CHAPTER 3
METHODOLOGY

Methodology overview

This study estimates the CO_2 emissions from root respiration and microbial respiration in a dry dipterocarp forest at the King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Ratchaburi Province by using an automated-chamber. Both of the soil respirations are important along with the carbon cycle in the terrestrial ecosystem, especially in the forest ecosystem. The trenching method was used to separate R_m and R_b. This method has been widely used to partition soil respiration (Bowden, *et al.*, 1993; Kelting, *et al.*, 1998; Epron, *et al.*, 1999; Hanson, *et al.*, 2000; Bond-Lamberty, *et al.*, 2004; Jiang, *et al.*, 2005). It has advantage that it has relatively less disturbance to remaining trees than other methods, and the relative ease of the experimental implementation. Moreover, the biotic and abiotic factors were observed for explanatory correlation of CO_2 production, diurnal and seasonal variation from both of soil respirations such as plant growth, litter production and decomposition, soil property, soil temperature and moisture, precipitation (Figure 3.1).

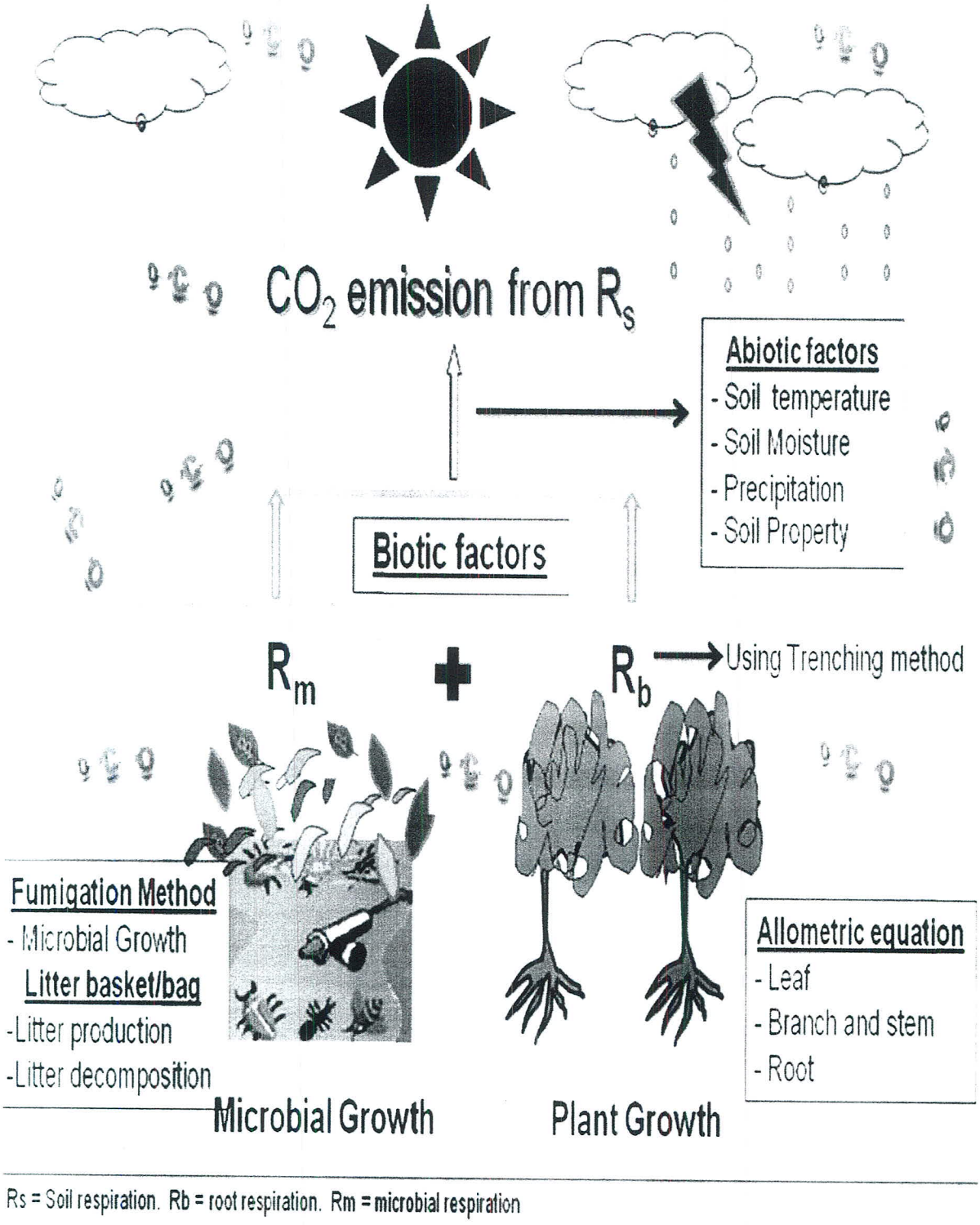

Figure 3.1 Soil respiration and related parameters that are included in the current dissertation study.

3.1 Site description

The experiment was performed in the dry dipterocarp forest at the King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Ban Ranbua, Tambon Rangbua, Chombung District, Ratchaburi Province (13° 35' 13.3" N, 99° 30' 3.9" E, elevation of 110 m above mean sea level) (Figure 3.2). The total area of the dipterocarp forest used in this study covers 187.2 ha. This area has been kept as the dipterocarp forest for approximately (more than) 50 years (Figure 3.3). Communities around this forest have utilized it for energy (wood and charcoal), timber, and other products such as mushrooms and local hunting. As a result, most of the trees are those from the re-generated ones after being cleared occasionally by villagers.

In 2011, aboveground trees were 6-7 years old with the average height and diameter of 5.97 m and 6.59 cm, respectively. Since 2005, the area has been preserved, and cutting of trees is no longer permitted. According to Phiancharoen *et al.*, (2008) there are about 77 tree species found in this study area. The main species are *Dipterocarpus intricatus*, *D. obtusifolius*, *D. tuberculatus*, *Shorea obtuse and S. siamensis* (Dipterocarpaceae). This forest ecosystem is unique, while the aboveground biomass is periodically cut by villagers, the belowground biomass stays intact. Therefore, the aboveground to belowground biomass ratio for most of dominant species is <1.

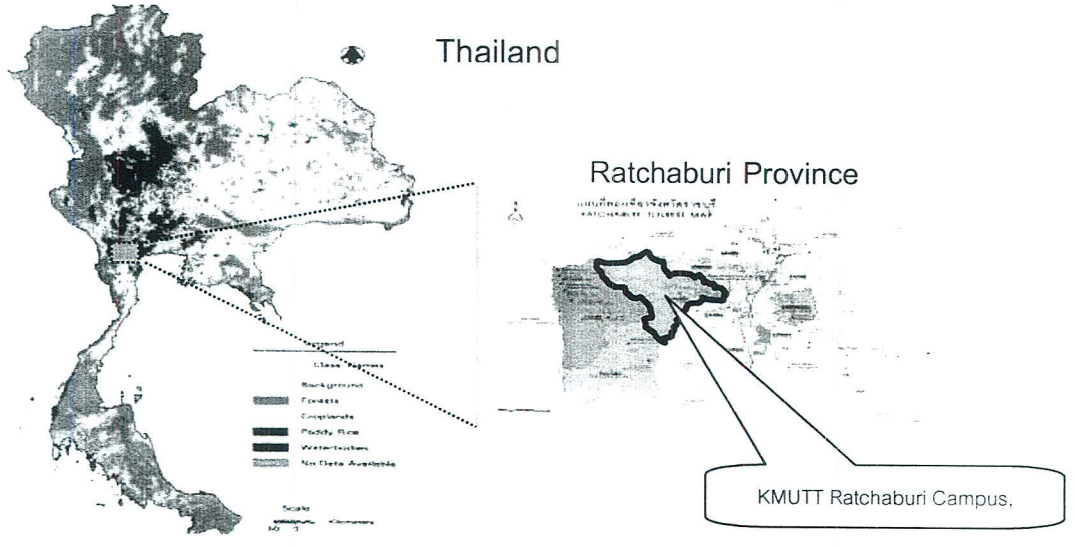

Figure 3.2 Location of the study site at KMUTT Ratchaburi Campus, Rang Bour Subdistric, Chom Bueng District, Ratchaburi Province in the west of Thailand (Sanwangsri, 2011).

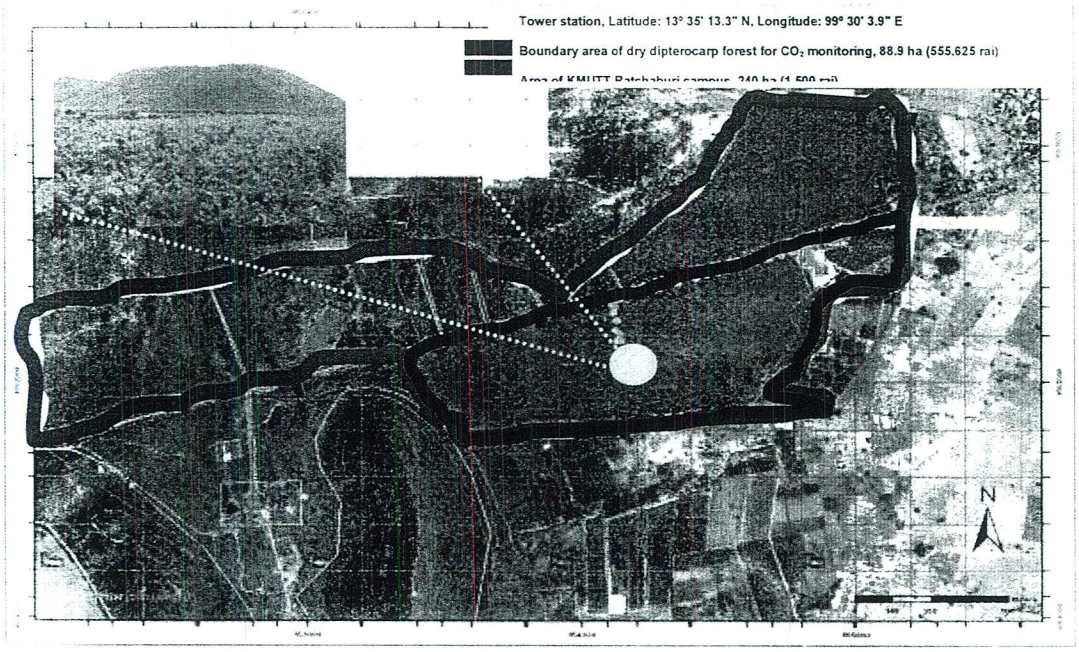

Figure 3.3 Ratchaburi flux observation site in dry dipterocarp forest ecosystem

3.2 Preparation of microbial and root respiration study plots

There were three sampling plots for each microbial and root respiration treatment. To separate the roots from microbial respiration, the trenching method (making a trench around a patch of soil to prevent root penetration) was used. This method has been widely used to partition soil respiration into R_m and R_b (Bowden, *et al.*, 1993; Kelting, *et al.*, 1998; Epron, *et al.*, 1999; Hanson, *et al.*, 2000; Bond-Lamberty, *et al.*, 2004; Jiang, *et al.*, 2005). This method has been promoted in the last few years due to less disturbance to remaining trees than other methods, the maintenance of most field conditions (daily and seasonal soil temperature evolution, rain events, litterfall, soil texture, etc.), and the relative ease of the experimental implementation. In addition, this method can block carbon supply from trees to the soil so as to estimate relative contributions of autotrophic (root) and heterotrophic (microbe) respiration to the total soil respiration. For this method, roots were severed by digging a trench to a depth of 100 cm (20-90 cm below the rooting depth) around the plots (1 x 1 m) and with a protection belt of 20 cm. The trenches were backfilled after lining with corrugated fiber glass sheets and aluminum nets to prevent root ingrowths (Luo & Zhou, 2006).

The trenching method provides reliable and consistent estimates of both soil CO_2 efflux components (Bond-Lamberty, *et al.*, 2004; Hanson, *et al.*, 2000; Jiang, *et al.*, 2005). Trenched plots are an inexpensive and general method for separating the R_s flux into its components. Moreover, Bond-Lamberty, *et al.* (2010) found that no significant changes in soil temperature or moisture were detected after trenching in a boreal black spruce plantation. However, one aspect needed to be considered when using this method is the timing of the measurement. It is difficult to determine because the rates of root decomposition in each site are different. For example, time required for the plot to reach steady state after being disturbed by trenching varies from one to other ecosystems, ranging from 4 months in a boreal black spruce forests to 9 months after trenching in 80-years mixed hardwood forest at Massachusetts (Ewel and Cropper, 1987; Bowden, *et al.*, 1993; Kelting, *et al.*, 1998; Bond-Lamberty, *et al.*, 2004).

For this experiment, a trenching plot was prepared on September 10, 2008, the fiber glass sheets and aluminum nets were installed on November 6, 2007 (Figure 3.4). The measurements of *in situ* CO_2 emission from microbe respiration were started on February 2008. The time allowed for dead root decomposition was 5 months. Bond-Lamberty, *et al.* (2004) studied root decay by using a trenching plot in a black spruce plantation in the United States. They found that trenched areas had significantly lower R_s than the control plots (no trenching), with differences appearing ~ 100 days after trenching. This is considered sufficiently longer than 100 days after trenching, so that CO_2 emissions from the plots with trenching are considered to be released solely from microbial respiration. After making a trench, soil samples were taken regularly for inspection. Living roots started to decline significantly after 2 months. Later on, no living root was observed.

Before (September 10, 2007) **After** (November 6, 2007)

Figure 3.4 Trenching areas for measuring CO_2 flux from microbial respiration

3.3 *In Situ* gases flux measurements and instrument setup

3.3.1 Soil respiration using closed-automated chamber

Soil respiration was measured by using the automated-chamber technique (Figure 3.5). The measuring system consisted of a chamber operation system and data-storing unit (data logger). The chamber for soil respiration consists of 2 parts: the cover and the base. The cover is made of acrylic of 0.3 m width x 0.3 m length x 0.3 m. height, and the base is made of stainless steel with dimensions of 0.3 m (width) x 0.3 m (length) x 0.15 m (height). The base was permanently inserted into the soil, where gas sampling was taken. To monitor the net CO_2 exchange through soil respiration and to prevent the effects of photosynthesis, the opaque chamber was used and installed in an area without plants. The automated-chambers were linked to a digital thermometer and data logger to collect soil and chamber temperature.

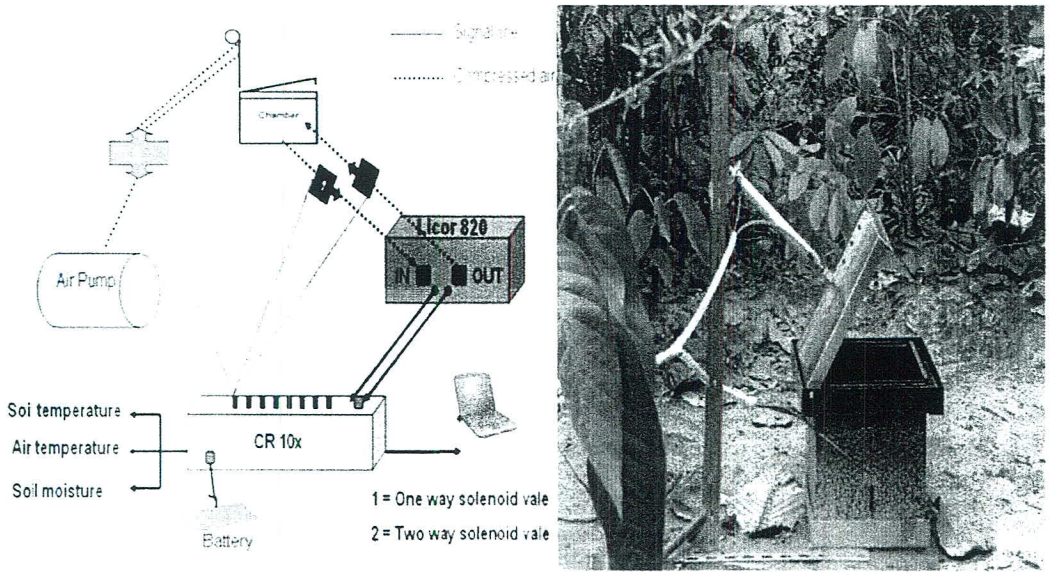

Figure 3.5 Schematic diagram of CO_2 flux measurement setting and picture of automated-chamber used in this study.

The chambers were closed and opened by a hydraulic system which was controlled by a program on a datalogger (CR10X, Campbell Scientific, Logan, Utah, USA) and a two-way solenoid valve. The details of the operation and the measurement system are described in Table 3.1. At any given time, the CR10X commands the two-way solenoid valves to close the chamber, and another one-way solenoid valve is set open. Then, the air sample inside the chamber is pumped (1.0 l/min) into the measurement unit, where CO_2 concentration is determined by a NDIR detector in Licor 820 analyzer (Licor Corporation, Lincoln, Nebraska, USA). The data generated is stored in the datalogger. Additionally, after analysis of CO_2 concentrations, the air is channeled to another one-way solenoid valve to be returned to the same chamber as shown in Figures 3.6. Thus, soil respiration is repeatedly measured once per hour for every chamber. In the present study, there are 6 chambers (3 for control and 3 for microbial respiration). The chamber temperatures are also measured in parallel.

Table 3.1 Summary of chamber operating and CO_2 measurement system

Time (min)	Chamber action	Operation
1-15	All open	Pump drawing the ambient air for background CO_2 measurement
16-18	All open	Pump drawing standard gas from CO_2 tank
19-25	Chamber 1 is closed; the rest are open.	Air is drawn from headspace of Chamber 1 for CO_2 concentration measurement
26-32	Chamber 2 is closed; the rest are open.	CO_2 measurement from Chamber 2
33-39	Chamber 3 is closed; the rest are open.	CO_2 measurement from Chamber 3
40-46	Chamber 4 is closed; the rest are open.	CO_2 measurement from Chamber 4
47-53	Chamber 5 is closed; the rest are open.	CO_2 measurement from Chamber 5
54-60	Chamber 6 is closed; the rest are open.	CO_2 measurement from Chamber 6. New cycle starts from the 1st minute again.

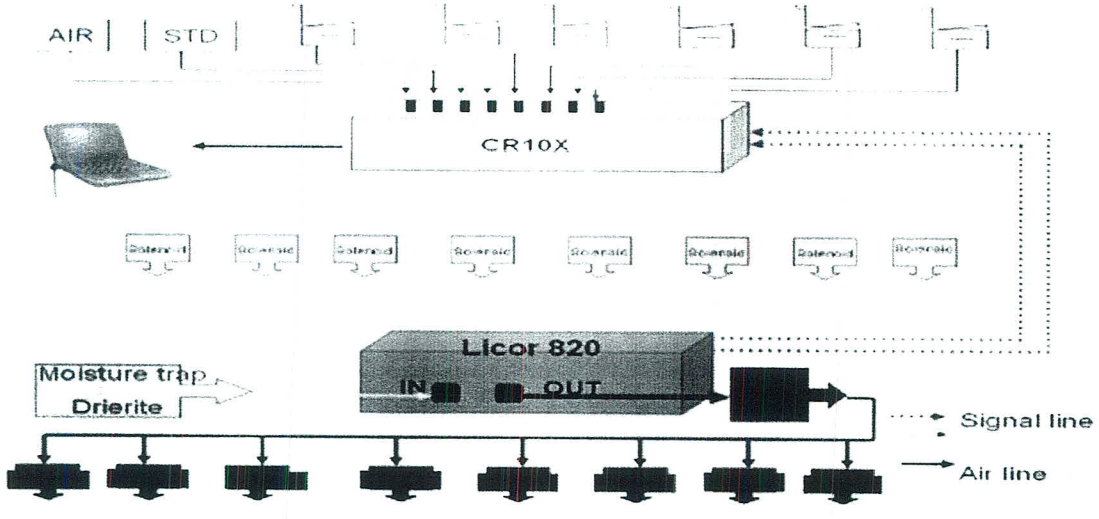

Figure 3.6 Schematic diagram of CO_2 flux measurement setting

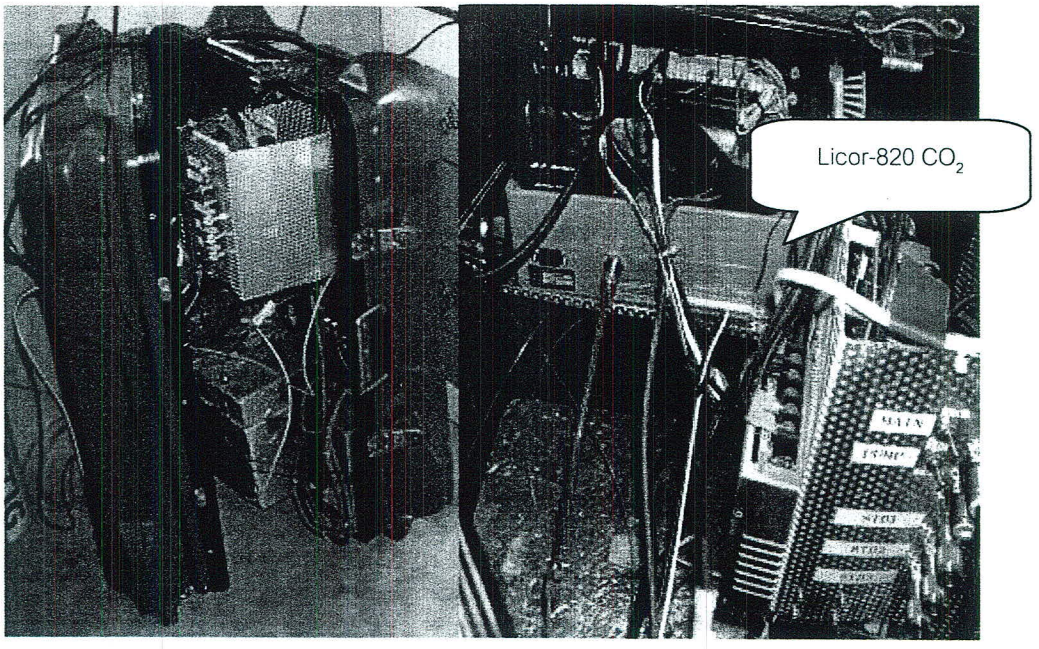

Figure 3.7 The suitcase-size box to determination of CO_2

3.3.2 Flux calculation

Soil respiration rates (CO_2 flux) were calculated using the linear portion of the gas concentration change during the chamber closing period as mentioned above. Only data showing a significant correlation of the measurement points (Pearson correlation coefficient of concentration data versus time was significantly >0 at the $p \leq 0.05$) were taken into account to calculate the CO_2 flux. CO_2 fluxes are expressed in terms of mass per unit area per unit of time (mg CO_2 m^{-2} h^{-1}). Firstly, the mixing ration or concentration obtained from the chamber headspace is converted to a mass or molecular basis using the ideal gas law, thus depending on temperature and pressure of the enclosed air as shown in equation 3.1:

$$Ci = \frac{qiMP}{RT} \qquad \textbf{(Eq. 3.1)}$$

Where: Ci = mass / volume concentration (g CO_2 m^{-3})

q_i = volume / volume concentration (m^3 / m^3)

M = molecular weight of CO_2 (44 g mol^{-1})

P = atmospheric pressure (1 atm)

R = gas constant (8.2058×10^{-5} m^3. atm $K^{-1}mol^{-1}$)

T = average temperature inside the chamber (K)

Normally, linear and non-linear models have been proposed to describe the relationship between trace gas concentration and time. If the enclosure dimensions, development period, and measurement protocol are suitably matched to the rate of trace gas exchanges and site characteristics, a linear model may be adopted. Thus, in this study, CO_2 flux was calculated using the linear portion of the gas concentration change over time following Equation 3.2:

$$F = \frac{dc_i}{dt}\frac{V}{A} \qquad \textbf{(Eq. 3.2)}$$

Where: F = flux on the aerial basis (g m^{-2} min^{-1})

V = volume of the chamber's headspace (m^3)

A = area of soil enclosed by the chamber (m^2)

dc_i / dt = rate constants of CO_2 concentration increase with time (g CO_2 m^{-3} min^{-1})

3.4 *In Situ* gases concentration measurements and instrument setup on soil profiles

3.4.1 Gas sampling by using closed chamber

For primary observation of soil respiration throughout the profile, CO_2 concentration from 5 layers: 0, 15-35, 70-90, 135-155, and 215-235 cm (at level 0 cm was used automated-close data) were measured. In February, 2011, the same layers of five soil profiles were measured the concentration of CO_2 every a month until January 2012 at every first week (during 1 to 7 day), using static chamber (Figure 3.9). The static chambers were made from stainless steel and acrylic. It had dimensions of 0.3 m width x 0.3 m length x 0.3 m height and the stainless steel base had dimensions of 0.3 m width x 0.3 m length x 0.15 m height (Figure 3.8).

When staring with the measurements, the lid is used to make a closed system. Gas is extracted by sticking an injector needle through the rubber septum, and collecting it by means of a gas-tight syringe with a stopper or a vacuum vial every minute around 5 min after enclosure. Gas sample was collected with a 25 ml-syringe and immediately kept in

vacuum. Vacuum vials can be made by manual method. Analyze the CO_2 concentration with GC as described below.

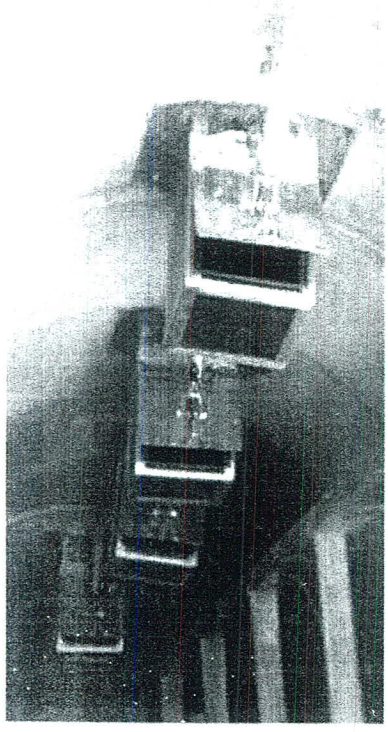

Figure 3.8 Installation of CO_2 profile measurements

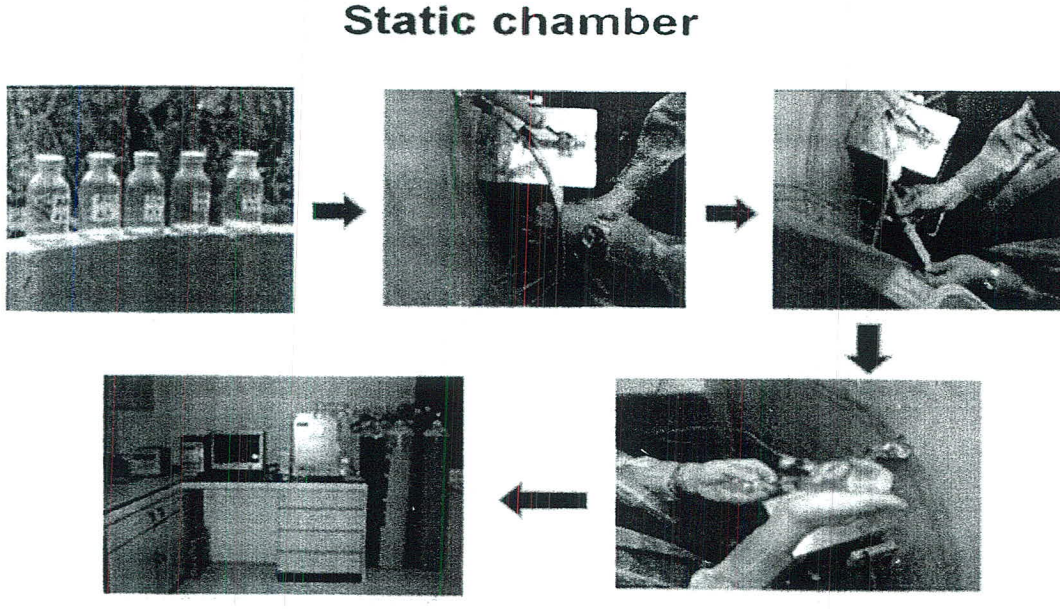

Figure 3.9 The collection of soil respiration in soil profiles by using static chamber method

3.4.2 Determination of CO_2 concentrations by Gas Chromatography

Concentrations of CO_2 from different soil layers were measured using a gas chromatograph. Carbon dioxide was analyzed with a Shimadzu Gas Chromatograph equipped with a FID detector (Porapak Q column) and methanizer (GC-FID). The GC operating conditions were as follows: column temperature at 100 °C; injection temperature at 120 °C; detector temperature at 150 °C; carrier gas include air and purify helium. The flow rate carrier of gases was 27 mL/min. A sample of chromatogram of gas components is showed in Figure 3.10.

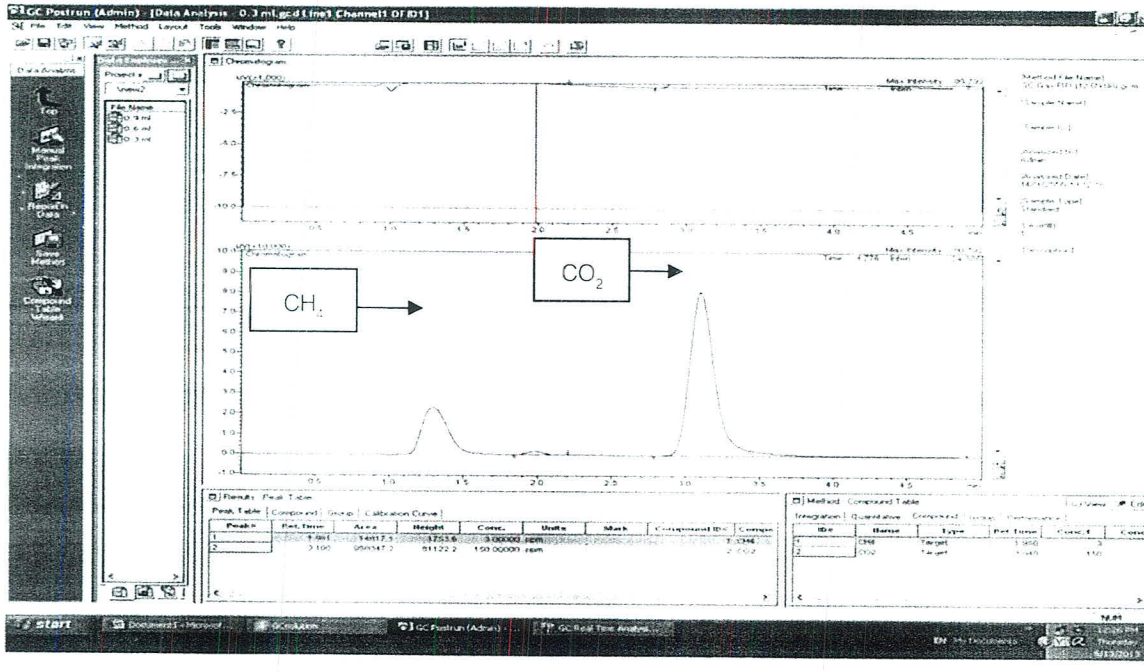

Figure 3.10 Example of chromatogram of gas sample analysis. Each peak identifies components of the gas sample and the magnitude of the peak is proportional to the concentration of CO_2.

3.5 Biotic factors observation

3.5.1 Plant growth and litter production

3.5.1.1 Allometeric equation for biomass estimation

This study estimated the carbon stock in a dry dipterocarp forest from aboveground and belowground plant biomass. The aboveground and belowground biomass of the tree was estimated from the relationship between the diameter at 130 cm from

ground level (D), height (H), dry weight of stem (W_S), dry weight of branches (W_B), dry weight of leaves (W_L), and dry weight of roots (W_R). In this study, the relationship between plant growth (diameter and height) and dry weight ($W_S + W_B + W_L + W_R$) were measured. Six dominant species were studied by digging around 16 trees using a backhoe engine and different plant biomass fractions were separated (leaves, root, branch) (Figure 3.11). Plant samples were weighed and oven dried at 80 °C to a constant weight, and then weighed each composition of all trees as stem, branch, leaf, and root. These masses are reported as dry mass per tree of each plant species in Table 3.2 (Lichaikul, 2010).

Generally, total aboveground biomass can be estimated from the allometric equations of stems (W_S), branches (W_B), leaves (W_L), and roots (W_R). This equation in each forest is different because trees of different species may differ greatly in tree architecture and wood density. Brown, *et al.* (1989) analyzed data from five studies in the humid tropics. A total of 168 trees were cut and weighed and an allometric equation for prediction biomass was obtained. Despite the fact that a sample of 168 trees is not likely to be representative of the many different tree species and forest types present in the humid tropics, the biomass equation fitted to these data is widely used (Anderson and Ingram, 1993 and Hairiah, *et al.*, 1999). This might not lead to large errors when the same equation is used to estimate the growth rates because the repeated measurements overtime at the same site assume a relatively constant forest composition and site specificity. Hence, allometric equations in this forest were measured in field study for reducing error estimation. Normally, the allometric equation used for calculation of biomass is expressed as Eq. 3.8:

$$Y = aX^b \qquad \textbf{(Eq. 3.8)}$$

$$\text{or } \log Y = \log a + b \log X$$

where: Y = dry weight of stems, branches, leaves and root (kg)

X = independent variables expressed as the relationship between diameter at breast height and height (D^2H)

a and b = constant

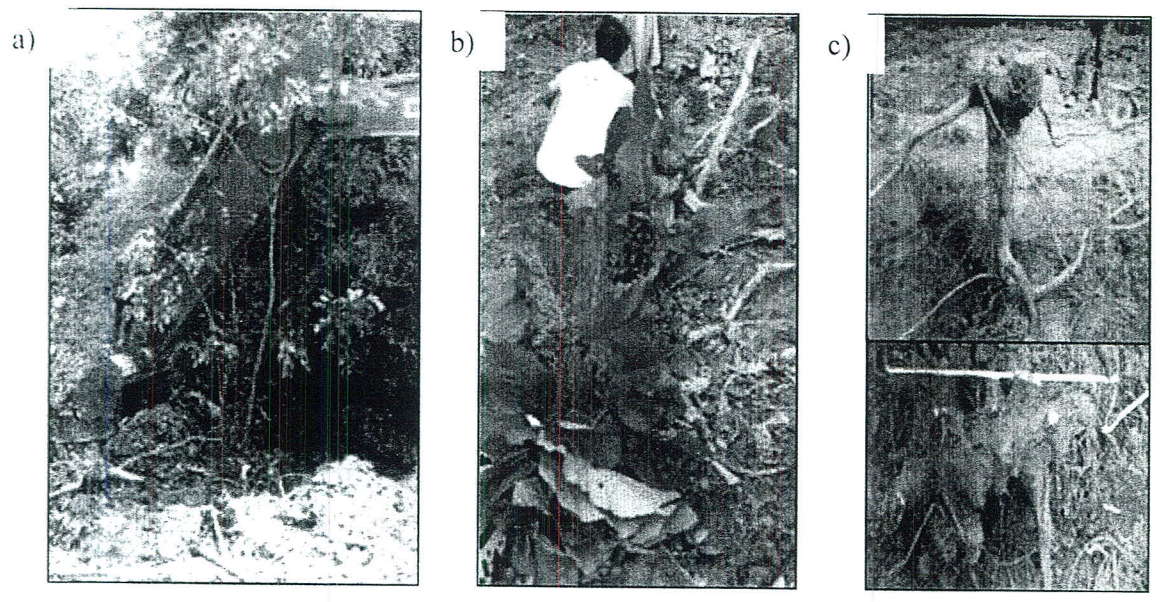

Figure 3.11 The processing estimated carbon stock of all biomass in a dry dipterocarp forest. Backhoe engine dig soil for (a) cutting the tree and (b-c) separated composition of the plant.

Table 3.2 Diameter, height and dry weight of stem, branches, leaves and roots of 6 dominant species in a dry dipterocarp forest, Ratchaburi Province (Lichaikul, 2010).

Name	Diameter (cm)	High (m)	Dry weight (kg)			
			W_R	W_S	W_B	W_L
Barringtonia acutangula Lin	2.00	5.5	37.4	13.5	5.78	3.98
	2.54	5.78	21.3	18.37	6.12	3.98
	2.03	4.9	28.4	9.92	3.46	1.9
Lannea coromandelica (Houtt.) Merr	2.03	4.91	8.8	10.16	3.09	3.08
	2.40	5.38	26.4	12.7	2.54	2.48
	2.26	5.8	8.15	14	4.4	3.49
Dipterocarpus tuberculatns Roxb	.2.28	6.03	25.88	13.86	1.61	2.18
	2.28	5.95	38.5	16.5	5	2.9
	2.64	6.17	14.4	19.26	3.12	2.18
	2.34	6.2	29.15	16.92	3.08	2.14

Name	Diameter (cm)	High (m)	Dry weight (kg)			
			W_R	W_S	W_B	W_L
Shorea siamensis Miq.	1.82	5.47	24	9.3	1.02	1.34
	3.55	7.48	15.6	11.38	2.99	2.24
	1.82	5.95	15.5	9.3	1.02	2.38
	1.72	6.25	17.3	20.68	4.01	1.62
Afzelia xylocarpa Kurz	1.82	6.16	16.5	7.84	1.77	1.6
	1.71	6.33	25.8	8.89	1.07	2.12
	2.88	11.67	22.9	11.23	3.08	1.89
Gardenia sootepensis Hutch	1.06	4.4	8.2	8.99	1.72	1.14

For the relationship between D^2H and the estimation of the dry weights of the stems, branches, leaves and root, a variation of 53-72% was found in the dry biomass of the 6 dominant species (*Barringtonia acutangula* Lin, *Lannea coromandelica* (Houtt.) Merr, *Dipterocarpus tuberculatns* Roxb, *Shorea siamensis* Miq, *Afzelia xylocarpa* Kurz, and *Gardenia sootepensis).* The diameter at breast height (DBH) at 130 cm from ground level and height of the plants were used to estimate the biomass stock in this forest. Consequently, the relationship between D^2H and the dry weight of stems, branches, leaves, and root estimation are presented in Figure 3.12.

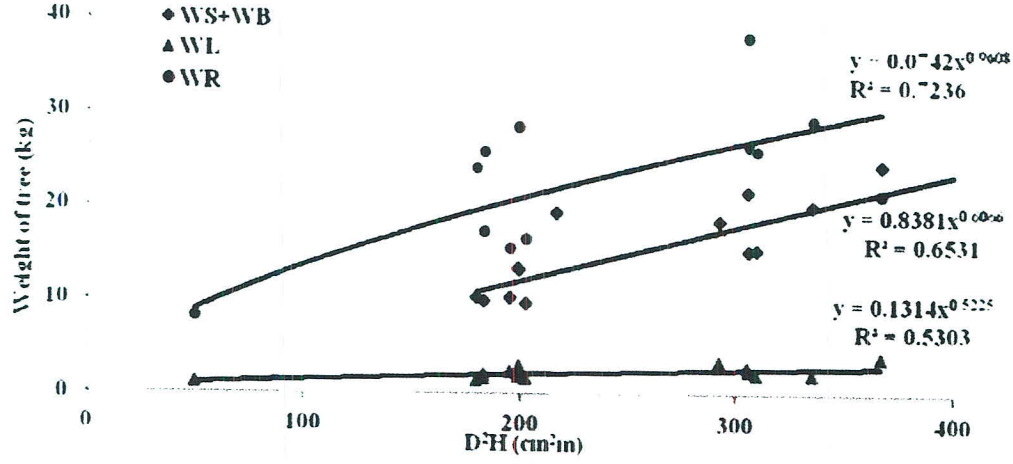

Figure 3.12 Allometric relationships for tree components dry weight of 6 dominant species

In sum, the biomass content of 6 dominant species was estimated from the relationships between diameter (D), height (H), and dry weight of stems and branches (B_{S+B}), dry weight of leaves (B_L) and dry weight of roots (B_R),. These were derived from the relationship described in Figure 3.12 and summarized as follows:

$$\log B_{S+B} = 0.6066 \log(D^2H) - 0.0767 \qquad r^2 = 0.65 \qquad \textbf{(Eq. 3.9)}$$

$$\log B_L = 0.5225 \log(D^2H) - 0.8814 \qquad r^2 = 0.53 \qquad \textbf{(Eq. 3.10)}$$

$$\log B_R = 0.9608 \log(D^2H) - 1.1296 \qquad r^2 = 0.72 \qquad \textbf{(Eq. 3.11)}$$

where;

B_{S+B} = Dry biomass of stem and branch (kg tree^{-1})

B_L = Dry biomass of leaf (kg tree^{-1})

B_R = Dry biomass of root (kg tree^{-1})

D = Diameter at breast height (cm)

H = Height of rubber tree (m)

These equations (Eq. 3.9-3.11) were applied to evaluate the plant biomass in this forest. The measurements of the diameters (D) and the heights (H) of 26 replication trees were carried out for every 3 months during May 2009 to February 2012 by using diameter tape (Figure 3.13). Then, the aboveground and belowground biomasses were calculated (kg dry mass of tree month^{-1}) by these allometric equations. In addition, the tree density in the forest was also estimated. Three study plots were setup at a terrain with slop <20% and collected the size of each plot of 10 m x 10 m. DBH more than 4.5 at height 1.3 m was used to separate tree and sapling plant. The number of individual trees and sapling in DDF were 1,724±66 tree ha^{-1} and 2,586±101 tree ha^{-1}. The density of sapling or young tree was higher than tree about 60% because this forest was regenerating forest then the tree were not older.

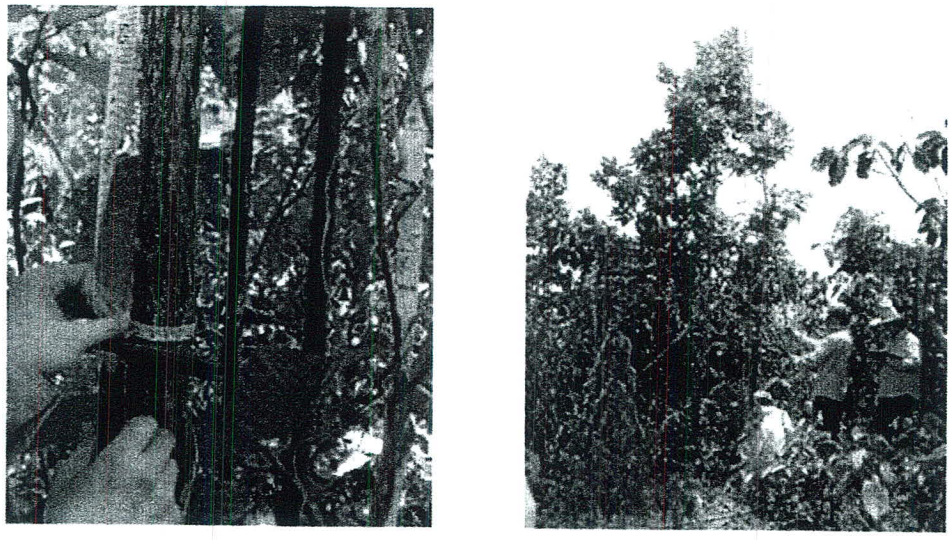

Figure 3.13 Measurement of circumference (calculate diameter at breast height: DBH) and height of 26 trees every 3 months.

3.5.1.2 Estimating root biomass by using soil core samples

Root biomass was estimated by taking soil core samples (Figure 3.14). The vertical distribution of the root mass was determined by collecting the soil samples at 0-60 cm depth and by using the size of the soil core as 30 cm (length) x 30 cm (width) x 60m cm (depth). The samples were cut into segments according to soil depths of 0-20, 20-40, and 40-60 cm. Three replicates of soil cores were taken in every month from September 2010 to August 2011. The roots were picked out from the soil layer and the adhering soil particles were brushed off. The root was washed through a 2-mm mesh screen. It was distinguished by eye based on color and consistency. Actively growing roots are usually light or brown-green colored and succulent whereas dead roots are black or dark colored and dry texture. Live roots are far more resilient than dead ones and are not easily broken if twisted. The sorted materials were rewashed with tap water, oven dried at 80 °C, and then each of the sizes of the roots, <2, 2-10, and > 10 mm, were weighed (Figure 3.14). The root mass values are reported as dry mass per volume of soil.

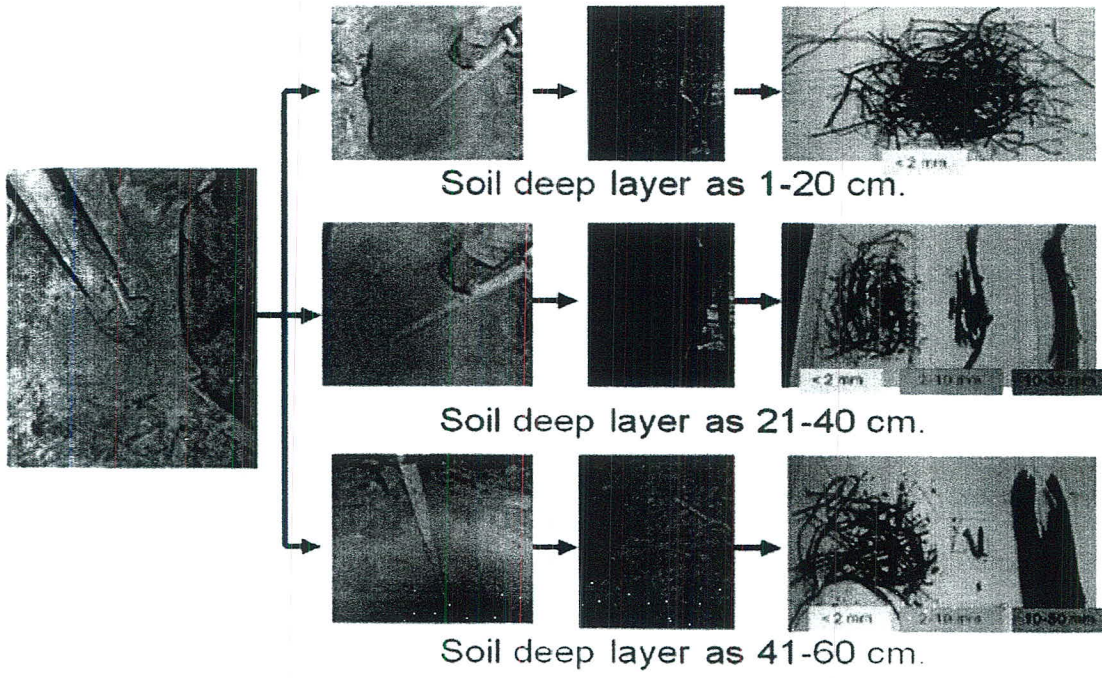

Figure 3.14 Estimating root biomass by taking soil cores and separates according to the sizes of the roots.

3.5.1.3 Monitoring root growth and senescence by using root windows

Root growth and physiology were directly studied by using a scanner at soil depths of 0.15, 0.75, 1.50, and 2.50 m (Figure 3.15). Two wells with 1 m diameter and 3.2 m deep were built in April 2009 while root measurement was started on 3nd September 2010. This facility allows the direct observation of root growth dynamics using so-called "root windows", through which roots can be observed at regular time intervals (McDougall, 1916). Normally, root physically separated from soil following core washing can be measured using a wide range of methods, ranging from manual line-intersection counting methods (Newman 1996; Tennant 1975) to automatic scanning the root image. The automatic method is fixing a flat bed scanner in each soil profiles for continuously observed root growth and decomposition in seasonal scale.

After scanning the root pictures, the images were analyzed by Gim and WinRHIZO^{TM} software. Gim is available as free-ware and open-source software for using draw the root growth and senescence before measured the root length and diameter by WinRHIZO^{TM} (Regent Instruments Inc., Canada). WinRHIZO^{TM} has now presumably become the most widely used commercial package specifically designed for the analysis of

scanned images of root samples physically separated from soil by washing (Bouma, *et al.*, 2000; Himmelbauer, *et al.*, 2004; Magalhaes, *et al.*, 2011). The advantage of this method is incomparably more convenient, faster and replicable than manual technique (Zoon and van Tienderen, 1990; Kaspar and Ewing, 1997; Dowdy, *et al.*, 1998).

A total of seven openings were thus created, encompassing a soil depth range of 3.2 m by 0.5 m increments. The walls were lined with molded cement rings of 50 cm height readily available from the local building materials outlet. To allow subsequent setup of root windows, opens of 40 cm wide and 30 cm high was created at the four soil profiles by using an electric diamond disc saw. Each root window included a specifically designed metallic frame supporting, on its upper side, a piece of 1-cm thick glass (25 x 30 cm) pressed against the soil at a 45° angle by means of two threaded rod actuators; on the frame's lower side, and two guide rails allowed the insertion of a flatbed scanner (Gonkhamdee, *et al.*, 2009) were prepared. The scanning plot was prepared on September 2, 2010, after set up about 4 months to avoid disturbance. Root monitor was achieved following a procedure similar to that described by Gonkhamdee, *et al.*, 2009 and Pierret, *et al.*, 2013. Eight pictures from both wells were scanned for 4 layers in every month. After that each picture was analyzed for the activity of fine root length and root growth and death by using Gimp 2.6 (Free Software) and WinRHIZO Basic Software Version (Regent Insturments INC, Canada). Gimp Software was used to draw the line of fine root growth and death before estimate the root width and length by using WinRHIZO. Using this access-well, it was able to estimate roots length density and the decay of root tissues at various soil depths.

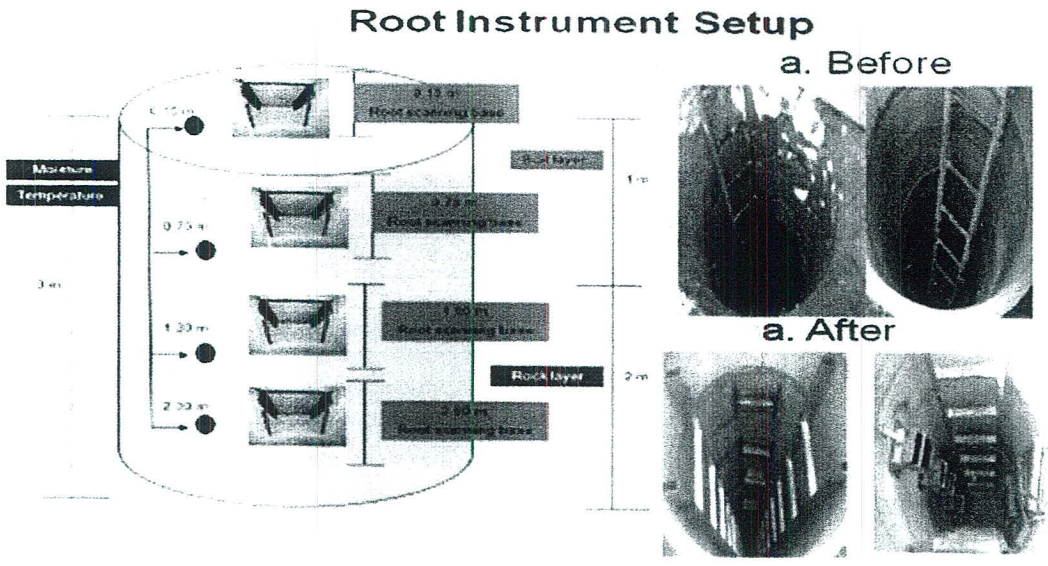

Figure 3.15 Illustration of belowground instrument setup for root monitoring and soil environmental property at different soil depths.

3.5.1.4 Litter production

Litterfall was measured for 3 years (May, 2009 – April, 2012). In order to collect the litterfall, thirteen square litter traps with the size of 1 m (width) x 1 m (length), made from nylon net, were set up (Figure 3.16). Each trap consisted of 1-mm mesh nylon netting (on a PVC frame) suspended from a wire hoop and was raised 1 meter above the ground. All accumulated litterfall in traps was collected once a month during three years period. The collected litter was sorted into leaf, branch, and other compositions (bark, seed, flower, and unidentified fraction). The sorted litters were weighed before and after oven drying. The oven temperature was set at 80 ^{o}C and the litter was incubated around 48 hours or until a constant weight was achieved.

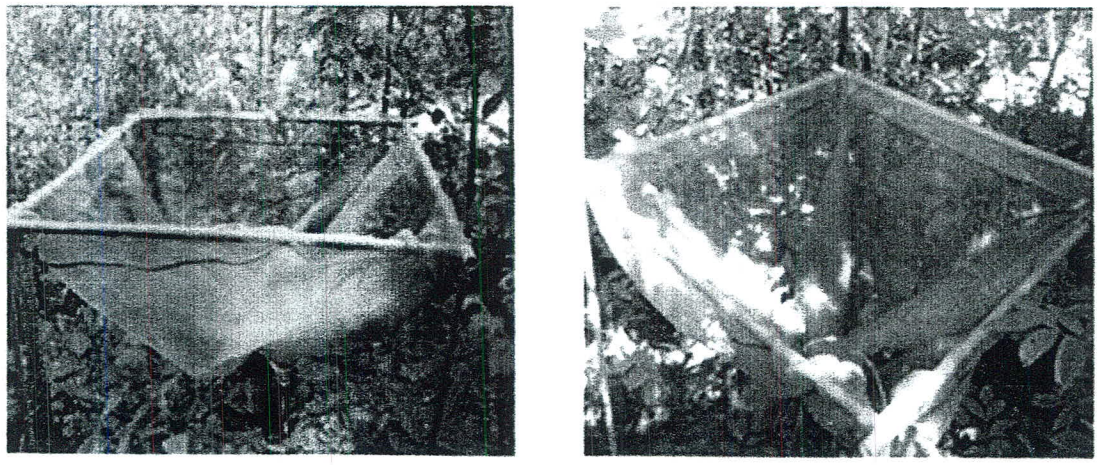

Figure 3.16 Litter trap in dry dipterocarp forest at Ratchaburi Province

3.5.2 Microbial growth and litter decomposition

3.5.2.1 Estimating microbial biomass by using fumigation method

The microbial biomass was measured by using a fumigation-extraction method (Jenkinson and Powlson, 1976) (Figure 3.17). Soil samples of about 10 g from soil different levels of soil depth, 0-20, 20-40, 40-60 cm were moved into 50 ml beakers. After that, it was moved into a desiccator, with a small beaker of about 25 ml alcohol free chloroform ($CHCl_3$). The desiccator was evacuated for 2 minutes at room temperature, started to count after the chloroform and begun to boil vigorously. Then, it was sealed under negatively pressurized conditions for 24 hours. The soil sample was then extracted with 100 ml 0.5 M K_2SO_4 for 30 min on a shaker at 250 rev min^{-1} and filtrated through filtration paper (Whatman No.42). The ratio of 1 part of soil to10 parts of extractant (w/v) was used. Dissolved organic carbon (DOC, μg C_{mic} g dry $soil^{-1}$) in fumigated and nonfumigated samples was measured by the Total Organic Carbon Analyzer (Shimadzu TOC 5000, Japan). The microbial biomass of this method represents the living microbial biomass in the soil and it does not affect the non-living organic matter; therefore the flush exclusively derives from the microbial biomass. Mean microbial carbon content was calculated for both fumigated (subscript F) and non-fumigated (subscript NF) treatments at each site according to Eq. 3.12.

$$C_{Mic} = (DOC_F - DOC_{NF})\, K_{EC} \qquad \textbf{(Eq. 3.12)}$$

Where; K_{EC} is the extractable part of the microbial biomass C after fumigation. K_{EC} was set at 2.22 (Inubushi *et al.*, 1991; Joergensen *et al.*, 1996). The dissolved organic carbon was corrected for sample dry weight and calculated for each layer.

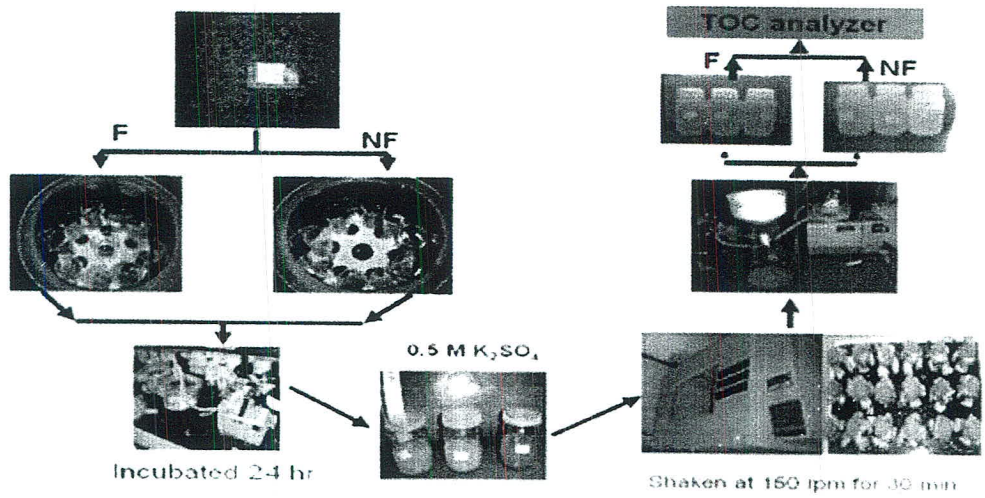

Figure 3.17 Microbial biomass measured by using fumigation-extraction method

3.5.2.2 Estimating litter decomposition by using litter-bag

Branch and leaf litter decomposition was studied using the standard litter-bag technique (Falconer *et al.*, 1933). Freshly fallen leaf litter was collected and air dried for decomposition study in April 2009. Leaf and branch decomposition of 4 dominant species in dry dipterocarp forests *Afzelia xylocarpa* Kurz , *Barringtonia acutangula* Lin, *Dipterocarpus Obtusifolius* Teijsm, and *Dipterocarpus tuberculatns* Roxb were measured at two layers: the soil surface and at a 10 cm depth, with three replications (72 bags per sample type of leaf and branch) (Figure 3.18). The biggest size of leaves and branches of the 4 dominant species were *Dipterocarpus tuberculatns* (26 wide x 40 lenght cm) and the smallest of the leaves and branches belonged to *Afzelia xylocarpa* (4.5 wide x 6.5 lenght cm). About one-hundred grams of air dried leaf and branch litters were placed inside 30 cm x 30cm nylon bags with 1.0 mm mesh.

Litterbags of each treatment (three plots) were randomly placed on the surface of the forest soil in June 2009. These bags were attached to both the forest floor and a 10 cm soil depth by metal pins to prevent movement and to ensure contact between the bags and the litter layer. Forty-eight litter bags per month were brought back to the

laboratory. The collected litters were oven-dried at 70 °C for 48 hours and brushed to clean out the soil contamination. The residual litter in each litter bag was weighed to find out the weight of the biomass left or lost.

The study of leaf decomposition measures the lost weight of leaf litter through a period of time. The simple model used to describe the decomposition rate is the exponential model or often called Olson's model [Olson, 1963] and the decomposition rate constant value (k) also can be calculated from below equation (Eq. 3.13). Nevertheless, decomposition rate values may be shown in the form of a percentage of annual decomposition by calculating the initial amount of litter and the amount of residual litter in the period of study time to show the value in the form of percentage of decomposition.

$$Y_t = Y_o . e^{-kt} \qquad \textbf{(Eq. 3.13)}$$

Where Y_o = initial weight of litter,

Y_t = weight of litter at time t

k = decomposition rate constant

e = natural logarithm

t = time of decomposition

Figure 3.18 The litter bag from litter decomposed study

3.6 Abiotic factors observation

3.6.1 Soil temperature

Soil temperature was measured at a depth of 5 cm with two averaging Soil Thermocouple Probes (TCAV, Campbell Scientific, Inc., USA.).

3.6.2 Soil moisture

Soil moisture was measured at a depth of 5 cm with two averaging Soil Water Content Reflectometers (CS615, Campbell Scientific, Inc.). The moisture sensor meter showed soil moisture content in units of percent water-filled pore space (%WFPS). The conversion of volumetric water content (data of CS-615) to %WFPS is shown in equation 3.14. SWC is the soil water content, BD is the bulk density (1.42 g cm^{-3}) and PD is the particle density (2.68 g cm^{-3}).

$$\%WFPS = [(SWC/BD)/(1-(BD/PD))] \qquad \textbf{(Eq. 3.14)}$$

3.6.3 Precipitation

Rainfall was measured at 11 m from ground level by using TE525 Tipping Bucket Rain Gauge (TE525, Campbell Scientific, Logan, Utah, USA).

3.6.4 Soil properties analysis

In this experiment, soil samples were collected from both root and non-root plots to analyze soil physical and chemical properties. These included soil texture, bulk density, total organic carbon content, and pH. Soil samples of each site were divided into different depth layers as follows: 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, and 91-100 cm, respectively.

3.6.4.1 Soil texture

The soil texture was determined by using the hydrometer method (Bouyoucous soil particles size analysis method) on air-dried soils that had been passed through a 2-mm sieve to remove small rocks, roots, pebbles, and debris. This method determined the physical proportions of three sizes of primary soil particles including clay (diameter: < 0.002 mm), silt (diameter: 0.002 – 0.05 mm), and sand (diameter: 0.05 – 2.0 mm) as determined by their settling rates in an aqueous solution using a hydrometer (Daniel, 2004). The hydrometer method of estimating particle size (sand, silt and clay content) was based on the dispersion of soil aggregates, using a sodium

hexametaphosphate ($Na(PO_3)_6$) solution and subsequent measurement based on changes in suspension density (Gee, *et al.*, 1982).

3.6.4.2 Bulk density

Determinations of the bulk density were done by measurement of volume and weight (Equation 3.10) The soil bulk density is the ratio of mass of dry solids to the bulk volume of the soil (Blake and Hartge, 1986). The bulk volume included the volume of the solids and of the pore space. The determination of bulk density consisted of drying (105 °C), for no less than 48 hours, and weighing a soil sample, the volume of which is known (core volume method) or must be determined (Lichaikul, 2004).

$$\text{Bulk density, } (g\ cm^{-3}) = \frac{\text{Weight of oven dry soil (g)}}{\text{Total volume of soil } (cm^3)} \qquad \textbf{(Eq. 3.10)}$$

3.6.4.3 Organic carbon content (%OC)

Organic carbon content in soil was determined by using the Walkley and Black Method (Walkley and Black, 1934). The method is used to determine the organic matter content (nitrogen, phosphorus, carbon, etc) in soil. However, we can convert this into % organic carbon following Equation 3.11 (Office of Science for Land Development, 2004):

$$\%\ \text{Organic matter} = \%\ \text{Organic carbon} \times 1.724 \qquad \textbf{(Eq. 3.11)}$$

3.6.4.4 Soil pH

Determination of the soil pH was made by using a glass-electrode pH meter on a 1 : 1 ratio of soil water suspension of air-dried soil samples, which had been passed through a 2-mm sieve (Jackson, 1967).

3.7 Statistical analysis

In this experiment, the Pearson product moment correlation coefficient was used to determine the relationship between CO_2 concentration in the chamber and sampling times. Only those that showed a significance at 95% were selected for flux estimates. Correlation and regression analysis were also used to test the relationship between soil respiration and environmental factors (soil temperature and moisture)