used in agriculture to control soilborne pathogens, nematodes and weeds in many vegetable, fruit, nut and nursery crops worldwide (Cao et al., 2013; Desaeger et al., 2008; Santos et al., 2006). Methyl bromide is a versatile and highly effective fumigant which has been widely used for preplant soil fumigation. However, it has to be phased out by 2015 in developing countries owing to its stratospheric ozone depletion potential.

**Abbreviations:** Pic, chloropicrin; 1,3-D, 1,3-dichloropropene; DMDS, dimethyl disulfide; DZ, dazomet; MBN, microbial biomass nitrogen; DAA, dissolved amino acids; PN, potential nitrification; PDN, potential denitrification; WAF, week after fumigation; wk, week.

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Therefore, other fumigants are being investigated to replace methyl bromide and are selected based on their efficacy towards target organisms. Chloropicrin (Pic), 1,3-dichloropropene (1,3-D), dazomet (DZ) and dimethyl disulfide (DMDS) are alternative preplant soil fumigants for the control of soilborne pests and diseases of agricultural crops (Duniway, 2002; Wang et al., 2009). However, the fumigant does not act solely on its target organisms and can affect the soil microbial community and activity of non-target microorganisms (Jacobsen and Hjelmsø, 2014), such as soil nitrifiers and denitrifiers which are largely responsible for the production of nitrous oxide ( $\text{N}_2\text{O}$ ) in soil.

$\text{N}_2\text{O}$  is a significant greenhouse gas, and also contributes to ozone depletion in the stratosphere (Ravishankara et al., 2009). Agricultural activities are the most significant source of  $\text{N}_2\text{O}$ , and soil plays an important role in releasing  $\text{N}_2\text{O}$  to our atmosphere (Del Grosso, 2010). Although a wide range of processes have the potential to produce  $\text{N}_2\text{O}$ , its production in soil is primarily attributed to nitrification and denitrification (Bremner, 1997; Kool et al., 2011). It has been reported that application of fumigants altered the production of  $\text{N}_2\text{O}$  (Spokas et al., 2005) and fungal denitrification under aerobic conditions was identified as the primary process for Pic-induced  $\text{N}_2\text{O}$  production in soil (Spokas et al., 2006). Many herbicides (Das et al., 2011) and fungicides (Kinney et al., 2004) also affect the dynamics of  $\text{N}_2\text{O}$  emission from the soil. In addition to dynamic changes in soil  $\text{N}_2\text{O}$  following fumigation, there are also alterations in soil microbial nitrification and denitrification which are two principal processes responsible for  $\text{N}_2\text{O}$  emissions from soils. Various fumigants caused significant depression in soil nitrification (Stromberger et al., 2005; Yan et al., 2013) and affected soil denitrification processes (Ajwa et al., 2003). Although some non-traditional nitrification and denitrification processes may be involved in  $\text{N}_2\text{O}$  production in soil (Marusenko et al., 2013; Zhu et al., 2013), soil fumigation reduced the overall microbial populations and biomass.

Due to mineralization of soil microbial carbon and nitrogen after fumigation,  $\text{N}_2\text{O}$  emission would be expected to increase following fumigation. In addition, the impact on soil nitrification and denitrification after fumigation would also alter the dynamics of  $\text{N}_2\text{O}$  production in fumigated soil. The objective of this study was to quantify the dynamic change in  $\text{N}_2\text{O}$  production after fumigation and to determine the interaction between nitrification, denitrification and  $\text{N}_2\text{O}$  production in fumigated soils.

## 2. Materials and methods

### 2.1. Laboratory incubation of field soils

Soil samples were collected from the top 20 cm of greenhouse soil in Guojiawu village, Yufa town, Daxing district, southwest of Beijing; GPS coordinates are  $116^\circ 24' \text{E}$ ,  $39^\circ 28' \text{N}$ . The field study site is not relevant to any endangered or protected species. Soil samples were taken with the authorization of the Institute of Daxing Agricultural Science (Beijing City), and no other specific permissions were required for the field site. Soil characteristics were as follows: 64.1% sand, 28.5% silt and 7.4% clay; soil pH 7.2 and 1.8% organic matter. The soil was sieved through a 5 mm screen and pre-incubated for 7 days at room temperature in the dark, before any treatments were applied. Laboratory incubations were carried out within 2 weeks of soil collection, to avoid decreases in microbial activity due to storage. The experimental design consisted of four fumigant treatments (chloropicrin, Pic; 1,3-dichloropropene, 1,3-D; dimethyl disulfide, DMDS; dazomet, DZ) and a control in three replicates. 150 g soil samples were placed in 250 ml flasks, treated with  $(\text{NH}_4)_2\text{SO}_4$  (equivalent to  $100 \text{ mg N kg}^{-1}$  soil) and mixed thoroughly. Fumigants were added into the flasks at typical field

application rates for each chemical (Pic  $53 \text{ mg kg}^{-1}$ , 1,3-D  $39 \text{ mg kg}^{-1}$ , DMDS  $68 \text{ mg kg}^{-1}$ , DZ  $54 \text{ mg kg}^{-1}$ ) (Spokas et al., 2007). The flasks were sealed with rubber stoppers and left for 7 days in the dark at  $25^\circ \text{C}$ . After 7 days fumigation, all the flasks were taken to a ventilation hood to remove the fumigants, and then soils were mixed thoroughly before sampling. During a further incubation period at  $25^\circ \text{C}$  for 8 weeks, soils were stirred and aerated for 10–15 min every day and sprayed with deionized water according to the weight loss, in order to maintain aerobic conditions and constant moisture.

### 2.2. Dissolved N, MBN, PN and PDN in fumigated soil

After 7 days fumigation, all the flasks were taken to a ventilation hood to remove the fumigants, and the soils were mixed thoroughly. Soil samples were collected 1, 4, and 8 weeks after fumigation. Soil mineral nitrogen ( $\text{NH}_4^+-\text{N}$  and  $\text{NO}_3^--\text{N}$ ) levels were measured with a continuous flow analytical system (Futura Continuous Flow Analytical System, Alliance instruments, France) after extraction with 2 M KCl. Microbial biomass nitrogen (MBN) was estimated by the chloroform fumigation method (Brookes et al., 1985). Dissolved amino acids (DAA) were measured by the ninhydrin reaction method (Joergensen and Brookes, 1990). Potential nitrification (PN) rates were measured by adding ammonium sulfate to catalyze the nitrite formation (Berg and Rosswall, 1985). Potential denitrification (PDN) rates were measured by acetylene inhibition of  $\text{N}_2\text{O}$  reduction (Ryden et al., 1979).

### 2.3. Change in $\text{N}_2\text{O}$ in fumigated soil

To study the nitrous oxide production in fumigated soil, soil was treated with fumigants in the same manner as above. The flasks were then tightly sealed with rubber stoppers with an outlet port for syringe sampling and incubated at  $25^\circ \text{C}$ . Three duplicate incubations of each fumigant treatment were performed. For the determination of  $\text{N}_2\text{O}$  concentration in fumigated soil, 10 ml gas subsamples were collected from the sampling ports using gastight syringes at 1, 2, 3, 4, 5, 6, 7, and 8 weeks after fumigation. After each sampling, the remaining gas in all flasks was evacuated by vacuum pump, fresh air was added, and the flasks were incubated at  $25^\circ \text{C}$ . The gas sample was injected into a 20 mL clear headspace vial which was immediately crimp-sealed with an aluminum cap and teflonfaced butyl-rubber septum (Agilent). Finally, the amount of  $\text{N}_2\text{O}$  in the gas samples was analyzed using an Agilent 7890A gas chromatograph coupled with an Agilent 7694E headspace sampler and a micro electron capture detector.

**Table 1**  
Dissolved N and MBN in fumigated soil at 1WAF.

Treatment	$\text{NH}_4^+-\text{N}$ ( $\text{mg kg}^{-1}$ )	$\text{NO}_3^--\text{N}$ ( $\text{mg kg}^{-1}$ )	DAA ( $\text{mg kg}^{-1}$ )	MBN ( $\text{mg kg}^{-1}$ )
Pic	100.7 a	228.9 b	29.5 a	42.2 c
1,3-D	88.9 ab	233.6 b	26.4 ab	48.3 c
DMDS	83.8 ab	229.5 b	22.8 ab	73.7 bc
DZ	75.3 b	224.9 b	21.0 b	91.0 ab
Control	20.7 c	260.1 a	2.1 c	120.3 a

Pic; chloropicrin, 1,3-D; 1,3-dichloropropene, DMDS; dimethyl disulfide, DZ; dazomet, WAF; week after fumigation, DAA; dissolved amino acids, MBN; microbial biomass nitrogen.

Means within columns followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's new multiple-range test.

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