2.1. Study area

The study had been carried out across forest covers of Gujarat, a state falling in the western part of India, lying between 20°07' – 24°41' N latitude and 68°10' – 74°28' E longitude. The total geographical area cover of the state is 1,96,024 km^2, which is 6% of total land cover of the country (Gujarat Forest statistics report, 2010-2011). At the time of field data collection, there were 26 administrative divisions called districts (Fig. 7). The study plots were laid down accordingly. Currently (2016), the state is divided into 33 districts (*http://gstfc.gujarat.gov.in*). Human population of the state has increased from 5.07 (2001) to 6.04 crore (2011) during past decade (Census of India, *http://www.census2011.co.in*). Rural and urban division of this population is nearly 50%. The human density of Gujarat state has increased from 258 per sq km in 2001 to 308 per sq km in 2011. The detail analysis of Population Census 2011 published by Govt. of India for Gujarat state reveal that population of Gujarat has increased by 19.2% in this decade compared (2001-2011) to past decade (1991-2001). Mean growth rate of human population over the past few decades is ~20.2%. Increasing population and their per capita consumption creating tremendous pressure on land use land cover (LULC) of the state.

Gujarat state experiences three distinct seasons in a year, viz. Summer (March – June), Monsoon (July – October) and Winter (November – February). Mean annual precipitation (MAP) of the state is 699 mm (ranges between 380 – 1957 mm) across 26 districts (India Meteorological Department, 1960–2010 data). The monthly minimum and maximum temperatures recorded in the state range between 2–15 °C and 38–45 °C across the state.

Figure 7: 22 selected districts (highlighted with yellow colour) of Gujarat state (INDIA) as on 2009.

Geologically, the state consists of flows of basaltic rock surrounded by a fringe of alluvium. Major soil type is alluvial (sandy, sandy loam, sandy clay loam in texture). Soil colour ranges from light brown to dark brown, yellowish red to black. Soils are little acidic, neutral to highly alkaline in nature (data from Gujarat State Agricultural Marketing Board, http://agri.gujarat.gov.in). Bulk density of the soils at different depths ranges from 1.14 g cm^{-3} at surface to 1.39 g cm^{-3} at 1.25 m. The soils are slightly acidic with pH ranging from 6.60 to 6.96 (for further description see pH measurements). The state has a forest cover of 9.76% of its total geographical area (year 2011, Forest Survey of India (FSI), http://www.fsi.org.in; Forests and Environment Department, http://www.envforguj.in; Gujarat Forest Statistics, 2010-2011). More than 90% area of this recorded forest cover is occupied by trees (Forest Survey of India (FSI), http://www.fsi.org.in; Forests and Environment Department,

http://www.envforguj.in). Both geographically and climatologically the state has distinct regions as vast saline desserts of Rann of Kutcchh district, Banni grasslands of Kutcchh district, Scrub lands of central and south Gujarat, Dry deciduous and moist deciduous forests of north and south-east Gujarat. As per the FAO classification, the forest cover is broadly categorised as tropical moist deciduous forest, tropical dry deciduous forest, tropical thorn forest and tropical littoral and swamp forest. Forests in the Gujarat state are unevenly distributed. The spread corresponds to the MAP received by each zone.

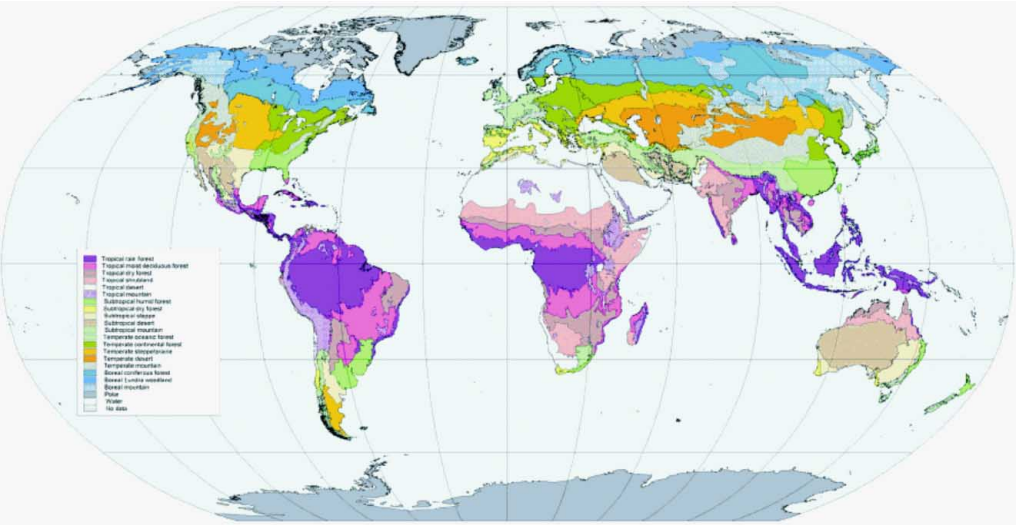

Figure 8: A world map illustrating global classification of forests by FAO. (Image source: http://www.fao.org/docrep/004/y1997e/y1997e1g.htm)

India ranks 6th position among the 12 mega biodiversity centres of the world and Gujarat is rich in floral diversity of species, habitats and ecosystems that represents nearly 13% of the floristic diversity of the country (http://gujaratflora.com/). The state has four National Parks and twenty-one Wildlife Sanctuaries which are managed by the Forest Department of the Government of Gujarat. The Biological Diversity Act, 2002 has defined three types of Biodiversity namely: Ecosystem

diversity, Species diversity and Genetic diversity. The Gujarat is covering Ecosystem diversity like different Forests Ecosystems, Desert Ecosystems, Wetland Ecosystems, Coastal & Marine Ecosystems and Agro-ecosystems. Gujarat state has about 2,200 naturally occurring plant species, which also include about 252 indigenous tree species (http://www.gsbb.in/gujarat-biodiversity.php). All the tree species, observed in the study area, have been listed (botanical name, vernacular name, family name) in appendix. According to forest statistics, *T. grandis* is the most dominating tree species with nearly 28% of the total tree population, followed by *T. crenulata* (7.9%). Both these species are timber yielding. *L. coromandelica* (4.5%), *B. monosperma* (3.7%), *A. catechu* (2.8%), and others (like *D. melanoxylon*, *A. latifolia*, *L. parviflora*, *M. indica*, *P. marsupium* etc) are the other dominating species found across the state.

Reflecting strong fundamentals and resilience, the Indian Economy posted robust growth rate of 8.4% during 2010–2011, thereby emerging as one of the fastest growing economies among the developing countries. Gujarat is one of the leading and fast developing states in India with an average compound annual growth rate (CAGR) of GDP >9% during the last decade 2001–2011 (Socio-Economic review of Gujarat state, 2011–2012). It is one of the most industrialised states of India with 60 special economic zones (SEZs) and ~200 industrial areas, contributing to about 22% of the total Indian export. Massive activities by industries like Petrochemicals, Chemicals, Pharmaceuticals, Agricultural, Food–processing, Mineral–based, Plastic, Textile, Automobiles are spread across the state. To support these activities, infrastructure (roads, buildings) activities have increased tremendously. All these are radically altering the LULC utilization. Human pressure is very much evident on the existing forest cover. The state forest department has taken up massive reforestation programs. At many parts species like *T. grandis*, *D. strictus*, *B. monosperma*, *D.*

melanoxylon, *E. globulus*, *A. catechu* have been extensively planted. Various forest management schemes like Social forestry programme, Joint forest management (JFM) are implemented by different government / non-government organisations (NGOs). The management programmes include schemes for different working plans, afforestation, and re-habilitation of degraded farmlands and creation of public awareness towards conservation of forests. More than 5 % of the total forest area in Gujarat has been afforested till date by the State Forest Department (Forests and Environment Department records). Nearly 130 years ago, *P. juliflora* was planted in few parts of the state to minimise the spread of desertification (Tewari et al., 2001). Over a period of time, this species spread widely and has become an invasive species. Nearly 700 km^2 area is occupied by this species, having a dubious distinction of ranking first in terms of distribution. Keeping the dominant impact of MAP on AGB aside, these activities also are likely to have an impact on AGB of these existing forest covers.

The study area, comprising of 22 districts of Gujarat state has been classified into four major rainfall zones (RFZ–1,2,3,4) (Fig. 10) based on MAP. Four districts falling in the north-west arid zone (<450 mm MAP) have been excluded as the vegetation cover over there was negligible. MAP (coming from 50 year meteorological dataset, 1960–2010; Indian Meteorological Department, http://www.imd.gov.in) received at each zone (1–4) is 543 (474–633), 665 (518–788), 875 (818–951), 1590 (965–1957) mm respectively (Table 3). RFZ–1 includes 4 districts (Jamnagar, Porbandar, Rajkot, Surendranagar), RFZ–2 has 6 districts (Ahmedabad, Amreli, Bhavnagar, Gandhinagar, Junagadh, Sabarkantha), RFZ–3 has 5 districts (Anand, Bharuch, Dahod, Kheda, Panchmahals) and RFZ–4 has 7 districts (Dangs, Narmada, Navsari, Surat, Tapi, Vadodara, Valsad). Collectively, these 22 districts cover ~66% of geographical area of Gujarat (Fig. 10). Geographical area

occupied by each RFZ (1–4) is 19, 22, 12, and 13% respectively (Gujarat Forest Statistics, 2010-2011).

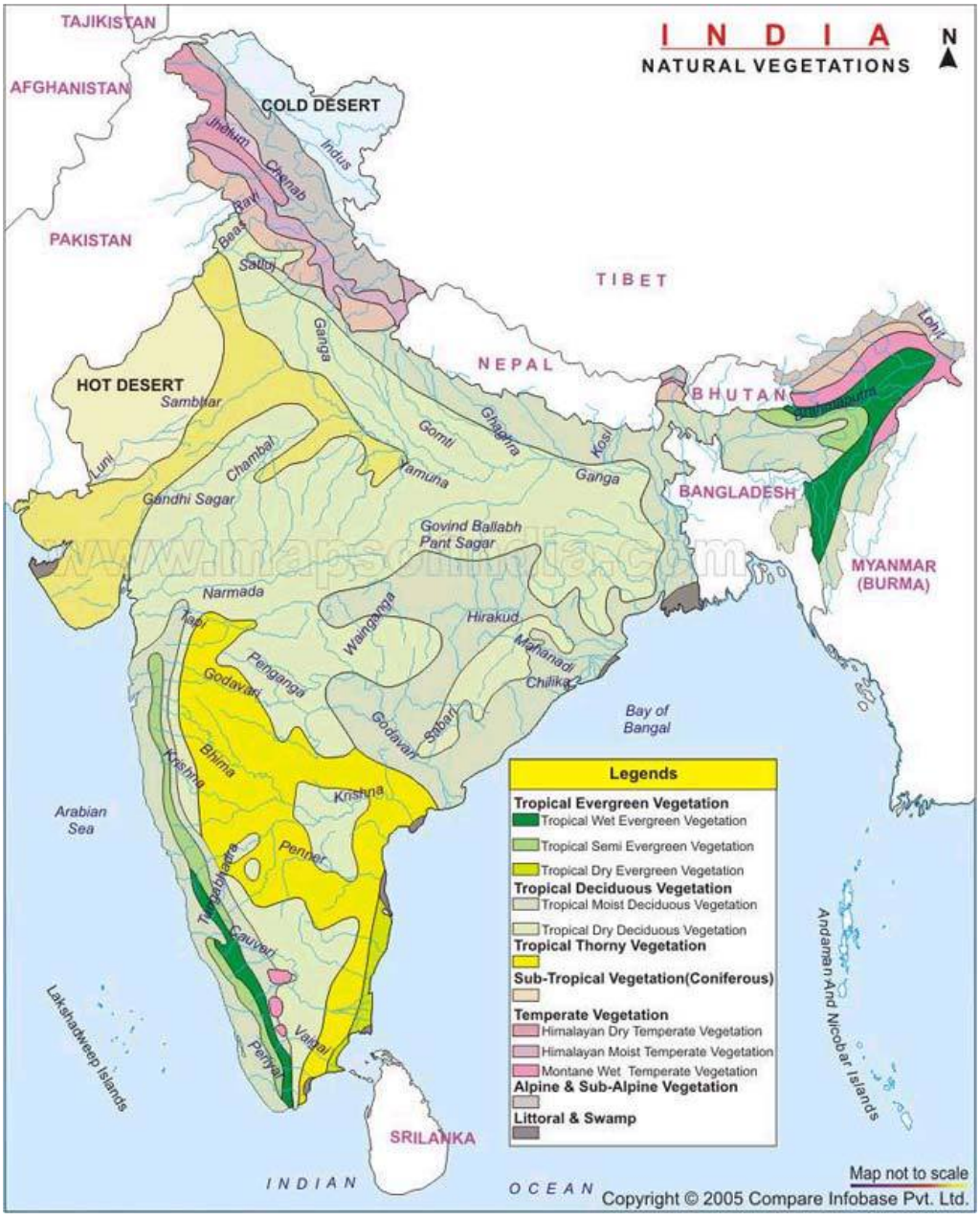

Figure 9: Figure illustrates how natural forest vegetation has been classified across India (Image source: https://environmentofearth.wordpress.com/2008/03/11/45/)

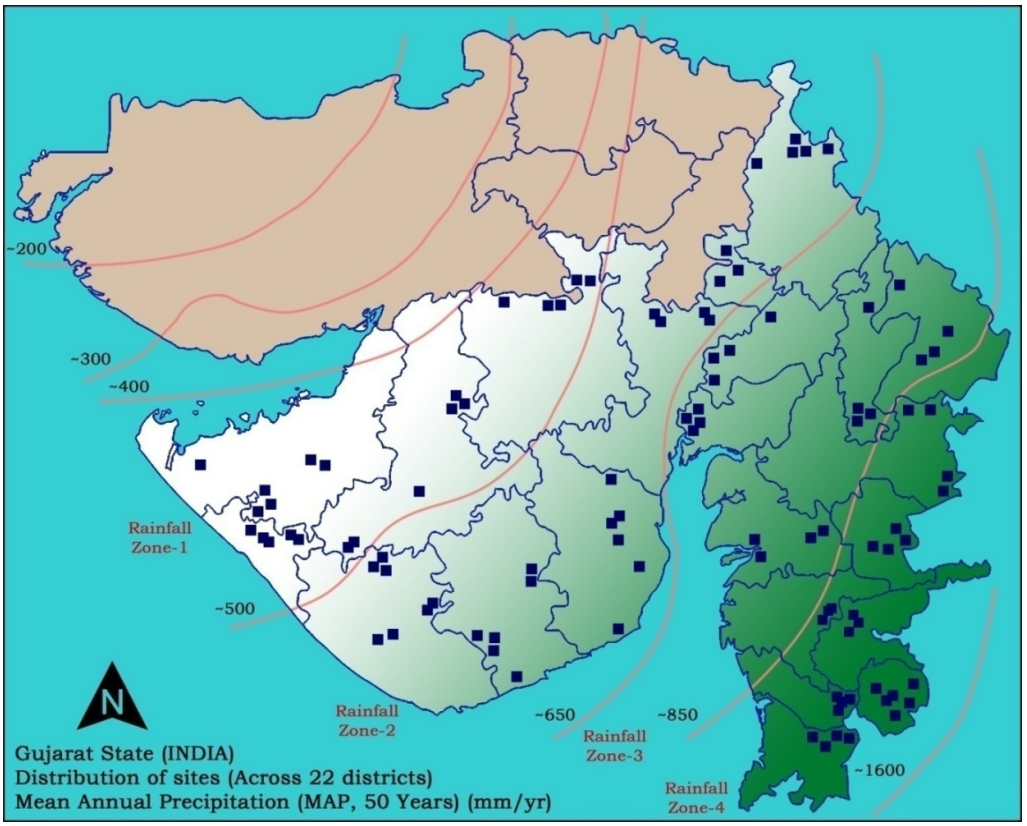

Figure 10: Map of study area (Gujarat) showing distribution of 95 plots (blue bullet points) and mean annual precipitation (MAP, mm yr^{-1}) of each RFZ (Rainfall data: Indian Meteorological Department data).

Table 3: Number of districts selected, mean annual precipitation (MAP, mm yr^{-1}) of past 50 years, number of plots (250 m^2) and quadrats (0.1 ha) laid down across all RFZs.

RFZ	No. of Districts	MAP (1960 – 2010) mm yr^{-1}	MAP (2007 – 2011) mm yr^{-1}	Plots 250 m^2	Quadrats 0.1 ha
1	4	543	968	22	88
2	6	665	969	31	124
3	5	875	1069	20	80
4	7	1590	1315	22	88

2.2. Field data collection

Following the design developed, by the research team of National Vegetation Carbon Pool Assessment project (NVCPA, IIRS, Dehradun), sample plots of 250×250 m having NDVI values (came from MODIS data of 2006–2008) ranging from 0.05 to 0.65 across 22 districts of the study area had been marked. Latitude and longitude inputs for all these points, across the state, were given by NVCPA team (IIRS, Dehradun). A total number of 95 sample plots were laid down across the state for this study (Table 3).

Field visits and data collection were carried out for 2 years (2009–2011). 2–4 days were spent at each plot during the field study. Four representative quadrats of 0.1 ha (31.62×31.62 m) had been randomly identified within each 250×250 m plot (Fig. 11). A total of 380 (95×4) 0.1 ha quadrats were laid down across the four RFZs. Each quadrat was demarcated by a measuring tape. Density and diversity of vegetation in each quadrat was noted. As per the protocol developed for the assessment of vegetation carbon pool of the entire nation, all individual trees having >10 cm GBH (girth at breast height) were included in the sampling (Field manual 2008, NVCPA project, IIRS-NRSC, ISRO Geosphere Biosphere Programme). Biophysical parameters for trees such as GBH and height and spread of canopy cover were measured. Girth (cm) over the bark of each tree was measured by a measuring tape at a height of 1.37 m from the ground level. Height (m) of the tree was measured by using Vertex Hypsometer. Spread of canopy cover was measured in 4 opposite directions from the bole surface to the peripheral end of canopy in respective directions. Many of the representative trees were marked by metal strips for long-term monitoring. Total number of primary, secondary, tertiary (or more according to the branching pattern) branches of individual trees were noted down

and their length and circumference were measured. Ultimate and penultimate twigs (5–7 cm girth) with intact leaves were collected as a part of the semi harvest method for AGB estimation. Girth of these twigs (few centimetres above base) and length were recorded to obtain their volume. Two 5×5 m (diagonal corners of 0.1 ha quadrat) and five 1×1 m (4 corners and one at the centre of 0.1 ha quadrat) quadrats were demarcated within each 0.1 ha quadrat for the sampling of shrubs and herbs respectively (Fig. 11). Procedure followed for biophysical measurements of shrubs was nearly the same as of trees. Out of five 1×1 m quadrats laid down for herbs, two quadrats were sampled for biomass calculations. All the collected plant samples were brought to the laboratory in sealed bags. After washing, the samples were oven–dried at 70°C until they showed a constant weight for further biomass estimations.

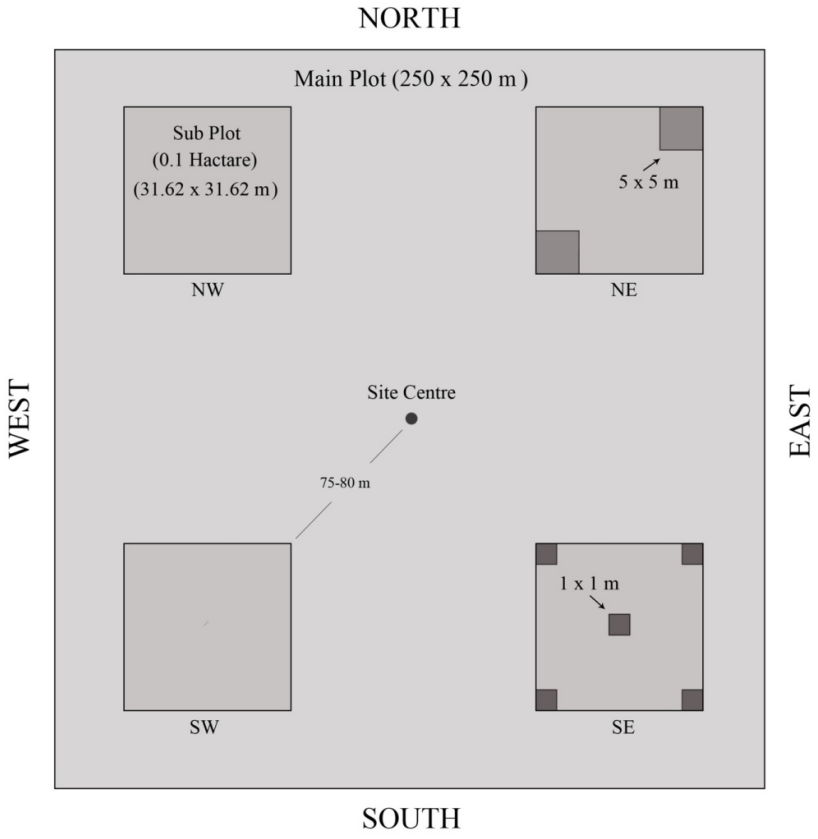

Figure 11: Plot outline (250 m^2) and four sub-plots (quadrats) of 0.1 ha (31.62 m^2).

Soil samples (up to 25 cm depth at 5 cm interval) were collected from each of the 0.1 ha quadrat. They were collected by following the trench method (Dinakaran and Krishnayya 2008). Where, samples were collected from 4 different locations randomly placed in the 0.1 ha quadrat. Each location represents the type of vegetation cover seen in the 0.1 ha quadrat. These 4 points were dug out to collect soil samples up to 25 cm depth with 5 cm increments from the surface. Based on our preliminary survey, 3 soil samples were collected sequentially for the depths 0–5, 5–10, 10–15, 15–20, and 20–25 cm. Samples coming from a particular depth of a quadrat were pooled together. This was treated as a composite sample for the soil at that particular depth. Litter debris found in soil samples, across all depths have been excluded. For each 250×250 m plot, there are 4 composite samples coming from the 4 quadrats of 0.1 ha. Total number of soil samples collected for all the plots were 95×4×5 (plots×quadrats×number of depths). Collected soil samples were brought to the laboratory in sealed covers. Samples were air dried and subsequently stored in a dry (humid free) environment until further analysis.

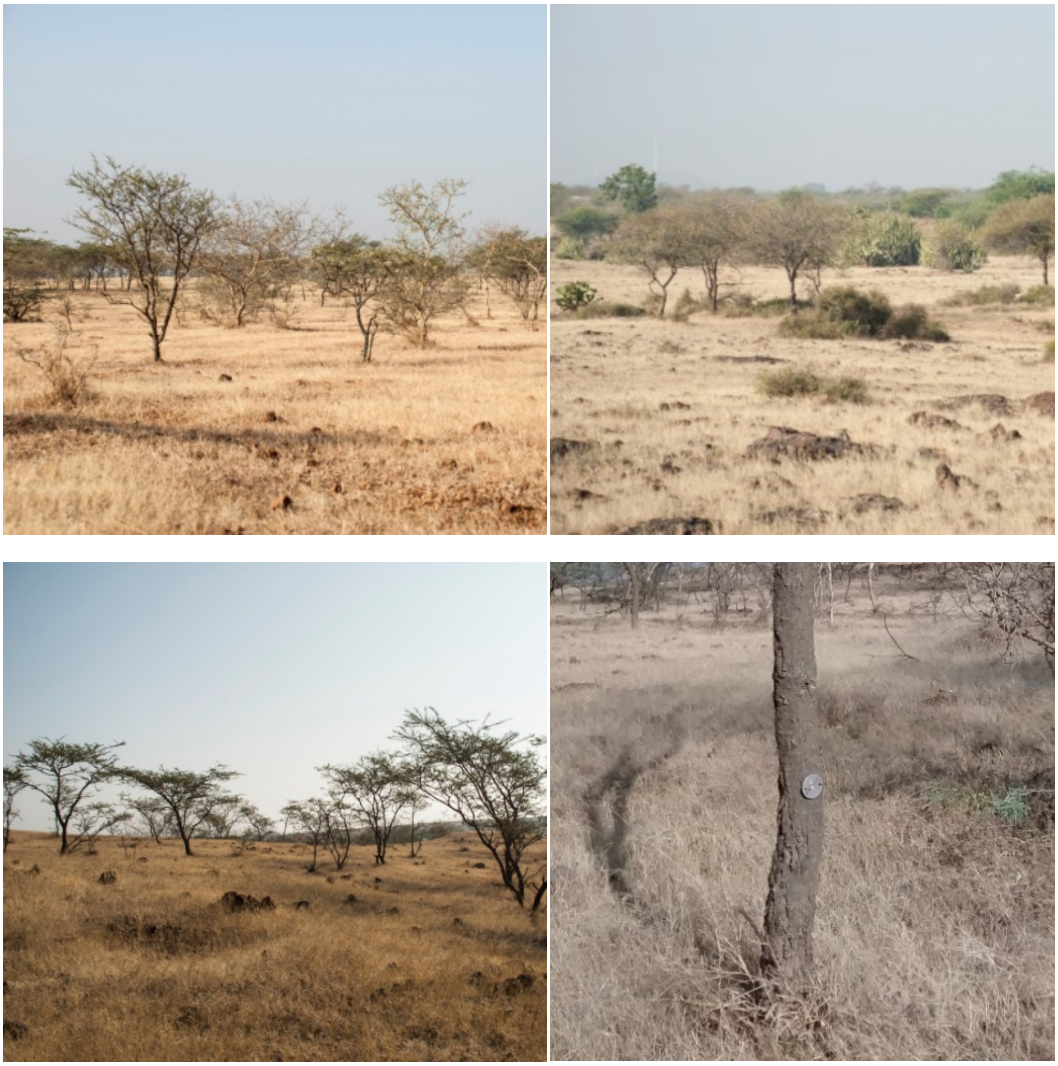

Figure 12: Pictures of study area across RFZ–1 showing vegetation spread, tagged trees.

Figure 13: Pictures of study area across RFZ–2 showing vegetation spread, tagged trees.

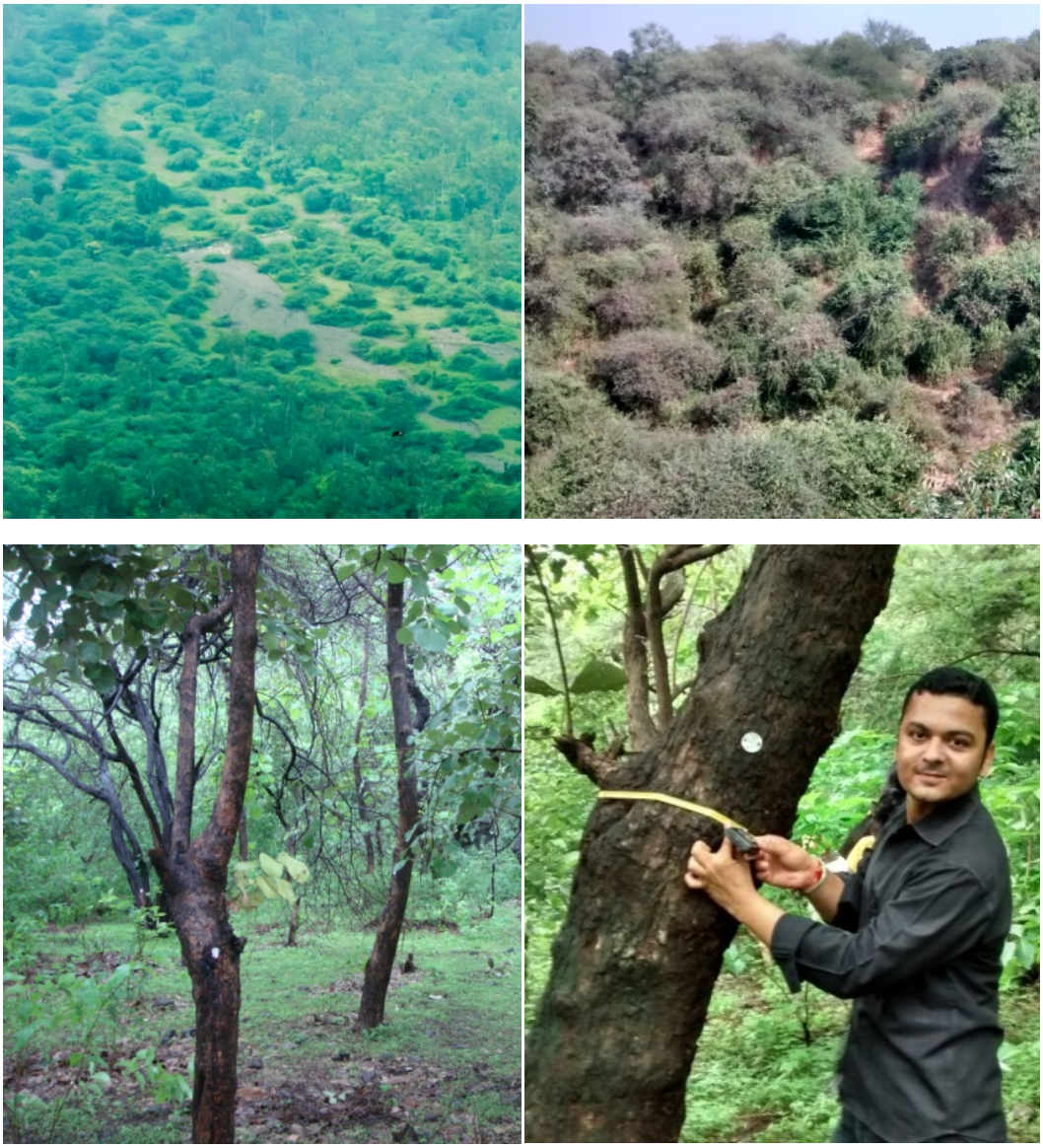

Figure 14: Pictures of study area across RFZ–3 showing vegetation spread, tagged trees.

Figure 15: Pictures of study area across RFZ–4 showing vegetation spread, tagged trees.

2.3. Above-ground biomass (AGB) calculations

Estimation of biomass was done by following semi harvest method developed in the National Vegetation Carbon Pool assessment program. In this method, the collected ultimate and penultimate twigs (5–7 cm in girth) were oven dried till constant weight obtained. Volume and biomass ratio was obtained. This ratio was utilised to convert the measured volume of a tree into AGB of the tree. Similar procedure was followed for estimating AGB of shrubs. Values of tree biomass were compared with values generated by using region and species–specific volumetric equations (developed and published by Forest Survey of India, FSI, 1996; Patil et al., 2012). Error values in these estimations were less than 10%. To minimise the uncertainty associated with AGB estimates, we included GBH, height, and specific gravity in the calculations (as suggested in the 'biomass-diameter-height regression model' by Chave et al. 2005). The equation followed was:

$$V = a + b\, D^2H$$

Where V=Volume (m^3) under bark; D=Diameter at breast height (m) over bark (calculated by using GBH data); H=Height of tree (m); a & b are statistical constants.

Diameter at breast height (DBH) for the biomass calculations was calculated by dividing GBH values with 3.14 (value of π). Region and species–specific gravity (wood density, g cm^{-3}) values for trees (provided by Indian Institute of Remote Sensing, IIRS, Dehradun, India) were used to convert volume into biomass of each tree. Pooled AGB values of trees in a quadrat were considered as AGB of the quadrat. These were extrapolated to express AGB as Mg ha^{-1}.

2.4. Leaf characteristics and litter decomposition experiment

To analyse the carbon movement in soil through the decomposition process, leaf litter decomposition experiment was carried out at one of the districts, Vadodara, the site of the litter decomposition study, which lies at 22°19′15.26″N, 73°10′47.63″E at an altitude of 37 m above mean sea level. Three protected plots in Vadodara (Botanical garden, Arboretum, Farm house) having similar vegetation covers as well as similar soil characteristics were identified for the litter decomposition study. The selected species are trees (*Tectona grandis* L., teak; *Madhuca indica* J.F.Gmel., mahua and *Mangifera indica* L., mango), perennial grass (*Dendrocalamus strictus* Nees., bamboo), shrubs (*Hibiscus rosa-sinensis* L., hibiscus; *Datura stramonium* L., datura and *Bougainvillaea glabra* L., bougainvillea) and herbs (*Cyperus rotundus* L., cyperus; *Spinacia oleracea* L., spinach and *Catharanthus roseus* L., vinca). Leaves of chosen species have larger differences in leaf area, and in the proportion of structural carbohydrates. These measurements were done in newly senesced leaves and leaves on the verge of senescence. Contents of senescent leaves indicate quantities of biochemicals available to the microbial organisms in the soil.

Leaves (newly senesced and on the verge of senescence) of selected plant species were air dried in the laboratory. We placed 25 g of dried leaf material (intact or broken) of each species in a standard perforated litter bag (1 mm mesh size). These bags were placed in the soils at the three points of similar climate. Normally soil biological activity (of micro flora, micro fauna and other microbes) is more in the top layers and gets decreased as the depth increases mainly because of variations in the availability of easily decomposable material. Litterbags were kept at two depths (0–5 cm and 15–20 cm) to find out whether the soil biological activity shows any difference (up to 25 cm depth) in affecting litter decomposition. Sets (n=3) of each

species were kept at each site, at both the depths (3×3×2). Each set has triplicate samples/bags. Total number of litter bags placed for the 10 selected species were 540 (10×3×3×3×2). A uniform distance was maintained between the sets at each depth to avoid disturbance during sampling. The litter bag experiment started in the month of June (2009). Soil moisture content is the lowest in this month and we assumed that soil biological activity would be minimal. The experiment was terminated when the data showed that half of the species had <20% material left over. At 90 days one set (10×3×3×2) of litter bags was removed and brought to the laboratory. Bags were cleaned by removing adhered soil carefully (by using a brush and magnifying glass). Remaining litter was weighed after drying. This step was repeated at 180 days and at 270 days. Remaining litter (at 90 days, 180 days and 270 days) was subjected to chemical analysis (structural and non structural carbohydrate constituents). Soil samples were collected from the three sites before placing the litter bags in the field and also after picking up decomposed samples (at 90, 180 and 270 days). These samples were analyzed for MBC by the fumigation method (Witt et al. 2000) explained in detail in subsequent section.

Leaf area was measured by a leaf area meter (CI-203 Area Meter, CID-Bioscience). Dry weights of leaves were measured and specific leaf area (SLA) was calculated (leaf area/leaf dry weight). Analytical precisions for leaf area and leaf weights are ±0.001 cm^2 and ±0.1 mg respectively. Structural carbohydrate (lignin and holocellulose [cellulose+hemicellulose]) and non structural carbohydrate constituents were estimated by following the method of Booker et al. (1996). In this method, pre-weighed dried and powdered leaf samples were extracted with 1 ml of 50% methanol for three times. Subsequently the residues were rinsed with methanol:chloroform:water (53:26:21) mixture for two times. It was followed by another rinsing with phenol:acetic acid:water (51:25:24) mixture. Later the residues

were washed with ethanol. All the remained residues were carefully collected in pre-weighed crucibles and were oven dried at 70^{o}C for overnight. Difference in the weight from initial weight is considered as non structural components. The residue (left out after solvent extraction) was treated with 5% H_2SO_4, kept in boiling water for 1 hour, and then centrifuged at 4500 rpm for 10 minutes. The resulting pellet was suspended and washed two times sequentially with hot water, 95% ethanol and acetone. Samples were centrifuged and the pellets formed were dried. After oven drying at 70^{o}C, the pellet was mixed with 72% H_2SO_4, incubated for 2 hour at 20^{o}C, and then diluted with distilled water. These samples were kept in boiling water for 2 hours. The solution was filtered through pre-weighed Whatman no.1 filter paper. The residue on the filter paper was oven dried at 70^{o}C. Difference in weights is considered as lignin content. Cellulose+hemicelluloses (Holocellulose) were calculated by subtracting the non structural components and lignin content from the total amount of sample. Lignocellulose index (LCI, lignin/lignin+cellulose, Moorhead and Sinsabaugh, 2006) was calculated for the litter remaining in the litter bags (at 0, 90, 180 and 270 days) to look at the degradability of the material (LCI value towards 0 indicates high decomposition and towards 1 indicates less decomposition of the material).

2.5. Soil analysis

2.5.1. Measurements of soil characteristics

Soil characteristics measured are i) soil pH and ii) particle size distribution. To check the differences of these soil characteristics up to 25 cm depth across the four RFZs, analysis have been done for composite soil samples of 0–25 cm depth in soil. Soil pH was measured at a soil:water ratio of 1:5 (weight/volume) in a digital pH meter ANALAB (PHCAL) (2010-11/134). Analytical precision for soil pH was 0.01. Variations in the proportion of particle size were used to define soil texture. Particle

size separation of the soil samples was done by pipette method (Kilmer and Alexander, 1949). Briefly, the air dried samples were first screened to remove all the plant materials and roots. Then the soil sample was passed through a 2 mm sieve, weighed and transferred into 250 ml beaker. About 50 ml of distilled water and 5 ml of 30% H_2O_2 were added into the beaker for removing the organic matter. Samples were stirred continuously. This step was repeated up to the cessation of frothing. Beakers were kept on a hot plate at 70^0C for 30-45 minutes and ensured that organic matter was completely removed from the sample by adding extra H_2O_2 if necessary. The supernatant was decanted carefully. After that, 100 ml of distilled water along with 10 ml of calgon solution (Sodium hexameta phosphate (35.7 g) and sodium carbonate (7.94 g) dissolved in 1000 ml of distilled water) was added to the samples and kept for overnight. The samples were filtered through 53 µm sieve and the leftovers were washed with water. The particles on the 53 µm sieve were transferred into petri plates covered with aluminium foil and it was oven dried at 70^0C overnight and weighed. The filtrate (particles passed through the 53 µm sieve) was transferred into a 1000 ml measuring cylinder and filled up with distilled water to the 1 liter mark. The suspension was stirred vigorously for about half a minute, so that the particles were evenly distributed throughout the cylinder. The 2 µm particles (clay) are pipetted at a 10 cm depth after a predetermined settling time (usually six to six and half hours). Pipetted sample was transferred into a pre-weighed aluminium foil covered petriplate. The petriplate was oven dried at 70^0C overnight and weighed. The percentage sand, silt and clay was calculated by the following equations

Sand (%) = weight in grams of fractions on 53 µm sieve / Total dry weight of sample × 100

Silt (%) is obtained by subtracting the sum of the percentage of sand and clay from 100.

Clay (%) = A-B / 10 × 100

Where, A= weight in grams of pipetted fraction, B= weight correct for dispersing agent (in grams), 10 is the initial weight of sample.

Soil bulk density was calculated by using an online soil calculator (CENTURY, model 4.0, http://www.nrel.colostate.edu/projects/century/) by incorporating soil particle size data.

2.5.2. Soil organic carbon (SOC) estimation

SOC analysis was performed for collected soil samples from all the five depths separately. Estimation of SOC content in the collected soil samples (from RFZs 1–4 sites and litter decomposition experiment) had been done by wet oxidation method (Walkley and Black, 1934). This method has long been a standard method for measuring organic carbon in forest soil samples. Prior to wet oxidation the air dried soil samples were passed through 2 mm sieve to remove any plant materials and grounded into fine powder with the help of mortar and pestle. A known amount (0.5 g) of soil was taken in a 250 ml conical flask and it was treated with 10 ml of 0.167M potassium dichromate ($K_2Cr_2O_7$) and 20 ml of concentrated sulphuric acid (H_2SO_4). The mixture was swirled gently and allowed to stand for 30 minutes in a dark place. Subsequently 100 ml of distilled water was added into the mixture. Afterwards, 10 ml of orthophosphoric acid (85%, H_3PO_4), and 0.2 g of sodium fluoride (NaF) was added into the mixture. Then few drops of ferroin indicator (1,10 phenanthroline (3.71g) and ferrous sulphate (1.74g, $FeSO_4 . 7H_2O$) dissolved in 250 ml of distilled water) were added into the mixture. Finally the mixture was titrated against 0.5 M ferrous ammonium sulphate (FAS) ($Fe (NH_4)_2 (SO_4) . 6H_2O$) solution. During each titration, the colour of the solution was yellow-orange to light green at

first and changed to turbid grey and finally to a burgundy colour at the end point. The percentage of easily oxidizable organic carbon was calculated as follows:

$$\text{Easily oxidizable organic carbon (\%)} = \frac{(B\text{-}S) \times M \text{ of FAS} \times 12 \times 100}{\text{Gram of soil} \times 4000}$$

Where B is ml of ferrous ammonium sulphate solution used to titrate the blank, S is ml of ferrous ammonium sulphate solution used to titrate sample, M is the molar of ferrous ammonium sulphate solution used and 12/4000 is miliequivalent weight of carbon in grams.

A correction factor (1.32) has been applied to correct the incomplete oxidation of the sample (Lettens et al., 2007). SOC content of each depth (g C m^{-2}) was calculated as (Schlesinger, 1990).

$$\text{SOC content (g C m}^{-2}) = \sum_{i=1}^{k} OC_i \times BD_i \times D_i \times 10{,}000$$

Where OCi is the organic carbon content (%) in layer i, BDi is the bulk density (g cm^{-3}) of layer i, Di is the thickness (m) of the layer i and 10,000 is the factor used to convert the SOC value from 1 cm^2 to 1 m^2 (100 × 100 cm^2 =1 m^2). Replicates of each sample were estimated and mean values were calculated. Thus SOC carbon content finally converted into mega gram per hectare (Mg C ha^{-1}) for each depth (from surface up to 5, 10, 15, 20, 25 cm) by again multiplying with 10,000 (100 × 100 m^2 =1 hectare). For better understanding of the behaviour of deeper SOC with respect to AGB values, we extrapolated SOC_{25} (SOC values up to 25 cm depth) to

SOC_{100} (SOC content up to 100 cm depth in soil) by using the following equations (Jobbagy & Jackson, 2000, 2001; Yang et al., 2011).

i) $Y = 1 - \beta^{d}$

ii) $SOC_{100} = [(1 - \beta^{100}) / (1 - \beta^{25})] * [SOC_{25}]$

Where, *Y*=cumulative proportion of the soil carbon pool from the soil surface to depth *d* (cm); β=the relative rate of decrease in the soil carbon pool with soil depth; SOC_{100}=the soil carbon pool in the upper 100 cm (Mg ha^{-1}); SOC_{25}=the soil carbon pool in this study up to 25 cm depth (Mg ha^{-1}). β value was calculated by equation-1 based on data generated in this study. β value differed for each sample plot (depending on the rate of decrease in SOC from one soil depth to another). Our β values ranged between 0.83–0.99.

2.5.3. Soil microbial biomass carbon (MBC) estimation

Soil microbial biomass carbon (MBC) estimation was done for the replicates (n=3) of collected soil samples (from RFZs 1–4 sites and litter decomposition experiment) by chloroform fumigation extraction method (Witt et al. 2000) to evaluate changes in belowground microbial activity across the soil profile. The method estimates organic carbon coming from active soil microbes. Obtained values indicate changes in the activity of soil microbial community across the soil profile. In this method, 20 g of dried soil samples were taken in 250 ml Schott bottles. Nearly 10 ml of distilled water was added for moistening and triggering microbial activity. Microbial carbon content was extracted immediately in one set of bottles. To these bottles 0.5 M K_2SO_4 was added. These were placed on a shaker for an hour. The solution was filtered and carbon content of the filtrate was estimated by wet oxidation method (Walkley and Black, 1934) described in previous section. To another set of bottles 3

ml of ethanol free chloroform was added and sealed. After incubating for 24 hours in darkness, the bottles were opened to evaporate chloroform. 0.5 M K_2SO_4 was added as mentioned earlier and carbon content was estimated by wet oxidation method (Walkley and Black, 1934) described in previous section. MBC was calculated as the difference in organic carbon content between fumigated (C_f) and unfumigated soils (C_{uf}).

$$MBC\ (mg\ kg^{-1}) = C_f - C_{uf}$$

2.6. Data analysis

One way ANOVA had been carried out using SPSS software to check whether the differences seen in the measured parameters (tree density, species diversity, AGB, SOC, MBC) across RFZs 1–4 are significant or not. Simple regression analysis was carried out to describe the pattern of relationship (linear or logarithmic) between the measured variables (AGB, SOC, and MBC). Coefficient of correlation (Pearson´s) was calculated to look at the relationships between variables across all the 22 districts and across all the 4 RFZs. Litter decomposition constants, *k* values ($g\ g^{-1}\ yr^{-1}$) were calculated by using exponential decay equation (Olson, 1963). Linear regression analysis was performed between measured structural and nonstructural carbohydrates of leaves and their respective *k* values.

$$k_t = (1/t)\ \ln\ (X_o/X_t)$$

Where, t denotes unit time (90, 180, 270 days), k_t is the decomposition rate when time is t, X_o is the weight of litter at initial time, X_t is the mass remaining when time is t.