3.1. Production of biochar

Tea pruning litter and mixed wood chips were used as feedstocks for biochar production. Three different techniques: pyrolysis, gasification and conventional method (using kiln) were used for production of the biochars. Tea pruning litter were collected from Dhekiajuli tea estate of Sonitpur district of Assam, India (26.7027°N, 92.4651°E). Whereas the mixed wood chips were collected from Napaam sawmill of Sonitpur district, Assam, India (26.7003°N, 92.8308°E). We are using the following terminology for the produced biochars:

1. TLC (tea pruning litter conventional)
2. TLG (tea pruning litter gasified)
3. TLP (tea pruning litter pyrolyzed)
4. WCC (mixed wood chips conventional)
5. WCG (mixed wood chips gasified)
6. WCP (mixed wood chips pyrolyzed)

To obtain TLP (tea pruning litter pyrolyzed) and WCP (mixed wood chips pyrolyzed) biochars, air-dried ground feed stocks (15 g each) were placed into a pyrolysis unit with a fixed-bed tubular reactor (height = 30 cm, internal diameter = 2.47 cm) made up of quartz glass in which the temperature was controlled by Ni-Cr thermocouple placed in the centre of electrical furnace (Image 3.1 (a)). Feedstocks were then heated with a temperature rise of 40°C per minute until it touched 650°C under constant nitrogen flow rate of 100 ml per minute. A condenser was attached at the outlet of the reactor to condense the vapours coming out of it where water was used as cooling medium. The yields of biochar and other liquid products were determined by weighing. Time required to produce one batch of biochar in the aforementioned pyrolysis unit was approximately 40 minutes.

Chopped feedstocks of about 2-3 inch were put into a gasification unit of reactor diameter 130 mm and nozzle height 650 mm (Image 3.1 (b)) to produce TLG (tea pruning litter gasified) biochar. Constant temperature of 650°C was uphold throughout the production duration of 4 hours. During the process, air was used as gasification agent and condensate are collected with the help of fabric filters. Whereas, WCG (mixed wood chips gasified) biochar was obtained from a biomass

gasification company, Infinite Energy Pvt. Ltd., Faridabad, India. Its production temperature and burning time were similar to that of TPG biochar (650°C, 4 hour).

TLC (tea pruning litter conventional) and WCC (mixed wood chips conventional) biochars were produced using a tin kiln (drum) of size 58 cm in diameter and 87 cm height (Image 3.1 (c)). Dried feedstocks were put into the kiln and ignited. After ignition, the kiln was closed and sealed with clay to prohibit oxygen passage inside it. Then the feed stocks were allowed to burn for 3 hours. Documented maximum production temperature for TLC and WCC was 350°C.

Produced biochars were stored securely in air sealed containers in the laboratory. Laboratory analysis pertaining to characterization of biochars were accomplished in the department of Environmental science and sophisticated analytical instrumentation centre (SAIC) of Tezpur university, Assam.

3.2. Characterization of biochars

3.2.1. Biochar yield

Total biochar yield was calculated using the equation shown below:

$$\text{Biochar yield} = \frac{M_b}{M_f} \times 100$$

where,

M_b = mass of biochar produced (kg)

M_f = mass of feedstock used (kg)

3.2.2. Proximate analysis

Proximate analysis was performed following ASTM D1762-84 method [1] use for the analysis of wood charcoal.

Procedure:

Crucibles and lids were heated to 750°C, cooled, and weighed prior to their use for proximate analysis. Moisture content (MC) of the sieved samples were determined as the percent mass loss on heating the samples at 105°C using a hot air oven for two

hours. Volatile matter (VM) content was measured as percent mass loss between 105°C and 950°C in a furnace. The percent ash content was calculated from the remaining mass after combustion of the samples at 750°C for 6 hours.

Moisture, volatile matter, ash and fixed carbon content were calculated using the following formulas:

(i) $Moisture$ (%) = $(A - B)/ A \times 100$

(ii) $Volatile\ matter$ (%) = $(B - C)/B \times 100$

(iii) $Ash\ Content$ (%) = $(C - D)/ C \times 100$

(iv) *Fixed Carbon (%) = 100 - (Moisture + Volatile matter + Ash)*

where,

A: Air-dried sample (gm)

B: Sample after drying at 105°C for 2 hours (gm)

C: Sample after drying at 950°C for 6 minutes (gm)

D: Residue after drying at 750°C for 6 hours (gm)

3.2.3. Ultimate analyses

Ultimate analyses were accomplished in a CHNS-O analyser (Perkin Elmer, USA model: 2400 series 2). The ultimate analyses involved the measurement of the weight percentage of carbon, nitrogen, hydrogen, oxygen and sulphur in the samples.

C/N, H/C, O/C ratios were calculated by dividing the results with the atomic mass of the specific elements. Labile carbon (LC) was obtained by subtracting fixed carbon (FC) from total carbon (C).

3.2.4. Calorific value (CV)

The calorific value (CV) of the biochars were determined in a bomb calorimeter (auto bomb calorimeter, SE-1AC/ML). 0.5 g of sample was dried in an oven at 105°C for 24 hours. Samples were then sieved through 2 mm sieve before using in bomb

calorimeter. The following equation was used to obtain the calorific values of the biochar samples:

$$Q = \frac{E(t - t_0)}{G}$$

where,

Q = calorific values of samples ($J\ g^{-1}$)

E = energy released during combustion (J)

t = endpoint temperature (°C)

t_0 = initial temperature (20°C)

G = mass of samples (g)

3.2.5. pH and electrical conductivity (EC)

Procedure:

Air dried sample (5.0 g) was taken in a conical flask and after adding distilled water (50 ml in biochar (1:10 ratio); and 25 ml in soil (1:5 ratio)) it was shaken for 30 minutes in a rotary shaker. The pH and EC were then measured by placing the electrode of the pH and conductivity meter (Model: HI98130, Hanna Instruments) in sample suspension [2].

3.2.6. Surface acidity and alkalinity

Procedure:

Boehm titration was carried out to determine the surface acidity and alkalinity using the method given by Cheng and Lehmann [3].

Biochar (0.15 g) was added to 15 ml of either 0.1 N NaOH or 0.1 N HCl solution and shaken thoroughly with an end-over-end shaker for 30 hours then it was filtered through filter paper of Whatman no. 40. An aliquot of 5 ml of NaOH filtrate was transferred to 10 ml 0.1 N HCl solution followed by back titration with 0.1 N NaOH using phenolphthalein as an indicator. Similarly, surface alkalinity was estimated by

directly titrating an aliquot of 5 ml of the HCl with 0.1 N NaOH. The base or acid uptake of biochar was considered as surface acidity or basicity of the biochars and expressed as mmol g^{-1}, respectively.

3.2.7. Cation exchange capacity (CEC)

CEC of the biochar samples were determined following the method of Yu al el., 2014 [4] with slide modification.

Procedure:

Biochar weighing 5g was taken in 250 ml conical flask where 100 ml of 1 N sodium acetate solution was added and shaken for 30 minutes. After that, the solutions were centrifuged at 2400 rpm and decanted the supernatants. The solution was shaken again after adding 35 ml of ethyl alcohol and the supernatants were decanted. After repeating the process for 3 times the samples were filtered. Now, the filter paper was placed inside the bottle and added 100 ml of 1N ammonium acetate and shaken for 30 minutes. Finally, the filtrates were transferred to a volumetric flask and the volume was made up to 1 L. The filtrates were then analysed in flame photometer.

3.2.8. Determination of functional group

Fourier transform infrared spectrometer (FTIR) (IMPACT 410, OMNIC E.S.P.5.0, Nicolet, USA) was used to determine the existence of functional groups in the air-dried amendments.

3.2.9. Determination of Poly Aromatic Hydrocarbons (PAHs)

PAHs in biochar was determined by the procedure given by Fabbri et al. [5] with little modifications.

Sample extraction:

For extraction of PAHs, the soxhlet extraction apparatus was used. Biochar sample (5g) was placed into the soxhlet extractor. Extraction was carried out with 160 ml of extraction solvents (tolune/cyclohexane (1:1, v/v)) for 5 cycles. During the extraction process, the soxhlet apparatus was covered with aluminium foil to avoid the photo degradation of PAH. The extract was then stored in amber colour bottle for GCMS analysis.

GCMS analyses:

Extracted solution was analysed in a GCMS (Agilent 8890, MS library: apci_msms_nist) to determine the presence of PAHs in the biochar samples. Extracted sample of 1 µl was injected under split less conditions (1 min, split ratio 1:50 to the end of analysis) keeping the injector temperature at 280°C. The thermal program of the used capillary column was: 50 to 100°C at 20°C min^{-1}, then from 100°C to 300°C at 5°C min^{-1}, then a hold for 2.5 min at 300°C. Results obtained from GCMS library was taken into consideration for determination of PAHs in the biochar samples.

3.2.10. Surface area and image of biochar surfaces

Surface area (SA) of the biochars were determined by BET method of isotherm analysis (Model: Quantachrome, Nova 1000E) and the images of the surface, and pore structures of the biochars were taken in a scanning electron microscope (JSM 6390LV). Air dried, sieved (2 mm) biochar was used to analyse this parameter.

3.2.11. Water holding capacity (WHC)

WHC of the amendments and basic experimental soil was estimated following the method of Tripathi [6].

Procedure:

The weight (W1) of empty kin box with a filter paper (Whatmann no. 42) at the bottom of it was taken. Half of the box was filled with sieved air-dried amendments or experimental soil and recorded the weight (W2). Box was then kept on a soaking dish and water was poured on the dish until the water level came to 1 cm above the base of the box. Dish was then protected to avoid evaporation and kept undisturbed for 12 hours. An empty box without any sample was also kept and its weight was taken (W3). After 12 hours, the box was carefully removed from the dish and water on the outer surface was wiped and the weight of the box (W4) was measured. Finally, the box was oven dried at 110°C for 24 hours and its weight was recorded (W5).

Calculation:

For calculation of WHC the following calculations were made:

Total water in wet soil (S1): W4-W1-W3

Weight of oven dried soil (S2): W5-W1

$$Water\ holding\ capacity\ (\%) = \frac{S1}{S2} \times 100$$

3.2.12. Adsorption potential of the biochars

Adsorption potential of the biochars was estimated by calculating the methylene blue (MB) uptake following the method developed by Yadav et al. [7].

Biochar (0.2 g) was added in 50 ml of 20 mg l^{-1} MB solution and shaken in a magnetic stirrer (JSGW, 14412) for 24 hours at room temperature. The absorbance of the suspensions was noted in every 15 minutes at 630 nm in a UV visible spectrophotometer (model-Eppendorf Kinetic Bio Spectrophotometer). The equilibrium adsorption of MB by the biochars were calculated using following equation:

$$A_b = \frac{(C_0 - C_t) \times V}{W}$$

where,

A_b = adsorption of biochar (mg g^{-1})

C_0 = initial adsorbate concentration (mg/dm^3)

C_t = final adsorbate concentration (mg/dm^3)

V = volume of MB (dm^3)

W = mass of the biochar (g)

3.2.13. Recalcitrance potential (R50 value) and carbon sequestration potential (CSP)

The R50 values of the biochars were obtained from the TGA data following the method of Harvey et al. [8] and the CSP was determined using the calculation developed by Zhao et al. [9].

Recalcitrance potential and thermal degradation of the biochars were determined in a thermo-gravimetric analyser (DSC-TGA, Netzsch, Germany). An aluminium crucible was used during the analysis. Air dried biochars were grounded and sieved before analysis and particle size were made less than 250 μm in diameter. Biochar sample (5 ug) was heated in air from 35°C until it reached the maximum temperature treatment of 1000°C with heating rate of 10°C per minute.

R50 values of the biochars were then calculated following the equation shown below:

$$R50_x = \frac{T50_x}{T50_{graphite}}$$

where,

$T50_x$ = temperature at which 50% of biochar was degraded (oxidised)

$T50_{graphite}$ = temperature at which 50% of graphite was degraded (oxidised). The data for oxidation of $T50_{graphite}$ have been derived from Harvey et al. [8].

Carbon sequestration potential (CSP) of the biochars were determined by the following equation:

$$CSP = \frac{M \times B_y \times C_b \times R50}{M \times C_f}$$

where,

M = mass of feedstock (g)

B_y = biochar yield (%)

C_b = C content of biochar (%)

R50 = recalcitrance potential of the biochar

C_f = C content of the feedstock (%)

3.2.14. Elemental (macro, micro and heavy metal) concentration

Total elemental concentration in biochars were estimated using atomic absorption spectroscopy following Tripathi [6]

Procedure:

Sample (1 g) was taken in a 100 ml volumetric flask and 10 ml of di-acid mixture (9:4; HNO_3:$HClO_4$) was added to it. The flask was placed on hot plate at low heat (40-50ºC) in a digestion chamber for 30 minutes. The flask was then heated at high temperature (100ºC) until the production of red fumes ceased. The content was evaporated till the volume reduced to 2-3 ml and the liquid became colourless. After cooling, 30 ml of double distilled water was added and filtered using Whatman no. 42 filter paper and the volume was made up to 100 ml. The elemental content in the filtrate were quantified using Inductively Coupled Plasma-Optical Emission Spectroscopy (Optima 2100 DV, Perkin Elmer, USA).

3.3. Experiment on seed germination performance

To study the impact of produced biochars on seed germination performance and seedling growth a study was carried out during the month of November, 2020 at Department of Environmental science, Tezpur University.

3.3.1. Collection and preparation of seed and soil

Seeds of french bean (*Phaseolus vulgaris* L. variety Arka Anoop) and Indian mustard (*Brassica juncea* L. variety TS 38) were collected from the Krishi Vigyan Kendra (KVK), Sonitpur, Assam, India.

Germination test was performed in 70% alcohol sterilized disposable dishes. Dishes were made ready for the treatments with the treatment tags. Seeds were washed with distilled water followed by dripped in 2% sodium hypochlorite (NaClO) solution for surface sterilization. After 30 minutes the seeds were ringed with distilled water to remove the coating of NaClO.

Garden soil was collected (at 15 cm soil depth) from an agricultural field situated near Tezpur University, Assam. Visible roots, debris and other unwanted particles were separated from the soil soon after collection. Basic soil properties were tested (table 3.1). Properly mixed (100 g) uniform particle of soil was taken in each pre sterilized disposable dish.

3.3.2. Experimental design for seed germination

Biochar at two doses 10 t ha^{-1} (5.54 g kg^{-1}) and 20 t ha^{-1} (11.08 g kg^{-1}) was added to aforementioned dishes containing 100 g soil (calculated using metric conversions and bulk density of the soil). Three replications were taken for each treatment. Twenty seeds of each crop were allowed to germinate in one dish. Equal amount of distilled water (to control contamination) required for germination was sprayed on every dish.

Treatment undertaken

1. **TLC10:** tea pruning litter conventional (10 t ha^{-1})
2. **TLC20:** tea pruning litter conventional **(**20 t ha^{-1})
3. **TLG10:** tea pruning litter gasified (10 t ha^{-1})
4. **TLG20:** tea pruning litter gasified (20 t ha^{-1})
5. **TLP10:** tea pruning litter pyrolyzed (10 t ha^{-1})
6. **TLP20:** tea pruning litter pyrolyzed (20 t ha^{-1})
7. **WCC10:** mixed wood chips conventional (10 t ha^{-1})
8. **WCC20:** mixed wood chips conventional (20 t ha^{-1})
9. **WCG10:** mixed wood chips gasified (10 t ha^{-1})
10. **WCG11:** mixed wood chips gasified (20 t ha^{-1})
11. **WCP10:** mixed wood chips pyrolyzed (10 t ha^{-1})
12. **WCP20:** mixed wood chips pyrolyzed (20 t ha^{-1})
13. **C:** control

3.3.3. Germination Parameters

Germination performance was observed till tenth day of germination (after emergence of radicle and plumule). Number of seeds germinated in each day (24 hour) was noted and the lengths of plumules and radicals were recorded at the end of the experiment. On completion of germination, the germinated seeds were uprooted, and soil samples were collected for further analysis.

To evaluate the germination performance of the tested seeds under applied biochar treatments, percent germination was calculated following Benech Arnold et al. [10]. Germination index, percent inhibition of germination, and vigour index were obtained as given by Sarma et al. [11] and calculated using following formula:

3.3.3.1. Percent germination

$$\text{Percent germination} = \frac{No.of\ seeds\ germinated}{Total\ no.of\ seeds} \times 100$$

3.3.3.2. Germination Index (GI)

Germination Index (GI) = (10 x n1) + (9 x n2) + (8 x n3) + (7 x n4) + (6 x n5) + (5 x n6) +(4 x n7) + (3 x n8) + (2 x n9) + (1 x 10)

where, n1, n2, n3 are the no. of seeds germinated on 1st, 2nd and subsequent days until 10^{th} day.

3.3.3.3. Percent inhibition of germination

Percent inhibition of germination = $100 - \frac{\text{GI of treatmnet}}{\text{GI of control}} \times 100$

3.3.3.4. Vigour index (VI)

Vigour index (VI) = (Radicle length + Plumule length) × Percent germination

3.3.3.5. Seedling biomass

Uprooted seedlings were washed properly to remove the dart and wiped carefully to dry. The fresh weight of the seedlings was recorded using a digital analytical balance (ATY224, Shimadzu). Then the seedlings were oven dried to remove the moisture and dry biomass weight was recorded.

3.3.3.6. Influence of biochar on seed bed

Short term Influence of biochar on basic soil parameters were documented using the methods described in the sections of 3.2. and 3.7.

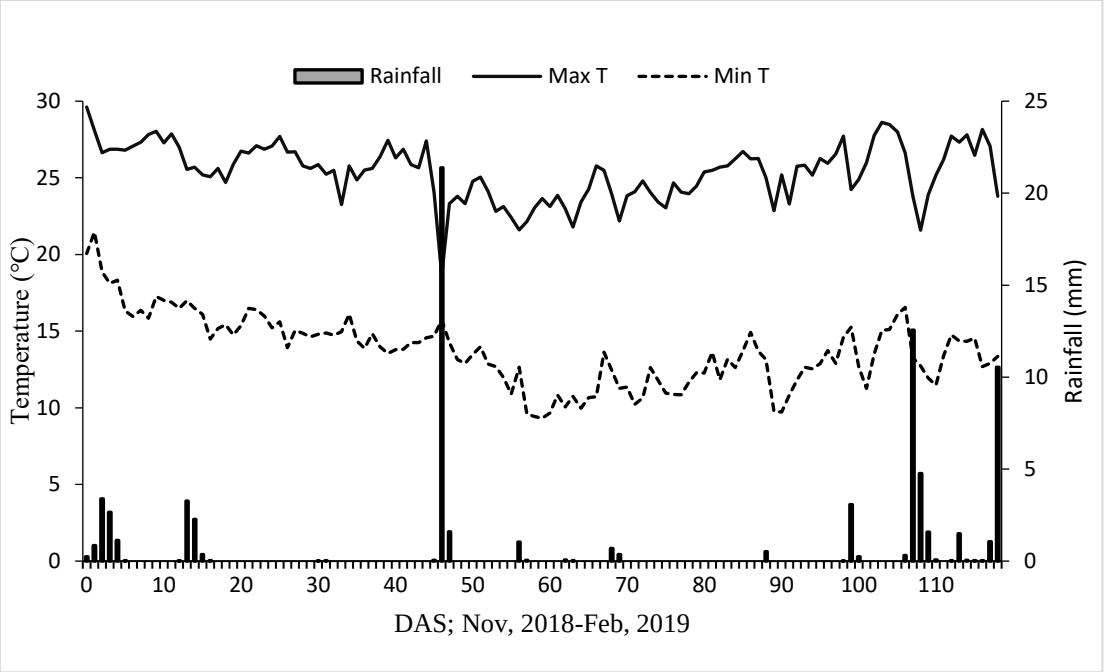
3.4. Field experimental

3.4.1. Experimental site

The field experiments were conducted at experimental field of Tezpur University, located at north bank plain agro-climatic zone of Assam, India (Figure 3.1). The topographical position of the site is 26º69′ N and 92º82′ E. The area falls under subtropical climatic region with a monsoon type of climate. Average prevailing temperature of the site is 23 to 33ºC during summer (June to September) and 13 to 27ºC in winter (October to February). It receives a yearly rainfall of 1898 mm with peak during monsoon period (June to September). The average minimum and maximum temperature and total rainfall during the experimental period were displayed in Figure 3.2

Figure 3.1. Experimental site: Tezpur University, North bank plain agro-climatic zone, Assam, India

A



B

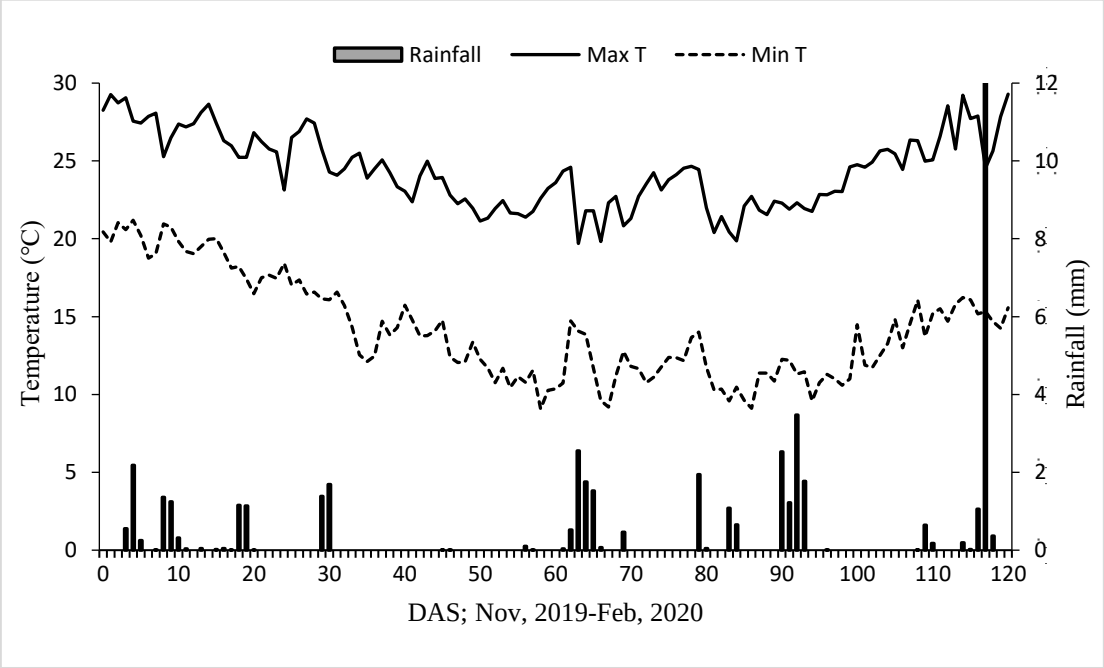


Figure 3.2. Meteorological graph showing maximum and minimum air temperature (°C) and rainfall (mm) during the crop growth period (A) November, 2018 to February, 2019 and (B) November, 2019 to February, 2020

3.4.2. Treatment details of field experiments

To achieve the objectives 2 and 3, field experiments were conducted for two consecutive years during November to February of 2018-2020. Tea pruning litter conventional (TLC), tea pruning litter gasified (TLG), mixed wood chips conventional (WCC), mixed wood chips gasified (WCG) and farmyard manure (FYM) were used as soil amendments whereas, commercial NPK fertilizers were used as inorganic nutrient source. The properties of gasification and pyrolysis made biochars were similar, therefore only gasification based biochars were used for the field experiment. Total twenty (including control) treatments were selected for the present study. Each treatment was replicated thrice and arranged as per factorial randomized block design (factorial RBD). The size of each treatment plot was 2 m^2 and a buffer zone of 1 ft. was kept restricting the water flow between treatment plots.

Treatments undertaken

1. **TLC10:** tea pruning litter conventional at 10 t ha^{-1}
2. **TLG10:** tea pruning litter gasified at 10 t ha^{-1}
3. **WCC10:** wood chips conventional at 10 t ha^{-1}
4. **WCG10:** wood chips gasified at 10 t ha^{-1}
5. **FYM10:** farmyard manure at 10 t ha^{-1}
6. **NPKR:** NPK fertilizer at 100% recommended dose
7. **TLC5:** tea pruning litter conventional at 5 t ha^{-1}
8. **TLG5:** tea pruning litter gasified at 5 t ha^{-1}
9. **WCC5:** wood chips conventional at 5 t ha^{-1}
10. **WCG5:** wood chips gasified at 5 t ha^{-1}
11. **TCFYM:** tea pruning litter conventional at 5 t ha^{-1} + FYM at 5 t ha^{-1}
12. **TGFYM:** tea pruning litter gasified at 5 t ha^{-1} + FYM at 5 t ha^{-1}
13. **WCFYM:** wood chips conventional at 5 t ha^{-1} + FYM at 5 t ha^{-1}
14. **WGFYM:** wood chips gasified at 5 t ha^{-1} + FYM at 5 t ha^{-1}
15. **TCNPK:** tea pruning litter conventional at 5 t ha^{-1} + NPK at 50% of recommended dose
16. **TGNPK:** tea pruning litter gasified at 5 t ha^{-1}+ NPK at 50% of recommended dose

17. **WCNPK:** wood chips conventional at 5 t ha^{-1} + NPK at 50% of recommended dose
18. **WGNPK:** wood chips gasified at 5 t ha^{-1} + NPK at 50% of recommended dose
19. **FMNPK:** FYM at 5 t ha^{-1} + NPK at 50% of recommended dose
20. **C:** control

3.4.3. Crops

Indian mustard (*Brassica juncea* L, variety TS 38) and French bean (*Phaseolus Vulgaris* L. variety Arka Anoop) crops were used for the study. The seeds of both the crops were collected from Krishi Vigyan Kendra, Sonitpur, Assam.

3.4.4. Soil amendments

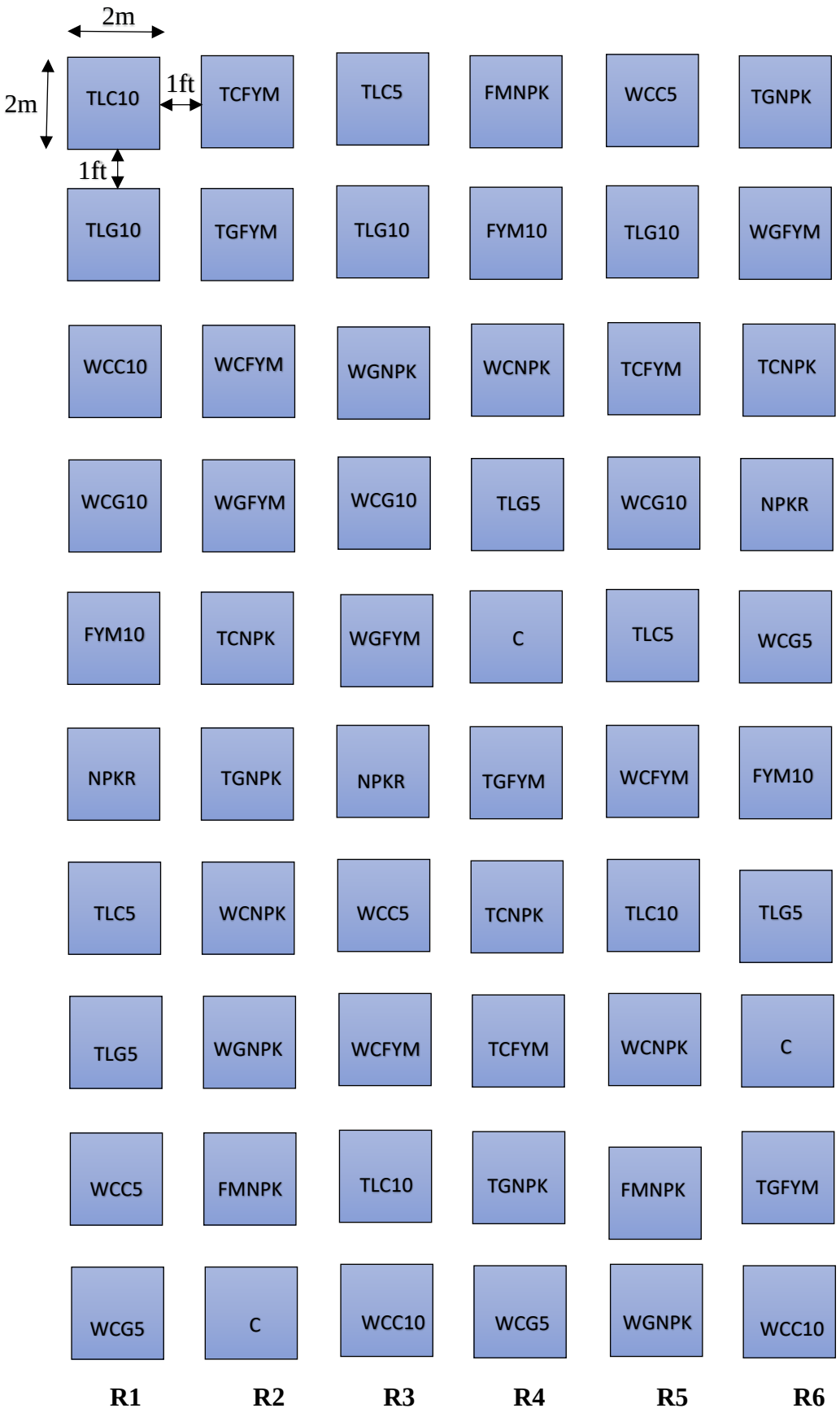
Biochar and farmyard manure (FYM) were used as soil amendments. Gasification and conventionally made biochars were used for field experiment. Whereas, FYM was collected from a cattle farm of a village situated nearby the experimental site. The used FYM was a mixture of cow dung and garden waste (ratio of 3:1).

3.4.5. Characterization of farmyard manure (FYM)

The basic physico-chemical properties of the farmyard manure were analysed following the methods described in section 3.2 and 3.7.

3.4.6. Application of soil amendments and inorganic fertilizers

Application rate and time of both the soil amendments (biochar and FYM) and inorganic fertilizers were performed following the package of practice recommended jointly by Assam Agricultural University, Jorhat and Government of Assam, India. The recommended application dose of soil amendments for both the crop for the region is 10 t ha^{-1}. Soil amendments were applied five days before seed sowing and mixed thoroughly (ploughed) with soil.



Experimental layout of all the treatments to achieves objective 1 and 2

3.4.7. Inorganic fertilizer

Commercially available fertilizers i.e. urea [$CO(NH_2)_2$], single super phosphate [$Ca(H_2PO_4)2.H_2O$] and muriate of potash [KCl] were used as inorganic nitrogen, phosphorus and potassium source.

The recommended dose of NPK for mustard are:

- Urea = 130 kg ha^{-1}
- Single super phosphate (SSP) = 253 kg ha^{-1}
- Muriate of potash (MOP) = 67 kg ha^{-1}

Fertilizers (100%) were applied as basal dose one day before sowing the seeds.

The recommended dose of NPK for french bean are:

- Urea = 132 kg ha^{-1}
- Single super phosphate (SSP) = 281 kg ha^{-1}
- Muriate of potash (MOP) = 68 kg ha^{-1}

50% of urea along with 100% of SSP and MOP were applied as basal doses one day before sowing the seeds. The remaining 50% of the urea was applied at 30 days after sowing.

3.4.8. Seed sowing

Mustard seeds (variety TS 38) were sown at the rate of 10 kg ha^{-1} during the first week of November and harvested on the first week of February in both the years. Thinning was carried out after three weeks of seed germination to maintain plant to plant distance of 10 to 15 cm. Irrigation was provided during the flowering stage of the mustard crop.

French bean (variety Arka Anoop) seeds were sown in line with a spacing of 40 cm × 30 cm (placed one healthy seed at each space) from first week of November to first of February. In absence of rain for continuous five days, watering was provided to each plant at three days interval. Disease and pest incidents were not detected in both the crops.

3.5. Gas sampling from the fields

Among the potent greenhouse gases (CH_4, N_2O, and CO_2) from agricultural field. We have selected N_2O and CO_2 as both the experimented crops (mustard and french bean) were upland and aerobic in nature. Possibility of CH_4 production only exist under anaerobic conditions as the methanogens are strictly anaerobic.

3.5.1. N_2O sampling and analysis

Gas samples were collected using the static chamber technique following Buendia et al., [12]. Rectangular chambers made up of acrylic sheets (6 mm thick) of size of 50 cm × 30 cm × 70 cm was used for sampling. Rectangular aluminium channel of size 50 cm × 30 cm was fitted in the soil during field preparation (before sowing the seeds). The acrylic box was placed on the top of the aluminium channel during gas sampling. Battery-operated fan was fixed inside the chambers to homogenize the air and a thermometer was installed to record the temperature. Gas samplings were done at ten-days interval from seed germination till harvesting of the crops. Samples were collected using 20 ml leakage-free syringe fitted with the three-way stopcock. Gas samples were collected between 08.00 am and 12.00 pm of Indian standard time at fixed intervals of 0, 15, 30 and 45 minutes after placing the acrylic sheet chambers on aluminium channels.

Analysis of N_2O flux

Collected gas samples were analysed using a gas chromatograph (Varian, 3800 GC) fitted with an electron capture detector (ECD) and a 50 cm long stainless steel chromopack capillary column of 0.53 mm outside and 1 µm inside diameter. Column, injector and detector temperatures were maintained at 80, 200 and 300°C, respectively. Nitrogen (99.999% purity) was used as carrier gas with a flow rate of 15 ml min^{-1}. Moisture, oxygen and ascrite traps were used to filter out moisture, O_2 and CO_2 from the samples. GC responses were calibrated using certified N_2O standard. N_2O fluxes were calculated following the equation of Parashar et al. [13].

$$F = \frac{\Delta C_{N20}}{10^6} \times BV(STP) \times \frac{44 \times 10^3}{22400} \times \frac{1}{A} \times \frac{60}{t}$$

where,

F = efflux of N_2O in µg N_2O-N m^{-2} hr^{-1}

ΔC_{N2O}= change in concentration of N_2O inside the chamber from '0' to 't' minutes (ppmv)

A = area converted by the chamber

T = time (minutes)

BV_{STP}= chamber volume at standard temperature and pressure in cm^3

$$BV_{STP} = \frac{BV \times BP \times 273}{(273 + T) \times 760}$$

where,

BV = chamber volume (cm^3)

BP = barometric pressure (mm Hg)

T = chamber temperature at the time of sampling (ºC)

Cumulative N_2O emission or seasonal integrated flux (E_{sif}) for the whole crop growing season was computed by the equation given by Ma et al. [14]:

$$\text{Seasonal } N_2O \text{ emission} = \sum_{i=1}^{n=1}(Fi \times Di)$$

where,

n = total number of the N_2O sampling made during the experimental period

F*i* = N_2O flux in i^{th} sampling interval

D*i* = number of days between two sampling intervals i.e. 10 days interval

3.5.2. Sampling and analysis of soil CO_2 flux

Soil respiration or soil CO_2 flux was recorded during seedling (0-20 DAS), vegetative (20-40 DAS), flowering (40-60 DAS) and maturation (70-80 DAS) stages of mustard. Similarly, for french bean the CO_2 measurement was noted at seedling (0-20 DAS), vegetative (20-45 DAS), flowering (45-70 DAS) and maturity (70-90 DAS) stages. CO_2 flux measurement sites for each treatment replication was marked with a flag. Sampling for each plant growth stages was done between morning 10.00 am to 12.00 pm. An automated CO_2 flux system (LI-8100A, LICOR, USA) was used for soil CO_2 flux estimation and the recorded results were viewed through an instrument operating

device (HP-Ipaq). The chamber of the system was 11 cm high and 20 cm in diameter. CO_2 measurement ranged from 0-20000 ppm at operating temperature of 20-40ºC. Soil collars made up of short PVC plumbing pipe sections that are sharp at one end were inserted at a depth of 2 cm during sampling.

3.5.3. Global warming potential (GWP)

IPCC 2020 factors (for 100 years of emission of N_2O) were taken to calculate GWPs for the mustard and french bean fields using the equation developed by Jain et al [15]:

$$GWP = N_2O \times 298$$

3.5.4. Carbon equivalent emission (CEE)

CEE was calculated following the formula given by Bhatia et al. [16]

$$CEE = \frac{12}{44} GWP$$

3.5.5. Amendment effect index

Amendment effect index (AEI) on N_2O emission and soil CO_2 flux was calculated using the formula given by Zhang at el. [17].

$$AEI\ (\%) = \left(\frac{QA - QC}{QC}\right) \times 100$$

where,

QA = quantity emission of treated soil

QC = quantity emission from control

3.6. Sampling for plant parameters

To measure the rate of photosynthesis and transpiration, the fully expanded young leaves from the top of the plant was selected. For mustard crop, it was recorded at vegetative (20-40 DAS), flowering (40-60 DAS) and maturity (70-80 DAS) stages of the crop. Similarly, photosynthetic rate and transpiration for french bean plants grown under different soil treatments were recorded at vegetative (20-45 DAS), flowering

(45-70 DAS) and maturity (70-90 DAS) stages of the crop. An harvest, the plants were uprooted and recoded the biomass and yield.

3.6.1. Photosynthesis and transpiration

Photosynthetic and transpiration rate of the plants were recorded with an infrared gas analyser (portable photosynthesis system; LI-6400, Li-Cor, Lincoln, USA) under ambient environmental conditions. Measurements were recorded between 10 am to 12 noon. The photosynthetic and transpiration rate were expressed as μmol CO_2 m^{-2} s^{-1} and μmol H_2O m^{-2} s^{-1} respectively.

3.6.2. Plant biomass and seed yield

Plants were uprooted carefully during harvest and washed with running tap water to remove soil from the outer surfaces of the plants. The fresh weight of the plants was recorded and then the plant biomass was kept in an oven (BDI - 50, labotech universal hot air oven, India) at 70 ºC for 72 hours to get the dry biomass. The weight of both fresh and dry plant biomass (shoot and root) was recorded using a digital analytical balance (ATY224, Shimadzu).

3.7. Soil sampling and analysis

To analyse the basic soil properties, soil sample was collected (before application of treatments) from the experimental field. Moreover, soils from each treatment plots (including replications) were collected separately after harvesting the crop. Collected soil samples were mixed (treatment wise) to get a homogenous sample. Now, one part of each sample was air dried. The dried soil samples were sieved through 2 mm sieve and stored in plastic zipper bags for analysis of different soil physico-chemical parameters and the second part was stored at -4 ºC for study the soil biological parameters.

3.7.1. Ultimate analysis

Ultimate analysis of the soil collected from each treatment were done as explained in the section 3.2.3.

3.7.2. Soil physico chemical parameters

Soil pH, and electrical conductivity (EC), cation exchange capacity (CEC), and soil water holding capacity (WHC) were determined as described in the section 3.2.5. and 3.2.7.

Whereas soil bulk density was estimated following Baruah and Borthakur [18] using the following procedure.

Procedure

Initially, weight (W1) and volume (V) of the empty bulk density bottles (weight with the stopper) were noted. The bottles were then filled with the soil and amendments till the rim and continuously tapped for about 15-20 times to produce the strength of packing. After capping the bottles, final weight (W2) was recorded.

Calculation:

$$Bulk\ density\ (g\ cm^{-3}) = \frac{\mathrm{W2} - \mathrm{W1}}{\mathrm{V}}$$

3.7.3. Available N

Available N in soil was determined following alkaline potassium permanganate procedure using Kjeldahl distillation unit (Kelplus Distyl Emva) [19].

Procedure:

Air dried sample of 0.5 g was taken in a distillation tube and 25 ml of 0.32% $KMnO_4$ was added into it. The tube is then loaded in distillation unit where 25 ml of 2.5% NaOH was added automatically. At the end of the delivery tube, 20 ml of 2% boric acid with mixed indicator in a 250 ml conical flask was positioned to trap the N distilled. With the absorption of ammonia, the pinkish colour turns to green. Distillation was continued for 9 minutes till a distillate of about 100 ml was collected in the conical flask. Then the collected distillate (trapped ammonium-N + nitrate-N) was titrated with 0.02 N H_2SO_4 till the colour changed back to pinkish. A reagent blank without soil sample was also analysed for calculation.

Calculation:

$$Available\ N\ (mg\ kg^{-1}) = \frac{R\ (mL\ titrant\ in\ sample - blank) \times\ acid\ normality\ \times atomic\ wt.of\ N\ \times\ extractant\ (mL) \times 1000}{Sample\ weight\ (g)\ \times 1000}$$

$$Available\ N\ (mg\ kg^{-1}) = \frac{R\ \times 0.02 \times 14 \times 100 \times 1000}{Sample\ weight\ (g) \times 1000}$$

3.7.4. Soil nitrate nitrogen

Soil nitrate nitrogen was determined using the method produced by Narayana and Sunil [20].

Procedure:

Pipetted out 10 ml of nitrate stock solution (0.7220 g potassium nitrate dissolved in distilled water and volume made up to 100 ml) to a beaker. 5 ml of conc. HCl and 2 ml of Zn/NaCl granular mixture were added into it, mixed thoroughly and kept undisturbed for 30 minutes then the solution was filtered using Whatman no 41 filter paper and diluted to 100 ml. Aliquots of stock solution containing reduced nitrate were transferred into series of 10 ml standard flask. Then the solution was mixed with 1 ml of 0.5% sulfanilic acid and 1 ml of 2 mol L^{-1} HCl solution, shaken for 5 minutes. Then, 1 ml of 0.5% methyl anthranilate and 2 ml of 2 mol L^{-1} sodium hydroxide solution were added to form an azo dye and the contents were made up to the volume 10 ml adding distilled water. The absorbance of the red coloured dye was measured at 493 nm against the corresponding reagent blank.

3.7.5. Soil ammoniacal nitrogen

Determination of ammoniacal nitrogen or exchangeable ammonium in soil extracts was done following the method described by Wang and Oien., 1986 [21]

Soil was extracted with 2M KCl and ammoniacal nitrogen was estimated by colorimetry using the indophenol method.

Procedure:

Soil sample (1.0 g) was shaken in a rotary shaker for 1 hour with 100 ml 2M KCI, then filtered through 9 cm Whatmen GF/C glass microfibre filter. After that the 10 ml of soil extract was transferred into a 60 ml test tube and 10 ml NaOH-tartrate solution was added and mixed well. Then 7 ml alkaline phenol solution and 5 ml sodium hypochlorite were added. The same procedure is followed with standard solutions (standard solutions of 0, 0.5, 2, 5, 8, and 10 mg $NH_4N\ l^{-1}$ in 2M KCl was made diluting 100 ml of stock solution containing 100 mg of NH_4-N L^{-1} to 1 L with 2M KCl) and blank. The absorbance was examined at 635 nm in a UV visible spectrophotometer (model-Eppendorf Kinetic Bio Spectrophotometer).

3.7.6. Available P

Available P was determined by the procedure given by Bray and Kurtz [22].

Procedure:

Air dried sieved (2mm) soil sample of weight 2.5 g was taken in a flask where 25 ml of Bray's extractant (NH_4F in HCl) was poured. The solution was shaken in the rotary shaker for 5 minutes and then centrifuged at 6000 rpm (for 10 minutes). From it, 10 ml of supernatant was taken in 50 ml of volumetric flask. To it 10 ml of molybdate reagent (Ammonium molybdate in HCl) was added and then the solution was diluted to about 40 ml with distilled water. To the volumetric flask 2 ml of stannous chloride ($SnCl_2$) solution (10g of $SnCl_2$ was dissolved in 25 ml of conc. HCl) was pipetted out and volume was made up to 50 ml with distilled water. After 10 minutes absorbance was recorded at 660 nm. One blank was prepared without sample. For the standard curve, 1, 2, 3, 4, 5 and 10 ml of 2 ppm solutions were taken in 50 ml volumetric flask and same procedure as sample was (+10 ml molybdate reagent + 2 ml $SnCl_2$ + distilled water = 50 ml and OD at 660 nm) was carried out. Once the linear calibration curve was developed, the slope of the curve was determined and then the concentration of the unknown solution was calculated by using the equation y = mx + c.

$$Available\ P\ (mg\ kg^{-1}) = Sample\ concentration\ (mg\ g^{-1}) \times dilution factor$$

where, dilution factor = volume of extractant/sample weight (g)

3.7.7. Available K

Available K was determined following the method given by Jackson [23]

Procedure:

2.0 g of air dried sieved (2mm) soil sample was taken in a conical flask and 20 ml of 1N ammonium acetate of pH 7.0 was added to it. The suspension was kept for 30 minutes and then filtered using Whatman no.1 filter paper followed by taking the reading in flame photometer in K filter.

Calculation:

$$Available\ K\ (mg\ kg^{-1}) = \frac{R \times Volume\ of\ extractant}{Sample\ weight\ (g)}$$

where, R is the mg g^{-1} of K in extract (photometer reading).

3.7.8. Elemental (macro, micro and heavy metal) concentration

Soil elemental concentration were measured using the method displayed in the section 3.2.14.

3.7.9. Soil organic carbon

Organic carbon percentage was analysed following dichromate wet oxidation method developed by Walkey and Black [24] with a minor modification.

Procedure:

Air dried soil (1.0) g was taken in a 500 ml conical flask. In the flask, 10 ml of 1N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 was added. The flask was then allowed to stand for 30 minutes for complete oxidation. After oxidation, 200 ml of distilled water was added and subsequently 10 ml of conc. H_3PO_4 was added. Mixture was then titrated using 1.5 ml of diphenylamine indicator with 0.5N ferrous ammonium sulphate (FAS) till brilliant green colour was attained. A reagent blank was run with each set without soil.

Calculation:

$$Organic\ carbon\ (\%) = \frac{Titrant\ in\ blank\ (mL) - Titrant\ in\ sample\ (mL) \times 0.003 \times 100}{Titrant\ in\ blank\ (mL) \times sample\ weight\ (g)} \times 100$$

3.7.10. Fulvic acid carbon (FAC), humic acid carbon (HAC)

The FAC, HAC were determined conferring to the process described by Page et al. [25]

Procedure:

Soil sample (5.0 g) was taken in a 250 ml conical flask where 100 ml of 0.1N sodium pyrophosphate solution was added and placed in a rotary shaker for 30 minutes (flask was covered with aluminium foil before shaking). The flask was then kept overnight and next day filtered with Whatman no. 1 filter paper (supernatant I). pH of the filtrate was adjusted between 2-3 using Conc. H_2SO_4 and again kept overnight. Next day, solution was again filtered through Whatman no. 1 filter paper (supernatant II). The filtrate was analysed for fulvic acid carbon. The precipitate in the filter paper was washed with 20 ml of 0.1N NaOH. The optical density of the solution was noted in 465 nm (E4) and 665 nm (E6) using spectrophotometer (Eppendorf BioSpectrometer). Then the solution was analysed for humic acid carbon. Organic carbon content (FAC and HAC) of the solutions was determined by dichromate oxidation method as described in section 3.7.9.

3.7.11. Microbial biomass carbon

Soil microbial biomass carbon (MBC) was estimated using $CHCl_3$ fumigation-extraction method given by Vance et al. [26]

Procedure:

Weight of soil samples 10 g each at three sets were taken. The first set was kept in oven at 105°C for estimation of moisture content and the second set was fumigated and the third set was kept in refrigerator for further required calculations.

Fumigation: 10 g of fresh soil sample was weighed and placed in beaker and put it in a vacuum desiccator with $CHCl_3$. The desiccator was sealed with Vaseline (a commercial cosmetic product with lubricant properties) to avoid leakage and incubated at 25 ± 2°C for 48 hours.

Extraction: 10 ml of 0.5M K_2SO_4 was added to both the fumigated and non-fumigated samples and kept for 10 minutes for extraction. The content was then filtered with Whatman no. 42 filter paper.

Oxidation: 8 ml of the extract was taken in 250 ml conical flask and 2 ml of $K_2Cr_2O_7$ was added to it. A 15 ml of digestion mixture was added to the extractant and allowed to stand for 30 minutes. After 30 minutes, 25 ml of distilled water followed by 2-3 drops of ferroin indicator were added and the contents were mixed. The content was then titrated with 40 mM ferrous ammonium sulphate (FAS) till brick red colour was obtained. A reagent blank was run with each set without soil.

Calculation:

Soil moisture content (MC%)

$$MC\ (\%) = \frac{weight\ of\ wet\ soil\ (g) - weight\ of\ oven\ dried\ soil\ (g)}{weight\ of\ oven\ dried\ soil\ (g)} \times 100$$

Weight of soil sample (oven-dry weight equivalent) (MS g)

$$MS = \frac{weight\ of\ wet\ soil\ (g)}{\{100 + MC(\%)\}} \times 100$$

Total volume of solution in the extracted soil (VS ml)

$$VS = wet\ soil\ weight - oven\ dry\ soil\ weight + extractant\ volume$$

Determination of extractable C (Ext C in μg ml^{-1})

Volume of $K_2Cr_2O_7$ consumed (Y ml)

$$Y = \frac{Normality\ of\ FAS \times Titrant\ volume}{Normality\ of\ K_2Cr_2O_7} \times 100$$

Volume of $K_2Cr_2O_7$ consumed for oxidizing easily mineralizable C in 10 ml of extractant = 2-Y ml

Amount of extractable C

$$Ext\ C\ (\mu g\ mL^{-1}) = \frac{600 \times (2 - Y)}{10}$$

Total weight of extractable C (µg g^{-1} soil) in fumigated (CF) and non-fumigated (CNF) samples

$$CF\ or\ CNF = Ext\ C \times \frac{VS}{MS}$$

Microbial biomass carbon (µg g^{-1} soil or mg kg^{-1} soil)

$$MBC\ (mg\ kg^{-1}\ \text{soil}) = \frac{\text{CF} - \text{CNF}}{\text{K}}$$

where, K = 0.25 that represents extraction coefficient of microbial biomass carbon

3.7.12. Soil enzyme activity

3.8.12.1. Dehydrogenase activity

Soil dehydrogenase activity was estimated following the method of Garcia et al. [27] with little modification.

Procedure:

Fresh soil (1 g) was taken in a 30 ml beaker and into that 0.2 ml of 0.4% 2-*p*-iodo-nitrophenyl-phenyltetrazolium chloride (INT) was added, incubated for 20 hours at 22°C. After 20 hours 10 ml of 95% ethanol was added and agitated vigorously for 1 hour. The mixture was filtered through Whatman no.1 filter paper. Absorbance of the red colour of iodo-nitro-tetrazolium-formazan (INTF) was observed at 490 nm. For preparation of the standard graph 0.1, 0.2, 0.3, 0.5, 0.8 and 1 ml of the Nitorphenyl Formazan standard solution was taken in 50 ml volumetric flask and the volume was made up with ethanol. Dehydrogenase activity was calculated from the standard graph and expressed as µg INTF g^{-1} soil h^{-1}.

Calculation:

$$Dehydrogenase\ activity\ (\text{mg INFT } g^{-1}\ h^{-1}) = \frac{Amount\ of\ INTF\ from\ standard\ graph\ (\mu g)}{Soil\ weight\ (g) \times incubation\ time\ (hour)}$$

3.7.12.2. Urease activity

Soil urease activity was estimated by hydrolysis reaction as described by Tabatabai and Bremner [28].

Procedure:

0.5 g of fresh soil was weighed in a 50 ml volumetric flask. To it, 0.2 ml of toluene was pipetted followed by addition of 9 ml of Tris-HCl buffer of pH 9.0. Sample was kept in the shaker for 15 minutes to mix the contents and then 1 ml of 0.2M urea was added to the mixture. Flask mouth was covered by a stopper and kept in BOD incubator at 37°C for 2 hours. After 2 hours, the volume was made up to 50 ml with KCl-Ag_2SO_4 solution. The contents were swirl for few seconds and allowed to stand the flask until the content cooled to room temperature. The supernatant was used for the estimation of ammonia. From it, 1 ml of supernatant was taken into a test tube where 1 ml of Phenol-Pentacyano-nitrosyloferate solution was added followed by 1ml of alkaline hypochlorite solution. The reaction was kept for 5 minutes at 37°C and 7 ml of water was added to it before taking the absorbance at 625 nm. For the control (without soil), 1 ml of 0.2M urea was added after addition of KCl-Ag_2SO_4 solution. A standard curve was prepared by using ammonium chloride as a standard (10 g of NH_4Cl was dissolved in 100 ml distilled water that forms a 28 µg of NH_3-N of stock solution). Urease activity was calculated from the standard curve and expressed as µg NH_4^+ g^{-1} h^{-1}.

Calculation:

$$Urease\ activity\ (\mu g\ NH4-N\ h^{-1}\ g^{-1}\ dw\ soil) = \frac{Amount\ of\ NH_4^+\ from\ standard\ curve\ (\mu g)}{Soil\ weight\ (g) \times Incubation\ time\ (hour)}$$

3.7.12.3. Phosphatase activity

Soil phosphatase activity was estimated by the method described by Tabatabai and Bremner [29]

Procedure:

Fresh soil sample of weight 1.0 g was taken in a 15 ml centrifuge tube and 4 ml of 0.1 M acetate buffer was poured into the tube. In this suspension, 1 ml of 20 mM p-nitro

phenyl phosphate was added, and the content was kept in BOD incubator at 37 ± 1°C for 1 hour. After 1 hour, 4 ml of 1M NaOH and 1 ml of 0.5M $CaCl_2$ pipetted to the tube to stop the reaction. The mixture was then centrifuged at 5000 rpm for 10 minutes and absorbance was measured at 400 nm. A calibration graph was prepared with standards containing 10, 20, 30, 40 and 50 µg p-nitrophenol (PNP) and the enzyme activity was expressed as µ mol PNP g^{-1} h^{-1}.

Calculation:

$$Phosphatase\ activity\ (\mu mol\ g^{-1}\ h^{-1)} = \frac{Amount\ of\ PNP\ from\ standard\ curve\ (\mu g)}{Soil\ weight\ (g) \times molecular\ weight\ of\ PNP\ (g)}$$

3.7.13. Soil microbial count

Soil bacteria were isolated and count by serial dilution method using aseptic techniques.

Procedure:

Fresh soil 0.5 g was weighed and put in a conical flask containing 50 ml of sterile agar (1%). It denotes 10^{-2} dilution. The content was shaken vigorously for 1-2 minutes. From this content, 0.5 ml was pipetted to a vial containing 4.5 ml of 0.1% agar (It denotes 10^{-3} dilution) using a sterile pipette and shook vigorously for 1-2 minutes. Procedure (0.5 ml pipette out to 4.5 ml 0.1% agar) was repeated for three other dilutions (10^{-4}, 10^{-5} and 10^{-6}). From the highest dilution 10^{-6}, 1 ml of the mixture was pipetted to each of 3 petri plates containing about 10 ml of PDA. Using a sterile glass stirring rod it was spread over the entire surface. The same step was followed for the remaining dilutions (10^{-5} and 10^{-4}). Petri plates were sealed with parafilm and incubated in room temperature. Number of colonies grown were recorded after 24, 48 and 72 hours and expressed as log CFU g^{-1} dry soil.

3.8. Statistical analysis

Statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, IL, USA) software package. Analysis of variance (one-way ANOVA) and Duncan's multiple range test (DMRT) was executed at $p \leq 0.05$ to process the data and to determine statistical difference between treatment means. Variations in collected data were presented as standard deviation ($p \leq 0.05$). Three-way analysis of variance (ANOVA) was executed in the seed germination study under different studied biochar (seed

germination test objective 2) to estimate significance between biochar type, biochar application rate and seed and their respective interactions. Pearson's linear correlations between the variables were performed to estimate their relationships. In addition, PCA plot was performed using Jamovi software. Figures were developed using Graph Pad Prism 8.3.0.

A

B

C

Image 3.1. Images showing (A) pyrolysis, (B) gasification and (C) conventional apparatus (kiln) used for biochar production.

References

[1] American Society for Testing and Materials. *Standard Test Methods for Nails*. ASTM International, 2007.

[2] Baruah, N. Gogoi, N. and Farooq, M. 2020. Influence of biochar and organic soil amendments on bioavailability and immobilization of copper and lead to common cocklebur in acidic sandy loam soil. *Journal of Environmental Chemical Engineering*, *8*(6):104480, 2020.

[3] Cheng, C. H., and Lehmann, J. Ageing of black carbon along a temperature gradient. *Chemosphere*, *75*(8): 1021-1027, 2009.

[4] Yu, H., Huang, X., Ning, J., Zhu, B., and Cheng, Y. Effect of cation exchange capacity of soil on stabilized soil strength. *Soils and Foundations*, *54*(6): 1236-1240, 2014.

[5] Fabbri, D., Rombolà, A. G., Torri, C., and Spokas, K. A. Determination of polycyclic aromatic hydrocarbons in biochar and biochar amended soil. *Journal of Analytical and Applied Pyrolysis*, *103*: 60-67, 2013

[6] Tripathi N. Analysis of fertilizers for major and micronutrients. In: Tandon, H. L. S., editor, Methods of analysis of soils, plants, waters, fertilizers and organic manures, pages 153-182, Fertilizer Development and Consultation Organisation: New Delhi, 2009.

[7] Yadav, A., Ansari, K. B., Simha, P., Gaikar, V. G., and Pandit, A. B. Vacuum pyrolysed biochar for soil amendment. *Resource-Efficient Technologies*, *2*: S177-S185, 2016

[8] Harvey, O. R., Kuo, L. J., Zimmerman, A. R., Louchouarn, P., Amonette, J. E., and Herbert, B. E. An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). *Environmental Science and Technology*, *46*(3): 1415-1421, 2012.

[9] Zhao, L., Cao, X., Mašek, O., and Zimmerman, A. Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. *Journal of Hazardous Materials*, *256*: 1-9., 2013.

[10] Benech Arnold, R. L., Fenner, M., and Edwards, P. J. Changes in germinability, ABA content and ABA embryonic sensitivity in developing

seeds of *Sorghum bicolor* (L.) Moench. induced by water stress during grain filling. *New Phytologist*, *118*(2): 339-347, 1991.

[11] Sarma, B., Devi, P., Gogoi, N., and Devi, Y. M. Effects of cobalt induced stress on Triticum aestivum L. crop. *Asian J Agri Biol*, *2*(2): 137-147, 2014.

[12] Buendia, L. V., Neue, H. U., Wassmann, R., Lantin, R. S., and Javellana, A. M. Understanding the nature of methane emission from rice ecosystems as basis of mitigation strategies. *Applied Energy*, *56*(3-4): 433-444, 1997.

[13] Parashar, D. C., Mitra, A. P., Gupta, P. K., Rai, J., Sharma, R. C., Singh, N., and Sinha, S. K. Methane budget from paddy fields in India. 1994

[14] Ma, Y., Sun, L., Zhang, X., Yang, B., Wang, J., Yin, B., and Xiong, Z. Mitigation of nitrous oxide emissions from paddy soil under conventional and no-till practices using nitrification inhibitors during the winter wheat-growing season. *Biology and Fertility of Soils*, *49*(6): 627-635, 2013

[15] Jain, N., Dubey, R., Dubey, D. S., Singh, J., Khanna, M., Pathak, H., and Bhatia, A. Mitigation of greenhouse gas emission with system of rice intensification in the Indo-Gangetic Plains. *Paddy and Water Environment*, *12*(3): 355-363, 2014.

[16] Bhatia, A., Sasmal, S., Jain, N., Pathak, H., Kumar, R., and Singh, A. Mitigating nitrous oxide emission from soil under conventional and no-tillage in wheat using nitrification inhibitors. *Agriculture, Ecosystems and Environment*, *136*(3-4): 247-253, 2010.

[17] Zhang, A., Bian, R., Pan, G., Cui, L., Hussain, Q., Li, L., Zheng, J., Zheng, J., Zang, X., Han, X., Yu, X. Efects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a feld study of 2 consecutive rice growing cycles. *Field Crop Res*, 153:160–127., 2012.

[18] Baruah, T. C., and Borthakur, H. P. Soil Physics. In: Baruah, T. C., and Borthakur, H. P., A textbook of soil analysis, pages 28-31, *Vikas Publishing House Pvt. Ltd.*: New Delhi, 1997.

[19] Subbiah, B. V., and Asija, G. L. A rapid process for the determination of available nitrogen in soils. *Current Science India*, 25: 259-260, 1956.

[20] Narayana, B., and Sunil, K. A spectrophotometric method for the determination of nitrite and nitrate. *European Journal of Analytical Chemistry*, *4*(2): 204-14, 2009.

[21] Wang, L., and Øien, A. Determination of Kjeldahl nitrogen and exchangeable ammonium in soil by the indophenol method. *Acta Agriculturae Scandinavica*, *36*(1): 60-70, 1986.

[22] Bray, R. H., and Kurtz, L. T. Determination of total, organic and available forms of phosphorus in soils. *Soil Science*, 23: 39-45, 1945.

[23] Jackson, M. L. Soil chemical analysis, *Prentice Hall of India Private Limited*, New Delhi, India, 1973.

[24] Walkley, A., and Black, I. A. An examination of the method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 34: 29-38, 1934.

[25] Page, A. L., Miller, R. H., and Keeney, D. R. Methods of soil analysis. Part 2. *Soil Science Society of America*: Madison, 1982.

[26] Vance, E. D., Brookes, P. C., and Jenkinson D. S. An extraction method for measuring soil microbial biomass carbon. *Soil Biology and Biochemistry*, 19: 703- 707, 1987.

[27] Garcia, C., Hernandez, T., and Costa F. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. *Communications in Soil Science and Plant Analysis*, 28: 123–134, 1997.

[28] Tabatabai, M. A., and Bremner, J. M. Assay of urease activity in soils. *Soil Biology and Biochemistry*, 4: 479-487, 1972.

[29] Tabatabai, M. A., and Bremner, J. M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1: 301–307, 1969.