CHAPTER-3

MATERIALS &
METHODS
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MATERIALS AND METHODS

Details about the study area, materials used and methodologies adopted during the entire course of the present research study is elaborated in this chapter under the following broad heads -

3.1 Site
3.2 Community Analysis (Flora/ fauna)
3.3 Soil Physicochemical Analysis
3.4 Soil Microbial Biomass Estimation
3.5 Crop Productivity Estimation
3.6 Statistical Analysis
3.7 GIS Development

3.1 SITE

3.1.1 Location: The study area is located at the mid hills of Dimoria Block of Assam, Northeast India. The area is located between 91°56’ to 92°10’ E Longitude and 26°6’ to 26°10’ N Latitude (Fig. 3.1).

3.1.2 Climate: The climate is subtropical. May to July and December to February are the hottest and coldest months, respectively. Mean monthly rainfall trend during the years of study is shown in Fig-3.2. The total annual average rainfall were 1872 mm, 1737 mm, 1189 mm, 1889 mm and 1600 mm during the year 2004, 2005, 2006, 2007 and 2008, respectively (see fig 3.3). About 70 % to 90 % of the rainfall is received during the months of April to July. Winter rains was also recorded in the
months of November, December and February. The data is collected from Hydromet Division of Indian Meteorological Department.

3.1.3 Characteristics of the Study Area: The sites were located within an altitude of 238 to 260 meters above mean sea level. All the plots were located on the north eastern aspect. The topography of the plots is almost uniform. The slopes of the plots varied from $5^\circ$ to $35^\circ$ (see table-3.1).

Table: 3.1 Characteristics of the studied plots

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>4-yr jhum cycle</th>
<th>8-yr jhum cycle</th>
<th>Reference Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude (m amsl)</td>
<td>252</td>
<td>260</td>
<td>238</td>
</tr>
<tr>
<td>Aspect</td>
<td>North East</td>
<td>North East</td>
<td>North East</td>
</tr>
<tr>
<td>Topography</td>
<td>Uniform</td>
<td>Uniform</td>
<td>Uniform</td>
</tr>
<tr>
<td>Slope</td>
<td>$25^\circ$</td>
<td>$35^\circ$</td>
<td>$5^\circ$</td>
</tr>
</tbody>
</table>

3.1.4 Experimental Plot Description: To understand the impact of biological factors in nutrient cycling and soil organic matter buildup, 4-year cycle jhum fields, 8-yr cycle jhum fields and a forest plot were identified consulting the Mikir gaonbura for the study during October 2003. Finally in December 2003 fourteen plots (seven of 4-yr cycle jhum fields and seven of 8-yr cycle jhum fields) with similar topography and aspect were selected. Each plot was of 1.0 hectare (12.5 bighas). The plots are towards south from the NH-37. Another plot of 1.0 hectare inside a natural forest, regarded as sacred by the Karbi (a traditional community residing in Dimoria) was also selected to compare the results. A google earth satellite imagery of the study area is shown in Plate-1. Google earth imagery of the jhum hills is shown in Plate-2. A remote sensing imagery of the studied site is shown through Plate-3. Plate-4 to Plate 6 shows important changes in the jhum plots.
Fig-3.1: Map showing location of the studied sites at Dimoria Block of Assam
Fig-3.2: Mean monthly rainfall at Dimoria, Assam during the study period

Fig-3.3: Total annual rainfall at Dimoria, Assam during the study period
Plate-1: A Google Earth Satellite Imagery of the study area

Plate-2: A Google Earth Satellite Imagery of Jhum hills

Plate-3: Remote Sensing Imagery of the Studied Sites
Plate-4: Slashing and burning in a study area

Plate-5: Mosaic inside a jhum plot

Plate-6: Profuse growth of *Mikania micrantha* Kunth. during jhum redevelopment in Dimoria
3.1.5 Experiments Laid: During the study, seventeen experiments were laid under four major heads to understand the complex interactions between vegetation, soil and biological factors so that acceptable management strategies for soil, fertility management can be worked out. The list of the experiments is given below:

A) Vegetation and Biological Factors in Jhum fields
Experiment-1: Observing the community structure in 4-yr, 8-yr old jhum forest and the reference site.
Experiment-2: Comparison of population of different soil organisms during various stages of jhum in 4-yr and 8-yr cycle with the reference site.
Experiment-3: Microbial diversity in 4-yr and 8-yr cycle.
Experiment-4: Economic yield of crops on 4-yr jhum and 8-yr jhum fields.

B) Soil Physical, Chemical, Nutrients and Biological Factors in Jhum Fields
Experiment-1: Comparison of soil physical and chemical properties of 4-yr and 8-yr cycle jhum with the reference site.
Experiment-2: Comparison of soil hydraulic properties of 4-yr and 8-yr cycle jhum with the reference site.
Experiment-3: Comparison of soil nutrients during various stages of jhum cultivation in 4-yr and 8-yr cycle with the reference site.
Experiment-4: Comparing the changes in ammonium and nitrate in 4-yr and 8-yr cycle with the reference site.
Experiment-5: Comparison of hydrology and amount of nutrient loss after burning in 4-yr and 8-yr cycle with the reference site.
Experiment-6: Changes in soil carbon nitrogen ratio during various stages of cropping in 4-yr and 8-yr cycle jhum fields and reference site.
Experiment-7: Soil microbial biomass study in 4-yr, 8-yr cycle jhum fields & reference site.
Experiment-8: Earthworms and ammonium and nitrate in 4-yr, 8-yr cycle jhum fields and a reference site.
Experiment-9: Soil respiration studies of 4-yr and 8-yr cycle jhum fields & reference site.
Experiment-10: Effect of perturbation on nutrient dynamics.

C) Jhum Redevelopment
Experiment-1: Changes in soil physical, chemical and biological parameters during 3 year after abandonment.
Experiment-2: Relationship of chemical and hydrological properties of soil with soil microbes in 4-yr and 8-yr cycle jhum fields after 3-yrs of abandonment.

D) GIS Development
Experiment-1: GIS application to understand jhum redevelopment.
recorded after consultation with the traditional communities. This was done to achieve active participation of the traditional communities and it facilitated the traditional communities to understand and accept our findings during the later part of the study.

Sampling for earthworm was done every Sunday of a month during the entire year. For each study site, three widely separated 10 m x 10 m plots were randomly selected. Sampling points were located at the corner and center of sampling plot and separated by a distance of 10 m. Earthworms were collected by conventional digging (25 cm x 25 cm x 30 cm) and hand sorting method. Worms were counted, weighed (with gut content) in a plastic physical balance and 5 worms were preserved in 4% formalin and others released back to the soil. Results were expressed in terms of biomass (fresh weight, g m⁻²) and frequency (%). Frequency of earthworms was determined following Dash (1993).

3.2.1 Density: Density refers to the numerical strength of plants in a community and is defined as the number of individuals of a species per unit area. It was calculated using the following formula -

\[
\text{Density of a species} = \frac{\text{Total number of individuals of the species}}{\text{Total number of quadrats studied}}
\]

3.2.2 Vegetation Community Structure: For community structure in the 4-yr, 8-yr and reference site only frequently occurring species of dominant, predominant, co-dominant, shrubs, herbs, grasses, orchids, parasites were recorded.
3.1.6 Layout of Experimental Plots: Each 4-yr cycle jhum and 8-yr cycle jhum fields were further subdivided into three equal blocks each of about 2500 m² following Gomez and Gomez (1984).

For all the experiments data collection were carried out in six stages

Stage – 1  Before Slashing (January to March)
Stage – 2  After Sowing (April to June)
Stage – 3  After Harvesting (July to September)
Stage – 4  1 year after abandonment (October to November 2005)
Stage – 5  2 year after abandonment (October to November 2006)
Stage – 6  3 year after abandonment (October to November 2007)

To monitor soil physical, chemical and biological parameters data were collected during October to December in 2005, 2006 and 2007. Attempt to integrate GIS was done in 2008-2010.

3.2 COMMUNITY ANALYSIS (FLORA/FAUNA)

Vegetations, micro fauna were analysed following quadrat method. Thirty 1 m² quadrats were laid randomly on each 1.0 hectare plot. Similarly 10 quadrates of 1 m² were laid on the sacred forest to estimate the coverage of the prominent exotic weed species. After carrying out reconnaissance survey inside the sacred forest only 10 quadrats were selected on the periphery of the sacred forest where disturbances actually occur to compare our results. Coverage of the prominent exotic weeds was
3.2.3 Soil Fungal & Bacterial Population and their Identification: Soil bacterial population have been estimated following Waksman (1952) using the nutrient agar medium with $10^5$ dilutions. On the other hand soil fungal population was estimated adopting dilution plate method following Johnson and Curl (1972) using Martin’s Rose Bengal agar medium with $10^3$ dilutions in distilled water. Petri-dishes inoculated and then incubated at $30\pm1^\circ$C for 24 hours and $25\pm1^\circ$C for 5 days for bacteria and fungi respectively. Bacterial and fungal population were estimated by counting the developed colonies with the help of colony counter and expressed as number of colony forming units (cfu) $g^{-1}$ dry soil. Representative isolates of fungi were identified under microscope with the help of standard manuals (Domsch et al., 1980; Barnett and Hunter, 1972). Representative isolates of bacteria were identified under microscope with the help of Lindquist (2001).

3.2.4 Shannon-Wiener diversity index: The Shannon-Wiener Diversity Index, $H$, was calculated using the following equation $H = -\sum P_i(\ln P_i)$ where $P_i$ is the proportion of each species in the sample.

3.3 SOIL PHYSICOCHEMICAL ANALYSIS

For soil physic-chemical analysis a bamboo soil auger (constructed manually using a 4 cm dia bamboo) was used to collect soil samples from 0-20 cm depth. 50 random samples were collected from each plot. Undesired materials like stones and other hard substances were removed from the samples and kept inside polythene bags.
3.3.3 **Bulk Density**: Sieved soil free from other solid debris was filled to the brim of a pre-weighed 50 ml glass beaker after tapping it about 5 times. It was then weighed. The exact volume of the beaker was recorded by filling it up with water using a burette. The bulk density in gm cm$^{-3}$ was then calculated by dividing the weight of the soil with volume of the soil.

3.3.4 **Soil pH**: In 10 mg of soil, 25 ml of distilled water was added and mixed with a glass rod and allowed to stand for 1 hour before measurement. The pH was then measured electrometrically with a portable digital pH meter.

3.3.5 **Organic Carbon**: A known quantity of soil is passed through a 0.5 mm sieve, and was treated with standard $K_2Cr_2O_7$ solution in presence of concentrated $H_2SO_4$. The excess of it was determined by titrating it against a standard $FeSO_4$ solution. The procedure is based on Walkly - Black procedure. Organic matter was calculated using the following formula: % Organic matter = Organic carbon x 1.724

3.3.6 **Total Nitrogen**: The digested sample was diluted to 250 ml and made alkaline with NaOH and distilled water in micro - kjeldahl distillation apparatus following the standard procedure as described in (Baruah and Borthakur, 1997). The liberated ammonia is absorbed in boric acid and is determined by titration with a standard sulphuric acid.

3.3.7 **Ammonium N**: Nitrate nitrogen of the soil was estimated by measuring the transmittance of the yellow orange coloured solution developed at 410 nm using a
and marked. Soil texture was measured and this value was used to calculate other soil hydraulic properties i.e. wilting point and field capacity in cm$^3$ cm$^{-3}$ following the software developed by Saxton (1986). Soil pH, moisture content, temperature and water holding capacity were estimated four times in a year following the procedure described by Singhal (1996) whereas, soil organic matter was calculated after estimating the soil organic carbon following Nelson and Somm.ers (1982). Microbial biomass carbon was determined using the fumigation extraction method as described by Vance et. al. (1987).

3.3.1 Soil Texture: Soil texture was determined by mechanical analysis as described in Baruah and Borthakur (1997).

3.3.2 Water Holding Capacity: Water holding capacity is the percentage quantity of moisture held by soils in the form of films when fully saturated. It is measured as the amount of water taken up by unit weight of dry soil when immersed in water under standardized conditions. Water holding capacity in per cent was estimated using the following formula:

$$\text{Water Holding capacity (\%)} = \frac{W_2 - W_3 - W_4}{W_3 - W_1} \times 110$$

where,
- $W_1 =$ weight of brass box + filter paper.
- $W_2 =$ weight of brass box + saturated soil.
- $W_3 =$ weight of brass box + oven-dry soil.
- $W_4 =$ Amount of water retained by the filter paper.
Spectronic-20 spectrophotometer following the procedure as described by Baruah and Borthakur (1997).

3.3.8 Nitrate N: Nitrate nitrogen of the soil was estimated by measuring the transmittance of the yellow coloured solution developed at 410 nm using a Spectronic-20 spectrophotometer following the procedure as described by Greenberg et. al. (1992).

3.3.9 Soil Phosphorus: Soil phosphorus in ppm was estimated following Olsen’s method (Olsen et al. 1954). 1gm of soil was weighed and a pinch of Darco-G60 and 200 ml of 0.5M NaHCO₃ solution was added, shaken and then filtered through Whatman No. 1 filter paper. 5 ml of this extract was pipetted out and 5 ml of ammonium molybdate solution added. After shaking, 1ml of SnCl₂ solution was added and the intensity of blue colour developed measured at 660 nm through a Spectronic – 20 spectrophotometer.

The observations recorded & calculations adopted are given below:

Weight of soil taken = 1gm
Volume of 0.5M NaHCO₃ = 20 ml
First dilution = 20/1 = 20 times.
Volume of extract taken = 5 ml
Second dilution = 25/5 = 5 times
Total dilution = 20 x 5 = 100 times

3.3.10 Potassium: Potassium in ppm was determined using a flame photometer as described by Anderson and Ingram (1993). 25ml of neutral N NH₄OAc solution was added to 5gm of soil and shaken for 5 minutes and the filtered through Whatman
NO.1 filter paper. The filtrate was fed into the automizer of the flame photometer, 100 of which was set with 40 parts per million K solution and the reading was recorded.

The observations recorded & calculations adopted are given below:

- Weight of the soil = 5 gm
- Volume of the neutral \(NH_4OAc = 25\) ml
- Reading of the flame photometer of the test solution = \(K\)
- Concentration (ppm) as read from the standard curve = \(C\)
- Dilution factor = \(25/5 = 5\) times
- Available K in soil (ppm) = \(C \times 5\)

3.3.11 Soil Respiration: The method described by MacFadyen (1970) was adopted to conduct soil respiration studies. Soil respiration was measured by incubating 500 gm of soil samples with 25 ml NaOH and 25 ml water for 24 h in sealed, rectangular 1 litre plastic jars. After incubation, the alkali was titrated against 1 N HCl using phenolphthalein indicator.

3.3.12 Litter Decomposition: Nylon-bag technique as described by Gilbert and Bocock (1960) was followed for litter decomposition studies. Paddy straws were collected, air-dried and 5 g each kept in 10 cm \(\times\) 10 cm nylon bags with mesh size of 1mm. Bags stitched with nylon thread and randomly kept in 4-yr, 8-yr and reference site at 10 cm depth and further covered with paddy straws. Thirty bags per plot were kept. Three bags were recovered at regular intervals until 90% decomposition was observed. Residual materials were separated and oven-dried at 80\(^\circ\)C for 48 hours and weighed. The net organic matter decay was calculated adopting the negative exponential decay model described by Olson (1963) \(X/X_0 = \exp(-kt)\), where \(X\) is the
weight remaining at time \( t \), \( X_0 \) the initial weight, \( \exp \) is the base of natural logarithm, \( k \) is the decay rate coefficient and \( t \) is the time (year). The time required to achieve 50\% (\( t_{50} \)) and 99\% (\( t_{99} \)) decay was calculated as \( t_{50} = \frac{0.693}{k} \) and \( t_{99} = \frac{5}{k} \). Litter decomposition was determined after deducting the mass remaining in the litterbags in each month from the initial fresh weight.

3.4 **SOIL MICROBIAL BIOMASS ESTIMATIONS**

Soil microbial biomass nitrogen and phosphorus were estimated by chloroform-fumigation extraction method following Brookes et al., (1982, 1984), while microbial biomass carbon was estimated adopting the chloroform-fumigation-incubation method as described by Jenkinson and Powlson (1976) with slight modifications as reported by Srivastava and Singh (1988).

3.5 **CROP PRODUCTIVITY ANALYSIS**

Under crop productivity analysis economic yield of the cultivated crops were calculated. For this, cultivated crops from five sq. meter area were harvested, weighed, kept separately. These were then dried in a oven at 50\(^\circ\)C for four days and then reweighed. Economic yield in kg ha\(^{-1}\) yr\(^{-1}\) was then estimated following Ramakrishnan (1992).

3.6 **STATISTICAL ANALYSIS**

Tukey’s test was carried out to compare the mean values of physico-chemical properties and microbial C, N and P between 4-yr, 8-yr and reference site. Least
Standard Deviation at 0.05 level was worked out to determine the variations in different parameters studied between 4-yr, 8-yr and reference site. Linear regressions were worked out following Zar (1974) to find out the impact of certain biological factors in nutrient cycling and organic matter build up in shifting cultivated hills.

3.7 GIS Development: The map of the study area was collected from the Sonapur Circle Office, jhum sites plotted and then scanned. This map was then geo referenced using Diva GIS software. The estimated soil organic matter content was entered into the geo referenced map through excel to deliver jhum redevelopment map in 4-yr and 8-yr cycle jhum fields abandoned after cultivation.