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Interactive effect of climate factors, biochar and insecticide chlorpyrifos on methane consumption and microbial abundance in a tropical Vertisol



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Keywords: Elevated CO ₂ Temperature Biochar Chlorpyrifos CH ₄ 16S rRNA	Climate change may increase the pest infestation leading to intensive use of insecticides. However, the effect of insecticide and climate factors on soil methane (CH ₄) consumption is less understood. A laboratory experiment was carried out to evaluate the effect of temperature (15 °C, 35 °C, and 45 °C), moisture holding capacity (MHC) (60%, 100%), biochar (0%, 1%) and chlorpyrifos (0 ppm, 10 ppm) on CH ₄ consumption and microbial abundance in a tropical Vertisol of central India. Methane consumption rate k (ng CH ₄ consumed g ⁻¹ soil d ⁻¹) varied from 0.065 \pm 0.005 to 0.608 \pm 0.018. Lowest k was in 15 °C-60% moisture holding capacity (MHC)-no biochar and with 10 ppm chlorpyrifos. Highest k was in 35 °C-100% MHC-1% biochar and without (0 ppm) chlorpyrifos. Cumulative CO ₂ production (ng CO ₂ produced g ⁻¹ soil d ⁻¹) varied from 446 \pm 15 to 1989 \pm 116. Both CH ₄ consumption and CO ₂ production peaked in the treatment of 35 °C-100% MHC-1% biochar. Chlorpyrifos inhibited CH ₄ consumption irrespective of treatments. Abundance of 16S rRNA of eubacteria (× 10 ⁶ g ⁻¹ soil) varied from 2.33 \pm 0.58 to 85.67 \pm 7.00. Abundance of 16S rRNA genes representing Actinomycetes (× 10 ⁴ g ⁻¹ soil) varied from 7.67 \pm 1.53 and pmoA gene (Methanotrophs) (× 10 ⁵ g ⁻¹ soil) varied from 1.23 \pm 0.59 to 34.33 \pm 6.51. Chlorpyrifos under climate change factors may inhibit CH ₄ consumption but the use of biochar stimulated the CH ₄ consumption, CO ₂ production and microbial abundance. Study highlighted that use of chlorpyrifos under climate change factors may inhibit CH ₄ consumption but the use of stochar may alleviate the negative effect of the chlorpyrifos.

1. Introduction

Climate change will affect soil ecosystem by impacting the microbial biomass, diversity, and their metabolic activities (Romero-Olivares et al., 2017). Therefore, understanding the function of microbes in response to the global change is important for maintenance of ecosystem. It is also predicted that use of insecticide will increase many fold in future because the prevalence of pests will increase under elevated atmospheric CO₂ and temperature (Yan et al., 2017). The insecticide chlorpyrifos (0,0-diethyl-3,5,6-trichloro-2- pyridylphosphorothioate) is a widely used for treatments of most of the crops, lawns, and ornamental plants (Gomez, 2009). This broad-spectrum insecticide is effective in controlling a variety of insects including mosquitoes (larvae and adults), flies, and ecto-parasite of cattle and sheep (Kumar1 and Kumar, 2007; Liu et al., 2005). The effects of chlorpyrifos on soil microbial biomass carbon and nitrogen, microbial activities including nitrogen cycling have been studied (Riah et al., 2014). However, little information is available on the impact of chlorpyrifos on soil methane consumption. Methane consumption is an important process for climate

change perspective. CH₄ consumption activity of soil reduces concentration of atmospheric greenhouse gas methane. Methane is an important greenhouse gas as its global warming potential is about 25 times higher than CO₂ (Kollah et al., 2017). CH₄ concentration in different soil compartments may vary from 1000 to 20,000 ppm which is emitted to atmosphere (Metz et al., 2007). Therefore, consumption of atmospheric CH₄ is crucial to regulate climate change. If the chlorpyrifos applied to soil inhibits CH₄ consumption, then its use will adversely affect the ecosystem. Therefore, strategies need to be identified to minimize the negative effect of chlorpyrifos on CH₄ consumption. However, there is lack of information on the impact of chlorprifos on CH4 consumption. It is also not clearly known how chlorpyrifos influences CH₄ consumption under the influence of climate factors (temperature and moisture).

It is estimated that at the current pace of CO₂ increase, even with measures to minimize CO₂ emissions, the atmospheric concentration of CO₂ will reach 550-700 ppm by 2050 and 650-1200 ppm by 2100 (Higgins et al., 2015). Due to the increase of atmospheric greenhouse gases (GHGs) the mean global temperature is predicted to rise by 2.5 °C

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or more by 2050 and up to $6.4 \,^{\circ}$ C by the end of this century (IPCC, 2007). Elevated temperature and CO₂ affects CH₄ consumption by reducing methanotrophs population. Elevated CO₂ and temperature influences certain aerobic microbial population.

Recently, biochar (BC) use in agriculture has been suggested as a potential win–win strategy for climate change mitigation and food production (Kollah et al., 2015). Biochar is a pyrolysed biomass of organic feedstock, produced by heating in an oxygen-limited environment (pyrolysis) at temperature of 400–900 °C (Lehmann, 2007). High cation exchange capacity (CEC) of BC enables it to absorb NH₄-N and other plant nutrients (Subedi et al., 2013). Biochar may also absorb various insecticides and reduce their toxicity (Diez et al., 2013). In addition biochar may enhance microbial groups and stimulate insecticide biodegradation (Atkinson et al., 2010).

Rise in temperature is likely to increase the water retention potential of atmosphere. This may lead to intense precipitation during wet season. Therefore, up-lands of tropics is expected to remain submerged in future climate. Thus, apart from increasing atmospheric CO_2 , climate variables like temperature and moisture will vary in future climate. Considering these facts, experiments were carried out to evaluate the influence of chlorpyrifos on soil CH₄ consumption and abundance of different microbial groups under the influence of temperature and moisture. Experiments were also undertaken to study the role of biochar on the effect of chlorpyrifos on CH₄ consumption in a Vertisol.

2. Materials and methods

2.1. Study site

Study site was located at the agricultural experimental field located at the Indian institute of soil science, Bhopal, Madhya Pradesh, India (23°18'N/77°24'E, 485 m above sea level) (Mohanty et al., 2015). The experimental field was maintained under national project on organic farming since 2004. Fields were planted with soybean (Glycine max L.) and wheat (Triticum aestivum L.) during the summer and winter seasons, respectively. Wheat variety HI 8498 and soybean variety JS 335 were grown at a spacing (cm) of 22.5×5 and 45×5 , with seeding rates of 100 kg and 80 kg ha⁻¹ respectively. Soils were collected during 2015 from the soybean field that received no fertilizer inputs. Sampling was done at the vegetative growth phase of soybean (45 days after sowing). A composite sample was prepared for this experiment by mixing 4 samples from corners and 1 sample from centre of the field. Soil were collected from 5 to 15 cm depth profile. The location has a humid subtropical climate, with a hot summer and a humid monsoon season. It experiences southwestern monsoon rains between July and September. Mean annual temperature remains about 25 °C. Highest temperature reaches near 45 °C during the mid summer (May-June). During winter (December-January) the average temperature remains about 15 °C, the average yearly precipitation is 1200 mm and air humidity is 65%.

2.2. Soil physico-chemical properties

The soil is a heavy clayey Vertisol (Typic Haplustert) and the experimental site was characterized with 5.7 g organic C, 225 mg available N, 2.6 mg available P, and 230 mg available K. Organic carbon (OC) was determined by wet digestion method (Walkley and Black, 1934). Available N was determined by alkaline KMnO₄ method (Subbiah and Asija, 1956). Available phosphorus was extracted by 0.5 N NaHCO₃ solution buffer at pH 8.5 (Olsen, 1954) and phosphorus in the extract was determined by ascorbic acid method (Watanabe and Olsen, 1965). Available potassium was extracted by shaking with neutral normal ammonium acetate for 5 min (Hanway and Heidel, 1952) and then K in the extract was determined by flame photometer (Lindsay and Norvell, 1978).

The electrical conductivity (EC) was 0.43 dS m^{-1} and the pH was 7.5 (1:2.5 of soil and water in w:v) (Smith and Doran, 1996). The mean

weight diameter of soil aggregates was 0.53 mm, and total, micro and macro porosity values were 51.7%, 32.1% and 19.6%, respectively (Yoder, 1936). The water holding capacity, bulk density and saturated hydraulic conductivity of the soil were 62% (w/w), 1.45 mg m⁻³, and 7.3×10^{-6} m s⁻¹ respectively. The textural composition of soil was: sand 15.2%, silt 30.3%, clay 54.5%.

3. Biochar preparation

The biochar (BC) was produced by slow pyrolysis from the stalks of pigeon pea (Cajanus cajan) grown in the experimental farm located at Central Institute of Agricultural Engineering Institute, Bhopal, India. The sun dried of pigeon pea stalks were shredded to 5–7 cm in length. The system used for BC generation was an unconfined insulated chamber made of mild steel. The chamber had inner diameter of 360 mm, height of 500 mm and wall thickness of 2 mm. Chamber temperatures was maintained externally and the heating rate to attain the pyrolysis process temperature (450 °C) was 6 °C min⁻¹. Charring process took about 4 h. The details about the BC unit and thermal degradation behaviour of char are given elsewhere (Gangil, 2014). Characterization of biochar was estimated by standard protocols (Nelson and Sommers, 1982). The pH (1:1.25, H₂O), electrical conductivity, ash content and bulk density of the BC were 9.57, 1.95 (dS m⁻¹), 15.5% and 239 kg m⁻³ respectively. The total C, N, P and K concentrations (%) of BC were 86.4, 0.40, 0.09, and 0.74 respectively. Total Ca, Mg, and Na concentration (mg kg $^{-1}$) was 92.2, 19.5, and 395 respectively. The BC was grounded manually and passed through 2 mm sieves to achieve particle size < 2.00 mm.

4. Experimental setup and soil incubation studies

Temperature of the location goes down to an average of 15 °C during winter and peaks to 45 °C in summer. The location receives heavy rainfall during monsoon and soil remains submerged. To mimic the condition of dry and wet season, the experiment was carried out by incubating soil at 60% moisture holding capacity (MHC) and 100% MHC. Usually the 60% MHC represent the general field moisture condition and 100% MHC represents flooded condition of wet season. Chlorpyrifos is applied at different concentration depending on the crop and intensity of infestation. Biochar is applied at 1-2% as soil amendment (Asai et al., 2009). Considering these agricultural field scenarios experiment was undertaken. The experiment used a factorial design to determine the impact of different climate factors, biochar and insecticide on CH₄ consumption. The factors were temperature (15 °C, 35 °C, 45 °C), moisture (60%, 100%), biochar (0%, 1%) and chlorpyrifos (0 ppm, 10 ppm). Each factorial combination (3 temperature \times 2 soil moisture \times 2 biochar \times 2 choropyrifos) was replicated 3 times, for a total of 72 experimental units. A 1000 ppm stock solution of chlorpyrifos (Sigma Aldrich, USA) was prepared using HPLC grade acetonitrile (Sigma Aldrich, USA). The chlorpyrifos stock of 0.1 ml was added to 130 ml pre-sterilized serum vials. These vials represented the treatments of 10 ppm (w/w) chlorpyrifos. Similarly, the vials added with 0.1 ml pure acetonitrile served as treatment of 0 ppm chlorpyrifos. To nullify the effect of solvent on microbial activity vials were kept open for overnight to evaporate acetonitrile completely. To each vial 10 g portion of air dried soil was placed. Biochar was added to vials at the level of 0% or 1% (w/w). Sterile distilled water was added to maintain 60% or 100% moisture holding capacity (MHC). The contents of the vials were mixed thoroughly, capped with rubber septa and sealed using aluminium crimp seal. One ml of pure CH4 was injected into the headspace of the vials for a final concentration of 1000 ppm. Vials were incubated at 15 °C, 35 °C or 45 °C in separate biological oxygen demand (BOD) incubators (Metrex scientific instruments Pvt. ltd., N Delhi, India). Vials were shaken at 100 rpm (rotation per minute) for 8 h per day. At regular intervals (~1 day), 0.1 ml of headspace gas was analyzed for CH₄. After each sampling, the headspace was replaced with an

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