



Does soil amendment alter reactive soil N dynamics following chloropicrin fumigation?

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HIGHLIGHTS

- The use of straw significantly decreased soil reactive N for three months after fumigation.
- Straw and manure alleviated detrimental effect of fumigation on microbes and enzymes.
- Following fumigation, biochar decreased protease, while increased arylamidase and L-glutaminase.
- DOC content in amendments played the important role on microbial activity.

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ABSTRACT

Chloropicrin fumigation had strong inhibitory effect on soil N cycling. Knowledge gap existed about the performance of reactive N in soil applied with different amendments used to improve the fumigation function or soil quality. In this study, we employed four amendments, i.e., wheat straw residue, manure, biochar and ammonium thiosulfate, incorporated into soil at the regular application rate. Simultaneously, bare soil was selected as control (CK). Based on a three months incubation assay, soil reactive N and activity of three enzymes governing N-mineralization was measured, i.e., protease, arylamidase and L-glutaminase, as well the soil fluorescein diacetate (FDA) hydrolysis, basal soil respiration, and dissolved soil organic carbon (DOC). Result showed that, compared with the bare soil, the addition of straw or manure to soil markedly enhanced the FDA and the resistance of arylamidase and L-glutaminase to the fumigation, while significantly decreased the concentration of DON, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. The addition of biochar to soil had no effect on the reactive N, but contrasting effects on the three enzymes, i.e., suppressed protease activity, and enhanced arylamidase activity. The ammonium thiosulfate showed an inert effect on the measured microbiological indices and reactive N except the enhanced concentration of $\text{NH}_4^+\text{-N}$. DOC content of amendments governed microbial activity under fumigation condition. In synthesis, our findings suggested that under chloropicrin fumigation the use of straw or manure enhanced the microbial abundance and the activity of N-mineralization enzymes, which may lead to low reactive N by the microbial N immobilization for a longer period.

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1. Introduction

Over the past 20 years, soil-borne diseases in China are getting more and more serious due to the expanding conserved land and the intensive mono-cropping cultivation system (Cao et al., 2017). Fumigation as a common effective technique is widely used to control the soil borne diseases (Ajwa and Trout, 2004; Desaege

et al., 2008; Haydock et al., 2010; Minuto et al., 2006; Santos et al., 2009). Methyl bromide (MeBr) was the predominant soil fumigant with a history more than 50 years (Ruzo, 2006). However, due to the detrimental effect on ozone, MeBr had been completely prohibited in agriculture system since the year of 2015 based on the framework of Montreal protocol. Instead of banned MeBr, chloropicrin was reported to be one of the most suitable alternatives (Gullino et al., 2003; Spokas et al., 2006) by its high efficacy in killing board-spectrum soil borne diseases through rapidly immersing into the tissues of biota and killing cell, and no depletion on ozone (UNEP, 1998).

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However, increasing studies found that fumigants including the chloropicrin have caused detrimental effects on non-targeting soil microorganisms and the governed nutrient cycling processes (Butler et al., 2014; Yan et al., 2013, 2017). For instance, Zhang et al. (2011) reported that the chloropicrin fumigation increased the proportions of dissolved organic N (DON) in total dissolved N. The study of Yan et al. (2013) showed that, soil nitrification was suppressed by addition of fumigants, i.e., chloropicrin, 1,3-dichloropropene, dimethyl disulfide and metham sodium. In which, chloropicrin showed stronger inhibitory effect on soil nitrification than other fumigants; soil nitrification restarted 10 weeks after chloropicrin fumigation relative to only one-week in soil with other fumigants. Wei et al. (2016) found that bacterial and fungal populations were notably altered by chloropicrin fumigation in a field trial. Furthermore, the inhibitory effect of chloropicrin fumigation on nitrification was found on many soil types (Yan et al., 2015).

However, the previous studies about the influence of fumigation on soil N cycling were only conducted on the bare soil, without considering the reality in agriculture such as the general use of soil amendments. In agricultural practices, several soil amendments have been used to reduce the volatilization of chloropicrin to enhance the fumigation efficiency. For instance, the study of Gao et al. (2009) reported that the loss mass of chloropicrin dramatically decreased while soil was amended with manure. By adding ammonium thiosulfate to soil, the volatilization of chloropicrin was decreased significantly compared with bare soil (Gan et al., 2000; Schwarzenbach et al., 1993; Wang et al., 2000), and potentially due to the formed non-volatile organic compound through nucleophilic reaction (Schwarzenbach et al., 1993). Qin et al. (2007) reported the half-life period of chloropicrin in soil applied with ammonium thiosulfate decreased to 5.5 h compared with 16.5 h of bare soil. The study of Ashworth et al. (2009) found that, by spraying ammonium thiosulfate on the surface-ground, the volatilization of chloropicrin was decreased by 26.1%. In addition, biochar with high porous media has been presented as a promising soil amendment due to the high sorption ability on pesticide (Yavari et al., 2015).

Up to date, however, the knowledge gap exists with lacking information on how fumigation affects critical N status in soil applied with amendments and the performance after fumigation. This knowledge would improve our holistic understanding following the contemporary agricultural practices and provide proposals for nutrient management. In this study, we chose chloropicrin as the fumigant, and four amendments, i.e., straw, manure, biochar and ammonium thiosulfate. The objective is to investigate how soil amendments affect the reactive N status following fumigation and the underlying mechanism by measuring critical activity of N-mineralization enzymes, basal soil respiration and fluorescein diacetate (FDA) hydrolysis. We hypothesized that, compared with the bare soil, the use of amendments may enhance the suppression on soil nitrogen transformation, e.g., ammonification or nitrification.

2. Materials and methods

2.1. Soil properties

Soil was collected from a 0–10 cm depth from a greenhouse at Dongxiaoying village, Tongzhou, Beijing. This greenhouse has a more than 10-year history of growing vegetables but has never been subjected to fumigation. After sampling, soil was sieved through a 2 mm mesh and then stored at 4 °C. The soil had a texture of sandy loam with 53.7% sand, 42.4% silt and 3.9% clay, 40.1 g kg⁻¹ organic matter, pH 7.5 (soil:H₂O, 1:2.5), total N 1.3 g kg⁻¹,

243.6 mg kg⁻¹ available P and 183.0 mg kg⁻¹ available K.

2.2. Soil fumigation and incubation

Prior to the fumigation, soil was adjusted to 40% of water holding capacity, and pre-incubated at 25 °C for 7 d. Afterwards, four materials, i.e., straw residuals, manure, biochar, and ammonium thiosulfate were added into soil. In addition, bare soil was prepared as the control treatment (CK). Each treatment was conducted in triplicate; one replicate contains 800.0 g soil in a 2 L plastic jar. The basic properties of four amendments were presented in Table 1. Briefly, wheat straw was oven-dried, crushed and sieved through a 2 mm mesh, and then mixed with soil at a rate of 2% (w/w); ammonium thiosulfate was firstly sprayed on soil with 2:1 mol ratio to chloropicrin, then stirred and mixed thoroughly; cow manure was bought from a vegetables production company in Beijing, air-dried, crushed and sieved through a 2 mm mesh, and then mixed with soil at a 5% rate (w/w); biochar was made from the wheat straw used in this study, pyrolyzed at 500 °C for 2 h in a muffle furnace, crushed and then sieved through 2 mm mesh, and finally mixed with soil at a rate of 2% (w/w).

Fumigation was conducted after adding the soil amendments, chloropicrin with 68 mg kg⁻¹ as the typical application rate was inserted into the middle of soil by 100 mL micro-syringes (Spokas et al., 2007), and then the jars were sealed immediately and stored at 25 °C in the dark. After 7 d fumigation, repeated evacuations were applied to remove the chloropicrin, and then two bottles containing 100 mL deionized water and 100 mL 1 M NaOH were put into the jars to maintain the humidity and adsorb the evolved CO₂ during incubation. These two bottles were replaced by two new every 2 d. Every day during incubation, soil was stirred homogeneously and aerated for 10–15 min to maintain the aerobic condition, afterwards soil was sprayed using the deionized water to maintain the constant moisture content based on the weight loss. After fumigation, the whole incubation lasted 84 d.

2.3. Soil sampling and analysis

80.0 g soil per jar was taken out at the end of pre-fumigation (–7), and at 0 (5 h after chloropicrin fumigation), 7, 14, 28, 56, and 84 d after chloropicrin fumigation. Triplicate subsamples with each 20 g were extracted with 0.5 M K₂SO₄ (1:4 of soil to K₂SO₄, w/v). In the extracts, the dissolved organic C (DOC) was measured following the method of Kalembasa and Jenkinson (1973). Total dissolved N (TDN) was measured by the persulfate oxidation (Hagedorn and Schleppi, 2000). The NH₄⁺-N and NO₃⁻-N concentration was measured. In detail, 10 g of fresh soil was weighted into a 150 mL flask with 50 mL of 0.0125 M CaCl₂, and then was shaken for 60 min at 30 rpm. Subsequently, the suspension was centrifuged at 2000 rpm for 2 min, the supernatant was extracted and stored at 4 °C for further measurement using an autoanalyzer (Auto Analyzer 3, Seal Ltd., UK). Dissolved inorganic N (DIN) was the sum of NH₄⁺-N and NO₃⁻-N concentration. Dissolved organic nitrogen (DON) was calculated by subtracting DIN from TDN.

Three critical N-mineralization enzymes were determined, i.e., protease, arylamidase, L-glutaminase. Briefly, protease and arylamidase were measured by the colorimetric method described by Ladd and Butler (1972) and Acosta-Martínez and Tabatabai (2000), respectively. L-glutaminase was measured following the H₂SO₄ titration method (Frankenberger and Tabatabai, 1991).

Furthermore, based on the measured three enzymes activities, the index of soil resistance was evaluated (Bécaert et al., 2006), as followed:

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