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Differential influence of legume and cereal crop residue incorporation on methane production and consumption in a tropical vertisol

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Crops and Soils Research Paper

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Abstract

Crop residue incorporation to the soil is an essential strategy to improve soil quality and crop productivity in order to attain sustainable development goals. Experiments were conducted to evaluate the differential effect of crop residues on CH_4 production and consumption in a tropical vertisol. Soils were incubated with residues of cereals (maize and wheat) and legumes (chickpea and soybean) at 1% w/w, under non-flooded and flooded conditions to estimate CH_4 consumption and CH_4 production rates, respectively. Rates of CH_4 production (ng CH_4 produced g/soil/day) varied from 0.068 to 0.107 with lowest in chickpea residue and highest in wheat straw amended soil. CH_4 consumption rates (ng CH_4 consumed g/soil/day) was highest (0.79) in wheat straw amended soil and lowest (0.53) in chickpea residue amended soil. Organic carbon (%) and available NO_3^- (mM) contents increased significantly ($P > 0.05$) in residue amended soils over control under both flooded (methanogenic) and non-flooded (methane consuming) conditions. Abundance of methanogens and methanotrophs was estimated as mcr and pmoA gene copies g^{-1} soil, indicated that both the microbial groups were stimulated significantly due to the amendment of crop residues. Linear models exhibited significant correlation among CH_4 production and consumption with organic carbon, available nitrate and microbial abundance. The study highlights that crop residues incorporation influences both CH_4 consumption and production potential of soil and this effect is more pronounced with biomass of cereals than legumes.

Introduction

Greenhouse gas (GHG) mitigation from agricultural soil is one of the key focal areas of current agricultural research. The major three GHG are – CO_2, CH_4 and N_2O depending on the role in global warming. Methane is the second most important GHG with a current ambient concentration of 1.8 ppm (Pittock, 2017). Atmospheric CH_4 concentration has increased over the years dramatically due to intensive agriculture and less measures to curb this GHG. For example, during early 2000s, atmospheric CH_4 concentration was rising from terrestrial ecosystem at 0.5 ppb (parts per billion) per year. But in the past few years, CH_4 concentration in air is rising at 9–12 ppb per year (Peng *et al.*, 2022). CH_4 affects the earth's atmospheric chemistry like ozone depletion due to its multifarious role in the earth's troposphere and stratosphere. Thus, in the current scenario mitigation of CH_4 from various sources mainly from agriculture is the most important task to curb GHG mediated global warming. Various agricultural practices influence soil biochemical properties which influence CH_4 cycling in soil ecosystem. The most commonly suggested strategy to improve soil quality is amendment of crop residues, including conservation agriculture (CA).

CA practices have been widely popularized across the globe to minimize GHG emission from agricultural soil (Lal, 2019; Pu *et al.*, 2022; Zhang *et al.*, 2022; Francaviglia *et al.*, 2023). There are several benefits of CA practices and many countries are adopting this approach in agriculture. For example, CA enhances water infiltration, reduce soil erosion, reduce compaction, increase surface soil organic matter and carbon content (Bilibio *et al.*, 2023) and improve soil aggregate formation (Nyambo *et al.*, 2022). At the global scale, CA is being practiced on 180 M ha (Francaviglia *et al.*, 2023; Reimer *et al.*, 2023). Among all countries, CA is practiced most intensively in northern USA (26.5 M ha) followed by Argentina (25.5 M ha), Canada (13.5 M ha) and Australia (17.0 M ha) (Chinseu *et al.*, 2019). CA is being practiced on more than 17.5 M ha in Asia (Kassam *et al.*, 2022) and 3.9 M ha in India (Thakur *et al.*, 2023) with a presumption of larger area in recent years. CA recommends retention of crop residues (at least 30%) in fields so that the residues get added into soil (Francaviglia *et al.*, 2023). Under such circumstances, left over crop residues undergoes

decomposition which leads to production of various organic compounds including organic acids, sugars. These organic compounds act as precursor molecule for CH_4 cycling.

CH_4 cycling is comprised of two processes, CH_4 production and CH_4 oxidation (consumption). Depending on the available C and N content, CH_4 cycling is influenced differently by the type of crop residue. In a vegetable–rice rotation, straw retention increased CH_4 emissions in the rice cultivation season (Qi *et al.*, 2023). Methane oxidation potential of paddy soil under three long-term (32 years) fertilization treatments evaluated with treatments of unfertilized control, inorganic fertilizers and wheat straw incorporation with inorganic fertilizers. The results showed that the methane oxidation potential in the straw with inorganic fertilizer treatment was significantly higher than those without residue treatments (Yang *et al.*, 2022). Incorporation of crop residue originating from both legumes and cereals can substantially enhance both CH_4 production and consumption (Zhou *et al.*, 2020). Literature on both CH_4 production and consumption in response to crop residue incorporation is limited. This information is essential to develop strategies to enhance GHG mitigation through CA. A previous study on CH_4 consumption in soybean–wheat, maize–wheat and maize–gram cropping systems under different tillage practice (Kollah *et al.*, 2020) indicated that no-tillage stimulated CH_4 consumption than conventional tillage irrespective of cropping system. CH_4 consumption potential was also highest in maize–wheat and lowest in maize gram. In order to better understand these mechanisms, the current experiment was undertaken to define how the residues of cereal (wheat, maize) are different than legumes (soybean, chickpea) in respect to influencing CH_4 cycling in soil ecosystem. We hypothesize that crop residues influence CH_4 cycling which depends on (1) C:N values of biomass, (2) mineralization and release of organic C and N compounds and (3) differential influence on methanogens and methanotrophs. Experiments were carried out with objectives to estimate CH_4 production and consumption in soil amended with residues of legumes and cereals, define the role of carbon and nitrogen content of crop residue in influencing methane cycling, and evaluate the microbial population in relation to CH_4 cycling in soil ecosystem.

Materials and methods

Experimental site

The study was carried out using soils collected from an experimental site located at the Indian Institute of Soil Science, Bhopal, India (23°18′N/77°24′E, 485 m above sea level). The location has a climate of humid sub-tropicalnature, with a hot summer and a rainy monsoon season. The location experiences south-western monsoon rain in July–September. During course of study the location had a mean annual temperature of 25.2°C with the highest of 43.9°C during mid-May and the lowest 4.4°C in January. The average precipitation was 1213.10 mm, and the humidity was 56.9%.

Experimental design, crop management and fertilizer application

The field experiment was laid in randomized block design with three replicates and four treatments. Treatments consisted of (1) un-amended control, (2) inorganic (chemical source) fertilizer, (3) organic fertilizer and (4) integrated (both inorganic and organic) fertilizer management. Inorganic source of N, P, and K were urea $(NH_2)_2CO$, single super phosphate Ca $(H_2OP_4)_2.H_2O$ and muriate of potash KCl, respectively. Organic fertilizers were amended to the fields on the basis of N equivalent, consisting of an equal amount (33.33%) of farm yard manure, vermicompost and poultry manure. The three organic sources contained nitrogen at 0.84, 0.97 and 1.97%, respectively, of dry weight biomass. Fertilizers were amended as single application at the day of sowing. Soybean (*Glycine max* L., var JS 335.) was cultivated during rainy (July – October) while wheat (*Triticum durum* L., var Sujatha C- 306) was cultivated in winter (Oct–March) season (rabi). Soybean was sown on 5 July 2019 at a spacing of 20 cm × 15 cm, while wheat was at 22 × 10 cm. Seed rates of wheat and soybean were at 100 and 80 kg/ha, respectively. Fertilizers were applied at the rate of 30:60:30 and 140:60:40 kg $N:P_2O_5:K_2O$ per ha in soybean and wheat, respectively, using urea, single super phosphate, muriate of potash as fertilizer sources. Soil samples were collected from the inorganic fields of soybean during the rainy season (kharif) 2019.

Soil sampling and processing

Soil samples were collected after 45 days of sowing (vegetative stage) from 0–10 cm depth using an auger (5 cm internal diameter). Soils cores of 0–10 cm were sampled from 4 corners and centre of fields. At each sampling point, upper soil layer (~3 cm) was removed to get rid of debris like plant biomass material (dead roots, leaves, and insects) and coarse gravels (stones and gravels). Soil cores were homogenized and mixed to form a composite soil sample. Collected soil samples were air dried under shade inside a room, then passed through 2 mm sieve and stored in plastic containers. Soils were used within 2 days of sampling to avoid issues related to change in soil properties due to storing.

Soil physico-chemical properties

The soil was characterized as a heavy clayey Vertisol (Typic Haplustert). The electrical conductivity (EC) was 0.38 dS/m and the pH was 7.78 (1:2.5 of soil and water in w:v) (Smith and Doran, 1996). The textural composition of soil was determined following standard method with values as sand 15.2%, silt 30.3%, clay 54.5%. Soil organic carbon was determined by wet digestion method (Bahadori and Tofighi, 2016). Available N was determined by standard alkaline $KMnO_4$ method (Sahrawat and Burford, 1982). Available P content of soil was determined by extracting P with 0.5 N $NaHCO_3$ buffer at pH 8.5 (Recena *et al.*, 2015) and P in the extract was determined by ascorbic acid method (Porto *et al.*, 2019). Available K content was determined by extracting soil by shaking with neutral normal ammonium acetate for 5 min and then K in the extract was determined by flame photometer (Culman *et al.*, 2019). Soil organic carbon content of soil was 0.82%, available N was 263 kg/ha, available P was 21 kg/ha and available K was 320 kg/ha.

Experimental set up

Experiment was carried out using 40 vials representing 2 CH_4 cycling (CH_4 production and CH_4 consumption) × 5 crop residues (control, maize, wheat, soybean and chickpea) × 4 replicates. Vials were laid out in completely randomized design to evaluate the differential role of crop residues on CH_4 cycling (Fig. 1).

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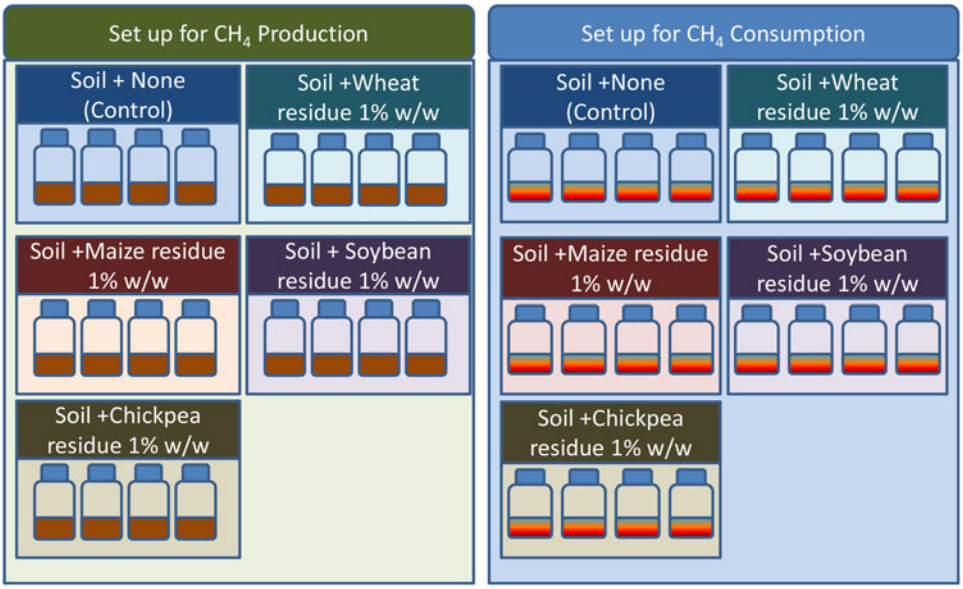

Figure 1. Experimental set up and layout for evaluating the effect of crop residues on methane production and consumption. Microcosms were prepared using serum vials containing mixture of soil and crop residues. The dried straw biomass of wheat, maize, soybean and chickpea were used in the study. For CH_4 production, the vials were flooded with sterile distilled water and closed using butyl rubber septa and sealed using aluminium crimp seal. For CH_4 consumption, soils were added with water to maintain 60% moisture holding capacity. All vials were incubated at 30 ± 2°C. Experiment was conducted in four replicates.

Crop residue preparation

The crops selected for the experiment were maize (*Zea mays L.*), wheat (*Triticum durum* L., var Sujatha C- 306), soybean (*Glycine max* L., var JS 335) and chickpea (*Cicer arietinum L*). Crop residues were collected from farm after cultivation of crops. Residues were sun dried and chopped to 5–8 cm size manually and milled in a cutting mill (SM100, Retsch, Germany). The miller was fitted with a 6-disc stainless rotor and bottom sieves with trapezoid holes to get final plant material of 1 mm size.

CH_4 production

To evaluate CH_4 production, soil samples were incubated under flooded condition as described elsewhere (Luo *et al.*, 2022). Briefly, portions of air dried 20 g of soil samples were weighed into 130 ml serum vials (Fig. 1). Crop residues were added at 1% (w/w) an equivalent of 20 t/ha level to soil to examine the influence of crop biomass incorporation on CH_4 production potential. The dose was selected considering the CA practice where significant amount of crop biomass is incorporated into soil. The soil in vials was flooded with sterile distilled water of 50 ml. After flooding, the vials were closed with butyl rubber septa and sealed using aluminium crimp seal. Vials were kept in a biological oxygen demand (BOD) incubator (30 ± 2°C) in the dark condition. To estimate CH_4 production in the soils, vials were shaken for 10 min on a horizontal shaker at 150 rpm for 30 min to release soil-trapped CH_4, if any and 0.1 ml of the headspace gas was analysed for CH_4. CH_4 concentration was analysed using a gas chromatograph (CIC, India) equipped with an flame ionization detector (FID) and a Porapak Q packed column (2-m length, diameter 2/8″, 80/100 mesh, stainless steel column) as described elsewhere (Mohanty *et al.*, 2017).

CH_4 consumption potential

Incubation experiment was carried out following methods as described elsewhere (Mohanty *et al.*, 2015; Kollah *et al.*, 2020). Briefly, a portion of 20 g soil placed into 130 ml sterilized serum vials (Fig. 1). Crop residues were added at 1% (w/w) level to soil. Soils were moistened with 5 ml sterile distilled water to attain about 40% moisture holding capacity. The contents of the vials were mixed thoroughly, capped with rubber septa and sealed using aluminium crimp seal. Pure CH_4 was injected into the headspace of the vials for a final concentration of 1000 ppm. Vials were incubated at 28 ± 2°C in a biological oxygen demand (BOD) incubator (Metrex scientific instruments pvt ltd, N Delhi, India). At regular intervals (~1 day), 0.1 ml of headspace gas was analysed for CH_4. After each sampling, the headspace was replaced with an equivalent amount of high purity helium (He) to maintain atmospheric pressure. The gas He was used because of its inert chemical nature. Vials were incubated till headspace CH_4 was completely consumed. The rate constant of CH_4 consumption (k) was determined from the slope of log-transformed values of CH_4 *v.* time during the rapid decline phase.

CH_4 estimation

The injector, column and detector were maintained at 120, 60 and 300°C, respectively. Under these parameters of GC, the retention time of CH_4 was 1.3 min. The GC was calibrated for accurate measurement, before and after each set of measurements using different mixtures of CH_4 in N_2 (Sigma Gases, New Delhi, India) as primary standards (CH_4 100 ppm).

Organic carbon and available NO_3 estimation

To estimate NO_3^{1-} content, samples were extracted with $CaSO_4$ (0.1 M) and then by reacting with phenol disulphonic acid following standard method (Sahrawat and Burford, 1982). Organic carbon was estimated by digesting soil with potassium dichromate ($K_2Cr_2O_7$) and 20 ml of concentrated sulphuric acid (H_2SO_4). The excess dichromate that was not reduced in the reaction was determined by volumetric titration using ferrous ammonium

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sulphate [$Fe(NH_4)_2(SO_4)_26H_2O$)] as described elsewhere (Bahadori and Tofighi, 2016).

DNA extraction

After experiment, about 0.5 g soil samples were taken out from vials to extract DNA using the ultraclean DNA extraction kit (MoBio, USA) following the manufacturer's instructions. The DNA concentrations were determined in a biophotometer (Eppendorf, Germany) by measuring absorbance at 260 nm (A260), assuming that 1 A260 unit represents 50 ng of DNA per µl. DNA extraction was further confirmed by electrophoresis on a 1% agarose gel. The extracted DNA was dissolved in 50 µl TE buffer and stored at −20°C until further analysis.

Real time PCR quantification of methanogens mcr and methanotrophs pmoA genes

Real time PCR was performed on a Step one plus real time PCR (ABI, USA) to quantify the genes of representative microbial species. Reaction mixture prepared by adding 2 µl of DNA template, 10 µl of 2X SYBR green master mix (Affymetrix, USA), 200 nM of primer (GCC Biotech, N Delhi). Final volume of PCR reaction mixture was adjusted to 20 µl with PCR grade water (MP Bio, USA). Primers targeting *pmoA* gene (particulate methane monooxygenase) of methanotrophs were used to quantify abundance of methane oxidizing bacteria. The primers for *pmoA* were A189F (5- GGN GAC TGG GAC TTCT GG-3) and mb661R (5- CCG GMG CAA CGT CYT TAC C-3) (Mohanty *et al.*, 2017). This primer set targeted methanotrophs belonging to both type I and II groups including *Methylobacter or Methylosarcina, Methylococcus, Methylosinus* group, *Methylocapsa, Nitrosococcus* (Kolb, 2009). Primer set used for quantifying methanogens were mcr1f (5-AAA GAC GCG GTA CAA GCA AC-3) and mcr1r (5-GCT GAA CAT ACA CGG CAC AG-3) (Li *et al.*, 2017). The amplicon length was about 213 base pairs. Thermal cycling was carried out by an initial denaturizing step at 94°C for 4 min, 40 cycles of 94° C for 1 min, 52°C (pmoA) or 60°C (mcr) for 30 s, 72°C for 45 s; final extension carried out at 72°C for 5 min. Fluorescence was measured during elongation step. Data analysis was carried out with Step one plus software (ABI, USA) as described in user's manual. The cycle at which the fluorescence of target molecule number exceeded the background fluorescence (threshold cycle [C_T]) was determined from dilution series of target DNA with defined target molecule amounts. C_T was proportional to the logarithm of the target molecule number. The quality of PCR amplification products were determined by melting curve analysis with temperature increase of 0.3°C per cycle. Standard for the genes was made from series of 10 fold dilutions of purified amplified products and data presented as number of cells/g of soil.

Statistical analyses

All statistical analyses were carried out using the Microsoft excel and 'agricolae' package of the statistical software R (2.15.1) (Ihaka and Gentleman, 1996). Results for the experiments were presented as arithmetic means and standard deviation of replicated observations. Arithmetic mean and standard deviation were calculated by Microsoft Excel. Tukeys honestly significant difference (HSD) test was performed to define the significant difference among treatments at $\alpha = 0.05$. Linear correlation models among factors were evaluated by Excel.

Results

Ch_4 production from soil under the influence of crop residue

Production of CH_4through methanogenesis varied temporally with amendment of crop residues. Methanogenesis occurred after 5 days of incubation and headspace CH_4 concentration increased steadily over the incubation period of 35 days (Fig. 2). In the control treatment, methanogenesis was very low compared to other treatments. Methane production rate varied with the residue of crops and it followed with a trend of wheat > maize > soybean > chickpea > none (no amendments). CH_4 production rate (ng CH_4 produced g/soil/day) was highest 0.107 in wheat followed by 0.092 in maize, 0.085 in soybean and lowest of 0.068 in chickpea.

CH_4 consumption in soil under the influence of crop residue

CH_4 consumption was estimated as the decline in the headspace CH_4 concentration over incubation period. Variation in CH_4 consumption was due to different crop residue is shown in Fig. 3. CH_4 consumption initiated mostly after 2 days of incubation and continued over the incubation period of 13 days. Headspace CH_4 declined to ambient air values in soil amended with crop residue. CH_4 consumption rate estimated as ng of CH_4 consumed g/soil/day. The trend of CH_4 consumption followed as wheat > maize > soybean > chickpea > control. CH_4 consumption rates were as follows: 0.79 ng CH_4 consumed g/soil/day in wheat, 0.77 ng CH_4 consumed g/soil/day in maize, 0.60 ng CH_4 consumed g/soil/day in soybean, 0.53 ng CH_4 consumed g/soil/day in chickpea and 0.31 ng CH_4 consumed g/soil/day in control.

Organic carbon and available nitrate

Organic carbon and available nitrate in soil samples were estimated after the end of incubation for CH_4 production and CH_4 consumption (Fig. 4). Organic carbon increased after incubation for the CH_4 cycling processes. After CH_4 production, organic C content was 1.43% in wheat, 1.02% in maize, 0.84% in soybean and 0.73% in chickpea. Similarly, after CH_4 consumption, organic carbon content was as follows: 0.93% in wheat, 0.84% in maize, 0.76% in soybean and 0.70% in chickpea. Organic carbon in control soil varied from 0.54–0.58%. Available nitrate content varied with crop residue and increased after incubation. The trend of available nitrate was opposite of organic carbon. Nitrate content of soil was higher during CH_4 consumption than CH_4 production. Nitrate content varied from 0.65 mM in chickpea to 0.39 mM in wheat after CH_4 production. Available nitrate content varied from 1.45 mM in chickpea to 0.85 mM in wheat. In control soils available NO_3 was 0.32 mM after incubation for CH_4 production and 0.68 mM after incubation for CH_4 consumption.

Microbial abundance

Abundances of different microbial groups comprising methanogens and methanotrophs increased with crop residue amendment (Table 1). Abundance of methanogens was in the range of 84×10^3 *mcr* gene copies /g soil to 11×10^3 *mcr* gene copies/g soil. Lowest was in un-amended control and highest in the soil amended with wheat residue. Methanogens abundance increased 7.6 fold in wheat and 2.45 fold in chickpea than control. Methanotrophs abundance was also stimulated in soil due to crop residue.

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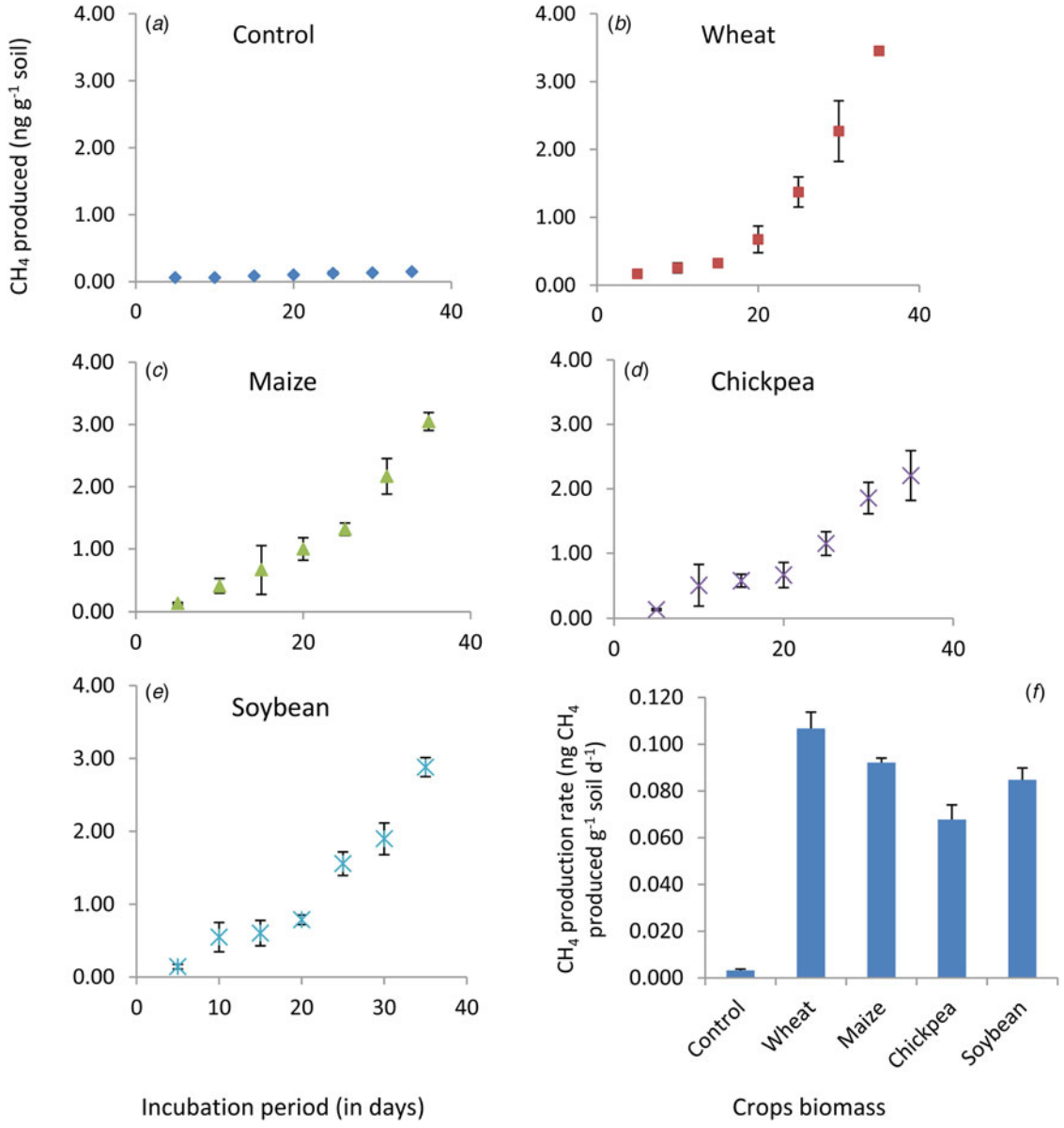

Figure 2. CH_4 production from soil under the influence of biomass of different crops. The crops were wheat, maize, chickpea and soybean. Water of 50 ml was added to soil and incubated as mentioned in the text. Headspace CH_4 was measured at regular intervals. Panel a–e : *Y* axis represents CH_4 production from soil where *Y* axis represents ngCH_4 produced g^{-1} soil and X axis represents incubation period in days. Panel f :CH_4 production rate of soils under the influence of different crop biomass, where, *Y* axis represents ngCH_4 produced g/soil/day and *X* axis represents biomass of crops. Each data point is arithmetic mean ± standard deviation of four replicated observations.

Methanotrophs were stimulated highest in wheat and lowest in chickpea. Methanotrophs were 15×10^4 *pmoA* gene copies /g soil in un-amended control. Their abundance increased 3.26 times by wheat and lowest 1.2 times by chickpea residue amendment.

Linear regression analysis

Linear regression models of CH_4 production and CH_4 consumption in respect to soil parameters indicated that change in soil parameters were in accordance to CH_4 cycling processes (Fig. 5). CH_4 production was linearly modelled as follows:

$$\text{organic carbon} = 6.414 \times CH_4 \text{ production rate} + 0.4628, \quad r^2 = 0.562) \tag{1}$$

$$\text{available nitrate} = -6.409 \times CH_4 \text{ production rate} + 1.0881, \quad r^2 = 0.836) \tag{2}$$

$$\text{methanogens } mcr \text{ gene abundance} = 3.1315 \times CH_4 \text{ production rate} + 0.2744, r^2 = 0.766) \tag{3}$$

Methanotrophs gene abundance was linearly modelled as follows:

$$\text{organic carbon} = 0.711 \times CH_4 \text{ consumption rate} + 0.3277, \quad r^2 = 0.7974) \tag{4}$$

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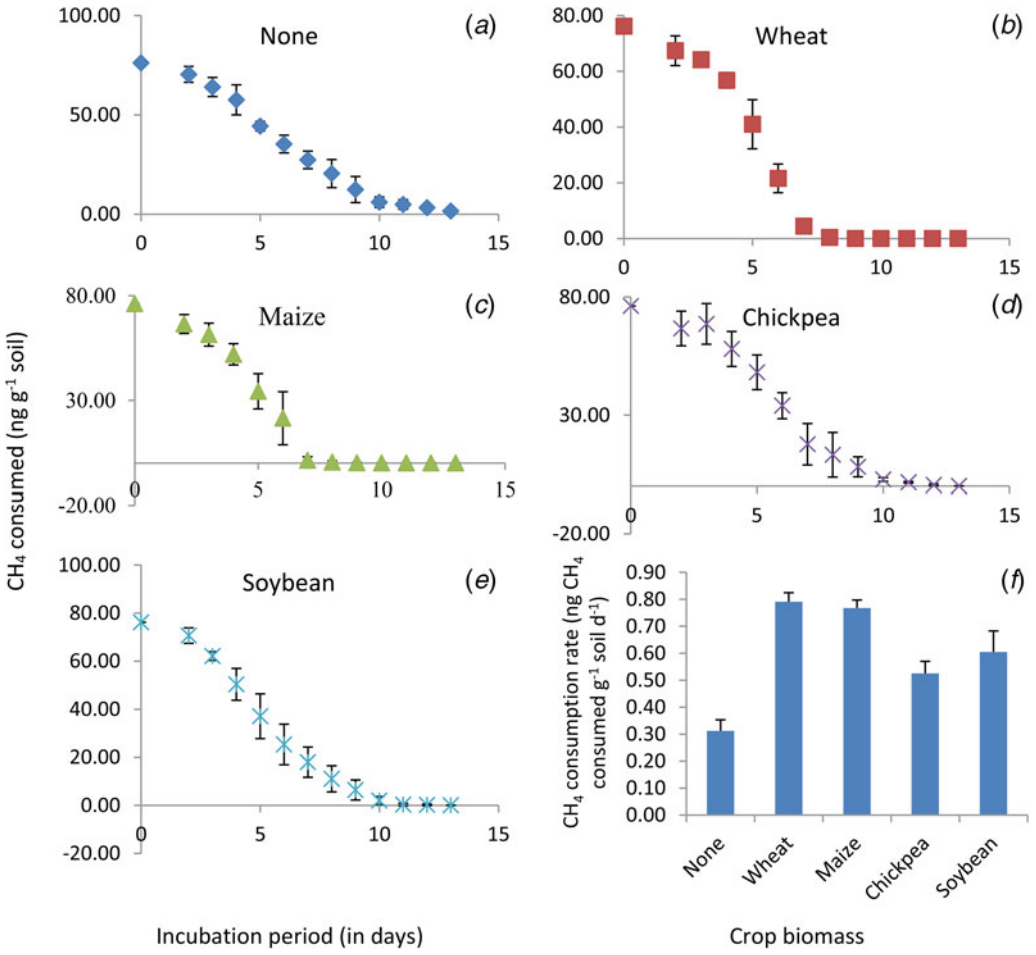

Figure 3. CH_4 consumption in soil under the influence of biomass of different crops. The crops were wheat, maize, gram and soybean. Headspace CH_4concentration was measured at regular intervals. Panel a–e : *Y* axis represents change in CH_4concentration in the headspace of vials and *X* axis represents incubation period in days. Panel f : CH_4 consumption rate where, *Y* axis represents ngCH_4 consumed g/soil/day and *X* axis represents biomass of crops. Each data point is arithmetic mean ± standard deviation of four replicated observations.

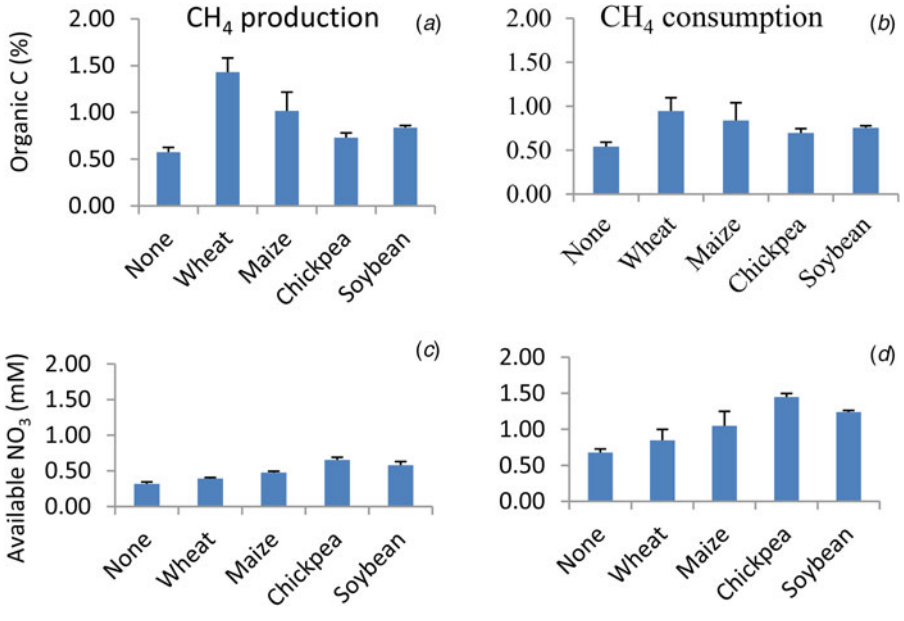

Figure 4. Organic carbon content (%) and available NO_3 concentration (mM) in soil after CH_4 production and CH_4 consumption under the influence of amendment of different crop biomass. Panel a and b represents organic carbon content in soil. Panel c and d represents available nitrate content. Soils after incubation were used for analysis. Each data point is arithmetic mean with standard deviation (error bar) of four replicated observations. *Y* axis represents organic carbon (%) or available NO_3 (mM). *X* axis represents biomass of crops.

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Table 1. Abundances of methanogens and methanotrophs in soil amended with different crop biomass after methanogenic and methanotrophic metabolism

Crop biomass	Abundance of methanogenic (*mcr* gene copies 10^3/g soil)	Abundances of methanotrophs (*pmoA* gene copies × 10^4/g soil)
None	11 ± 2.45^e	15 ± 2.87^d
Wheat	84 ± 3.70^a	49 ± 5.62^a
Maize	68 ± 4.55^b	36 ± 4.27^b
Chickpea	27 ± 4.65^d	18 ± 3.10^d
Soybean	40 ± 4.27^c	26 ± 4.27^c
Tukeys HSD (*P* 0.05, df error 12)	2.53	3.11

Each data is arithmetic mean ± standard deviation of four replicated observation. Values followed by same letter were not significantly different (*P* 0.05)

$$\text{available nitrate} = -1.7768 \times \text{CH}_4 \text{ consumption rate} + 2.3402, \quad r^2 = 0.777) \tag{5}$$

$$\text{methanorophs } \textit{pmoA} \text{ gene abundance} = 62.609 \times \text{CH}_4 \text{ consumption rate} - 8.7314, r^2 = 0.733) \tag{6}$$

Discussion

To define the role of different crop residues on the CH_4 cycling processes, soils were amended with crop residue at 1% w/w, equivalent to level of crop residue amendment practiced under agricultural practice (Diacono and Montemurro, 2015). Moreover, in CA 30% of crop residue is left in the field to improve soil carbon. Total carbon and nitrogen content of the crop residue was as follows: maize 39% C and 0.5% N (Xu *et al.*, 2013), wheat 44.8% C and 0.57% N, chickpea 37.1% C and 1.20% N and soybean 36.6% C and 1.09% N (Reddy *et al.*, 2008). To evaluate CH_4 production, soils were maintained under flooded conditions as saturated moisture facilitates anaerobiosis. There was no CH_4 production in control soil, but addition of crop residue stimulated CH_4 production. Headspace CH_4 concentration constantly increased due to accumulation of CH_4 produced during methanogenesis. Variation in CH_4 production was due to crop type. Wheat stimulated at highest level followed by maize, soybean and least by chickpea. This was due to the C: N content of crop residue. C:N of maize 66.3 (Feng *et al.*, 2012), wheat is about 78.6, soybean 33.6 and chickpea 31 (Reddy *et al.*, 2008). Crop residue driven CH_4 cycling was outlined to define different mechanisms (Fig. 6). A high carbon content of wheat stimulated CH_4 production at highest level, while low C:N in chickpea resulted lowest CH_4 production. Organic carbon was measured after the end of incubation to evaluate the mineralization of residue. Crop residue enhanced

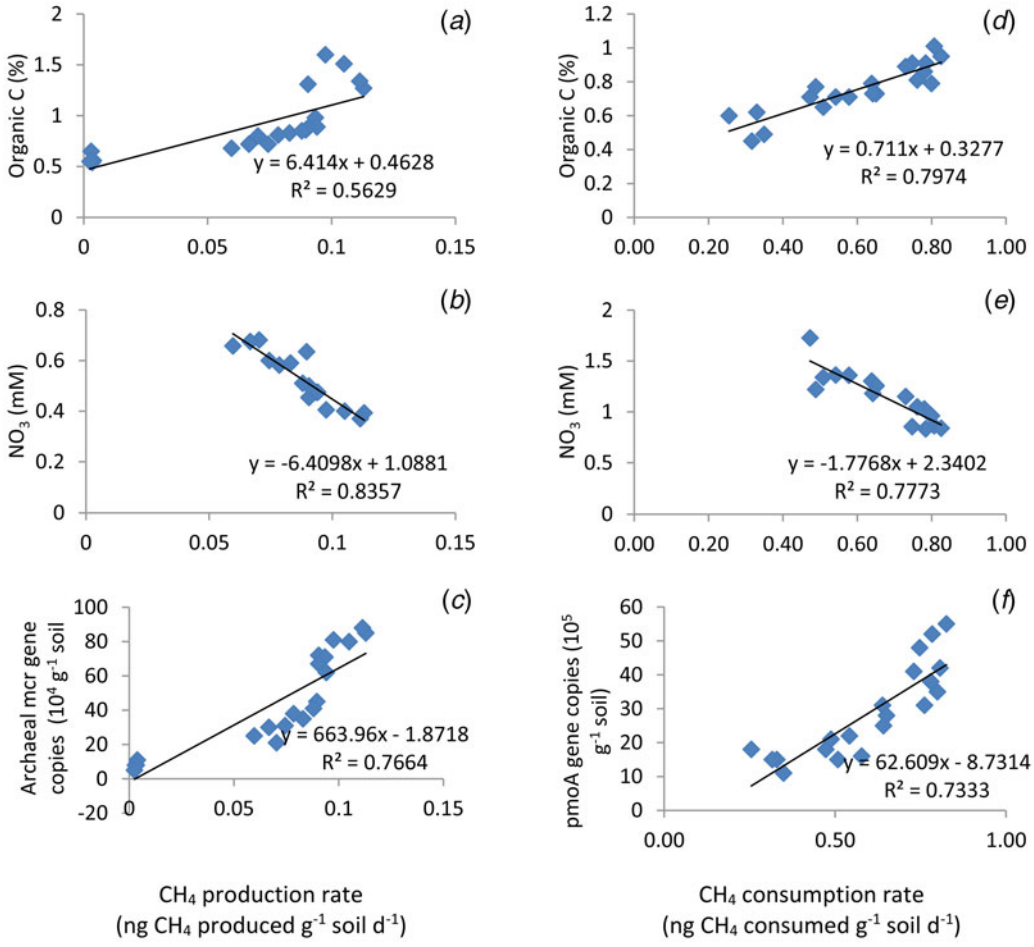

Figure 5. Linear regression models predicting CH_4 production and CH_4 consumption rates from soil parameters. Left panels (a, b and c) represents linear regression models for CH_4 production and right panels (d, e and f) represents regression models for CH_4 consumption rates. The parameters were organic C, available NO_3, and abundance of methanogenic archaeal *mcr* gene copies and methane oxidizing methanotrophs *pmoA* gene copies.

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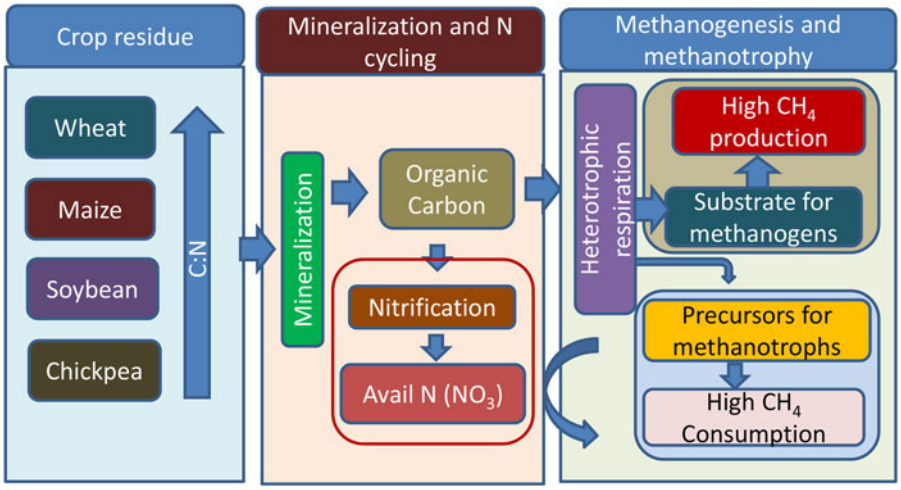

Figure 6. Hypothetical illustration of the mechanism of crop residues driven methanogenesis and methanotrophy. The crop residues were wheat, maize, soybean and chickpea. Residues of cereals (wheat, maize) had higher C:N values than legumes (soybean, chickpea). Residues of cereals stimulated both methanogenesis (CH_4 production) and methanotrophy (CH_4 consumption or oxidation) than the legumes. Crop residues undergo mineralization leading to the production of readily available carbon like organic carbon and available nitrogen (NO_3). Mineralization leads to the production of organic carbon and metabolism of heterotrophs produces CO_2 which may act as substrates for methanogenesis. Products of mineralization and CO_2 stimulated methanotrophy (CH_4 consumption). Probably metabolites of heterotrophs and CO_2 stimulated CH_4 consumption. Methanotrophs also carry out nitrification due to which NO_3 production was correlated with CH_4 consumption.

organic carbon content of soil and it followed the trend of wheat > maize > soybean > chickpea. Organic carbon acts as substrates for methanogens. Crop residues having higher carbon content stimulated methanogenesis than the lower carbon containing crop residues. Rate of CH_4 production significantly correlated with organic carbon content of soil ($P = 0.05$, $r^2 = 0.562$). Available nitrate content of soil was increased by amendment of crop residues. However, the nitrate content (mM) was highest in chickpea and least in wheat. CH_4 production correlated significantly with methanogens *mcr* gene copy numbers ($r^2 = 0.766$). However, the relation between CH_4 production and available NO_3^- was negative. Probably, CH_4 production was a way to lower the C:N value for stabilization under anaerobiosis (Arianti *et al.*, 2022). In a study on stimulating higher methanogenesis, substrates like straw of rice or wheat with higher C:N were bio-augmented (Luo *et al.*, 2023). Methanogens were higher in soil amended with wheat residue and lowest in chickpea residue amended soil. Higher abundance of methanogens and CH_4 production was due to higher available carbon content of crop residue.

CH_4 consumption rate was highest in soil amended with wheat and lowest with chickpea. High C:N of crop residue stimulated most to CH_4 consumption. CH_4 consumption rate correlated positively and significantly with soil organic carbon. In a study on global meta-analysis, and process-based modelling, it was observed that soil organic carbon constituted an important variable that governed the CH_4 uptake potential of soil (Lee *et al.*, 2023). CH_4 consuming microbial groups including methanotrophs use CH_4 as carbon source. However, this study indicated that soil organic carbon stimulated CH_4 consumption. Based on this finding it was proposed that CH_4 consumption potential of soil depends on the heterotrophic metabolism of other soil microbial groups. In a study on linkage between soil erosion and CH_4 consumption rates, it was observed that loss of heterotrophic microbial diversity affected CH_4 uptake potential (Schnyder *et al.*, 2023). However, it is unclear from the present study that how heterotrophs regulate CH_4 consumption activity in soil. Probably, higher heterotrophic activities released CO_2 which in turn stimulated CH_4 consumption. In a recent study, it was highlighted that CO_2 favoured CH_4 consumption (Noyce *et al.*, 2023). Available nitrate content measured after CH_4 consumption was at a higher level than CH_4 production. Most methanotrophs exhibit nitrification activities (Kollah *et al.*, 2023). Probably, this property of methanotrophs stimulated nitrification and enhanced available nitrate content in soil. Available nitrate content correlated significantly with CH_4 consumption. Methanotrophs abundance positively and significantly correlated with CH_4 consumption potential, as these groups of organisms consume CH_4. Study highlighted that crop residue having higher C:N stimulated CH_4 cycling processes including CH_4 production and CH_4 consumption. Carbon content was the most important property than nitrogen content to regulate CH_4 cycling.

Conclusions

The current study evaluated CH_4 cycling (both production and consumption of CH_4) in a tropical vertisol amended with residues of legumes (soybean, chickpea) and cereals (wheat, maize). Carbon content of crop residue was the most important factors to shape both CH_4 production and CH_4 consumption. Organic carbon acts as substrate for CH_4 production, but how organic carbon stimulated CH_4 consumption, seeks further research to elucidate the mechanism.

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Authors' contributions. MS carried out chemical analysis. BK contributed in statistical analysis and drafted manuscript. RP and MHD collected soil samples from experimental fields and processed for incubation studies. AB, NA and AS performed soil and microbial analysis. GD carried out molecular analysis. SRM conceptualized the experiment and prepared final draft.

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Ethical standards. Not applicable.

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