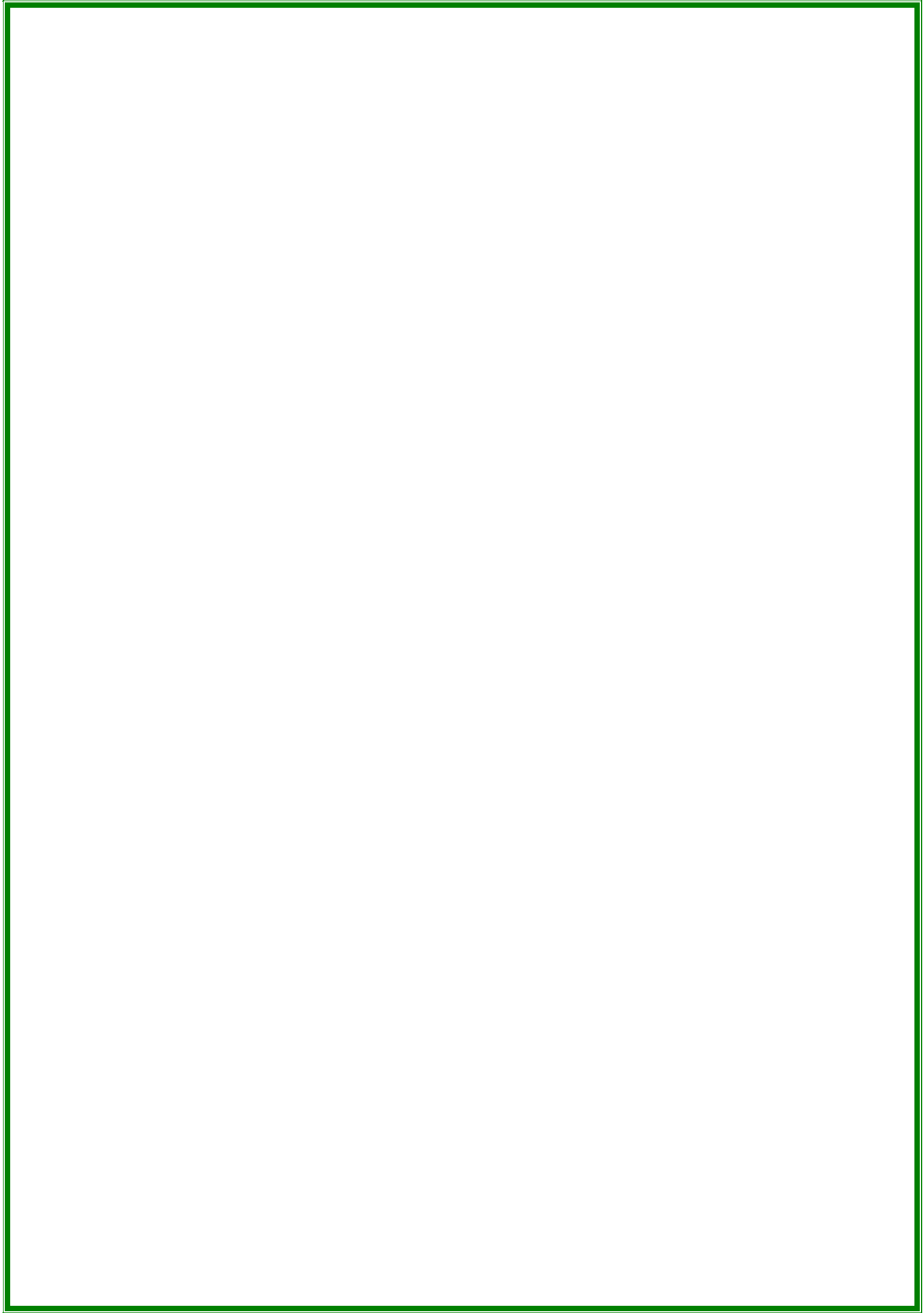# Measuring Tropical Forest

Carbon Allocation and Cycling v3.0

A RAINFOR-GEM Field Manual for Intensive Census Plots

Measuring Tropical Forest Carbon Allocation and Cycling: A *RAINFOR-GEM* Field Manual for Intensive Census Plots

Medición de la Distribución y Dinámica del Carbono en los Bosques Tropicales: *RAINFOR-GEM* Manual de Campo para las Parcelas de Inventario Intensivo

Medição da Distribuição e Dinâmica de Carbono em Florestas Tropicais: *RAINFOR-GEM* Manual de Campo para Parcelas de Inventários Intensivos
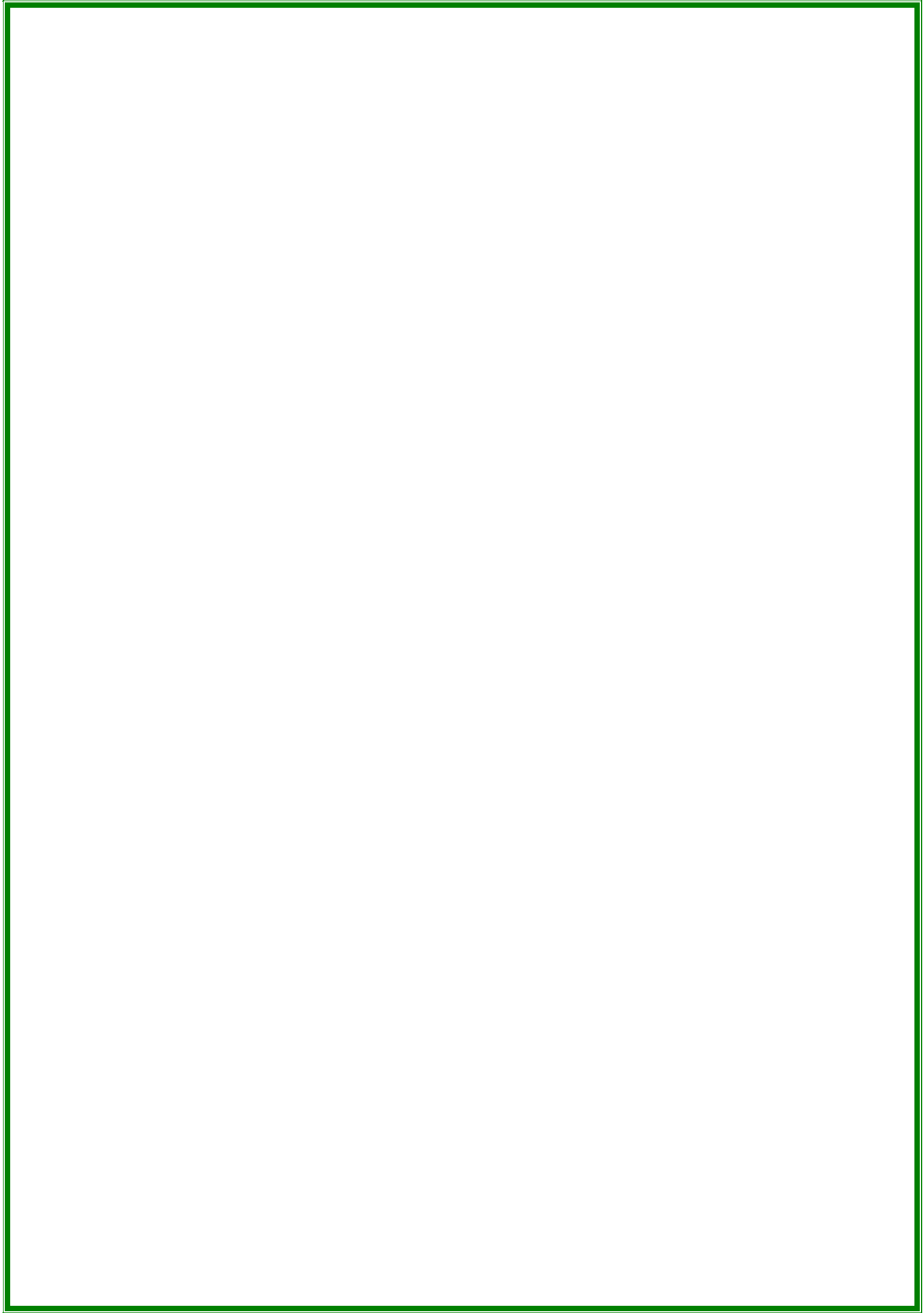
Mesure de l'Allocation du Carbone et de la Dynamique du Carbone dans les Forêts Tropicales: *RAINFOR-GEM* Manuel de Terrain pour les Parcelles Recensement Intensif

Pengukuran Peruntukan dan Kitaran Karbon Hutan Tropika: Garis Panduan Kerja Lapangan *RAINFOR-GEM* untuk Plot Pembancian Intensif

Version 3.0
July 2014

Toby Marthews, Terhi Riutta, Imma Oliveras Menor, Rocio Urrutia, Sam Moore, Dan Metcalfe, Yadvinder Malhi, Oliver Phillips, Walter Huaraca Huasco, María del Carmen Ruiz Jaén, Cécile Girardin, Nathalie Butt, Russell Cain and many further contributions from colleagues across the *RAINFOR* and *GEM* networks

Contents

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The *RAINFOR-GEM* Intensive Carbon Monitoring plots

RAINFOR (http://www.rainfor.org/): Español '**Red Amazónica de INventarios FORestales**'; Português '**Rede Amazônica de Inventarios Florestais**'; English '**Amazon Forest Inventory Network**'. *GEM* (http://gem.tropicalforests.ox.ac.uk/): '**Global Ecosystems Monitoring**' Network.

Global tropical rainforests play a critical role in regulating atmospheric levels of the greenhouse gas carbon dioxide (CO_2), and hence the rate of global climate changes (Malhi & Phillips 2005). Tropical rainforests also display considerable spatial variation in forest structure and function from the slow-growing, long-lived forests of the lowland Amazon to the highly dynamic ecosystems along the fertile Andes (Ghazoul & Sheil 2010). Networks of forest census plots in the tropics such as *ForestGEO-CTFS* (Condit 1998) and *RAINFOR* (Malhi *et al.* 2002, 2006, Phillips *et al.* 2004) have provided key insights into the underlying processes controlling forest spatial heterogeneity and powerful evidence for concerted changes in forest processes across regions over the last few decades, likely driven by increases in atmospheric CO_2 levels. However, it is revealing to take into account all key components of the ecosystem carbon (C) budget both above- and below-ground in order to assess correctly what changes in stem growth mean for overall ecosystem C storage and release. For this reason **a global network of 'intensive' monitoring plots ('*GEM*' plots)** has been established consisting of permanent sample plots across the tropics where all key ecosystem C stocks and fluxes are regularly monitored.

Version 1.0 of this manual established a set of standardised protocols to carry out plot installation and subsequent monitoring (Metcalfe *et al.* 2009) and versions 2.0-2.2 revised and extended these methods, incorporating modifications from experience in existing intensive plots (from many field workers across South America, notably all the authors of Girardin *et al.* 2010) as well as experiences in the new Asian (Terhi Riutta, Toby Marthews, Walter Huaraca Huasco) and African (Sam Moore, Maricarmen Ruiz Jaén) plots initiated in 2011. With version 3.0 we consolidate these methods, adding recent developments that have become necessary since 2012 and some clarifications of existing methods that have come out of our experience giving protocols workshops in Peru, Ghana and Gabon (2012) and Brazil and India (2014).

The data collected from this network - the *Global Ecosystem Monitoring (GEM) Network* http://gem.tropicalforests.ox.ac.uk/ - provides essential baseline estimates of current forest C storage, and allows us to track ongoing changes in forest C cycling. This research contributes both to local scientific capacity by training a large number of South American, African and Southeast Asian students in specialist ecological measurement and analysis techniques and to the development of the next generation of coupled atmosphere-biosphere models that will play a key role in shaping international climate policy.

Left: Dan Metcalfe (lead author of version 1.0 of this manual, Metcalfe *et al.* 2009; photo N. Butt); Right: Toby Marthews (lead author of versions 2.0 through to this version).

The *RAINFOR* network was set up in tropical South America in 2000-02 as described in Malhi *et al.* (2002) and Phillips *et al.* (2004), although it includes many plots that were being censused since the 1980s. *RAINFOR* is a joint effort between Oliver Phillips's group at Leeds, U.K. (the *Ecology and Global Change* Research Cluster http://www.geography.leeds.ac.uk/research/ecology-and-global-change/), Yadvinder Malhi's group at Oxford, U.K. (the *Ecosystems Lab* of the *Environmental Change Institute, School of Geography and the Environment, University of Oxford, U.K.*, http://www.eci.ox.ac.uk/research/ecodynamics/) and a kaleidoscope of partner institutions in countries across all three main tropical zones (see http://www.rainfor.org/en/partners for a complete list).

A similar plot network was initiated in tropical Africa called the African Tropical Rainforest Observation Network (*AfriTRON*, Lewis *et al.* 2009, http://www.geog.leeds.ac.uk/projects/afritron/) and there are now many sites there following the same protocols. *RAINFOR* protocols have been applied to a number of plots in tropical Asia and Australia since 2002 and, finally, the Tropical Biomes in Transition project (*TROBIT*, http://www.geog.leeds.ac.uk/groups/trobit/) initiated several forest/savanna ecotone plots on three continents that have now been incoporated into these monitoring networks.

The first *RAINFOR-GEM* intensive plots in Southeast Asia were installed in 2011 and, additionally, there are now temperate sites in the UK and Chile as well. For all the latest news, see the *Global Ecosystem Monitoring (GEM) Network* webpage at http://gem.tropicalforests.ox.ac.uk/ and the latest *RAINFOR-AMAZONICA* newsletter at http://www.rainfor.org/en/news/newsletters.

What are the RAINFOR-GEM protocols?

The **RAINFOR-GEM protocols for carbon monitoring of forest ecosystems** - including this manual - are all available in several languages online, freely downloadable from http://gem.tropicalforests.ox.ac.uk and http://www.rainfor.org/en/manuals:

The carbon balance approach to ecosystems dates back to the late 1960s (see e.g. Odum 1968, Lieth 1975, Whittaker & Marks 1975, Thornley 1976:ch.6, Kira 1978) but it really 'came of age' with the papers of Clark *et al.* (2001a, b) and Chambers *et al.* (2001, 2004). Forest monitoring programmes following this approach are ongoing now in a huge range of ecosystems worldwide (Malhi *et al.* 2009, Honorio Coronado & Baker 2010 and see Landsberg & Sands 2011:ch.5 for a general review) and are 'Tier 3' methods, meaning an approach conforming to the highest available standards for transparency, completeness, consistency, comparability and accuracy (GOFC-GOLD 2010). These protocols involve quantifying primary productivity, autotrophic and heterotrophic respiration component by component at all sites (see Malhi *et al.* 2009, 2014), with the emphasis on simplicity and robustness, enabling these measurements to be carried out by local field technicians over full annual cycles.

In addition to the *RAINFOR-GEM* protocols (Phillips *et al.* 2009, Metcalfe *et al.* 2009, Honorio Coronado & Baker 2010 and the other manuals above), there are various alternative protocols that are followed for carbon monitoring in tropical ecosystems including **the *ForestGEO-CTFS* protocols** (Condit 1998, Muller-Landau 2008) and the methods followed by **Winrock International** (Pearson *et al.* 2005a, b, Walker *et al.* 2012), the **Alternatives to Slash-and-Burn (ASB) Partnership for the Tropical Forest Margins** (Hairiah *et al.* 2001, 2011), Hoover (2008), Ravindranath & Ostwald (2008), the **Global Terrestrial Observing System (GTOS)** Programme (Law *et al.* 2008), the **Tropical Ecology, Assessment and Monitoring (TEAM)** Network (TEAM 2010, 2011) and the Woods Hole Research Center (Walker *et al.* 2011). There is also some overlap with the protocols for plant trait measurement recommended by **TRY** (Cornelissen *et al.* 2003, Pérez-Harguindeguy *et al.* 2013). The *RAINFOR-GEM* protocols presented here below differ from these alternatives in some essential respects, but bear much in common because they have been formulated in response to similar needs in the conservation and research communities.

GEM
Global Ecosystems Monitoring Network

NATURAL ENVIRONMENT RESEARCH COUNCIL

OXFORD MARTIN SCHOOL

UNIVERSITY OF OXFORD

eci

Projects

Andes to Amazon transect

Asian Forests

East African Forests

ECOFOR

European Forests Wytham

Global investigations

Lowland Amazon Forest

Southern Temperate Forests

Terrestrial LiDAR

West African Forests

Resources

Protocol documents

What is GEM?

The Global Ecosystem Monitoring network (GEM) is an international effort to measure and understand forest ecosystem functions and traits, and how these will respond to climate change.

An innovative approach to science

Our team is now spread over three continents. We are using this site as a tool to coordinate our efforts and experiment with networking science, a more efficient way of working as an international team. This site also allows you get closer to our scientists: from the corridors of Oxford University to the remote terrains of the rainforest, get our latest scoops, communicate directly with the team, and delve into our photo diaries.

Latest News

Erika Berenguer
14 days ago
Dendrometer bands
Hi all,

Is there any reason for the preference of plastic dendrometer bands instead of metallic ones?

Imma Oliveras
4 months ago
Dear all, greetings from Nova Xavantina. We are trying to analyze LAI images with Can Eye, but we can't make it work. Any tips on how to specify the parameters... More

More news

Where are we working?

(Map) ● - Site   ● - Plot   ● - Field station

What are Intensive Carbon Monitoring Plots?

To be part of the *RAINFOR*, *AfriTRON* or *GEM* networks, a census should be taken of all trees and lianas above 10 cm diameter on a regular basis and the data uploaded to *ForestPlots.net* in Leeds (Lopez-Gonzalez *et al.* 2011). What we call **Intensive Carbon Monitoring Plots** are census plots where all components of the carbon cycle are measured on a regular basis (e.g. stem CO_2 efflux, litterfall, root growth: see Malhi *et al.* 2009, 2014, Girardin *et al.* 2014 and Huaraca Huasco *et al.* 2014 for discussions of carbon cycle components). Intensive

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plots were first installed within the *RAINFOR* network in 2007 and have now been installed at locations across all three main tropical zones.

Map of **the *GEM* network** of intensive carbon monitoring plots (see above):
        http://gem.tropicalforests.ox.ac.uk/
Map of **the *RAINFOR* network** in tropical South America:
        http://www.rainfor.org/en/map
Map of **the *AfriTRON* network** in tropical Africa:
        http://www.afritron.net/en/map

Where can I get data from existing plots?

All census data for the *RAINFOR* and *AfriTRON* networks are held on an online application called http://www.ForestPlots.net/ [1] (Lopez-Gonzalez *et al.* 2011; shown above). This contains all up-to-date census information from more than 800 plots across the tropics[2], of which 500 have at least one measurement interval

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[1] Census data were initially held in a database (Peacock *et al.* 2007), which was replaced by *ForestPlots.net* in 2008 (Lopez-Gonzalez *et al.* 2011). This is a member database of *GIVD* (http://www.givd.info).

[2] n.b. all *RAINFOR* and *AfriTRON* plots have a unique code consisting of 3 letters and 2 numbers (e.g. TAM-05, CAX-06 indicating particular plots at Tambopata, Peru, and Caxiuanã, Brazil). As with all large databases there have been occurrences of inconsistent use of codes in the past, so all plot codes used in new research should be checked against *ForestPlots.net* to avoid confusion.

(two censuses). Censuses from a selection of these plots are available to registered users for view and download (229 plots as of April 2012; if you agree to the conditions on http://www.forestplots.net/UserAgreement.aspx then you can register at http://www.forestplots.net/RequestAccess.aspx).

Since February 2012 the *GEM* http://gem.tropicalforests.ox.ac.uk/ online repository has also been available to provide more information on the census plots where intensive carbon monitoring is underway.

How can I set up a new plot?

That is exactly what this manual is all about! Setting up an intensive carbon monitoring plot is a tough job and much depends on having a well-motivated team of *at least* 4 people who are familiar with the location where the plot is to be established. However, it is an advantage that the equipment required is almost entirely 'low-tech' (apart from the IRGA, Appx. II), which means that these protocols may be followed in virtually any forest - even if the host country has relatively basic infrastructure.

For specific information for the site(s) you will be visiting or working at (e.g. trail maps, rainfall data), please browse the contacts listed on *GEM* http://gem.tropicalforests.ox.ac.uk/ (where you will also find this manual and associated data sheets) and/or http://www.ForestPlots.net/. For how to start with the *RAINFOR-GEM* protocols, read on below ...
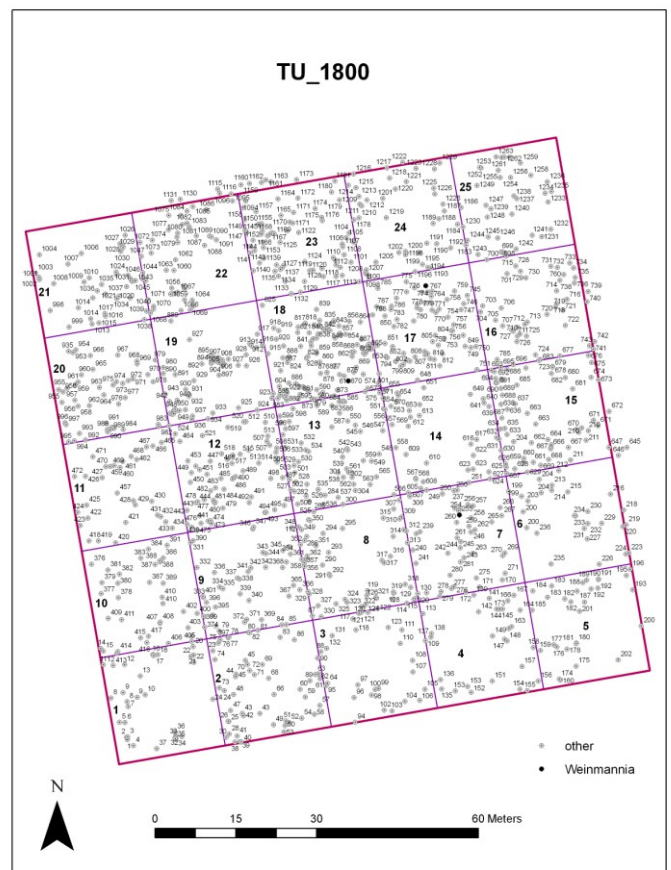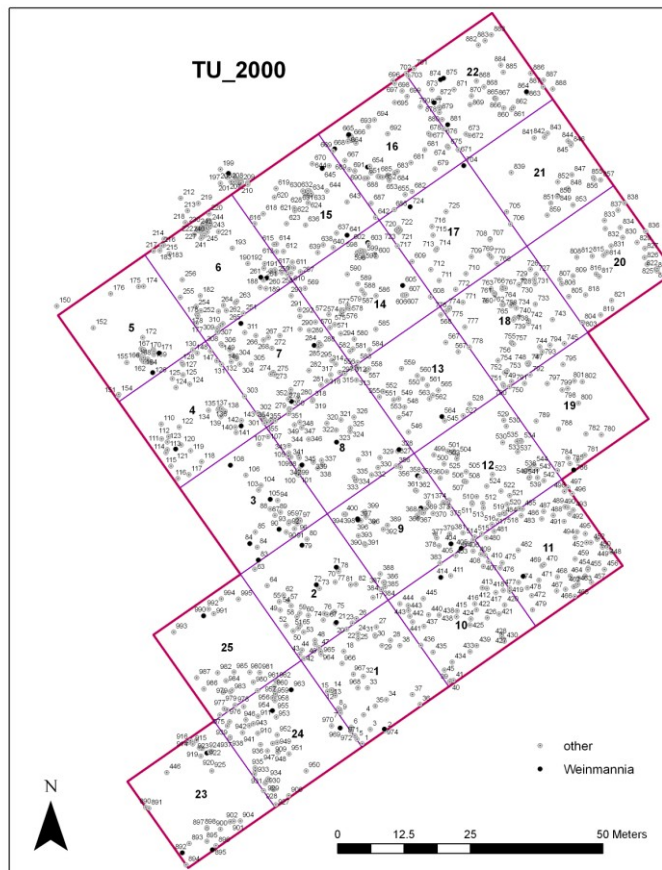
Two *RAINFOR-GEM* hectare plots in the Trocha Union, Cusco, Peru.

1. SETTING OUT THE PLOT and the TREE CENSUS:

1.1 Where to place a census plot?

The general strategy is to maintain sample forest plots across the edaphic range within each climatic zone and regional plot cluster (Phillips *et al.* 2009, Metcalfe *et al.* 2009). New plots should ideally be randomly located within local, geomorphological strata that satisfy certain logistical criteria. New plots should
  (1) be on reasonably homogenous soil parent material and soil type,
  (2) have adequate access,
  (3) have sufficient long-term security from human disturbance and
  (4) have sufficient long-term institutional support[3].
However, in most tropical research sites accurate habitat maps are still unavailable, which prohibits completely stratified sampling at large scales. Similarly, at local scales, identifying geomorphological strata is difficult if accurate soil maps are lacking. Satellite images help in identifying the range of vegetation types that might be found in any one area, but problems with the resolution and lack of ground-truthing limit the ability to accurately predict the exact distribution. Information from local residents and botanists who know the area can be very useful. Logistical constraints are also important: it is impractical to locate a plot more than 2-3 hours

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[3] These requirements are similar to those listed in Condit (1998).

from the closest vehicular access[4], and it can be difficult to fit a 1 ha plot into a forest that is dissected by too many tracks or a mosaic landscape of many land use types.

Effectively, the approach is to identify as wide an area as possible that fits both the logistical constraints listed above and also the requirements of the intentions for these new plots (e.g. if the intention is to sample swamp forest then identify the area of suitable swamp forest; if trying to sample degraded forest then map out the area of degradation). Within this area, identify local geomorphological strata and for each stratum (if possible) a plot should be initiated. Within each stratum, plots should be randomly located, to avoid 'majestic forest bias'[5] as much as possible (Malhi *et al.* 2002, Phillips *et al.* 2004), which can bias forest biomass estimates (Brown 1997), and TEAM (2010) also suggest to separate individual plots by at least 2 km. If maps are available, plot location should be randomly assigned prior to going to the field (e.g. Ravindranath & Ostwald 2008) or the position of the first plot corner can be randomised sufficiently by simply going to the middle of the identified stratum, choosing a random direction and going a random distance >20 m in that direction from the starting point. If good enough maps are not available (e.g. in a heterogeneous matrix of forest, open clearings and unmapped logging trails), the available area should be scouted out in the field and a location chosen that is as representative as possible[6].

Size: The coefficient of variation of basal area only really increases as sample plot size falls below ~0.4 ha (Phillips *et al.* 2009). Therefore, a plot size of 1 ha (planimetric) is commonly chosen because it is greater than the scale of typical tree fall events, but sufficiently small to sample individual soil types (although many *RAINFOR* plots are <1 ha). The plot should in *all* cases be divided into 20 m × 20 m subplots to follow the protocols below[7].

n.b. Circular plots are in use at many locations around the world (Ravindranath & Ostwald 2008, Maniatis & Mollicone 2010), e.g. by Winrock International (Pearson *et al.* 2005a, Walker *et al.* 2012). However, all current *RAINFOR*, *AfriTRON* and *GEM* plots are either square or rectangular or an irregular shape dictated by particular local circumstances (e.g. Trocha Union plots TRU-07 (irregular) and TRU-08 (square) in Peru shown above) and we currently strongly advise against using a circular design unless obliged to do so, because it cannot be divided easily into square subplots, which is necessary for our protocols (see §1.2)[8].

Orientation: N/S and E/W directions for the principal axes of the plot are the most convenient and square plots have a lower edge:area ratio than rectangular plots but steep slopes and/or local geology/soils (to maintain plot homogeneity) may mean that an irregular shape is necessary.

Timing: To minimise the errors caused by variation in stem water content between successive enumerations, plots should be measured over whole year intervals and at the time of year when there is least interannual variation in soil water availability. For plots in areas that experience severe interannual variation in rainfall (e.g. from El Niño events), the best time of year is during the wet season.

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[4] For example, TEAM (2010) advised no less than 100 m, and no more than 500 m, from any "trail or access point" (presumably they meant trails accessible by vehicle, not foot), although we would be happier being a bit further than 100 m away from vehicular access and up to 2-3 hours on foot is just about manageable (going further is possible, but necessitates creating a temporary camp such as has been done e.g. for the LF plot in Sabah, Malaysia).

[5] The primary purpose of an intensive carbon monitoring plot is to provide quantitative data on ecosystem carbon components. As is well known, these 'permanent field laboratories' can also be a great stimulus for scientific research in the area (Condit 1998) and can play a major role in biodiversity conservation. However, although extremely important, these considerations must remain secondary and should not be allowed to greatly affect the choice of plot location.

[6] Temporarily marking out the corners of a full hectare (without cutting any vegetation) may be necessary during the search for an appropriate area (as in the plot selection method of TEAM 2010).

[7] Subplots are called "quadrats" by *ForestGEO-CTFS* (Condit 1998).

[8] A circular plot is often difficult to lay out: GPS devices are not (yet) accurate enough for this under thick forest cover and there are usually too many lianas and other undergrowth to allow line of sight over more than ~10 m, which is required to lay out a consistent curved path. Using 'laser tape' is one option, although laser dots are difficult to see in bright sunlight or against dark surfaces such as wood or leaves at a distance of over 10 m even with the most powerful of these. Additionally, the grid-based measurement system used throughout this section §1 cannot be applied in a circular plot.

1.2 Setting out the plot

Subplot corner markers and Rostin Jantan and Matiew Tarongak in Sabah, Malaysia

Equipment (for 4 personnel): 2 measuring tapes (at least 30 m each, but preferably 50-100 m), 4 compasses, 2 clinometers, 2 machetes/pangas/parangs[9] (preferably 4), enough PVC tubes to mark each corner of each 20 m × 20 m subplot (i.e. 36 with maybe 5 spares; 2.5 cm diameter, 30 cm long is fine, painted red at the top (or some other vivid colour) for visibility), 2 rolls of rippable flagging tape, 2 permanent marker pens (plus a couple of spares; as with all easily-losable items it's advisable to tie flagging tape to each one)[10]. If you do not have permission to cut vegetation, ropes or straps may be required for pulling and tying vegetation temporarily out of sighting lines. If you have to reopen or improve the trails leading into the area of the new plot, you'll need extra equipment for this (e.g. ropes for handrails in steep sections). In areas where elephants occur - and/or other fauna who may remove corner markers such as these - an alternative corner marking approach will be essential, e.g. metal stakes sunk into the ground and labelled with flagging tape.

Remember to specify not only the size of the plot in hectares (ha) but also whether this is *planimetric* or *along the ground*. For example, a 1 ha plot *planimetric* will be exactly 1 ha of an orthorectified aerial photo or satellite image, but it may cover >1 ha of ground (with the excess being greater the steeper the topography). In this manual we assume that a 1 ha plot planimetric is being laid out and topographic information will be collected later to enable the on-the-ground area to be calculated (§9).

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[9] **We advise to discuss the use of machetes thoroughly with reserve management personnel** before any work begins. Even when permission to cut is given, any cutting should be kept to the absolute minimum because any impacts can affect growth of trees in the plot and the long term recruitment of new individuals (especially lianas).

[10] A handheld **compass hypsometer** is only slightly more accurate and, if cutting vegetation is not allowed, at least two extra assistants will be required to move branches and vegetation aside along each subplot side to take a sighting from corner to corner. If a **theodolite** with a stand is being used then the increase in accuracy can, however, be very great (Condit 1998). In the *ForestGEO-CTFS* network, many use a *Topcon Total Station* theodolite (and do not cut any vegetation) which makes for a much more accurate plot survey (to cm accuracy) but at the cost of more staff and time (e.g. surveying the Danum Valley 50 ha plot with a *Topcon Total Station GTS-239n* and two prism reflectors in Sabah, Malaysia, in 2011 took more than 6 months).

A **GPS device** is helpful for larger-scale maps, but beneath a forest canopy these are (currently) not accurate enough to be used for the actual marking out of a plot.

You will need to have with you a table of cosine numbers like the following:

If the slope rises/drops away at this gradient:	To ensure the side of the subplot is 20 m long horizontally it should measure this length along the ground, as illustrated below (= $\dfrac{20}{\cos(slope)}$ m, Condit 1998)
0°	20.0 m
5°	20.1 m
10°	20.3 m
15°	20.7 m
20°	21.3 m
25°	22.1 m
30°	23.1 m
35°	24.4 m
40°	26.1 m
45°	31.1 m
50°	34.9 m

Above 50° slope a subplot becomes very difficult even if it can be marked out - either workers will damage the plot sliding around during future censuses or ropes must be installed which would be a further disturbance: consider repositioning the plot to avoid these areas or modifying the layout to be irregular (e.g. the Trocha Union 7 plot in Peru shown above)
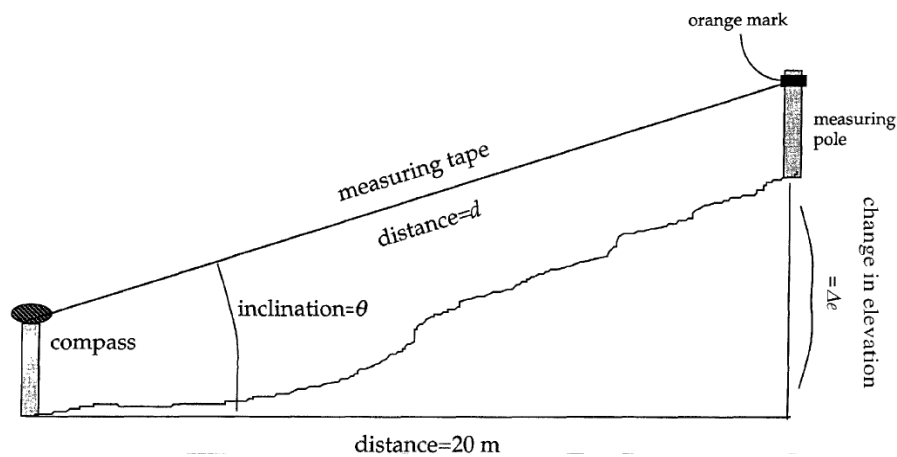
Condit (1998:Fig. 2.1.1) illustrating the difference between horizontal and straight-line distance.
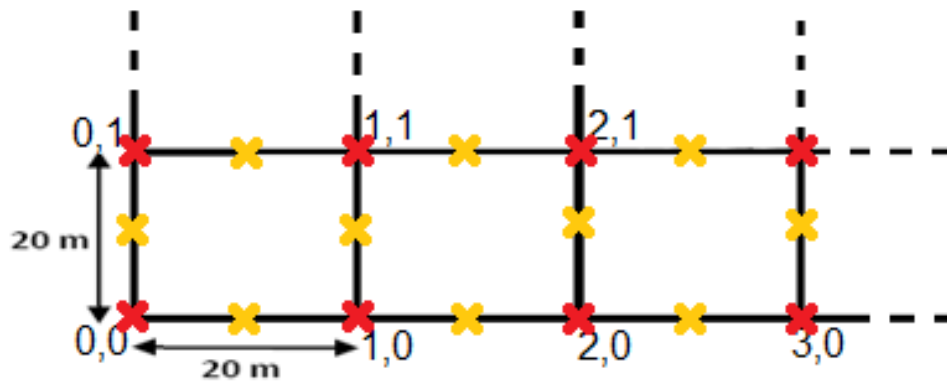
An example surveying path (cf. Condit 1998:Fig.2.1.2). Starting at the southwest corner (0,0), one team heads north and the other heads east (one person staying at 0,0 to hold the measuring tape and the other using a compass to head in the right direction and a clinometer and the table above to go the correct distance). After corners (0,1) and (1,0) and the first two 10 m points have been marked, both teams head for (1,1). If they do not meet within 20 cm of the same point, repeat the process. Thereafter, complete the first row of five subplots by one team holding the baseline (2,0)→(3,0)→(4,0)→(5,0) and the other proceeding across and down (2,1)→(2,0) then (3,1)→(3,0), etc. Subsequent rows are surveyed one row per team, with the team furthest from the first row always ensuring that they go at a pace slow enough to remain behind the other team (thereby checking correctly against all that team's points as they survey them).

Each red cross is a **permanent corner marker** (e.g. in the Fragment E plot in Sabah, Malaysia, these are 30 cm grey PVC tubes spray-painted red at the top with a stick cut from outside the plot inserted through it and flagging tape tied to the top of the stick[11]; the corner number is written *twice* with permanent marker on either side of the PVC tube *and also* written on the flagging tape) and each orange cross shows a **10 m marker** (e.g. in the Fragment E plot in Sabah, Malaysia, these are sticks cut from outside the plot inserted in the ground with flagging tape at the top saying "10 m")[12]. Finally, *ForestGEO-CTFS* also mark the corners of every 5 m × 5 m square (Condit 1998), but RAINFOR does not require this.

Divide into 2 teams, each with one measuring tape. Go round the subplots one-by-one following a surveying path such as that shown above. If allowed to cut vegetation, clear the sides of each subplot so that one can easily walk along the line (without damaging anything more than herb layer vegetation), otherwise you should carefully move plants aside to allow measurement along the side. Mark each corner and the 10 m points at the side of each subplot. There is no need to permanently install strings around each subplot (animals will more than likely move them in most forests anyway).

Timing: Marking out a plot like this in 1 ha of forest in a 4-person team takes 1-2 days with permission to cut vegetation. Without permission to cut, surveying can take much longer, for example *ForestGEO-CTFS* estimate 10-14 person-days in the field per ha (Condit 1998).

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[11] In areas where animals (e.g. elephants, warthogs) will search out and dislodge corner markers, something more robust will have to be used, e.g. metal pins sunk into the ground.

[12] If the marker position is occupied by a stream or small pond then attach as closely as possible but in such a way that the marker will not be washed away in the event of a flood. If a tree occupies exactly a spot that should be marked, either put the marker as close as possible to its real position (if the tree is <40 cm diameter) or attach the marker to the wood with a nail and flag as usual (Condit 1998).

1.3 Tagging large trees

*** Note that this job cannot be done during or after heavy rain because the paint will not stay on the tree ***

Walter Huaraca Huasco in the Fragment E plot, Sabah, Malaysia.

Equipment: (for more details on equipment, including suggested models and suppliers, please see the spreadsheet at http://gem.tropicalforests.ox.ac.uk/page/resources).

TEAM ONE (tagging team; preferably two people) 1 hammer, numbered aluminium tags [13] [14] (estimating max. 1000 stems ≥10 cm diameter per ha), at least the same number of aluminium nails[15] (ensuring they are of small enough gauge to fit through the holes of the tags, but with a head large enough that the tag does not fall off), 1 diameter tape (ideally, though can make do with a piece of tape 10 cm long), rippable flagging tape, extra write-your-own tags (to replace the small number of tags that will inevitably get lost during plot set-up) and 1 machete. 1 straight pole 1.6 m long cut in the field (from outside the plot) with a clear notch at 1.3 m.

TEAM TWO (measurement team) 1 diameter tape (and a spare; n.b. can make do with normal measuring tape but will have to divide all measurements by 3.1416 afterwards to convert circumferences into diameters), 1 set large tree callipers, 1 pad waterproof paper[16], pencil+sharpener or biro pen, weather-writer, list of codes in Appx. I (printed and laminated and *in the language of all field-workers* - which should be downloadable from the *RAINFOR* website manual page http://www.rainfor.org/en/manuals), rippable flagging tape, waterproof nontoxic red paint (or other vivid colour: either a spray timber paint, emulsion paint or

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[13] Pearson *et al.* (2005b) recommended to use an aluminium nail and a steel tag at sites where fire is prevalent.

[14] If there is a possibility that aluminium tags will be stolen, another suitable material should be used.

[15] Aluminium nails are not as strong as steel ones and bend if you are not careful, however we advise to use them anyway because there are no corrosion issues (even galvanised steel nails often rust after a year or two in the field: in humid environments sometimes after much less time). In Wytham Woods, UK, and at the Maliau Basin Conservation Area, Malaysia, the managers of the reserve supported the establishment of a plot but disallowed steel nails because they might damage sawmill machinery in the event of a small number of censused trees being cut in the future.

[16] See §10 for an example spreadsheet typed up from a pad like this. However, if possible it is more ideal to use the standardised field sheet templates used by *ForestPlots.net*, which are available to Principal Investigators and Field Leaders by emailing admin@forestplots.net.

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highway paint (highway paint is good but expensive and difficult to source): we use spray paint and estimate six 400 ml cans per ha), extra write-your-own tags and 1 machete.

Required for jobs that can be scheduled for a later date: an extendable ladder (as long as possible; preferably aluminium or some other material that is not too heavy to carry) and longer diameter tape for large trees (see below), a GPS to record positions of the corners of the plot and any prominent permanent features so that you can make a map of the new plot (e.g. existing trails, water courses; however please note the accuracy of the GPS points which may be only within ~10 m in forest). Best to do this *after* the plot has been completely laid out to avoid having to remeasure points.
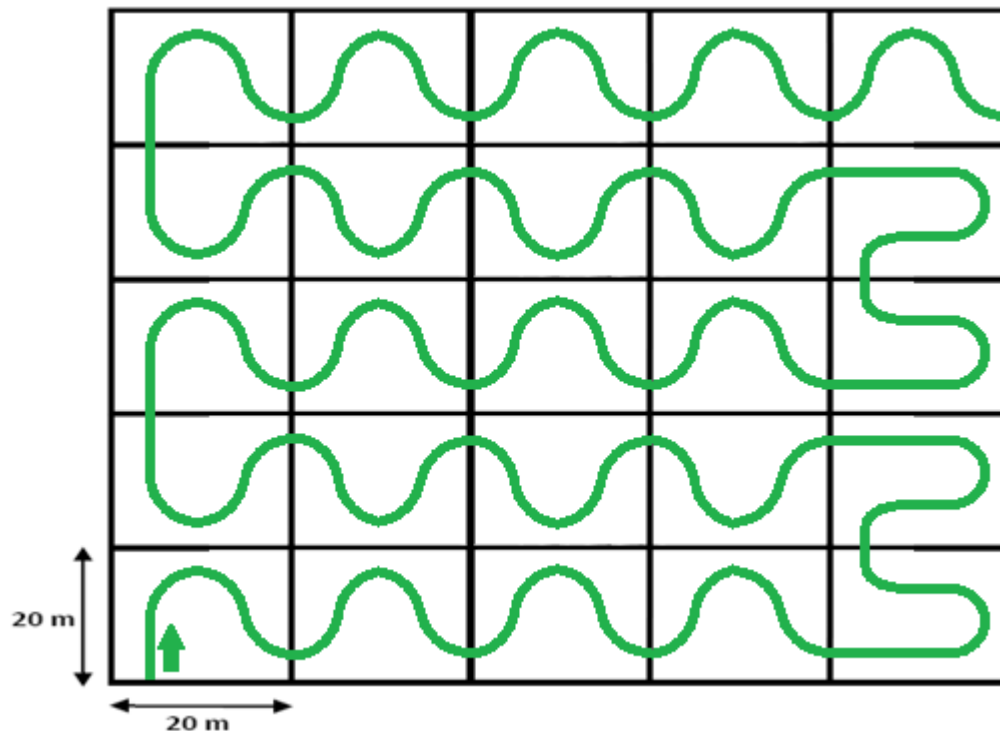
Team one starts and criss-crosses each subplot (e.g. doing the four 10 m × 10 m quarters of each 20 m × 20 m subplot one after the other following the green path above[17]) tagging all trees and lianas (all free-standing woody stems alive[18] and over 10 cm diameter at the Point of Measure - see Box 1). Ensure that the tag is *always* at 1.6 m along the stem by using the marked pole pushed firmly into the leaf litter to the mineral soil next to the tree (if using non-nail tag attachment methods such as a looped piece of twine, it may not be possible to ensure this but try to get it as close to 1.6 m along the stem as possible; if using nails knock them into the stem at an angle so that when the tag drops it slides towards the nail head rather than the stem). Tags should be consistently on the same side of the trees throughout the plot (e.g. always on the south side). For curved stems, measure that 1.6 m *along the stem* rather than vertically.

It's very important not to miss *any* stems so check gaps and overgrown areas thoroughly for trees that may be short, leaning or lying along the ground (newly broken or deciduous trees can be completely leafless so check carefully - e.g. if not in a strict conservation reserve cut the tree bark to see if the cambium is alive - and

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[17] It is important to follow a consistent route because it will make trees findable without a tree map (q.v. §9). In irregularly-shaped plots, decide the route and then number the subplots along that route.

  The illustrated search path is similar to TEAM (2010:Fig.12), but Condit (1998:Fig.2.2.2) used a different search path (with each quarter of each 20 m × 20 m subplot being searched in clockwise order starting with the SW quarter; within that, each section of each 10 m × 10 m quarter being searched in clockwise order starting with the SW section and; within that, each 5 m × 5 m subsection being searched clockwise).

[18] Standing dead stems (snags) should be excluded at this point and counted as part of the coarse woody debris survey in §4.2 (following Baker & Chao 2011). However, it is possible that in some censuses snags may have been tagged and included (following Metcalfe *et al.* 2009) so if the CWD survey is being undertaken by another team, check with them that this biomass component is not being either missed or double-counted.

if in any doubt, tag the tree anyway[19]). Palms, cycads, tree ferns and aloes should be censused along with all other trees and lianas. Woody bamboos and large herbaceous-but-woody monocots (e.g. dracaenids, aloes, Marantaceae, Araceae, Cyclanthaceae, Musaceae, Heliconiaceae and Strelitziaceae[20]) should all be included (they are excluded from the small herb survey in §7) and stranglers should *always* be included *whether or not* they are free-standing [21] [22]. Palms that have a diameter sufficient to be included but their main woody stem does not reach 1.3 m height should be excluded[23].

Woody bamboos often occur in large clumps (e.g. this *Bambusa arundinacea* clump in Mudumalai, India). If access makes it impossible to census each stem, choose a representative stem, measure and paint that, and record also the number of green, living poles greater than the minimum diameter at 1.3 m height in the notes [24]. The height estimate should be to the top of the whole clump.

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[19] Condit *et al.* (2014) suggested that in identifying a dead stem criteria "usually include 1. Leafless state in non-deciduous species, 2. Trunk broken, 3. Rotten wood encircling trunk, 4. Trunk entirely fallen or vanished". However, my advice is that, if there is any doubt, just call the tree alive (it is often unclear, e.g. in Wytham Woods there are several examples of trees called alive in 2008 and dead in 2010 that I found alive in 2012).

[20] See definition of "herb" in §7.

[21] This differs from the *ForestGEO-CTFS* protocols which exclude "species with pseudo-woody stems" and require stranglers to be free-standing (Condit 1998).

[22] In the case of a strangler encircling a host tree, the measured diameter will unavoidably include the host and thereby overestimate the live biomass of the fig. This happens for a very small percentage of stems, however, so we simply accept the overestimate and nevertheless do also measure the host in the tree survey if it is alive (or in the snag survey §4.2 if dead).

[23] Following Condit (1998): "The top of the stem is defined as the base of the lowest living leaf sheath. A difficulty arises when dead leaf sheaths persist, hiding the base of the lowest living sheath, so the location of the top of the stem must be estimated. When measuring the [diameter], dead sheaths are not removed, but are pressed as tightly as possible against the stem. Leaf petioles do not count as part of a stem even when they resemble a stem. Thus palms are often excluded even though their leaves are much taller than 1.3 m, and likewise for tree ferns or certain monocots with long petioles.".

[24] n.b. the RAINFOR protocols in Phillips *et al.* (2009) advise to tag each individual bamboo stem as for a normal tree, but in many cases this is impossible, which is why this modification has been suggested for v3.0 of this manual (July 2014) based on current practice followed in Mudumalai, India (H. S. Suresh *pers. comm.*).

Trees count as being inside a subplot if the stem base is inside (Phillips *et al.* 2009 specified ">50% of the roots"), even if most of the rest of the tree (and perhaps the POM) is outside. Make sure to include leaning trees and multi-stemmed trees (see Box 1), tagging each stem of all multi-stemmed trees individually (even branches that diverge horizontally, if they diverge below POM but reach a length long enough to be tagged)[25]. Tag buttress trees on the main stem (not the buttresses) and stilt rooted species (e.g. mangroves, species like *Socratea exorriza*) on the largest central root if the stem begins above 1.6 m. Lianas that have a maximum diameter ≥10 cm but do not reach 1.6 m above the ground (not 1.3 m) receive no tag.

If a stem has been missed then go back and tag it as soon as possible, but tell Team two that a tree will be out of numerical order (*do not* insert tree numbers like "62a" or "62.5" if a tree was missed by Team one between trees #62 and #63: use the next available number and make a note that this tree is out of number sequence). While doing this, Team one should check that there is flagging tape on all the corners and sides of each subplot (should have been correctly flagged while marking out the plot).

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[25] A couple of examples from Wytham Woods, UK, to make this clear and to show why we do this:

1. Tag numbers 4584, 4585, 4586 and 4587 were given to the stems of a multi-stemmed *Corylus avellana* tree in Wytham in 2008 (in order of size with #4584 the largest). This followed the RAINFOR-GEM protocols, but if the *ForestGEO-CTFS* protocols had been followed, all four stems would have received *the same tag number* 4584 and *stemtag* numbers 1-4 or A-D would have been assigned to differentiate the four stems (in *ForestGEO-CTFS* censuses, you need both the *tag* and the *stemtag* numbers to specify a particular stem, but in RAINFOR-GEM we don't use *stemtag* numbers). In RAINFOR-GEM we believe there are fewer database problems in the long run if we just use tag numbers, but Condit *et al.* (2014) defended the tag-and-stemtag system by saying "The preferred tagging system includes a principal sequence of numbers for individual trees and a subordinate sequence for stems; the two types of tag differ in form so are readily distinguished. So trees are numbered 1, 2, 3, . . . through many thousands, and stems within trees have letters 'A', 'B', 'C', etc. In this system, there are both tree tags and stem tags. There is a commonly used alternative in which all stems get a single sequence and one tag type. The disadvantage to the latter method is that there is no tag defining the tree, and we consider the former the CTFS standard. In the former method, stems are readily associated with the tree they belong with, because each is identified by both a tree tag and a stem tag.".

2. Following the *ForestGEO-CTFS* protocol, *tag* #17091 and *stemtag* #1 were assigned to a single-stemmed *Acer pseudoplatanus* tree in Wytham in 2008. In the following 2010 census, this stem had died but a second stem was found >10 cm on the same rootstock so it was *tag*ged #17091 also with *stemtag* #1 (because it was the new main stem). For analyses using one census only this is fine, but for multi-census analyses trees like #17091 are a problem because it didn't receive a separate tag for each stem.

Condit *et al.* (2014) alleged that it was a defect of the *RAINFOR* tagging method that we "cannot link multiple stems within a tree" because we do not use stemtags. In answer to this, it should be noted that (i) we can link them together through the x,y coordinates (at least to the nearest few metres), (ii) even following the *ForestGEO-CTFS* stemtag system it is not possible to link together by stemtag (i) individuals that branch below ground before breaking the surface (e.g. *Olea* spp.) or (ii) species that grow clonally (e.g. *Populus* spp.).

Finally, in Wytham, Terhi Riutta introduced a system of *group tag numbers* which involved no additional tag on the trees but an additional column in the census spreadsheet like this:

Tag number	Single/Multistem	Group tag number (if multistem)
4584	M	4584
4585	M	4584
4586	M	4584
4587	M	4584
4588	S	-
4589	S	-
4590	M	4590
4591	M	4590
4592	M	4592
4593	M	4592

Each multistem group is assigned a group tag number at first census (normally the tag number of the largest of the group) and this identifies that group in all subsequent censuses (even if the stem with that tag number subsequently dies or one of the other stems grows more quickly to become the largest of the group). This system has many advantages, but at present is not required in GEM censuses. The group tag number does not replace the tree tag, but is a piece of additional information that exists only on the spreadsheet and serves to enable further analyses.

Measuring a tree in Maliau Belian Plot, Malaysia (clearing off any loose bark, dried-up fronds (palms), moss or anything else that might obstruct the diameter tape and moving lianas and replacing afterwards). If it is not possible to move lianas like this and there is no appropriate liana-free POM, use 'large tree' callipers or estimate the diameter as closely as possible where lianas are minimal.

Team two follows, making the initial measurements of each stem. At each free-standing, tagged tree, write down tree number, Point of Measurement (POM), diameter at POM (DPOM) [26], code for tree form (from Appx. I) and an eyes-estimate of stem length (i.e. height[27]; see §1.5). At each tagged liana, write down tree number and the three POMs and other details listed in Box 1 (below). Also note the first and last trees in each subplot in the Comments field[28]. It can happen that a tree gets tagged and then, because of stem irregularities, the POM is rather high and the diameter is <10 cm: if so, do not remove the tag but record and include it.

If measuring also the coordinates of each stem within each subplot, it will be necessary to set up a rope around each subplot as you enter it and, for each stem, measure *lx* and *ly* the coordinates within the subplot parallel to those ropes (n.b. *lx* and *ly* are coordinates *along the ground* not planimetric, so values >20 m can occur on slopes). Move the rope to the next subplot as you complete each one.

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[26] *Do not* round down to the nearest cm (as used in the older literature such as Hamilton 1975): quote *to the nearest mm* (if in doubt, measure it again!). With leaning trees, make sure the diameter tape is taut and at right-angles to the stem.

[27] Requiring all workers to estimate *both* the **total height** (i.e. up to highest piece of foliage following the direction of the main stem) and the **bole height** (i.e. along the main stem as far as the 'crown point' or first major branching point) may help (see §1.5): even if the bole heights are not used, by recording both much confusion is avoided about the point to which height should be estimated. Note that if you are using the Yoneda equation in §3.2 you will need bole heights, which is another reason to estimate them here.

[28] See §10 for an example spreadsheet filled in with these data. However, if possible it is more ideal to use the standardised field sheet templates used by *ForestPlots.net*, which are available to Principal Investigators and Field Leaders by emailing admin@forestplots.net.

Tree #487 in Fragment E plot where the true POM was higher than the longest extensible ladder available locally (5.5 m) so a POM of 5.0 m was chosen instead.

We recommend that the largest trees (i.e. trees with a high POM (buttress trees or irregular trunks) for which a ladder is required) are simply noted on the first pass and censused all together at a later point, which is generally a more efficient use of personnel. Sometimes very large trees are encountered where even a ladder is inadequate (e.g. no clean, regular trunk below 8 m), in which case either use an optical method for assessing the diameter[29] or put the POM as high as you can reach and perhaps make some on-the-spot estimation to correct for over-/under-estimated biomass[30] (write all of this down clearly in the comments column).

Finally, don't forget to write down (i) the date(s) when all trees were measured (inc. perhaps a later date for ladder trees) and (ii) the full names of all people who were working on all those days.

Timing: Tagging trees like this in 1 ha of forest in a 4-person team takes a minimum of 2-3 days (depending on weather conditions).

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[29] This can be a little *ad hoc*, but if you can see where the appropriate POM would be above 8 m then you can take digital photo of the stem there and then also take a photo of some reference object at the same distance (e.g. a stem that you have already measured). By measuring apparent distance on the photos, a reasonable estimate of the DPOM may be derived. This method is certainly better than no measurement, although it will not be precise enough to estimate growth rates and should be used as a last resort only (this is the 'optical method' of Phillips *et al.* 2009). Perhaps a little better would be to use a Wheeler pentaprism.

[30] Ngomanda *et al.* (2012) proposed a method for estimating the biomass of tree buttresses, but currently, we do not follow this and rely on allometries at the analysis stage to estimate this component.

Javier Silva-Espejo measuring DPOM in Tambopata plot 3, Peru.

1.4 Tagging small trees

*** Note that this job cannot be done during or after heavy rain because the paint will not stay on the tree ***

Vianet Mihindou measuring a small tree at Lopé, Gabon.

Equipment: As for tagging large (>10 cm DPOM) trees, but instead of nails you will need ~1 mm diameter fishing twine (at least 100 m of it[31]). Also, you will need callipers (one each and a spare).

Following exactly similar methods to the main census, nominate **five 10 m × 10 m squares per ha** (i.e. 1.25 subplots per ha in total) to be 'small tree' squares (they should be clearly marked on all maps of the plot so that extra care is taken by workers when walking in these subplots). We recommend that these be one each in the central subplot and the four corner plots (or, in an irregular plot, as close as possible to this arrangement)[32]. In these subplots, all trees 2-10 cm DPOM[33] should be tagged and measured as for large trees **except that the tags are tied on instead of nailed on**. Tie the tags on the trees using a slip knot leaving plenty of extra twine to allow for stem growth (n.b. even slip knots do not slip very well after a year or two in the field). For DPOM

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[31] This should be fire-resistant in forests where fire is a regular occurrence (e.g. Mudumalai, India, where they use aluminium twine). Preferably not green in colour if leaf-cutter ants are present (*Atta* spp.) because it may be attractive to them (Condit 1998).

[32] This has been the standard for new census plots since ~2013 and was followed at Bobiri, Ghana, in 2011. Older plots, however, may still follow a different standard (e.g. Metcalfe *et al.* 2009:§3.3.4 instead specified one subplot/ha and at SAFE in Malaysia 5 subplots/ha are being censused for Small Trees).

[33] ForestGEO-CTFS census all stems down to 1 cm DPOM across the whole plot, so no separate 'Small Tree' subplots are set out.

measurements, make *two* measurements with the callipers in perpendicular directions[34] (North-South and East-West), record *both* and take the geometric mean DPOM=$\sqrt{(DPOM_1 * DPOM_2)}$[35].

Important: in order that people take extra care when walking in the small tree subplots, mark them clearly on the sketch map of the plot (§1.6).

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[34] n.b. *not* the maximum and minimum diameters.

[35] Condit (1998) advised to take the *maximum* calliper measurement around the stem (p. 47 therein, Rule 1), however this will overestimate woody biomass in stems of non-circular cross-section.

BOX 1: How to find the Point of Measure (POM[36]).
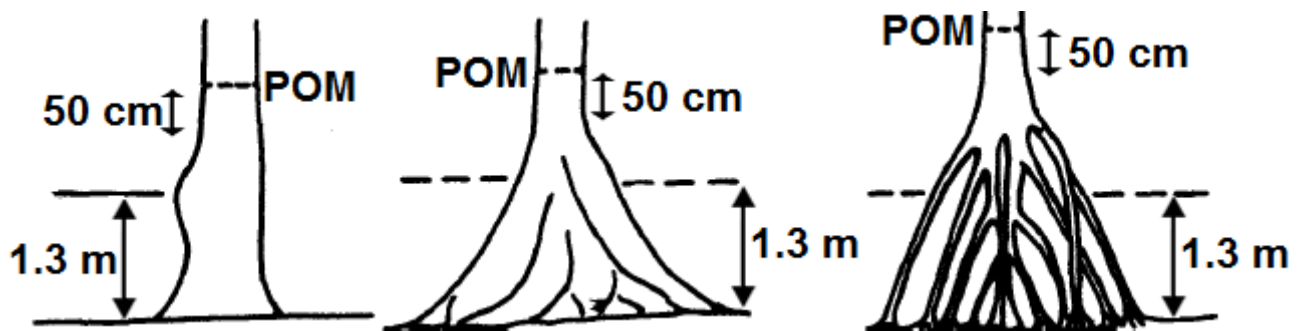
Illustrations modified from Hairiah *et al.* (2001).

The basic rule is:

Put the POM at 1.3 m above the ground or 50 cm above the top of the highest buttress[37] or stilt root (whichever is greater; Condit 1998)

but keeping below any major branching points (e.g. if the top of the highest buttress is 30 cm below a major branching then put the POM just below the branch point; if the buttresses extend up to or above the first major branch point (perhaps much higher than 1.3 m above the ground) then tag each of the branches as individual stems of a multistemmed tree).

For curved stems, these lengths are taken *along the stem* rather than straight. For non-liana stems that initially grow along the ground, take the start point of the aboveground stem to be where you can first completely encircle the stem. In the case of stems that are hollow lower down and seem almost dead, if the upper parts of it are alive then it's all alive so it's fine to put the POM on a hollow section. The diameter of the stem measured at the POM (over the bark) is called **DPOM** [38] [39].

However, if the tree has an irregular or leaning stem or it is a liana then there are a few more complications:

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[36] In CTFS terminology, this is *Height of Measure HOM* (CTFS uses the term *Point of Measure* to refer instead to the number of times the HOM has been changed: if the HOM changes in a subsequent census then this stem acquires a new measurement point so POM is increased by 1).

[37] Buttresses ascend tree stems over time (although at a maximum rate of only 3 cm per year, Condit 1998), therefore, the POM will change over time (see §1.7).

[38] **DBH = Diameter at Breast Height (over the bark)** is one of the most widely-used acronyms in forestry (with breast height taken to be 1.3 m above the ground, e.g. Geldenhuys *et al.* 1988, Condit 1998, although various other standard breast heights 1.3-1.5 m are still in use in some countries). However, because the Point of Measure (POM) is often not at 1.3 m height in primary forest, we are not technically measuring "DBH" but the diameter at the POM, here called "DPOM".

[39] Another point about DBH and DPOM: When you say you measured a tree with diameter tape to be "10 cm DPOM", what you've actually done is measure the girth (circumference) rather than the diameter and the diameter tape divides the measurement by 3.1416 (for a circle, circumference=π*diameter). In RAINFOR-GEM we follow widespread forestry practice (e.g. Hamilton 1975) and avoid use of the acronyms GBH or GPOM or (GPOM/π) even though for irregular stems you could argue that that would be more correct.
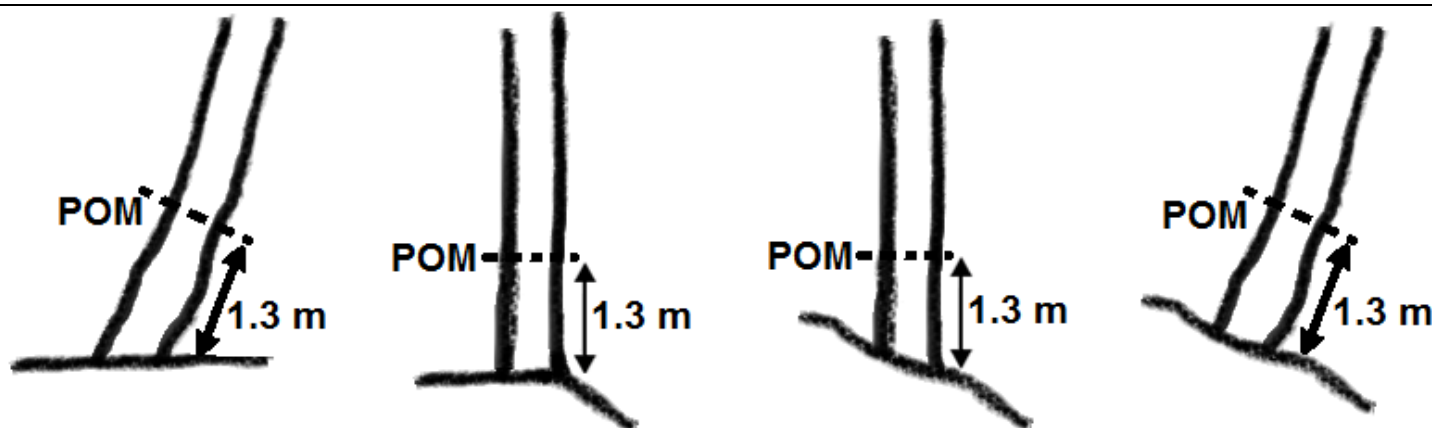
- If the tree is **leaning or bent** (i.e. a lean of ≥10° from vertical), the POM is measured 'underneath the lean' and if on a slope then on the downslope side[40] (trees very seldom lean upslope, but if you find one that does, measure underneath the lean on the upslope side[41]). If the ground falls away completely on the downslope side (e.g. a tree next to a stream) then this *doesn't* count as a slope.

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[40] This rule is a notable difference between *RAINFOR-GEM* (Phillips *et al.* 2009, Metcalfe *et al.* 2009) and other protocols such as those followed by Hamilton (1975), *ForestGEO-CTFS* (Condit 1998), ASB (Hairiah *et al.* 2001), Winrock (Pearson *et al.* 2005a, Walker *et al.* 2012), Köhl *et al.* (2006), Hoover (2008), Ravindranath & Ostwald (2008), TEAM (2010) and Walker *et al.* (2011) where the measurement is taken underneath the lean, but on the *upslope* side of a straight tree on sloping ground (i.e. the 3rd of the 4 trees illustrated here (and in Walker *et al.* 2011 also the 4th) would be measured on the opposite side and therefore acquire a higher POM).

This 'upslope' rule is based on noting that (i) the upslope side is closest to the ground in that case and (ii) sometimes erosion is greater at the stem base on the downslope side, but we follow a different rule because it actually leads to heavy reliance on a clinometer when measuring trees on mountainous or sloping terrain (e.g. think of where to measure two leaning trees on sloping ground if one is leaning just less than 10° downslope and another just over 10°). *RAINFOR-GEM* does a lot of work in montane forests, where because of the steep slopes trees very often lean increasingly over long periods of time, until eventually growing horizontally, which is why this rule makes more sense for our protocols. There is also a *mid-slope* rule, but this is rarely used in general and not used here (http://www.nativetreesociety.org/measure/girth_measurements.htm).

[41] This is very rare, but might occur e.g. as the result of a 'domino effect' treefall event or a storm where the winds happened to run mostly upslope.

A cloud forest where the majority of trees are leaning on steep terrain (Wayqecha, Peru).

- If the **stem is irregular**, search either slightly lower (but never lower than 1.25 m[42]) or higher (but remaining below the first major branch) to find a POM height where the stem is approximately circular (e.g. away from bulges, holes, deformities, unmovable lianas and any evidence of human activity such as tapping or wounds). This does involve a certain amount of subjectivity on how much irregularity is 'irregular', but this is unavoidable: just keep in mind that these diameters will be used to calculate wood volumes and try to avoid large overestimates or underestimates.

- If **roots are exposed** then you should assess whether this is a non-roots-exposed species (i.e. not a strangler and no stilt roots) where loose soil has been washed away for some reason (in which case the POM should be measured from the lowest point of the identifiable stem) or a roots-exposed species (in which case the POM should be measured from the soil surface).

- If the stem is either so **irregular** that it is never even approximately circular (e.g. cauliflorous *Cola* spp. which have stem protruberances) and/or the tree is **fluted** (e.g. *Aspidosperma* sp.) or **fenestrated** (e.g. *Platypodium elegans*), then set the POM at 1.3 m, measure around the irregularities and code the tree '**e**' (see Appx. I).

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[42] n.b. remember that if any woody stem fails to attain 1.3 m height, it should not be included in the survey (whether regular or irregular).
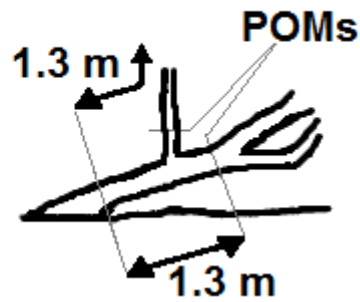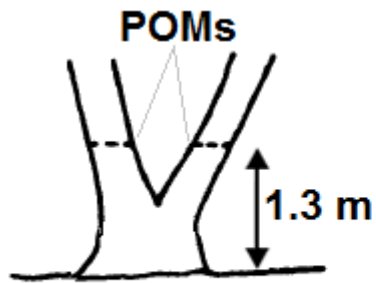
Multi-stemmed trees (for a real example of a tree similar to this, see Box 2).

- A **multi-stemmed tree** is taken here to be one that is single-stemmed up to a certain point but splits into more than one stem above that[43]. If the splitting point is below 1.3 m *along the stem* (or even below ground) then each stem ≥10 cm diameter should get a separate tag and all should be coded '**h**' (see Appx. I). If the splitting point is above 1.3 m along the stem then this counts as *one single tree* and does not receive code '**h**' (if the splitting point is between 1.3 m and 1.6 m then it is still a single tree but put the tag on the largest stem). Coppiced trees count as multi-stemmed trees (if any of the stems are large enough). Stilt roots must not be recorded as stems. For more about multi-stemmed trees, see footnotes in §1.3 above.

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[43] This protocol is in line with Hamilton (1975) and Geldenhuys *et al.* (1988:9).

Measuring a liana at three points in Maliau Belian Plot, Malaysia (tree #342)

  - **Lianas**: Any liana[44] or hemi-epiphyte[45] >10 cm diameter at any point along the stem within 2.5 m of the ground should be included in the census[46] (in Small Tree censuses §1.4, >2 cm diameter). Each liana stem should have its diameter recorded at the following 3 different points (Phillips *et al.* 2009; see Schnitzer *et al.* 2006 [47]):

(1) 1.3 m along the stem from the roots (DH)
(2) 1.3 m vertically above the ground (DV)
(3) at the widest point on the stem within 2.5 m of the ground (DMAX)

The tag will be at 1.6 m vertically above the soil (*not* 1.6 m along the stem as for non-lianas; if more than one point of the stem is 1.6 m above the ground then the tag will have been placed on the largest stem; *do not* tag multiple liana stems with individual numbers even if the stem is divided at 1.3 m vertically above the ground - *one tag per liana only*).

1. Firstly, search for the main rooting point of the liana (if the liana roots at more than one point, select "the last substantial rooting point before the stem ascends" following Schnitzer *et al.* 2008, even if adventitious side roots are present higher up; if there are several separate roots of similar size, measure and select the largest by diameter). This may well be several metres away from the tag of the liana (check that you have correctly identified a rooting point, i.e. the stem fully enters the mineral soil there). If the main rooting point occurs outside the plot, include the liana in the census provisionally but make a note of the situation (there may be a secondary rooting point found later within the plot).

2. Once you have the rooting point, measure the stem diameter at **1.3 m along the stem from that rooting point** (=**DH** =**"d1.3largo"**[48]). Record this and paint the point. If the tag is now clearly on the 'wrong' ascending part of the liana, move it to this part. The decision whether or not to include the liana in the census, by the way, is made based on the value of DH rather than DV or DMAX (because most allometries for lianas are based on DH).

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[44] i.e. a climbing plant that produces true wood, and germinates on the ground but loses its ability to support itself as it grows. This excludes climbing monocots (e.g. rattans and other climbing palms) and 'subwoody' dicots. See Gerwing *et al.* (2006) for details.
[45] Also see Gerwing *et al.* (2006).
[46] As mentioned in Clark *et al.* (2001a), lianas and hemiepiphytes whose stems do not descend to near the ground within the plot will be missed, but this is unavoidable and, in any case, rare.
[47] Schnitzer *et al.* (2006) gave the four "most common measurement locations for lianas" as the three listed above and also "20 or 30 cm from the roots or close to the ground" (we ignore this fourth one) and they must have preferred to use DH because they went on to base their allometries on it (they also noted that liana basal area can vary by 50% depending on which diameter is used to calculate it).
[48] This should *not* be called "DMIN" because it is not necessarily the minimum diameter of the liana.

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3. Next, measure the stem diameter at **1.3 m vertically above the soil** (=**DV** =**"d1.3altura"**; n.b. this may be several m from the rooting point; if more than one point of the stem is 1.3 m above the ground, choose the one closest to the tag along the liana; do not move up or down to avoid irregularities) and mark the measurement point with paint. If the liana is long enough to have a DH but never attains 1.3 m vertical height, record a null value for DV.

4. Finally, measure **the largest diameter the liana attains within 2.5 m of the ground** (i.e. over all parts of the liana that can be reached without a ladder) (=**DMAX**; most often this is close to the ground on a deformity or a branching node with anomalous growth). Record and paint the point (no need to record the height of that point). It's fine if this diameter is close to where DH is measured (in fact this will usually be the case because only a few species of liana 'bulge' significantly at points above the ground).

If these instructions[49] are still not clear, see Example 3 of Box 2 (below). Don't estimate the height of the liana (usually impossible). If it is clearly wrapped/looped around another tree, code it '**t**' for strangler as well as life-form code '**Fl**' (see Appx. I) and record the number of the host tree in the comments field (if the host is <10 cm in diameter or outside the plot, also record whether the host is alive or dead).

Cabled lianas: Some lianas are composed of separate cables (with the cables progressively splitting as the liana ages and each cable thickens, e.g. some Malphigiaceae). In these cases, diameter is estimated by tightening the diameter tape (and, if necessary, dendrometer), around all adjacent cables originating from the same root base. With board-like cables (like flat planks), don't push them together in a stack to minimise the diameter, but end-to-end (i.e. how they will have grown before separation). If the cables are too solid to move together[50] then measure the largest cable only (ignoring all others).

Lianas not circular in cross-section: Other lianas are clearly not circular in cross-section (e.g. 'monkey-ladder' *Bauhinia* spp.). These stems should be measured in two ways: conventionally (i.e. wrap the tape round the whole stem) and by twice measuring the linear distance of both the maximum and minimum dimension (i.e. to give four diameter measurements $d_{min,1}$, $d_{min,2}$, $d_{max,1}$, $d_{max,2}$) and taking the geometric mean (i.e. diameter=$(d_{min,1}*d_{min,2}*d_{max,1}*d_{max,2})^{0.25}$). Record *all four* diameters in the field at each census (to allow basal area calculations based on the individual d_{min} and d_{max} values and also because geometric means will seldom be calculated in the field). Therefore, enough space should be available in the census sheets for up to 12 diameters at a single liana stem.

Branching lianas: It can be difficult to decide where one liana ends and another starts. Lianas are sometimes connected to one another below ground but this can be hard to establish. Therefore, for ease of application we apply the criterion that any climbing stem that fully enters the mineral soil counts as an independent plant (an 'apparent genet'). If unsure, then tag the stem and comment that it may be the same as another stem. In cases where the liana plant branches, each stem that branches within 2.5 m vertical distance from the ground and attains ≥10 cm maximum diameter is measured. In practice it is rare for a branching liana to have two or more branches ≥10 cm diameter.

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[49] n.b. this protocol differs in some respects from what is advised in Gerwing *et al.* (2006) and Schnitzer *et al.* (2008).
[50] Tree #312 in the Maliau Belian plot, Malaysia, was a good example.

BOX 2: Five unusual trees.

Example 1: Here is tree #244 in the SAFE B North Plot (with Walter Huaraca Huasco measuring in July 2011). This tree has fallen over (you can see the large root tip-up mound to the right) and two branches from before it fell are now almost vertical and both are greater than 10 cm diameter if measured at 1.3 m vertically above the ground (the ground is slightly higher to the right at the tip-up mound). Note that this tree has been given *one* tag only and that is on the main stem 1.6 m from the stem base (*not* the branches, even though the whole main stem lies below 1.3 m vertical distance above the ground) and that it is coded '**d**' (fallen) and the height is measured *horizontally* along the stem not vertically.

All three are >10 cm diameter
at their lowest point

1.6 m

1.3 m

1.2 m ➡

Buttress   Main stem
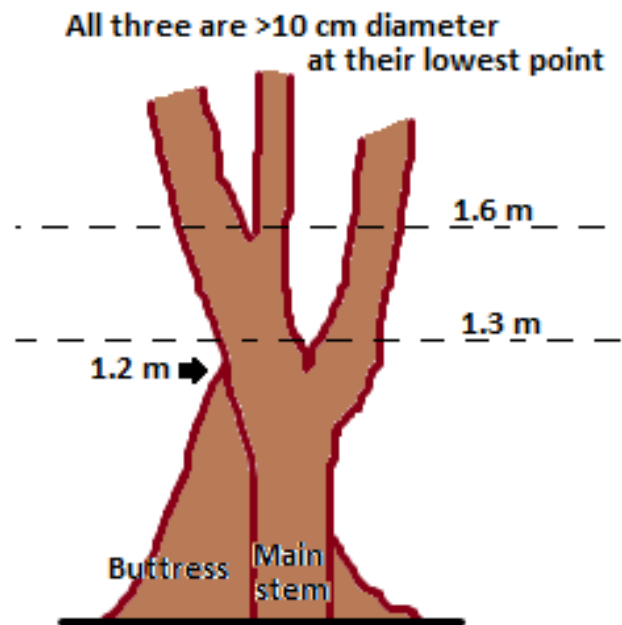
Example 2: Here is a drawing of trees #595 and #596 in SAFE Fragment B South Plot. First question: How many tags should this tree be given? At 1.6 m height it has three stems >10 cm diameter each, at 1.3 m only two and lower down only one. Also, does the buttress change anything? It's presence pushes the POM up to 1.7 m for the leftmost stem, but does this apply also to the other two stems? Answer: *two* tags because you look at how the tree is divided at 1.3 m (even if the POM is different from 1.3 m): on the left (tree #596) we kept the tags at 1.6 m and tagged the larger of the two stems. Second question: should the leftmost stem be measured at 1.7 m height (50 cm above the buttress)? No: because the POM should remain below major branching points (actually, instead of putting the POM at 1.5 m we avoided a small irregularity and put the POM at 1.3 m). Should the rightmost stem (tree #595) be measured at 1.7 m height (50 cm above the highest buttress, even if on the other side of the tree)? Well, to do this would have been a strictly correct interpretation of the protocols, but in this particular instance the buttress arguably doesn't affect stem #595 and we found the stem to be acceptably round at 1.4 m height so we put the POM there.
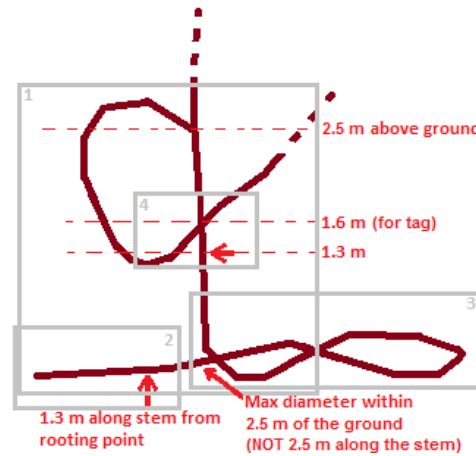
Example 3: Here is a large liana on Rhino Ridge Trail near the Danum Valley Field Centre, Sabah, Malaysia (the drawing of the tree shows where close-ups #1-4 were taken). This liana is corded (close-up #4) and has three stems at both 1.3 m and 1.6 m vertical height above the ground. Where to tag and measure this tree (if it were in a census plot)? Firstly, the liana would get *one* tag only and that on the main vertical stem (not the branch looping down). For the main rooting point: in close-up #2 three large cords enter the ground at three different points and of these we select "the last substantial rooting point before the stem ascends" (Schnitzer *et al.* 2008), in this case the one in the middle of the path. The DH diameter at 1.3 m along the stem from that rooting point can then be measured, although a little digging is required because the liana is growing along the ground at that point (i.e. passing the diameter tape around the stem below ground level). The DV diameter at 1.3 m is further along that stem, pushing the cords together as much as possible to make the measurement. Finally, the maximum diameter within 2.5 m of the ground can also be found, which here also occurs while the liana is on the ground.

Example 4: Trees that form extra stems out of thickened aerial roots: for example, these trees in India (upper at Khajuraho, lower at Mudumalai). In banyan groves there are usually several examples of such trees and a similar situation arises with cabled lianas where the cables are too solid to move together, and with trees where more than 1.3 m of roots have been exposed through soil movement. In such trees, you must basically find a ladder long enough to go above all the roots (inc. aerial roots) and both trees above would have been recorded as two-stemmed individuals and the DPOMs set on the two stems they combine into above the aerial roots. With such trees, remember that the primary purpose of these measurements is to estimate *biomass* and this method serves adequately for that[51].

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[51] As Condit (1998) put it: "There will inevitably be some trunks that simply defy measure, for instance when completely buried in a strangler fig. The best possible measurement must be taken—at least some estimate is better than none—and the location of the POM painted and recorded. As long as these cases are rare, the best estimate of the team leader is acceptable".

By the way, some trees in the world are as large as 1.5 ha on their own and these are certainly beyond our protocols as they stand (http://www.outreachecology.com/portfolio/The%20Worlds%20Largest%20Trees%20Cataloguing%20Indias%20Giant%20Banyans-

Example 5: Finally, a test. This tree is at Wittenham Clumps (Oxfordshire, UK). Where would you tag it? Which side would the tag go? Where's the POM and how would you measure the diameter? Answer at the end of §1.7.

by%20YD%20Bar-Ness-Outreach%20Ecology%20Report-Jun10.pdf). For me, these examples show that we've still got a long way to go in understanding forest ecology completely (!).

1.5 Tree height

Tree height should be measured or estimated for all stems >10 cm diameter in the tree census (§1.3). Having tree (total) height as well as DPOM allows the more accurate with-height allometric formulae of Chave *et al.* (2005) to be used for estimating biomass (recently updated by Feldpausch *et al.* 2011, 2012, Banin *et al.* 2012).
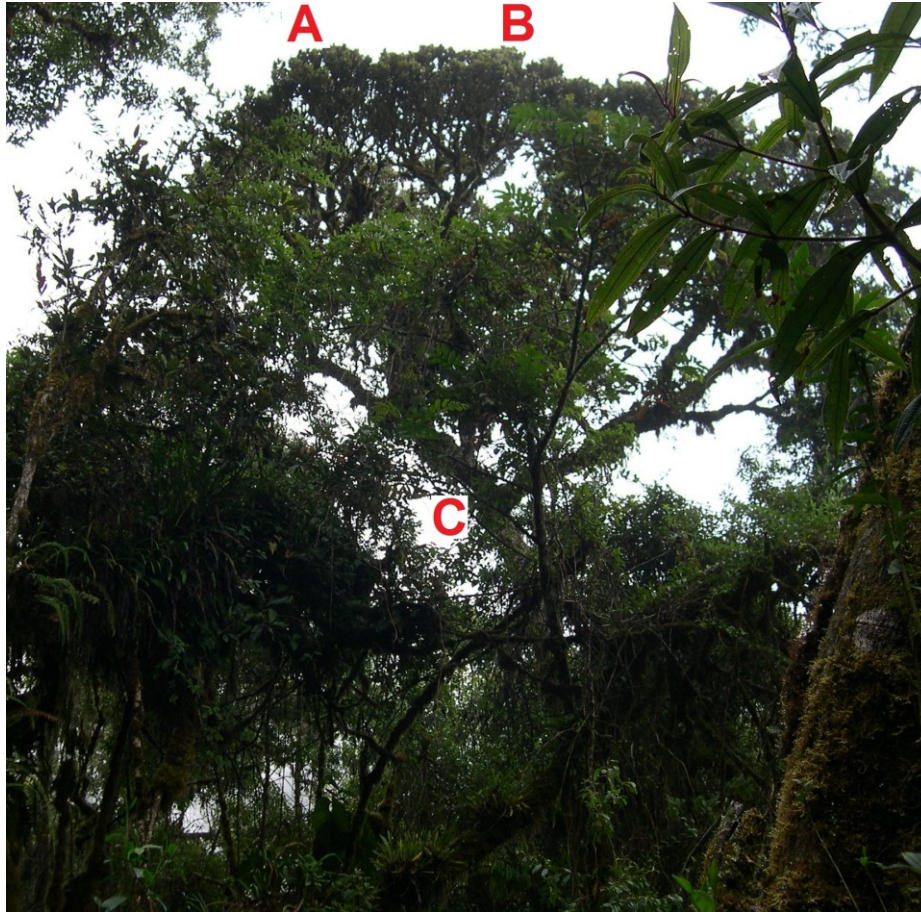
A view of a forest canopy individual from below (at Wayqecha, Peru). Note that for this tree the *bole height* is from the base to point C (the 'crown point' or point of first major branching), the *total height* is from the base to the last piece of foliage at point A (the *stem length* following the line of the stem) and the *vertical height* is from point B straight down to the ground[52] (the *crown depth* is the total height minus the height of the lowest piece of foliage, the *merchantable height* is the length of timber that may be made out of the tree, usually considered to go up to the 'timber point' where overbark diameter drops below 7 cm [53]). The protocols require only the **total height** to be recorded (though it would generally be of great interest to also record the bole height or crown depth or other measures). If the **crown is partially damaged** and most foliage ceases quite low down but one side branch continues upwards several m further then the recorded height is that of the side branch even though it may not account for a large fraction of the crown (this situation is quite common). For **palms**, record *both* bole *and* total height (defining the bole top as the base of the lowest living leaf sheath, Condit 1998). For **tree ferns**, **cycads** and **aloes** follow an approximation of the palm tree rule.

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[52] This differs from other definitions, e.g. the "plant height" of Cornelissen *et al.* (2003) and Pérez-Harguindeguy *et al.* (2013) is actually our *vertical height*, not *total height* (and the *ForestGEO-CTFS* protocols follow this convention rather than ours: see the 'crown traits' document at http://www.ctfs.si.edu/data///documents/Crown_traits_draft.pdf and Larjavaara & Muller-Landau 2013). We focus on *stemlength* in particular, however, because that is what determines the woody biomass of the stem and, therefore, is the correct height to measure for carbon assessment (i.e. *DO NOT* follow the *ForestGEO-CTFS* convention or Larjavaara & Muller-Landau 2013 in *RAINFOR-GEM* plots).

[53] Tree Volume Over Bark *VOB* is usually defined as the inventoried volume over bark of free bole, i.e. from stump or buttress to first main branch (e.g. Brown 1997, GOFC-GOLD 2010) and this implies a log of 'commercial', 'merchantable' or 'derbholz' height *L*, which will be the bole height less the height of the stump and any removal from the top either because of defects or diameter <7 cm (see Hamilton 1975 or http://www.fao.org/docrep/w5796E/w5796e06.htm). Especially in pasture/savanna trees (or other sympodial types), *L* can be much less than the total height of the tree.

A tall tree close to plot B North, Sabah, Malaysia (estimated 50-60 m tall).

In addition to the eyes-estimate of height recorded during tree censusing (see §1.3 and 'Method 2' in Chave 2005), as many trees as possible[54] should be measured more accurately with either a clinometer (see e.g. Hairiah *et al.* 2001, 'Method 1' in Chave 2005, Banin *et al.* 2012) or, preferably, a laser rangefinder (Banin *et al.* 2012). If using a rangefinder, you will find that it is difficult to fix on distant foliage so range to the highest visible branch (e.g. the smallest branch that can be fixed on) and adjust the height upwards by an estimated amount based on the position of this branch relative to the crown top.

Note that for trees where the top is not easily visible (e.g. the tall tree above), you should find a point far enough away that you can see the crown top[55] (e.g. Chave 2005 suggests distances ≤10 m for small trees, ≥30 m for tall emergents). For leaning trees and/or sloping terrain, range not only to the tree top but also - separately but without moving the tripod - to the tree base[56] and use trigonometry to deduce the stem length (Marthews *et al.* in prep.). If you have recorded both bole height and total height in the tree census (see §1.3), range separately also to the first major branch point. For the subset of trees measured more accurately, generate a regression of eyes-estimated height against true height and use this to identify outliers and then recheck those heights.

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[54] It has been suggested to follow a standardised sampling protocol, e.g. 50 stems per hectare (inc. both leaning and straight), 10 per size class (10-20, 20-30, 30-40, 40-50, >50 cm DPOM) plus the 10 largest stems, if not already included (this protocol is similar to those discussed in Banin *et al.* 2012). This usually equates to 2-3 days work.

[55] It is not necessary to measure every tree from the same distance (e.g. 15 m, 20 m, 30 m), but the distance to the base should be recorded for each tree.

[56] Here we take the "tree base" to be the centre of the stem base (differing from "the upslope side of the trunk base (where trunk and soil meet); hereafter referred to as the 'base of the tree'" in Larjavaara & Muller-Landau 2013 which we *do not* follow).
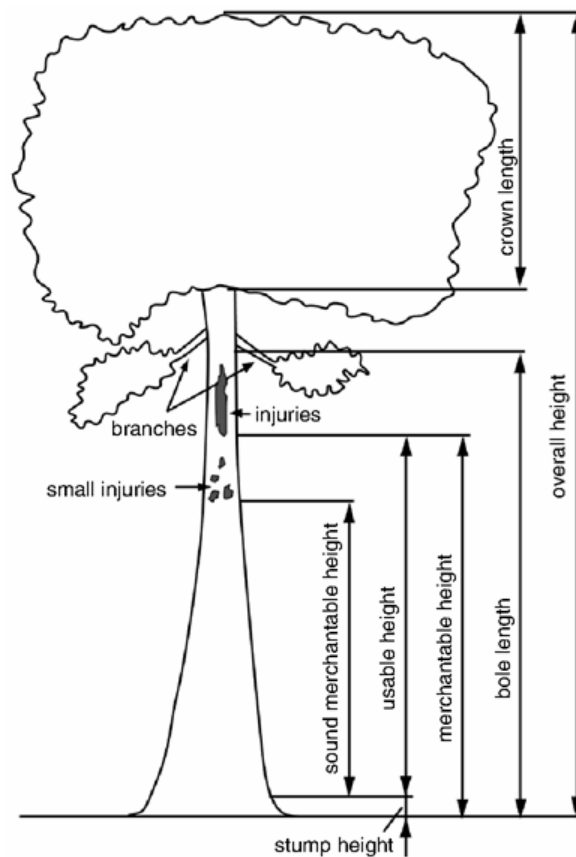
Tree height definitions from Köhl *et al.* (2006).

Height-diameter relationships derived from closely located plots (e.g. regional clusters) provide the best estimate of tree height for unmeasured trees. Where height data are not available, tree height may also be estimated in plots that lack plot-specific height models by applying continental (Banin *et al.* 2012) or regional height models (Feldpausch *et al.* 2011).

1.6 Sketch map of the plot

At this point it is useful to make a sketch map of the plot to show where the subplots are:
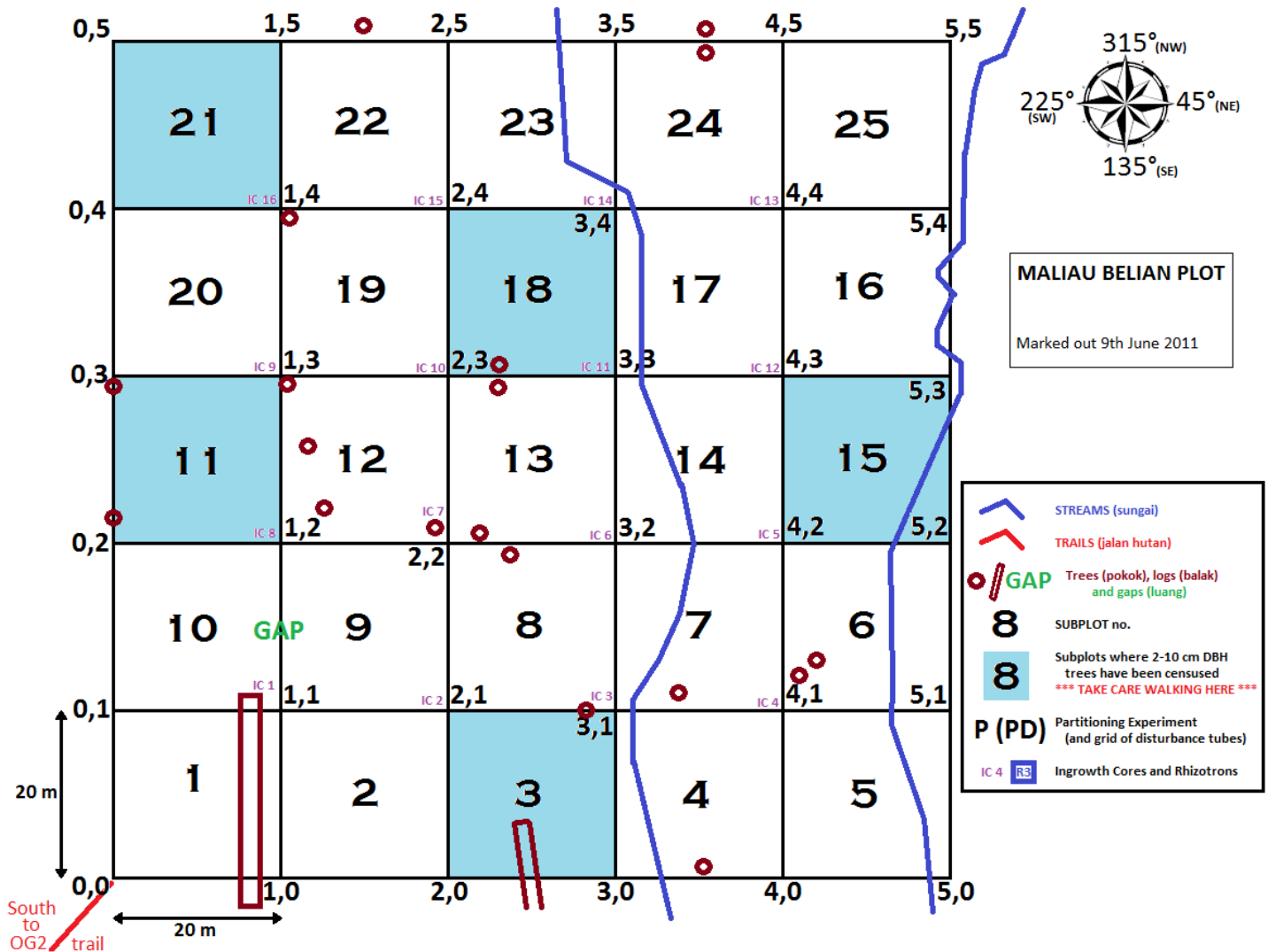
Sketch map of the Maliau-Belian plot, Sabah, Malaysia (actually, this sketch was made after the in-growth cores had gone in §2.1 but before the rhizotrons §2.2). Additionally, litter traps are sited at the centre of each subplot (not marked). Note that in this plot five full subplots were censused for small trees rather than the standard five 10 m × 10 m squares (§1.4).

1.7 Recensusing

A new plot census should be conducted **every year** in *GEM* plots to incorporate new recruits into the survey. Every recensus should be carried out as close as possible to the same time of year as the original census. When recensusing an existing plot, the protocols are essentially the same, however please note the following:

- Trees that have died since the last census need to be censused and coded (see Appx. I for details). Once dead, these stems will be measured in the CWD survey §4.2 (see Baker & Chao 2011).
- *'Unlikely' recruits*: Occasionally, relatively large trees of slow-growing species may 'appear' in the plot. We assume these were missed in the previous census and calculate their previous diameter using the median growth rate of the appropriate size class (10-20, 20-40 and 40+ cm) (Phillips *et al.* 2009).
- *Missing data*: This should be extremely unlikely to happen, but if necessary use linear interpolation to estimate diameters of trees that have been missed during intermediate censuses (see Appx. I).
- *Abnormal growth*: Correct obvious typos in previous census data in the field. Often, incorrect measurements show up when a plot has several censuses, as odd measurements in an otherwise steady sequence. In these cases interpolated values are used (Phillips *et al.* 2009).
- Over time, tree growth means that tags attached by nails may find their nails become partly 'eaten' by the tree and wire, thread, tape or twine may decompose (Condit 1998). Extra nails and/or twine should be taken along to allow some trees to be 're-tagged' during each census. Please avoid reusing tags unless absolutely necessary (e.g. never reuse the tag of a dead stem).
- When remeasuring trees, if the top of buttress has grown within 30 cm of the marked POM (sometimes called 'buttress creep'), in addition to measuring at original POM, measure diameter 50 cm above the first POM and paint the new POM, not the old POM. Discard low POMs as buttress extends over them with time. This procedure ensures that there is always a consistent, non-buttressed measurement of diameter growth (Phillips *et al.* 2009, Lopez-Gonzalez *et al.* 2011).
- When lianas slip to the ground or produce new adventitious roots, change the POM(s) accordingly (see comments in Gerwing *et al.* 2006, Schnitzer *et al.* 2008). Lianas that die due to death of the host but not immediately (i.e. there is a census when the liana is on the ground) should still be coded as multiple death (see Appx. I).

In all cases of correction, a record should be kept of the original measurement, the presumed error, and the correction made (see Flag 4, Appx. I). In the case of a change in POM, take one measurement at the original POM and another at the new POM (n.b. this only needs to be done in the year of the POM change, and in subsequent censuses only measure at the newest POM; for more information on *Managing Point of Measurement (POM) changes*, see Lopez-Gonzalez *et al.* 2011:Appx.S2).

Answer to Example 5 above: The POM should be 1.3 m up from the ground underneath the lean, which is a problem because the lower part of the stem is buried. Rather than digging underneath, take the point where the stem leaves the soil completely (just to my left in the photo) and go 1.3 m underneath the lean from there along the stem to place the POM. Another 30 cm along the stem is the tag point, although I would place the tag on the top side of the stem so that it is more visible. There wasn't, but if there had been any side-branch of the tree arising from the stem between the roots and that POM, it would have counted as a second stem and would have been tagged separately.

2. BELOW GROUND MEASUREMENTS

We start with roots. Because of the importance of the root component, we measure root biomass using two independent protocols: in-growth cores and rhizotrons. Both measure surface root biomass and growth (<30 cm depth): please note that both of these methods as described here require soil depth to be at least 40 cm (sometimes restrictive in mountainous areas) and not too rocky: if your soil is shallower than this, the in-growth cores and rhizotron windows must be made shorter (and the calculations appropriately modified).

2.1 In-growth cores

In-growth cores are a way of measuring fine root growth at ≤30 cm depth (Metcalfe *et al.* 2008a, b, Girardin *et al.* 2010, 2013).
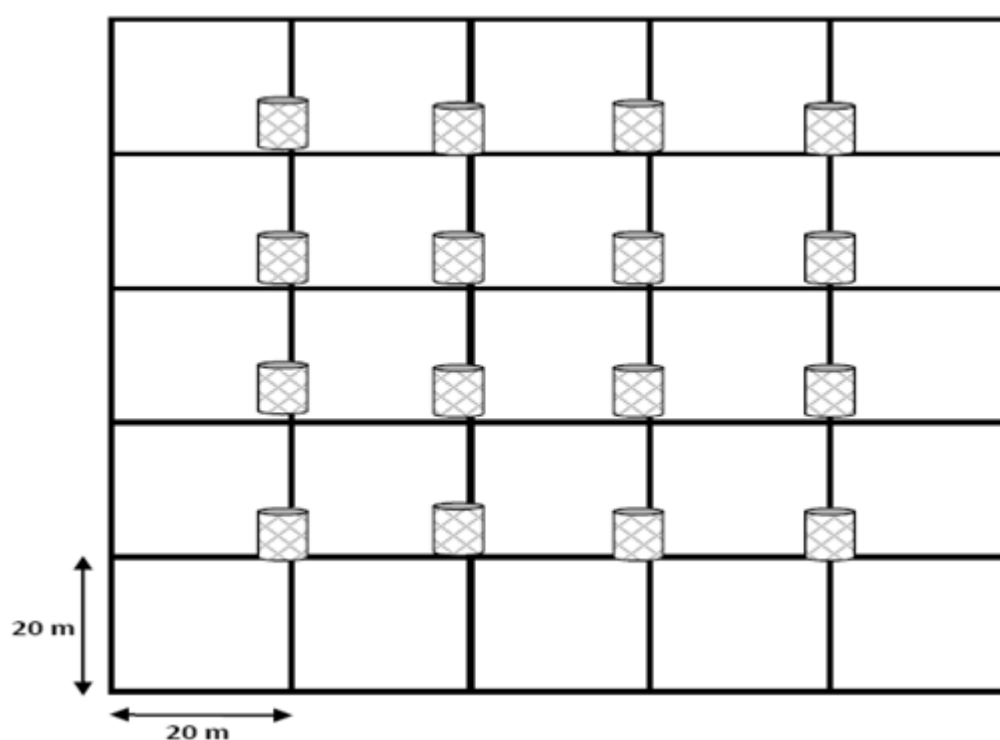
See Box 3 for how to construct in-growth cores.

In-growth core installation

Equipment: 6 bin liners (or similar large plastic bags), something to sit on, permanent marker pen (+spare) per team, 16 in-growth cores, map of where to put them (place them to avoid the transects for coarse woody debris surveys), weather writer, labelled sample bags (plastic sandwich bags (80 per ha; 10 cm × 20 cm or larger) labelled "IC 1 (1)", "IC 1 (2)", "IC 1 (3)", "IC 1 (4)", "IC 2 (1)", "IC 2 (2)", ..., "IC 16 (4)" grouped up into sets of 4 in larger sample bags labelled with the plot name, the date and "IC 1", "IC 2", "IC 3", ..., "IC 16"), post-hole digger (+spare) for each team, tape measure, soil moisture and temperature sensors, stopwatch for each team, scissors for cutting small roots, weighing balance (0.01 g resolution), duct tape, 8 fine (normal kitchen) plastic sieves for washing fine roots, paper bags (e.g. 0.5 kg of old newspapers per ha).

If the plot is a regular square, install the in-growth cores in a 4 × 4 grid as shown (if not square, get as close to this as possible; also try to position the cores so as to avoid where the transects for coarse woody debris surveys will be placed - see §4.2). Because of foot traffic along the subplot edges, place the cores 1-2 m away from the corner points. At each in-growth core location, remove and retain litter from a small 12 cm diameter area (place the cage down and cut around with a machete) then remove a core of soil with a post-hole digger. Place each core on a plastic sheet (if there are clear soil horizons, split across more than one plastic sheet) and manually remove the roots from the soil sample for a period of 40 min per sample *but* split the removal period into 10 min intervals (following Metcalfe *et al.* 2007c; do search the *entire sample during each interval (inc. all horizons)*). Whilst processing each sample, try to keep search effort constant (each sample should be processed by *one person only*: differences in effort between samples are no problem but differences within samples should be minimised). The purpose of searching by hand instead of using sieves is to avoid excessive alteration of soil texture. Sometimes you find fine roots inextricably growing through leaf fragments, in which case remove as much leaf as possible but retain the whole volume for weighing (the additional biomass from the leaves is usually minimal). Metcalfe *et al.* (2007c) commented "In addition, root samples collected in each time interval may be subdivided into categories (e.g. live/dead, mycorrhizal/non-mycorrhizal, fine/coarse, different species) to derive a more detailed assessment of root material present in soil samples.", but this remains optional.

If you have visibly clear soil horizons in your cores, do you separate them? Metcalfe *et al.*(2007c) homogenised their cores, but since 2012 we have tried to separate obvious horizons: do this by searching separately the horizons on your plastic sheet (however, the roots from each still go together into the same bag for each 10 min interval) and then returning them to the core in the same order afterwards. Do not search for 10 min in each horizon, however : search for 10 min across all horizons (i.e. total time will still be 40 min). Splitting the removal period into intervals allows estimation of the amount of root material remaining uncollected in the soil sample after 40 min (see *Analysis* below).

Place the roots collected from each interval into separately-labelled plastic bags (labelled with site, plot, date, sample number and interval), wash each back at camp (using the sieves and a bucket) and place in similarly-labelled paper bags (make up 64 bags/plot, each approximately 12 cm × 16 cm so that they can fit in the smaller sample bags), dry at 80°C until constant mass and weigh (separate into roots greater and less than 2 mm[57]) - all as soon as possible (if no oven is immediately available, store in a freezer until they can be dried, but for no longer than a week). After weighing, roots are currently discarded, but they may also be stored for further analyses (e.g. for scanning a subsample for establishing length/mass ratios). Sample dry mass from these first cores represents standing crop surface root mass.

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[57] 2 mm is the standard threshold between coarse and fine roots (see e.g. Cornelissen *et al.* 2003). However, remember that in-growth cores do not adequately sample coarse roots because roots larger than ~5 mm diameter are found very rarely (see §2.3): the point of this is just to improve comparability with other fine root estimates.

Installation of in-growth cores by Luiz Aragão and Magnologia in Rio los Amigos, Peru (top left) and Rostin Jantan in Sabah, Malaysia (top right).

Next, insert the cylindrical core into the hole, and replace the root-free soil in and around the core. Place leaf litter to ambient levels onto the surface of the core to mimic field conditions.

Timing: Estimate one person-hour per in-growth core in the absence of rain.
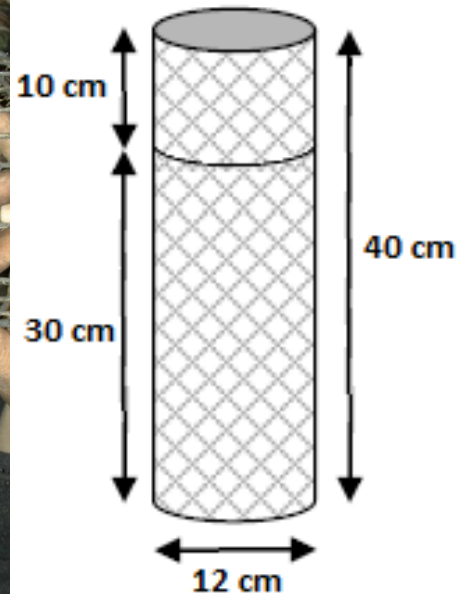
An in-growth core (photo W. Huaraca Huasco; core measures 12 cm in diameter by 40 cm long with the soil level 10 cm from the top as shown).

In-growth core repeated measurements

Equipment: 6 bin liners, something to sit on, permanent marker pen (+spare) per team, 16 in-growth cores, map of where to put them (place them to avoid the transects for coarse woody debris surveys), weather writer, labelled sample bags (plastic sandwich bags (80 per ha; 10 cm × 20 cm or larger) labelled "IC 1 (1)", "IC 1 (2)", "IC 1 (3)", "IC 1 (4)", "IC 2 (1)", "IC 2 (2)", ..., "IC 16 (4)" grouped up into sets of 4 in larger sample bags labelled with the plot name, the date and "IC 1", "IC 2", "IC 3", ..., "IC 16"), post-hole digger (+spare) for each team, tape measure, soil moisture and temperature sensors, stopwatch for each team, scissors for cutting small roots, weighing balance (0.01 g resolution), duct tape, 8 fine (normal kitchen) plastic sieves for washing fine roots, paper bags (e.g. 0.5 kg of old newspapers per ha).

After an interval of approximately 3 months (Metcalfe *et al.* 2008b), revisit each in-growth core. Before removing each core, take the CO_2 efflux measurement of the soil above the in-growth core with litter and then without litter (after removing the litter, wait a minimum of 5 min to stabilise before measuring the CO_2 efflux and only then remove the core). Record soil moisture and temperature inside all of the cores (without litter), extract the cores, and manually collect roots from the soil following the same procedure described above to estimate root mass production over the 3 month interval. Note the root dry mass collected from each time step in a datasheet. *Important*: record the exact dates of installation and collection of each ingrowth core each time.

Replacement rotation: Over the course of time, in-growth cores become degraded: some soil is lost at each excavation and repeated searching through the core fragments the soil structure. Arrange a rotation so that 3-4 of the in-growth cores are replaced with new cores (at least 1 m away) every three months (i.e. each in-growth core is in the ground for maximum 1 year; the advantage of changing 3 rather than 4 is so that the rotation shifts spatially each time). At changeover, excavate the old core as before but discard the soil into the old core hole, dig a new hole and set up a new in-growth core there using the same cage. This has the added advantage of giving regular root stock data from new locations each time.

Analysis

In-growth core calculations follow the method of Metcalfe *et al.* (2007c, 2008b). To estimate the total amount of fine roots in the in-growth core, a saturating curve is fitted to the cumulative fine root biomass over the 40 min search period. During each 10 min time step, progressively less root mass is extracted, therefore we can estimate the additional amount of roots that would have been found had the search time been longer. We

assume that after 120 min[58] we would have been able to extract all the roots in the sample. Possible extrapolation curves include the following:

$y = a*\text{LOG}_{10}(x)+b$	(Eq. 1, a **logarithmic curve**, Metcalfe *et al.* 2007c)
$y = a*x/(b+x)$	(Eq. 2, a **Michaelis-Menten asymptotic curve**, Metcalfe *et al.* 2007c)
$y = a*(x^b)$	(Eq. 3, a **power law curve**, Metcalfe *et al.* 2009)
$y = a*(1-\text{EXP}(-b*x))$	(Eq. 4, an **asymptotic exponential curve**)

In the equations, y is the cumulative root mass extracted by the end of each time step and x is the cumulative search time. After fitting the curve and deriving the parameters a and b for each in-growth core, the equation is solved to 120 min (*not* to infinity!), which is used as an estimate of the total root biomass in the sample.

Which curve to choose depends on which one provides the best fit. This can vary among study sites (Metcalfe *et al.* 2008b). It is possible that the same curve does not fit or does not converge for each in-growth core, in which case it is useful to test several different curves[59]. In the case that no curve gives an acceptable fit to one or more of the in-growth cores, apply an average 40 min → 20 min correction based on the other curves.

Finally, don't forget to calculate the volume of the in-growth core correctly when converting this mass to a per ha figure (see Box 3).

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[58] In Metcalfe *et al.* (2007c) the curves are solved to 120 min, and they recommended applying logarithmic or Michaelis-Menten curves. However, in Chris Doughty's MATLAB code a power law curve is solved instead to 100 min (which is also fine).
[59] If all curves converge, it is possible to compare the results (they will probably provide slightly different estimates). Terhi Riutta has investigated this using SAFE in-growth core data and is willing to share her results on request.

BOX 3: How to make an in-growth core

The mesh size is 1.5 cm × 1.5 cm here (though smaller ~1 cm is also fine). If this mesh comes in rolls 1 m wide you can cut into 50 cm (allow 10 cm for folding over the base of the core) × 43 cm (12 cm diameter[60] means 38 cm circumference plus a 5 cm overlap) pieces then tie up along the sides and base with ~1.1 mm diameter nylon fishing wire or plastic cable tie (making them like this you can estimate 25 cores from 5 m of mesh).

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[60] Metcalfe *et al.* (2008 a,b, 2009:§4.1.2.1.2) used ~14 cm diameter cores, but the ones in Peru are currently all 12 cm diameter. If you do *not* use 12 cm diameter cores, remember to note the diameter and modify the equations in §2.1 accordingly.

2.2 Rhizotrons

Root observation pits with screen rhizotrons are a method for measuring surface root growth (≤30 cm depth) in tropical field sites[61], allowing more regular readings than in-growth cores (monthly as opposed to three-monthly) (Metcalfe *et al.* 2008a, Girardin *et al.* 2010, 2013). In the Kosñipata valley, Peru, we have found close agreement between rhizotron and in-growth core methods (Girardin *et al.* 2010), but it is strongly advised to implement both protocols.
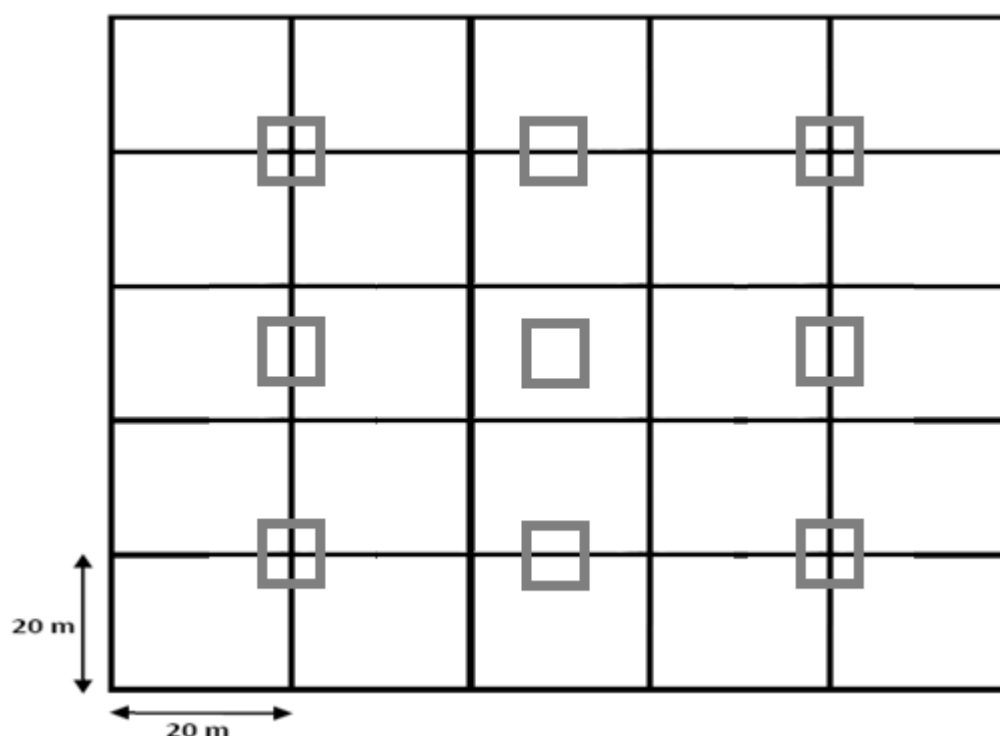
See Box 4 for how to construct rhizotrons.

Rhizotron installation

Equipment: 6 long (15 cm) nails per rhizotron (if using a metallic rhizotron made of MFCs, see Box 4), flat edged spade, post-hole digger, hammer, spirit level, plastic sheets (~1 m^2, e.g. bin-liners) and a metallic grid or grill (~70 cm × 70 cm) to prevent animals (or people) falling into the rhizotron pit

Ideally, installation should take place in the dry season otherwise high rainfall can soon erode the contact between the screen and the soil. If the plot is a regular square, install the rhizotrons in a 3 × 3 grid as shown (if not square, get as close to this as possible; also try to position the rhizotrons so as to avoid where the transects for coarse woody debris surveys will be placed - see §4.2). Because of foot traffic along the subplot edges, place the rhizotrons 1-2 m away from the edges (and the central one 1-2 m away from the middle). At the rhizotron location, permanently mark a 1.0 m × 1.0 m exclusion area (e.g. with flagging tape) which should remain strictly disturbance-free not only during installation of the rhizotron but also afterwards (*especially* no cutting!). Using the spade and trowel, dig a rectangular hole in front of the exclusion area that is ~30 cm across (as close as possible to the exact width of the rhizotron), 50 cm long (length of a forearm + 10 cm) and ~40 cm

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[61] Unfortunately, experience at the Wytham Woods plot, UK, has shown that this method is unsuitable for some clay-rich temperate plots because of the large amounts of soil contraction and expansion during the year. It is also problematic at sites with seasonally shallow water tables.

deep (as close as possible to the depth that brings the top edge of the perspex exactly in line with the surface of the organic/litter layer). If installing a rhizotron on a slope[62], the hole should be dug downhill from the exclusion area (and the downhill wall may be reduced or nonexistent; this will ensure that gravity keeps the soil pressed against the rhizotron screen). The rhizotron will be placed against the ~30 cm wall of the hole abutting the disturbance-free area so this wall should be as close to vertical as possible. Importantly, *do not* pull roots out of the walls: cut with either a knife or scissors as close as possible to the boundary. Soil from the hole should not be discarded but piled onto two separate plastic sheets: one for the organic layer (surface soil) and one for mineral soil (deeper soil, with the division judged approximately by soil colour; a couple of spadefuls of soil for each will be sufficient and the rest may be discarded well away from the rhizotron area).

Rhizotrons in use at Lopé, Gabon (left) and Sabah, Malaysia (right).

Next, remove and discard all large roots from the two soil piles (spend ~5 mins on each pile; only large roots need be removed because fine roots will decompose before first measurement). Then, cut one side of the hole to make it as flat as possible, using a flat-edged spade. Insert the rhizotron vertically against the ~30 cm wall (check with the spirit level; if necessary, remove the perspex protective cover with a craft knife) and secure both behind and to the sides with 6 long nails (if using a metallic, MFC rhizotron, Box 4) or by hammering the rhizotron along the side of the screen and into the soil (if using a steel rhizotron, Box 4). **Make sure that the top edge of the screen is level with the soil surface**. From the soil piles, back-fill behind the rhizotron screen with mineral soil, carefully compacting the soil down with a rod/stick so that the density of the soil is approximately the same as the soil of the walls of the hole at the same level (test the sides of the hole with your fingers to estimate the density). Following the visible horizons of the sides of the hole, back-fill with mineral soil and then organic soil, finally moving some litter from nearby to cover (inc. the screen). Next, place the insulating sponge against the rhizotron screen and prop it up with a stick (this minimises temperature variation and light and water entry). Finally, move the metallic grid (equipment list above) and the rhizotron roof to cover the hole[63], making sure that it covers the hole *but none of the exclusion area*, i.e. the top edge of the roof

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[62] A slight slope is advantageous as it ensures the soil keeps good contact with the rhizotron screen.
[63] There have been some issues in Tanguro, Brazil, with tapirs removing the covers and entering the rhizotron pits

should be directly above the rhizotron screen and *not* beyond it. Move the rhizotron cover so that it does not divert water onto the screen.

Rhizotron repeated measurements

Equipment: A4 transparencies, permanent fine marker pens (black, blue, red, green), soil temperature and moisture sensors

After leaving the rhizotrons for two months to recover from the disturbance of installation (Girardin *et al.* 2010, but cf. Metcalfe *et al.* 2008a who allowed nine months), root lengths should be recorded monthly at each

rhizotron either using protocol #1 or #2 (see below). Soil moisture and temperature should be recorded every session at the same point within 0.5 m of the rhizotron (but not near the rhizotron screen). If possible allow only one person to trace the roots, to avoid apparent changes in root dynamics due to changes between personnel. Transparencies of root tracings from the rhizotrons should be scanned (colour scan, jpg file format, 150 dpi) and saved in a file.

There are two different colour conventions for the root size classess[64] **black-red-blue-green** as used in South America (left stack and the photo: left to right Cécile Girardin, Liliana Durand, Walter Huaraca Huasco and Marlene Mamani Solórzano in Trocha Union plot 4, Peru) or **black-blue-red-green** as specified in Metcalfe *et al.* (2009) and used in Malaysia (right stack). Because of this, it is essential to note which convention is being followed with any rhizotron transparencies.

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[64] Metcalfe *et al.* (2009:§4.1.2.2.3) specified "<1 mm = black, 1-2 mm = blue, 2-3 mm = red, >3 mm = green" (correcting the typo that put "4 mm" for the last category). For mysterious reasons the blue and red colours were swapped at some point in South America. The current situation (2012) is that, because most intensive plots are in South America, the majority of rhizotron users across the network are using their colours as in the photo, but as long as good records are kept there is no problem using either colour convention.
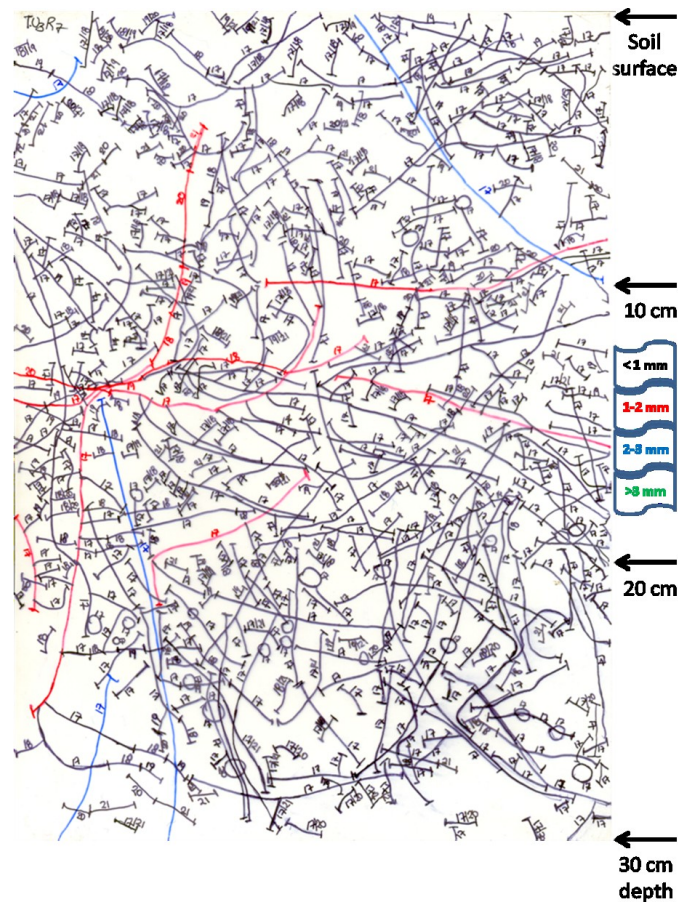
An example rhizotron transparency following protocol #1 (Kosñipata plots, Peru). Roots are traced onto a transparency placed against the rhizotron screen (the same one each time[65]). Different diameter classes are indicated by colour (black-red-blue-green convention) and root growth between two sessions is shown by tick marks (exclude roots/segments which branch after contacting the rhizotron screen). Roots labelled "17" or "17/" were first noted at session no. 17. Roots labelled "17/21" appeared at session no. 17 and were no longer present at session no. 21[66]. Circles indicate a white fungus (noted but not analysed). Note the transparency ID top left (plot code/rhizotron no.). *** PROTOCOL #1 IS ONLY INCLUDED FOR COMPLETENESS: SINCE 2013 ONLY PROTOCOL #2 IS RECOMMENDED IN RAINFOR-GEM PLOTS ***

[65] Cécile Girardin advises that it is very important not to let the number of transparencies accumulate: analyse as you go along.
[66] Bernier & Robitaille (2004) commented that attribution to the 'dead' category is often arbitrary: "one is also often left wondering whether the linear feature appearing on the screen is the root or a simple trace of remains".
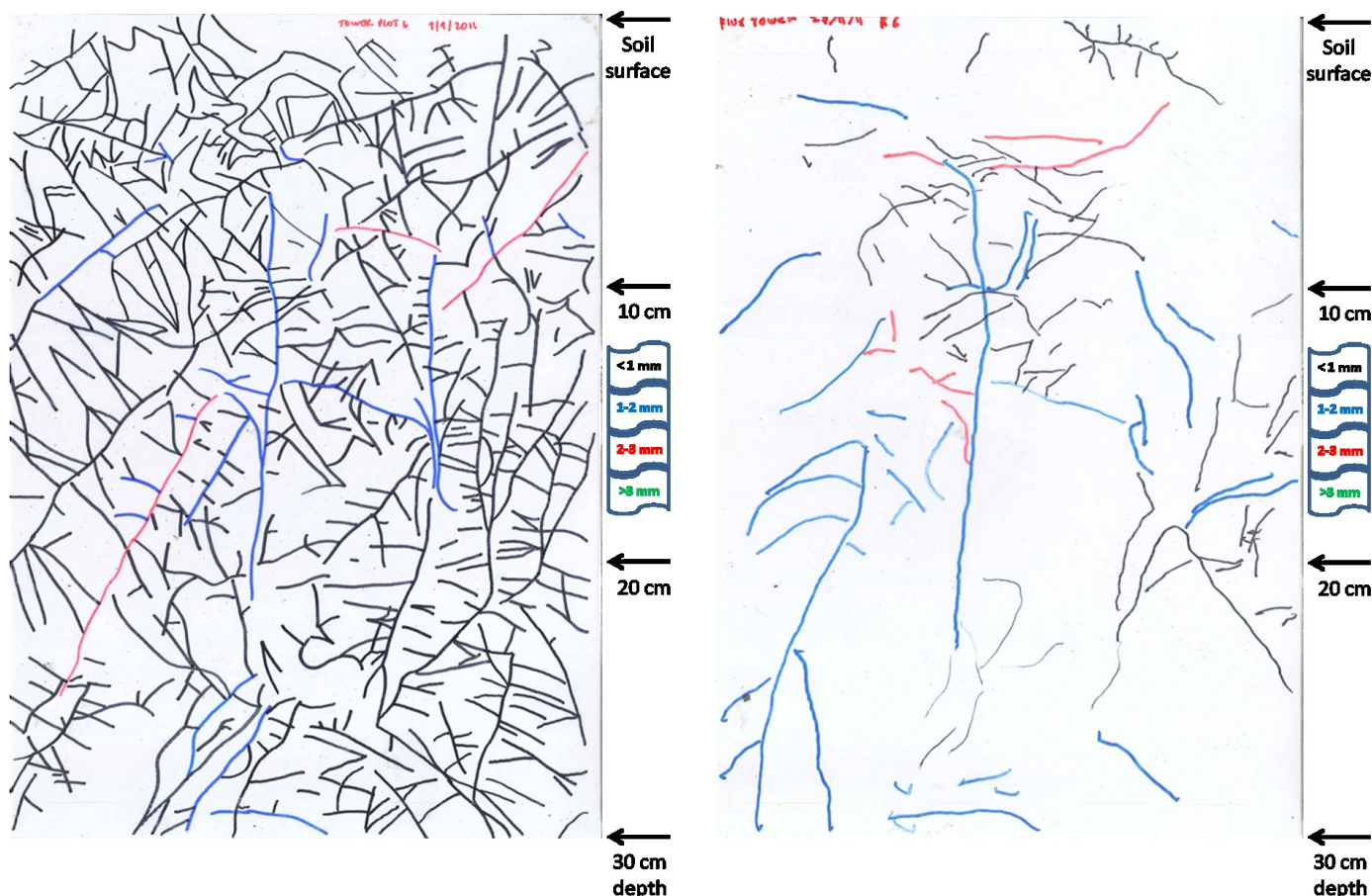
Two rhizotron transparencies following protocol #2 (method C. Girardin; example above from the SAFE plots, Malaysia). Roots are traced onto a transparency held against the rhizotron screen (a different one each time). Different diameter classes are indicated by colour (black-blue-red-green convention), but a new transparency is used each session and all roots are redrawn, which allows tick marks and session numbers to be omitted (exclude roots/segments which branch after contacting the rhizotron screen). Note the transparency ID top now includes the date (plot code/date of session/rhizotron no.). The right-hand transparency was drawn 2.5 months after the left-hand one, showing that (unusually) many fine roots have died between these two time points.

The advantage of protocol #1 is that much less time is spent drawing roots each session. The advantage of protocol #2 is that (a) transparencies can be simply scanned and therefore the image analysis step should be much quicker e.g. using WINRHIZO software http://www.regent.qc.ca/ [67]) and (b) roots moving within the soil between sessions are no longer a problem as they are when following protocol #1.

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[67] See examples at http://www.plantstress.com/methods/Root%20Methods%20PORTAL/Root%20length%20method%20PSU.htm. Other alternatives are RootLM (http://digital.cs.usu.edu/~xqi/RootLM/) or ImageJ (http://rsb.info.nih.gov/ij/, see §4.3).

3.6.1. RHIZOTRON DATASHEET

Plot identity: **Data collector:**

Session Number	Date (dd/mm/yy)	Soil Moisture	Soil Temperature	Session Number	Date (dd/mm/yy)	Soil Moisture	Soil Temperature

Analysis: Calculate the sum of expected elliptical cross-sections of observed roots that would cross the rhizotron screen (XSr, mm^2) using the following equations (Bernier & Robitaille 2004, Metcalfe *et al.* 2007b):

$$XSr = \frac{3.1416^2 * \sum r^2}{\sqrt{2}} \text{ where } r \text{ is:}$$

	Root diameter class			
	<1 mm	**1-2 mm**	**2-3 mm**	**>3 mm**
Assumed mean root diameter (mm)	0.5	1.5	2.5	3.5
so **Mean root radius *r* (mm)**	0.25	0.75	1.25	1.75

Count the number of 'hits' for each root size class (where a 'hit' is where a root has grown so that it would grow out through the rhizotron screen if the screen weren't there, e.g. 55 live root hits <1 mm diameter, 2 live root hits 1-2 mm diameter and 2 dead root hits <1 mm diameter; new root hits must be >0.5 cm to avoid possible confusion with previously-existing root lengths; for protocol option #2 images must be analysed for the number of hits by 'subtracting' the previous image), calculate the expected root cross-sectional area using mean root radii from the table above (e.g. XSr_{live}=(3.1416^2)*[(55*(0.25)^2) + (2*(0.75)^2)]/$\sqrt{2}$ =31.8 mm^2 live roots and XSr_{dead}=(3.1416^2)*[2*(0.75)^2]/$\sqrt{2}$ =0.9 mm^2 dead roots).

Root production (Pr, t/ha) for each rhizotron measurement session may be calculated using:

$$Pr = 2*10000*\rho*(1-F_C)*XSr*\frac{\sin\alpha\cos\gamma}{W}$$

i.e. (Alive root mass production Pr_{live} in t/ha)=2*10000*ρ*(1-F_C)*XSr_{live}*(sinα*cosγ/W) and (Dead root mass production Pr_{dead} in t/ha)=2*10000*ρ*(1-F_C)*XSr_{dead}*(sinα*cosγ/W), where the 10000 value converts g/mm^2 into t/ha, (mass density of fine roots ρ, assumed constant)=0.00029 g/mm^3 (if available, the value here should rather be obtained by dividing root volume by root mass recorded from the ingrowth core data §2.1), F_C is the soil coarse fraction (the fraction of soil composed of particles >2 mm; usually =0 in forest soils), α is the angle of the rhizotron observation screen relative to the ground (for a rhizotron inserted vertically, should =90°), γ is the ground angle relative to the horizontal (i.e. on a 1 in 10 slope put =atan(1/10) =5.7°), and W is the width of the rhizotron screen (mm). Finally, the multiplication factor of 2 is used because roots can only intersect with the rhizotron screen from the front (i.e. it is assumed that if there was not an empty space behind the rhizotron screen to allow for measurement and observation then an equal amount of roots would intersect from behind as well as from the front).

(see this calculation on an Excel spreadsheet in the 'Rhizotron equations exercises' spreadsheet at www.rainfor.org/upload/ManualsEnglish/Rhizotron%20equations.Corrected.xls).

BOX 4: How to make a rhizotron

There are currently two types of rhizotron in use in *RAINFOR-GEM* intensive plots: wooden ones and metallic ones. **Wooden rhizotrons** are generally better for areas where the topsoil is very loose (e.g. upper montane forests where there are root mats and an organic layer >20 cm deep), but if termites are present they eventually become damaged (even if anti-termite varnish is used). **Metallic rhizotrons** are termite-resistant and therefore generally better in lowland areas, but suffer from corrosion (even if anti-corrosive paint is used) and are difficult to attach securely when the soil is loose[68]. On balance, in the absence of other factors, we recommend metallic rhizotrons.
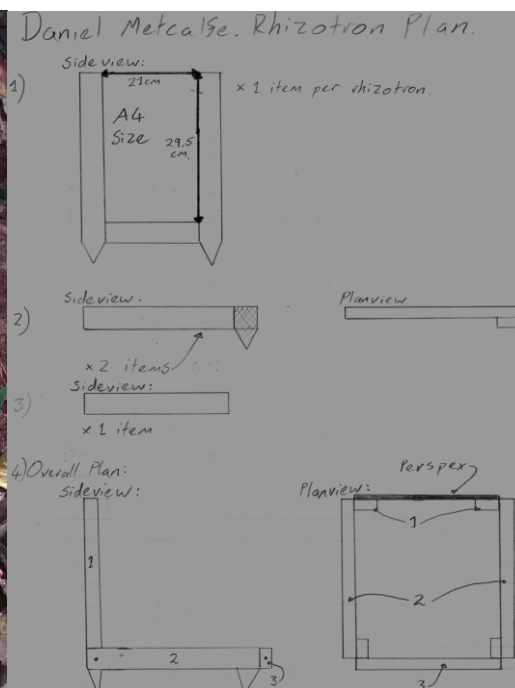
A wooden rhizotron in Wayqecha, Peru (photo W. Huaraca Huasco) and a construction plan (Dan Metcalfe). The polycarbonate here is 27 cm × 36 cm bolted to the wooden surround (2 cm × 4 cm planks) so that only an A4 screen is visible. The 'tray' at the front is optional for stabilising the construction in loose soil.

A wooden rhizotron used by Dan Metcalfe at Caxiuanã, Brazil, with and without the insulating cover (also showing the 'roof' behind that covers the rhizotron pit when not in use).

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[68] Also important considerations for choosing between metallic and wooden rhizotrons are what kind of workshop is locally accessible and the availability and relative cost of wood *vs*. MFCs and perspex *vs*. polycarbonate sheets.

A metallic rhizotron as in use at the SAFE plots in Sabah, Malaysia. This is constructed of four 378 mm Metal Framing Corners (MFCs), two 272 mm MFCs, a perspex rectangle 235 mm × 310 mm (or polycarbonate[69]), 6-9 nuts and bolts and six 15 cm nails with which to attach the rhizotron to the soil in the rhizotron pit (not shown).

A steel rhizotron (Stephen Adu-Bredu and Akwasi DGyamfi in Bobiri, Ghana) made of a single frame with groove into which the perspex screen slides after installation.

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[69] n.b. the metallic rhizotron design here deliberately avoids the necessity of drilling boltholes through the transparent screen because we had to use perspex (too brittle to allow holes to be drilled) rather than polycarbonate (as used in the wooden design above and the Peruvian design below).
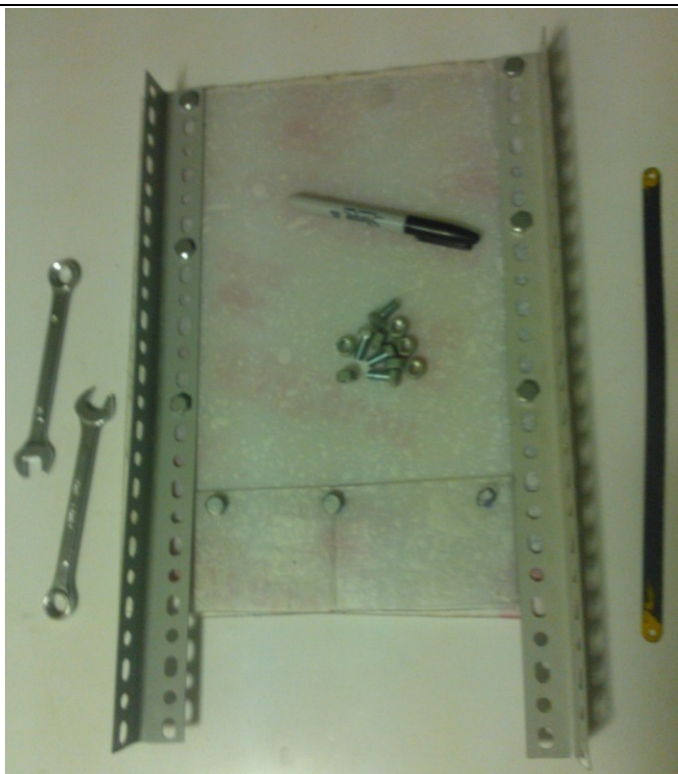
A rhizotron in use in Cusco, Peru, made of two MFCs (46 cm, to be cut down later as appropriate), two polycarbonate rectangles (275 mm × 350 mm and 211 mm × 80 mm) and 9 nuts & bolts, attached to the soil with 6 × 15 cm nails (photo B. L. Puma Vilca).

  - Whichever design is chosen, it is essential to ensure that the visible part of the perspex/polycarbonate screen is *exactly A4 size* (210 mm × 297 mm).

 - Each rhizotron needs an insulating foam sheet (27 cm × 39 cm). To make it waterproof, insert each into a plastic bag (e.g. the pedal-bin liners shown here) and tape closed (these are the equivalent of the pink sheet visible in the wooden rhizotron photos above).

 - Each rhizotron also needs a 1 m × 1 m 'roof' to cover the rhizotron pit. This roof is made of 2 cm diameter PVC tubing (small piece is 10 cm long, with a 1 cm slit cut at one end and a 1.5 cm 'shelf' at the other, the rest of the frame is made of 4 'elbow' pieces and 4 metre pieces, with two bent at an angle by softening the PVC with a candle and all assembled using fishing twine; suggest only to glue one side of each elbow piece to allow disassembly). The canvas sheeting was cut into 183 × 110 cm sheets and attached with staples.

2.3 Coarse roots and roots below 30 cm

Neither in-growth cores nor rhizotrons measure coarse roots much larger than ~5 mm in diameter because of (a) the impossibility of installing them where most large roots occur (i.e. 'butt roots' directly underneath tree stems), (b) large lateral roots away from tree stems are infrequent (~0.7% by volume in soil cores 30 cm deep, Metcalfe *et al.* 2008b) and (c) they are too slow-growing to invade in-growth cores in a 3 month interval. Because all known measurement methods for coarse roots are destructive (Clark *et al.* 2001a, Ravindranath & Ostwald 2008), there is currently no widely-accepted protocol for measuring this biomass component (Brown 2002). Therefore, we advise to use the root ratios in the table below. However, if the opportunity exists locally to estimate coarse root biomass more directly then this should be investigated (e.g. proximity to a logging area where a one-off survey may be conducted to measure a number of recently uprooted trees).

Table showing root proportions for selected biomes (n.b. root biomass excludes root exudates and mycorrhizae). Apply the first column to get the *fine* root biomass of the soil column from rhizotron or in-growth core data, then use the second column to get *total* root biomass and by subtraction you have the *coarse* root biomass.

Biome	Percent root biomass in top 30 cm of soil profile (assumed to apply to both fine and coarse roots)	Root:shoot ratio (R/S) (i.e. the ratio $BGLB/AGLB$)
Tropical rainforest or humid forest	69 (Jackson *et al.* 1996) [70] [71]	0.21±0.03 (Malhi *et al.* 2009)
Tropical dry forest	70 (Jackson *et al.* 1996)	0.56 (<20 t dry biomass/ha) or 0.28 (>20 t dry biomass/ha) (IPCC 2006 updated by GOFC-GOLD 2010:Table 2.3.3)
Tropical montane forest	-	0.27 (IPCC 2006:Table 4.4)
Tropical grassland savanna	57 (Jackson *et al.* 1996)	0.70 (Jackson *et al.* 1996 *
Temperate deciduous forest	65 (Jackson *et al.* 1996)	0.23 (Jackson *et al.* 1996)
Temperate grassland	83 (Jackson *et al.* 1996)	3.70 (Jackson *et al.* 1996 *)
Temperate coniferous forest	52 (Jackson *et al.* 1996)	0.18 (Jackson *et al.* 1996)

* cf. Table 3.4.3 of http://www.ipcc-nggip.iges.or.jp/public/gpglulucf/gpglulucf_files/Chp3/Chp3_4_Grassland.pdf .

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[70] i.e. if *B* is the biomass of roots in the top 30 cm of the soil, then total roots may be estimated as 45% more than *B* (=(1/0.69)**B*).

[71] As pointed out by C. Doughty, a value of 61% may be obtained from the IGBP land cover types using the following method: Take *depic*=0.30 (depth of ingrowth core into the soil in m) and *parama*=7.344 and *paramb*=1.303 (from CLM manual http://biodav.atmos.colostate.edu/kraus/Papers/Biosphere%20Models/clm%20manual.pdf p. 17 for IGBP land cover type 2 Evergreen Broadleaf forest, http://www.fao.org/forestry/4031-0b6287f13b0c2adb3352c5ded18e491fd.pdf Table 1). The fraction of roots contained in the top *depic* m of the soil profile is $f_{root} = 1 - \dfrac{e^{-parama*depic} + e^{-paramb*depic}}{2} = 0.61$ and, assuming a total soil depth of 2.4 m, $f_{allroots} = 1 - \dfrac{e^{-parama*soildepth} + e^{-paramb*soildepth}}{2} = 0.98$. Therefore, the fraction of roots in the top 30 cm is ($f_{root}/f_{allroots}$)=0.62.

However, there are other issues: if the soil is very shallow then trees cannot grow deep tap roots, but root biomass may be little different if there is a proportional increase in the lateral growth of coarse roots (e.g. for tree structural stability). If this is indeed the case, then root biomass will not scale simply with soil depth as in the calculation above (and, additionally, in shallow soils we should find that a greater proportion of coarse root respiration is captured in soil CO_2 efflux measurements, §3.1 and §3.3). Root biomass should also depend on soil moisture and nutrient levels as well as mean canopy height (higher canopies require more structural support, see e.g. "leaf support efficiency" measures, Leigh 1999:ch.6) and other factors. Therefore, we currently recommend simply to use the Jackson *et al.* (1996) value in the table above.

2.4 Soil properties

Equipment: Spades, post-hole digger, large sieves, 12 tarpaulins, large canvas bags, a square core 30 cm wide x 10 cm deep, saws, wooden planks, nails, weighing balance (resolution = 0.01 g), hanging balance.

Soil properties

The following soil sampling protocol is now recommended for all RAINFOR carbon plots by Martin Gilpin at Leeds (2014) [72]:

RAINFOR soil sampling protocol

ideal

ideal soil sampling protocol:	Depth (cm)
1 pit dug outside of plot (2m by1m area by 2m deep):	
12 nutrient samples	0-5
24 bulk density samples (3 per depth fraction to 200cm)	5-10
	10-20
	20-30
	30-50
	50-100
	100-150
	150-200
	200-250
	250-300
	300-350
	350-400
5 holes taken from 5 random subplots:	0-5
40 samples	5-10
	10-20
	20-30
	30-50
	50-100
	100-150
	150-200
76 samples	

Carbon, nutrients and bulk density are analysed from each sample (see e.g. Ravindranath & Ostwald 2008:176-178)). Sampling in the random subplots below 1 m depth may be regarded as optional if resources do not allow it. In all cases, if soil is shallower than 4 m then stop at the bedrock.

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[72] A more intensive soil sampling protocol using a mechanised auger and measuring a variety of properties is being carried out at many *RAINFOR-GEM* sites (see http://www.rainfor.org/upload/ManualsEnglish/Protocol_intensive_soil_sampling_EN.pdf), but this is not a required part of the carbon monitoring protocols. Soil chemical analyses such as those described in Malhi *et al.* (2009:suppl. info) are also highly desirable.

Roots

Randomly select at least one 1 m^2 area 10-15 m outside each plot (one area is adequate in relatively homogeneous plots, but if there is strong soil heterogeneity then more than one will be necessary). Remove the soil from the area onto separate tarpaulins for each of the following layers: 0-10, 10-20, 20-30, 30-40, 40-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400 cm (stop at bedrock if <4 m deep). For each layer, sample the roots with the sieves and separate into the following diameter classes: <0.5, 0.5-2, 2-5, 5-10, >10 cm (or a volumetric subsample of each layer if the whole layer cannot be searched). Do this by making a square core 30 cm wide, 10 cm deep and pushing it into the wall centred on depths 50 cm, 75 cm, 100 cm, 150 cm. Ultimately, this is to get a better estimate than the root:shoot ratios in §2.3. Place root samples from each diameter category into paper or canvas bags, dry at 80°C until constant mass and weigh. Use a fine balance for small root samples, and a hanging balance for particularly heavy roots.

Be extremely wary of portions of any pit collapsing whilst people are inside it. Construct wooden frames to reinforce the sides of the pit, and make sure than at least one person is present outside of the hole whilst anyone is inside. Afterwards, cover the hole with a wooden frame covered in plastic and rope off the entire area with highly visible marker tape.

3. CO₂ EFFLUX

3.1 Total soil CO₂ efflux
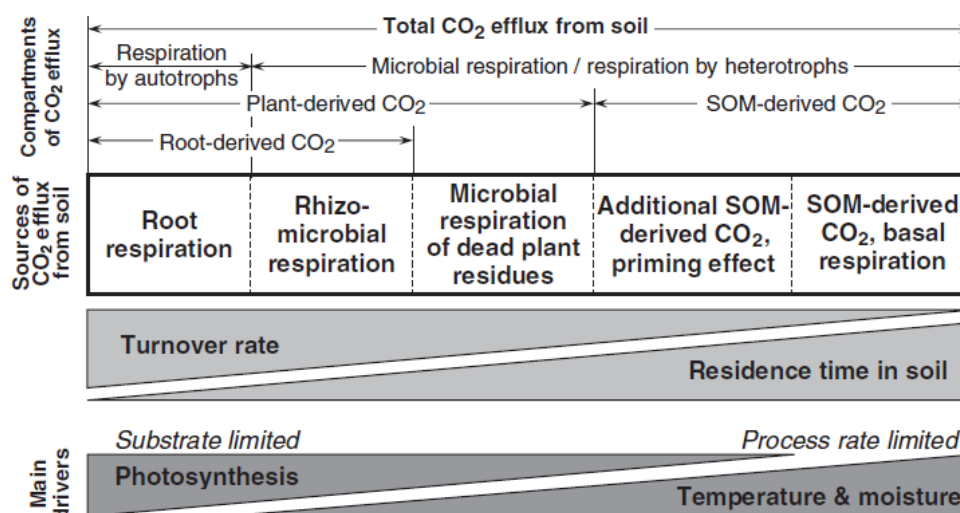
Note: for this experiment, it is necessary to be very clear exactly what is included in the term **heterotrophic respiration (CO₂ efflux)**: here we understand R_h to mean the respiration of heterotrophs (see Kuzyakov & Gavrichkova 2010:Fig.1 shown above) but *excluding* rhizo-microbial respiration[73] (mycorrhizae are part of the rhizosphere so this definition defines their respiration as a part of R_a not R_h even though mycorrhizal fungi are by definition heterotrophs). **Soil respiration** refers to the *total* soil CO₂ efflux (= heterotrophic respiration + root-derived respiration). Note especially that soil respiration includes the microbial respiration of all dead plant residues including fine litter and coarse woody debris even though these are generally considered to be *on* rather than *part of* the soil, i.e.

*** DO NOT CLEAR ACCUMULATED LITTER FROM WITHIN THE COLLARS BEFORE MEASURING SOIL RESPIRATION ***

(the partitioning experiment will provide details of what proportion of the CO₂ efflux is coming from the litter and what proportion from the soil below the litter layer). When studying soil respiration, it is also important to know about **soil priming effects** (reviewed in Sayer *et al.* 2011).

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[73] Plants take up most mineral nutrients through the rhizosphere where micro-organisms interact with plant products in root exudates. Plant root exudates consist of a complex mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions (e.g. HCO_3^-, OH^-, H^+), gas molecules (CO_2, H_2), enzymes and root border cells. Of these, soluble sugars, which are the most abundant components, are rapidly utilised by mycorrhiza and microorganisms in the rhizosphere and thus contribute to root-derived CO_2 (Kuzyakov & Gavrichkova 2010). Therefore, exudates may leave the roots, but they predominantly don't leave the rhizosphere so the transfer of C into the soil is minimal.
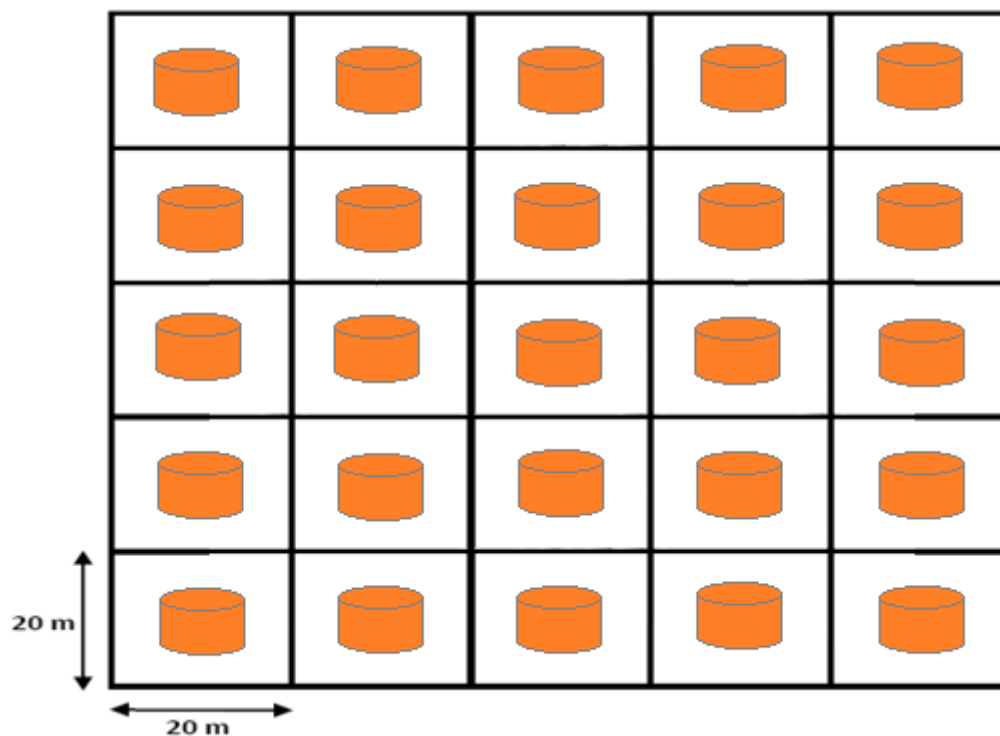
Insert 25 plastic collars (=tubes of about 10 cm length) per ha into the soil at the centres of each subplot (find the central point by having two people standing at adjacent corners using their compasses to direct a third person to the middle point). Install by simply hammering each gently into the soil, leaving over half (5-7 cm) above ground (this ensures a close seal whilst minimising soil and root disturbance) and labelling the collar "SR" for "soil respiration". After installation, leave all collars to 'settle' for at least a few days before first measurement.

Record CO_2 efflux (respiration) with the IRGA and SRC system from each collar every month (Appx. II). At each measurement, record the height of the portion extending out of the soil for each collar (to calculate V_a for the volume correction, Appx. II, allowing for settling of collars into the soil). After every measurement, record soil moisture and temperature (i) inside every collar and the depth of accumulated litter and (ii) outside every collar at least 20 cm away in a random direction (best is to measure both at three different points and average).

For all EGM calculations, see Appx. II.

Jeffry Amin with a soil collar in the Fragment E plot in Sabah, Malaysia.

Equipment: 25 PVC collars (10 cm long[74] and of a diameter to fit the SRC-1, see Appx. II), two compasses (if in a team of 3), a rubber-headed mallet, a piece of flat wood (better to hammer on that rather than hammer directly on the tube) and a permanent marker pen.

Time required: estimate half a day to do a 1 ha plot in a team of two.

n.b. As noted by Chambers *et al.* (2004) at their valley forest sites, surface roots in forests tend to aggregate at certain points, preventing installation of soil collars. This will slightly bias total soil CO_2 efflux readings towards lower values, but this effect may be assumed to be negligible.

n.b. (2) Relatedly, the efflux from coarse roots predominantly leaves the soil close to tree stems, which means that with the protocol described here we are missing the majority of coarse root efflux. Therefore, at the analysis stage an independent estimate of the coarse root component is added to the measured flux here to get 'real' total soil CO_2 efflux.

n.b. (3) Heinemeyer *et al.* (2011) highlighted the importance of the fact that surface fine roots are (necessarily) cut during collar insertion to 5 cm depth in the method described here. This not only artificially reduces soil CO_2 efflux but also removes the nocturnal peak in root respiration during moist periods measured when collars are not inserted. We need to use collars in order to ensure that the efflux is coming from the area underneath the chamber (without the collar, air may be drawn in from the surrounding soil), so this remains an unquantified uncertainty in our measurements here.

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[74] Metcalfe et al. (2009:§4.2.5.1.1) specified 4 cm length, but experience in Malaysia suggests that <10 cm the collars fall out too easily (and even 10 cm ones are problematic on slopes, T. Riutta *pers. comm.*). However, collars >10 cm cut too many surface roots so there is a compromise here (i.e. observe the situation at your site and go for slightly longer or shorter collars as appropriate).

3.2 Stem CO$_2$ efflux

First, choose the trees to have stem collars (from the census data) and generate the list of tree numbers: Select two trees >25 cm diameter per subplot randomly[75], i.e. 50 stems per ha[76]. If there are less than two trees >25 cm in any subplot, select randomly from the 20-25 cm trees or, if still not enough trees, leave that subplot with 0 or 1 stem collar only.

Next, in a team of 2 (one holding each collar on the tree, the other applying the sealant), find each selected tree. Position the collar at 1.5 m height (i.e. 10 cm below the tag; if the POM is at 1.5 m, install the collar above the tag at 1.7 m; *in areas where elephants occur, put at >2.0 m height* (record the height) following current practice in Gabon) but on the opposite side of the tree stem from the tag (if possible: if the stem is irregular, find a flat section of stem at 1.5 m height somewhere around the stem, avoiding buttresses). Before installation, clear loose bark, termite nests, ant trails (etc.) from the stem. Apply sealant and fix the collar to the stem (*above the bark*), ensuring that none is applied to the inside of the collar (i.e. all bark inside the collar is sealant-free as in the example collar above: this is very important for the volume correction in Appx. II) and that the seal between the collar and stem is complete without holes.

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[75] For example, if you have a list in a Microsoft Excel spreadsheet of 60 trees in a subplot and wish to randomly choose 2 of them over 25 cm diameter, this can be accomplished as follows: make a new column and put in the formula "=IF(*XXX*>25,RAND(),"")" where *XXX* stands for the diameter. Copy this formula down for all 60 rows and then just select the trees with the two highest numbers.

[76] If you make sure that this selection includes as many trees with dendrometer bands as possible, then a future study will be able to correlate growth with stem CO$_2$ efflux, which is very useful.

To measure stem CO_2 efflux (commonly called "stem respiration"[77]), place the SRC firmly onto the collar, maintain pressure on the chamber and commence IRGA measurement (Appx. II). Record CO_2 efflux with the IRGA and SRC system from each collar every month[78] (or as often as resources allow).

Equipment: 50 PVC collars (5 cm long and of a diameter to fit the SRC-1, see Appx. II; perhaps also a couple of spares), Fix-All sealant[79] (one 290 ml sealant tube can do 6-7 stems; if Fix-All is not available, use fume-free impermeable putty), an applicator/sealant gun (and two spares because they break easily), strong scissors, compass, map, perhaps a metal spoon (if possible; not necessary because you can smooth the sealant using a finger), a rag for removing excess sealant and a list of the tree numbers to be used.

Time required: estimate a day to do a 1 ha plot in a team of two.

CO_2 efflux measurements provide a mean value for each plot of CO_2 flux per unit area of stem surface (above the bark). To derive a rough estimate of stand-scale stem CO_2 efflux, per unit ground area, first calculate stem surface area for each individual tree (A_{stem} in m^2, which is the surface area of all above-ground wood except small twigs, not just the bole surface area A_{bole}) with either the equation for the surface area of a cylinder (for palms, tree ferns and other branchless tree forms):

$$A_{stem} = A_{bole} = 3.1416 * \frac{DPOM}{100} * H_{bole}$$

or for all other stems use equation 10 of Chambers *et al.* (2004)[80]:

$$A_{stem} = 10^{-0.105 - 0.686X + 2.208X^2 - 0.627X^3} \quad \text{where } X = \log_{10}(DPOM \text{ in cm})$$

where *DPOM* is the diameter at the *POM* of the tree and H_{bole} is the bole height of that stem (in m; i.e. up to the first major branch point except for palms where it is up to the base of the lowest living leaf sheath- see §1.5) [81]. Then sum **(1)** the surface areas of every stem >10 cm diameter[82] and **(2)** 25 * the average over the 'small trees' subplots of surface areas for small tree stems <10 cm[83]. Along with an estimate of branch area (based on branch dry mass, minimum and maximum branch diameter including bark), this gives estimated stem surface area per ha.

If more than one set of stem growth measurements are available, apply a linear regression of stem CO_2 efflux against growth rate for each tree (variation in stem CO_2 efflux for a particular tree over time is likely to be related to stem growth) to check for any unusual outliers. To calculate stand-scale stem CO_2 efflux, multiply the mean value per unit stem area by the estimated total plot stem area.

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[77] Most measured stem CO_2 efflux is likely to be true stem respiration, but a certain percentage can be attributed to CO_2 dissolved in the transpiration stream, ultimately coming from either root metabolism or soil heterotrophic respiration transported from the roots via the xylem (see McGuire & Teskey 2004, Robertson *et al.* 2010).

[78] Chambers *et al.* (2004) found no relationship between stem CO_2 efflux and stem surface (bark) temperature, so it is currently not required that temperature also be recorded for every stem respiration measurement (cf. §3.1). It may be that there is still a temperature dependence, however: e.g. stem CO_2 efflux may be more closely related to soil temperature than air temperature.

[79] Fix-All is fume-free sealant which means no CO_2 release as it dries. In trials in Malaysia it sticks very well in the field: it dries slowly enough that you can work it in to make an air-tight seal but still quickly enough to be solid within a minute.

[80] This is based on the Yoneda equation $A_{bole} = 3.1416 * \left(\frac{DPOM + D_{BoleTop}}{200} \right) * \sqrt{H_{bole}^2 + \left(\frac{DPOM - D_{BoleTop}}{200} \right)^2}$ (Yoneda 1993, Chambers *et al.* 2004).

[81] This approach (i) assumes that all parts of the bole emit CO_2 at the same rate (it's known that stem CH_4 emission predominantly comes from the base of the stem in tropical peatland trees, http://meetingorganizer.copernicus.org/EGU2012/EGU2012-1010.pdf, but whether CO_2 emission also declines as you move up the tree stem is not known) and (ii) ignores the known increase of branch CO_2 efflux rates with height in the canopy (Chambers *et al.* 2004).

[82] Rule of thumb: expect around 14000-15000 m^2/ha for large trees in forest.

[83] Rule of thumb: expect around 1700-2000 m^2/ha for small trees in forest.

3.3 Components of soil CO_2 efflux (the Partitioning Experiment)

The idea for this comes from the *DIRT* experiment at Harvard Forest, USA (Nadelhoffer *et al.* 2004), however, instead of applying the treatments over large areas of forest we apply it in small collars inserted into the forest floor (Metcalfe *et al.* 2008a). Note that all roots are removed during installation so all collars are initially root-free (corresponding to the "no roots" and "no inputs" treatments of the *DIRT* experiment) and the difference between rows 2 and 3 (protocol option #1) only develops over time in this design. This design includes a root-free-but-not-mycorrhizae-free treatment[84] (row 2) absent from the *DIRT* experiment.

Prepare the mesh collars (row 2, collars 2a-2c) by cutting two 3 cm × 3 cm windows (if square windows are not possible, 3.39 cm diameter circular windows[85]) along two opposite sides of twelve 40 cm long collars (i.e. four 3 cm × 3 cm windows on each collar[86]). The upper rim of the first window should begin exactly 5 cm along the collar, each window should be separated by 5 cm (if square, or 4.23 cm if circular) along the collar so that the windows are contained within the top 16 cm of the collar. Now cut the fine mesh into 5 cm × 5 cm squares, line the perimeter of the windows with glue/sealant and place a fine mesh square onto it. Do not extend the mesh over the lower ends of the collars.

Equipment: IRGA and SRC-1, PVC collars (all of a diameter to fit the SRC-1, see Appx. II), a rubber-topped mallet, a permanent marker pen, a tape measure, a stopwatch, a post-hole digger (and a spare in case it breaks), fine mesh, glue (or leftover sealant), fine nylon mesh cut into 5 cm × 5 cm squares (mesh size of 35-41 μm depending on availability), a knife/machete (and a spare), 3 large bags (e.g. black domestic bin-liners) and 3 smaller bags (or just use 6 large bags). You will also need a large sample bag (~3 L) of washed gravel (these

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[84] n.b. (1) In this experimental design only ectomycorrhizae that grow fungal mycelia across the soil matrix are considered and (2) It is well known that many mycorrhizae are symbiotic with particular species or genera of plants, but this design measures only the overall effect of all mycelial networks present in the soil without division into mycorrhizal species (see e.g. Cornelissen *et al.* 2003 for a summary of all types of mycorrhizae).

[85] If not possible, choose a diameter as close as possible and modify the spacing so that they remain positioned within the top 16 cm.

[86] = 4*9 cm^2 out of 35*2*3.1416*(11/2) cm^2 suface area below ground so these 'windows' collars reduce soil exposure by ~97%, but most roots are in the top few cm of the soil so in reality the reduction in root exposure is much smaller. Note that the bottom of the collar is not covered by mesh (the end of the collar away from the windows) because that will be in deep soil with few roots.

can be obtained e.g. from a local stream bed, which is preferable because it avoids introducing alien material to the plot).

A grid of soil collars for the partitioning experiment following protocol option #1. "C", "S" and "D" stand for "Control", "Sin hojarasca" and "Doble hojarasca" and rows 1, 2 and 3 are 10 cm collars (row 1), 40 cm collars with windows (row 2) and 40 cm collars without windows[87] (row 3)[88], with the B horizon collar slightly off the photo (10).

Partitioning Experiment installation

The partitioning experiment consists of several grids of collars per ha and an extra 10 collars in the central subplot. If the plot is a regular square, install the collars in the subplots shown in the diagram below (if not square, get as close to this as possible, i.e. each group separated by as much distance as possible within the plot; however, it is preferable to move the partitioning experiment into a neighbouring subplot if required to avoid placing one in a small trees census subplot). *Before collar installation*, insert collars (10 cm length) to ~5 cm depth at all locations (all separated by 50 cm as shown above), remove surface organic litter from inside each collar (place in a bag adjacent to each collar), leave them for a minimum of 5 min to stabilise, then record CO_2 efflux from all the collars (Appx. II). Afterwards place the surface organic litter back into their respective collars. The purpose of this initial measurement is to estimate pre-existing spatial variation between collars in each group.

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[87] It has been suggested to replace the window-less collars with window collars covered by 1 μm mesh to allow lateral flow and avoid the water-logging that deep collars often experience after heavy rain because of restricted lateral flow of soil water (Heinemeyer *et al.* 2011), however this has not yet been tested and is not (yet) part of the protocols.

[88] In Peru, numbers are used to label the collars (C1 → 1, S1 → 2, D1 → 3, C2 → 4, S2 → 5, D2 → 6, C3 → 7, S3 → 8, D3 → 9)

Sorting through for roots in two soil samples (Alizabat Rusilie and Rihan bin Rahim in Malaysia) and a soil collar in the process of being inserted into Row 2.

It is easiest to do this in a team of 3 (if you are lucky enough to have enough people for two teams of three, double all equipment above except the PVC collars). At present (2012), we follow protocol option 2 in Ghana and Gabon, but protocol option 1 elsewhere (concentrating on slightly different aspects of partitioning), but we recommend protocol option 2 for all future installations.
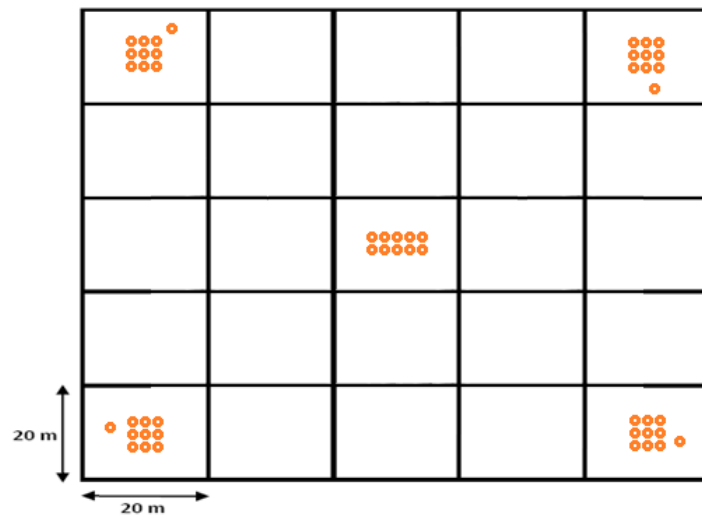
Partitioning Experiment protocol option 1: dividing all CO$_2$ efflux components

At each grid, designate the area for collar installation and nominate only two workers to be allowed within this area (all others remain outside to minimise disturbance, passing items to and from the nominated two as required during installation). Do the four grids of 10 collars first.
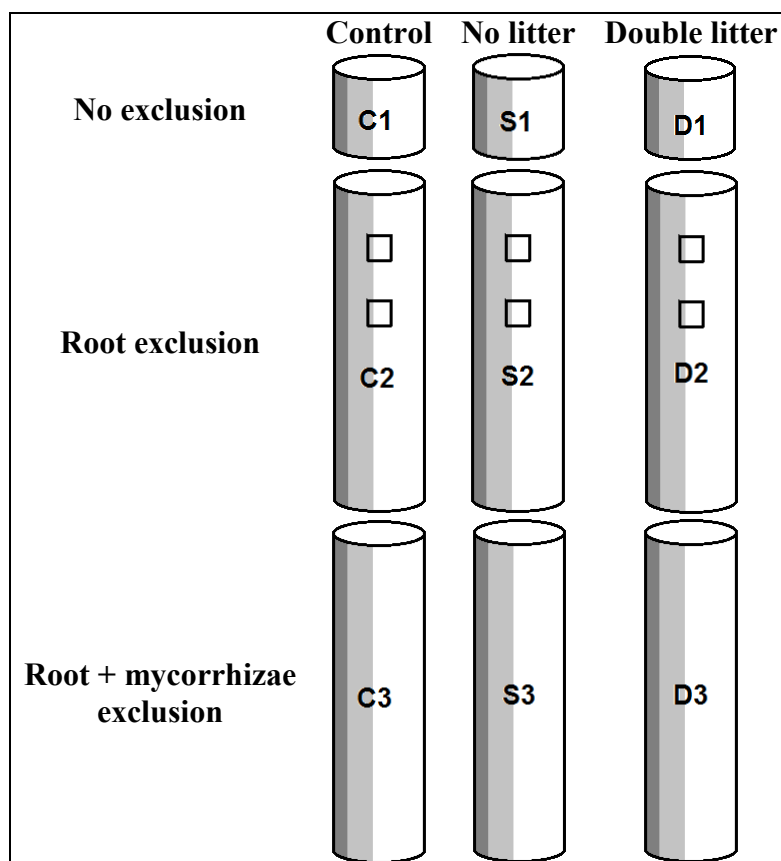
Start with the 'row 1' collars (C1, S1, D1): remove litter from C1 (place the collar on the ground and cut the litter with a knife all round the outside of the collar, then lift gently off and place on a small bag which is then placed outside the installation area). Likewise remove the litter from S1 and D1. Next, hammer the collars gently into the soil with the mallet, leaving 5 cm above ground. Replace the litter from C1 where it came from and then place the litter from both S1 and D1 combined onto D1. Replace the absent litter layer on S1 with gravel.

Next, row 2 (C2, S2, D2): remove the litter layers onto sample bags as before, then use the post-hole digger to dig three 35 cm deep holes, retaining all soil separately on three large bags. Next, time 20 minutes and have three people sorting through these three soil samples for roots (remove only roots: stones, twigs, leaves, earthworms or any other items remain in the sample; *only one person per sample*; these roots do not need to be weighed). Insert the long collars with windows into the three holes (windows at the top, but positioned so that the top of the upper window is just below the soil surface) and refill the hole with the root-free soil (compacting down slightly as required to approximate the density of surrounding soil). Finally, replace the litter onto C2, gravel onto S2 and the litter from both S2 and D2 combined onto D2. Be particularly careful with the mesh collars (row 2) to **(1)** ensure that soil is contacting the mesh windows on both sides, and **(2)** the mesh windows are not broken (by, for example, protruding roots or over-compacted soil). If there is not sufficient soil to fill the collars to the same level as the surrounding soil (or the gap between the collar and margin of the hole), gather extra root-free soil from outside the plot.

Row 3 (C3, S3, D3): exactly as for row 2 except that collars without windows are used.

The final 10 cm collar for this subplot should be labelled "10" and placed at least 50 cm away from the 3 × 3 grid in a random direction[89]. For this one, hammer it gently into the ground as for normal soil CO$_2$ efflux but then remove *both* the litter layer *and* the soil down to the mineral soil / "B" horizon[90]. Fill back to ground level with gravel.

All of the above is repeated at four sites per ha.

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[89] Thanks for Liliana Durand-Baca for the suggestion of adding this extra treatment to the design.
[90] In practice it is difficult to tell where the mineral layer begins, however it is sufficient to remove the top few cm of soil down to wherever a visible colour change occurs.

Partitioning Experiment protocol option 2: wider spatial sampling
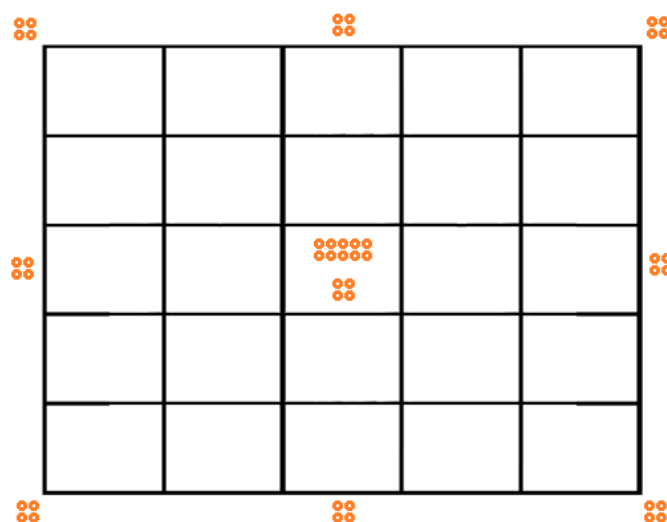
As above for the installation method, but 9 sets of 4 collars (C1, S1, S2 and S3), located in the central subplot and just outside the plot as shown: at each corner, at the middle of each 100 m side (every 50 m):

At each grid, designate the area for collar installation and nominate only two workers to be allowed within this area (all others remain outside to minimise disturbance, passing items to and from the nominated two as required during installation). Do the nine grids of four collars first.
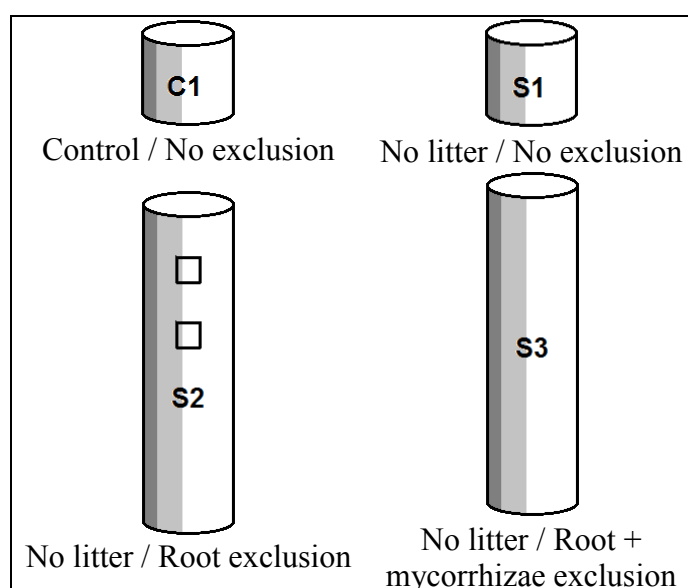
Start with the 'row 1' collars (C1 and S1): remove litter from C1 (place the collar on the ground and cut the litter with a knife all round the outside of the collar, then lift gently off and place on a small bag which is then placed outside the installation area). Likewise remove the litter from S1. Next, hammer the collars gently into the soil with the mallet, leaving 5 cm above ground. Replace the litter from C1 where it came from and discard the litter from S1. Replace the absent litter layer on S1 with gravel.

Next, collars S2 and S3: remove the litter layers onto sample bags as before, then use the post-hole digger to dig two 35 cm deep hole, retaining the soil separately on large bags. Next, time 20 minutes and have two people sorting through these two soil samples for roots (remove only roots: stones, twigs, leaves, earthworms or any other items remain in the sample; *only one person per sample*; these roots do not need to be weighed). Insert a long collar with windows into the S2 hole (windows at the top, but positioned so that the top of the upper window is just below the soil surface) and refill the hole with the root-free soil (compacting down slightly as required to approximate the density of surrounding soil). Insert a long collar without windows identically into

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the S3 hole. Finally, discard the litter and place gravel onto the soil in both collars. Be particularly careful with the mesh collar S2 to **(1)** ensure that soil is contacting the mesh windows on both sides, and **(2)** the mesh windows are not broken (by, for example, protruding roots or over-compacted soil). If there is not sufficient soil to fill the collars to the same level as the surrounding soil (or the gap between the collar and margin of the hole), gather extra root-free soil from outside the plot.

All of the above is repeated at nine sites per ha.

Control collars for both protocol options: The key advantage of the deep core insertion method chosen (i.e. extracting soil first and manually removing roots) is that there is no subsequent bias from decomposition of severed roots in the soil, as there would be if the collars had simply been directly inserted into the soil (the more obvious, common approach). However, because the soil disturbance associated with soil removal and manual mixing may alter subsequent CO_2 fluxes, we must quantify the CO_2 efflux that would occur if the collars had simply been inserted into the soil leaving severed roots within them.

Equipment: IRGA and SRC, 10 PVC collars (40 cm long and of a diameter to fit the SRC-1, see Appx. II), heavy, rubber-headed mallet, a piece of flat wood (better to hammer on that rather than hammer directly on the tube), post-hole digger, large plastic bags.

Insert these in the centre of the central subplot of each plot (see figure above). One row of five involves excavating five holes with a post-hole digger as before, but separated by 20 cm this time rather than 50 cm, removing the soil onto a large plastic bag, inserting a 40 cm collar (without windows, leaving 5 cm protruding out of the soil) and refilling with the same soil (manually mix the soil, *but do not remove any roots*). If there is not sufficient soil to fill the collars to the same level as the surrounding soil (or the gap between the collar and margin of the hole), gather extra root-free soil from outside the plot. Mark these collars "D1" to "D5" (there should be no confusion with collars D1, D2 and D3 in the 3 × 3 grids). The second row of five (displaced ~2 m away) are simply hammered into the soil directly without using the post-hole digger (you will need to spread the impact of the hammer across the surface of the collar with a strong piece of flat wood). If you encounter a thick root, withdraw the core and try installation at a nearby point. Mark these collars "ND1" to "ND5" (= No soil Disturbance). Finally, place within every collar a level of gravel (~2 cm depth) to maintain soil physical conditions.

Time required: estimate 1-2 days to do a 1 ha plot in a team of three (following either protocol option).

Partitioning Experiment repeated measurements

Record CO_2 efflux with the IRGA and SRC system from each collar every month (all partitioning grids and the control collars) using the procedure described in Appx. II. During each measurement, record for each collar the height of the portion extending out of the soil (e.g. 5.0 cm, 4.8 cm, 3.0 cm - this is to calculate V_a see Appx. II). After every measurement, record soil moisture and temperature (i) inside every collar and the depth of accumulated litter and (ii) outside every collar at least 20 cm away in a random direction (best is to measure both at three different points and average). For analysis calculations, see Appx. II.

Take the first CO_2 efflux measurements as soon as possible after installation, but remember that it will take a while for the collars to 'settle' after installation so a certain number of initial measurements will have to be discarded after a few months. For the control collars, calculate mean CO_2 efflux from the disturbed (D; DC_d) and undisturbed (ND; DC_{ud}) deep cores: the proportional change in CO_2 efflux attributable solely to soil disturbance is DC_{ud}/DC_d. Especially in the Root + mycorrhizae exclusion tubes, there is a possibility that the tube will be found flooded after heavy rain: in this eventuality, discard these measurements.

Partitioning Experiment protocol option 1: Data analysis

The partitioning experiment should allow estimation of the relative contributions of (1) surface organic litter, (2) roots, (3) mycorrhizae and (4) soil organic matter to total soil CO_2 efflux. Contributions are estimated from differences between collars subjected to different treatments, in excess of pre-existing spatial variation (*NV*).

Fine litter CO_2 efflux (decomposition): First calculate *NV* (proportion of ambient) for collars from the two litter treatments (zero and double litter level) from the CO_2 efflux measurements made on surface collars temporarily installed at all locations before collar installation:

> *NV for zero litter = (column C – column S) / column C*   (e.g. for S1 = (C1 – S1) / C1)
> *NV for double litter = (column D – column C) / column C*

After collar installation calculate litter CO_2 efflux (proportion of ambient) in excess of *NV* from the measurements after collar installation with:

> *Zero litter = NV value for zero litter – ((column C – column S) / column C)*
> *Double litter = NV value for double litter – ((column D – column C) / column C)*

Root CO_2 efflux (n.b. fine roots only, as explained in §3.1): Calculate *NV* (proportion of ambient) for the relevant collars (rows 1 & 2) from the CO_2 efflux measurements made on surface cores installed at all locations before collar installation:

> *NV = (row 1 – row 2) / row 1*   (e.g. for C2 = (C1 – C2) / C1)

Then, to remove any additional influence of the different levels of disturbance experienced by the soil in row 2 compared to the surface collars of row 1, calculate mean CO_2 efflux from disturbed (DC_d) and undisturbed (DC_{ud}) deep cores (above). Finally to calculate root CO_2 efflux (R_{root}, proportion of total ambient soil CO_2 efflux) in excess of *NV* and controlling for collar installation disturbance from the measurements after collar installation use:

> R_{root} = NV – (((row 1 – row 2) / row 1) * (DC_{ud}/DC_d))

Mycorrhizal CO_2 efflux: Calculate *NV* (proportion of ambient) for the relevant collars (rows 2 & 3) from the CO_2 efflux measurements made on surface cores installed at all locations before collar installation:

> *NV = (row 2 – row 3) / row 2*   (e.g. for C3 = (C2 – C3) / C2)

Now, to calculate mycorrhizal CO_2 efflux (R_{mycorr}, proportion of total ambient soil CO_2 efflux) in excess of *NV* from the measurements after collar installation use:

> R_{mycorr} = NV – ((row 2 – row 3) / row 2)

Soil organic matter CO_2 efflux: Calculate *NV* (proportion of ambient) for the relevant collars (rows 1 & 3) from the CO_2 efflux measurements made on surface cores installed at all locations before collar installation:

> *NV = row 3 / row 1*

Then, to remove any additional influence of the different levels of disturbance experienced by the soil in row 2 compared to the surface collars of row 1, calculate mean CO_2 efflux from disturbed (DC_d) and undisturbed (DC_{ud}) deep cores (above). Finally to calculate soil organic matter CO_2 efflux (R_{SOM}, proportion of total ambient soil CO_2 efflux) in excess of *NV* and controlling for collar installation disturbance from the measurements after collar installation use:

> R_{SOM} = NV – (row 3 / row 1) * (DC_{ud}/DC_d)

3.4 Coarse woody debris (CWD) CO_2 efflux

Equipment: IRGA and SRC, PVC collars (5 cm long and of a diameter to fit the SRC-1, see Appx. II), plastic sheet, strong adhesive tape, callipers, weighing balance (0.01 g resolution), drying oven

Take these measurements every two months (usually at the same time as the survey in §4.2). Collect 5 representative wood pieces (~5 cm long, i.e. small enough to fit into a PVC collar without sticking out) of each decomposition category from each plot (5 pieces per category * 5 categories = 25 pieces per ha), and place in appropriately marked sealed plastic bags (site, plot, date, piece number). Seal one side of a plastic collar with a plastic sheet secured with tape. Then place each individual piece of wood into the collar, place the SRC firmly onto the collar and commence IRGA measurement (Appx. II). Now measure for each wood piece: **(1)** wet weight, **(2)** dry weight, after drying to constant mass at 80°C, **(3)** diameter and **(4)** length. Ensure that the identity of each individual piece is noted, so that these measurements can be matched to the corresponding efflux value from the same piece.

Decomposition Level (following Harmon *et al.* 1995 [91])	**Leaves present**	**Fine twigs present**	**Intact bark**	**Firm wood**	**Soft wood**	**Very soft wood**
1 (little decay: bark cover extensive and leaves and fine twigs present)	**yes**	**yes**	**yes**	**yes**	no	no
2 (logs and snags relatively undecayed but with no leaves and few fine twigs and bark has started to fall off)	no	no	**yes**	**yes**	no	no
3 (logs and snags with no bark and only a few branch stubs remaining; although in this class the sapwood is decaying, the heartwood is relatively undecayed, and branch stubs do not move when pushed or pulled)	no	no	no	**yes**	no	no
4 (no branches or bark cover; outer portions of the wood are often case-hardened and the inner wood is decomposing)	no	no	no	no	**yes**	no
5 (logs are elliptical in cross-section (indicative of advanced decay) and often the wood is scattered across the soil surface)	no	no	no	no	no	**yes**

The mean difference between wet and dry weight for each decomposition category will provide a wood moisture correction necessary for the previous section. Estimate the surface area of each piece using (2 * 3.1416 * ((*diameter*/2)^2)) + (2 * 3.1416 * (*diameter*/2) * *length*). Dry weight and surface area can then be used to calculate CO_2 efflux per unit dry mass and surface area using the approach described above (also see Appx. II). These values may be scaled up to a per ha estimate of CO_2 flux using estimates of ground coarse litter mass and surface area (see §4.2).

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[91] n.b. these categories differ slightly from those in Baker & Chao (2011) (who only used 3 categories).

4. LITTER AND DEBRIS

More detail about sampling CWD is in http://www.rainfor.org/upload/ManualsEnglish/CWD_protocol_RAINFOR_2011_EN.pdf and Gurdak *et al.* (2014) and there are more equations for calculating volumes in Harmon *et al.* (1999:Fig.11.2). See http://www.rainfor.org/upload/ManualsEnglish/wood_density_english%5B1%5D.pdf for more detail on estimating wood density.

4.1 Ground fine litter mass (litter stock)

Equipment: Large paper bags, weighing balance (0.01 g resolution), drying oven, 0.5 m × 0.5 m wire square, knife.

Randomly place a 0.5 m × 0.5 m square in each subplot of the plot (with at least 20 m separation distance between measurement points). Cut around the margins of the square, collect all the fine organic litter (not including branches >2 cm diameter[92]) within each square, and place within an appropriately named paper bag (site, plot, date, measurement point number). Do not include litter which is so decomposed that it is not readily identifiable as material derived from leaves, fruit, flowers, seeds or wood (i.e. material that would be defined as humus). All the samples should be dried at around 80°C to constant mass and weighed. Dry mass should be noted in a datasheet. Where a single piece of litter constitutes a disproportionately large proportion of the total weight (e.g. a large seed, fruit), also note the individual weight of the piece in the observations column in the datasheet.

Repeat these measurements four times a year, if resources allow, because there can be strong seasonal cycles.

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[92] Slightly different from the 1 cm upper bound in Clark *et al.* (2001a).

4.2 Coarse woody debris (CWD) survey

Equipment: Strong plastic string (800 m), diameter tape, callipers, gloves, marker tape, machetes, 15 canvas bags (one for each decomposition/diameter category), large plastic bags, a hanging balance, graduated water flask, weighing balance (0.01 g), drying oven.

Coarse litter is here defined as all dead woody material over 2 cm diameter[93]. Standing dead wood material [94] [95] should be included in a **snag survey** (check that standing dead stems were indeed excluded from the main tree census in §1 [96]). In the **ground CWD survey** all lying CWD should be included excepting only debris that has fallen from dead stems[97] (the branches of dead snags are implicitly included in the allometric equation used to estimate snag biomass so to census these as well would be double-counting).

Establish **four 100 m long transects** along the four sides of a square just outside the boundary of the plot[98] (having two transects perpendicular to the other two allows sampling across the biases introduced by trees preferentially falling in one particular direction, see "orientation bias" in van Wagner 1982). Each transect should be 1 m wide, with each edge marked with plastic string secured to the ground surface every 20 m.

Cut all the dead wood pieces (>2 cm diameter) which intersect with the strings, except for particularly large pieces of dead wood (which cannot be easily lifted) which should only be marked with marker tape. Record diameter and length (within the transect) of all wood pieces >2 cm diameter encountered, note separately measurements from the following diameter categories: 2-5 cm, 5-10 cm, >10 cm. A piece of dead wood should only be measured if less than 50% of it is buried below the surface (Walker *et al.* 2012). A tree that fell since the last census and is now a log should only be remeasured if it was alive last census (otherwise it has already been recorded once as standing dead wood). Large logs that cannot be removed from the transect after measurement should be recorded and excluded from later surveys[99].

In the snag survey only include 'new' tree snags, i.e. trees declared as dead during this particular census only. Do not include any stems that have fallen but are still alive (which are still censused in §1). Check any strangle fig trees in the plot: if there is a dead host stem inside the fig then this counts as a snag and should also be measured.

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[93] n.b. the general division between coarse and fine dead wood is at 10 cm diameter (Harmon *et al.* 1995, Brown 2002), but not in these protocols.

[94] The *ForestGEO-CTFS* protocols at http://www.ctfs.si.edu/data///documents/Long_transects_protocol_2009_11_08.pdf differ in a few respects: notably, they exclude standing dead trees.

[95] Pearson *et al.* (2005a:§7.4.1) and Walker *et al.* (2012) used the following decomposition classes for standing dead wood:
   1   Tree with branches and twigs and resembles a live tree (except for leaves)
   2   Tree with no twigs but with persistent small and large branches
   3   Tree with large branches only
   4   Bole (trunk) only, no branches
However, please note that these are not used in *RAINFOR-GEM*.

[96] Standing dead stems should be included in the CWD survey (following Baker & Chao 2011). However, it is possible that in some censuses snags may have been tagged and included in the main tree census §1.3 (following Metcalfe *et al.* 2009) so if the main tree census is being undertaken by another team, check with them that this biomass component is not being either missed or double-counted.

[97] In steep areas where the majority of CWD is in 'trash' piles at the bottom of landslides it may be difficult to separate out the CWD from dead trees, but it should generally be possible.
   In the case of a stem that is alive but part of its canopy is dead, any branches dropped from the dead part of the canopy should be counted as CWD from a *live* stem.

[98] If transects cannot be laid out in this way because of mountainous terrain and/or an irregular plot layout, approximate as best as possible. If the transect intersects scrubby areas very difficult to access (e.g. large clumps of rattan) then estimate as best as possible from a subarea.

[99] At the moment, we do not monitor these as they decay.

Vianet Mihindou weighing coarse litter at Lopé, Gabon.

Retrieve all the wood pieces which are small enough to easily lift, and place them in canvas bags. Use a separate canvas bag for each of 5 decomposition categories (see table below[100]). When a bag becomes too heavy to easily carry along the transect, weigh the bag *in situ* with the hanging balance. Note the weight of each bag, then empty it and spread out the wood material evenly within the plot, *but outside of the transects*. Continue this process of collection, weighing and bag emptying until all small wood pieces have been removed from the transects and weighed. There are thus potentially 15 categories which should be weighed separately (3 diameter categories * 5 decomposition categories = 15 categories). Save 30 wood pieces from each decomposition category and place in well-sealed plastic bags (1 bag for each category; this does need to be done *at every sampling* - both for the initial estimate of CWD stock and the subsequent estimates of production

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[100] *RAINFOR* does not require the use of a penetrometer to measure decomposition level (partly because dead trees for which the heartwood rots before the sapwood cause difficulty).

- because the water content will change seasonally). Finally, record length (within the transect), diameter (record at three points and average) and decomposition category of all of the large, marked wood pieces which remain on the transect (see the Decomposition Levels table in §3.4). The values of both the mass of the small pieces in bags, and diameter of the individual large pieces should be stored in a datasheet.

To derive estimates of coarse woody debris dry weight, the additional weight of the canvas bags and the water within the wood (for the small wood pieces) and wood density (for the larger pieces) must be subtracted. Therefore, back at the lab weigh (**1**) the canvas bags, and (**2**) each individual wood piece saved (5 decomposition categories, 30 per category, 5 * 30 = 150 total) before and after drying at around 80°C to constant mass. Then place each wood piece into a graduated water flask half full with water and record (**3**) the volume of water displaced (remember 1 ml = 1 cm^3). Pieces from decomposition categories 4 and 5 should be wrapped in clingfilm before immersion to avoid saturation of airspaces within the wood (which would then lead to an underestimation of wood volume). These measurements should also be noted in the same datasheet, as wood mass and diameter.

Wood density may be calculated as wood dry mass divided by volume. The volume of the larger wood pieces which could not be removed from the transects may be estimated from the equation for a cylinder: $3.1416 * (diameter/2)^2 * length$. Mean wood density for the appropriate decomposition category[101], multiplied by the piece volume, gives an estimate of dry mass of each large wood piece. Total coarse litter mass per unit ground area may be calculated as the sum of all the weighed bags (minus the weight of water in the wood and the bags themselves) and the larger wood pieces which could not be removed from the transects.

Diameter and length measurements of each wood piece may be used to calculate **surface area** with: $(2 * 3.1416 * ((diameter/2)^2)) + (2 * 3.1416 * (diameter/2) * length)$. The sum of the surface areas of the pieces encountered gives an estimate of coarse litter surface area per unit ground area.

Coarse litter should be collected every 2-3 months from within all four transects. If there is a reasonably high production of dead branches, it is most efficient to collect the representative wood pieces required for §3.4 as part of the survey described here.

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[101] Wood density estimates have not been included here because they should be estimated in *all* cases on site. However, Tiepolo *et al.* (2002:Table 3) suggested the following as very general rules of thumb for wood samples:

General/Sound	0.47 t/m^3
General/Intermediate	0.34 t/m^3
General/Rotten	0.17 t/m^3
Palm/Intermediate	0.14 t/m^3
Palm/Rotten	0.09 t/m^3

4.3 Fine litter fall (litter accumulation)

Litter traps in Wytham Woods, UK (left) and in plot AA1, Alerce Andino National Park, Chile (right) and equipment for constructing a litter trap in Lopé, Gabon. Litter traps should be 50 cm × 50 cm (i.e. area 0.25 m²)[102]. Legs may be at the corners (e.g. 4 separate legs supporting a wire-frame tray) or in the middle of the sides[103] (e.g. a PVC frame with mesh cloth attached to the top) [104] or in between (e.g. the two above) depending on local availability of parts. The tray top should be at a height of 1 m above the midpoint of the trap area (requiring legs at least 1.5 m long) and it should be level (check with a spirit level). The netting mesh size should be as close as possible to 1 mm.

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[102] As used in Peru and Bolivia. n.b. this differs from the *ForestGEO-CTFS* standard size 71 cm × 71 cm (area 0.5 m²; Muller-Landau 2008, http://www.ctfs.si.edu/data///documents/Litter_Protocol_20100317.pdf) and the 1 m² traps of Metcalfe *et al.* (2008a).

[103] This design is necessary if three-way corner pieces of PVC tubing (i.e. , aka. "right angle outs") are not locally available.

[104] These designs are similar to the *ForestGEO-CTFS* trap designs: http://www.ctfs.si.edu/data///documents/Litter_Protocol_20100317.pdf.

Litter traps for areas where elephants occur (e.g. Gabon): Take the legs off the litter trap and place it directly onto the floor. As a back-up, clear an area of ground of all litter/debris and peg out a 50 cm x 50 cm square. If the littertrap basket has not been removed by elephants, collect sample from basket and remove (without collecting) all litterfall from within the pegged out square on the ground. If the littertrap basket has been tossed aside by an elephant, collect all the litterfall in the pegged out square on the ground. Ensure all litterfall is collected, ready for the next 2-week collection period.

Equipment: 25 litter traps per ha (one per subplot, designed as illustrated above), paper bags, tweezers, brushes, weighing balance (0.01 g resolution), spirit level, drying oven.

Litter traps should be emptied every two weeks[105] to minimise decomposition between visits. Fine litter is defined as all organic litter, but including only woody material less than 2 cm diameter[106]. Woody material over 2 cm diameter which enters the traps should be removed and spread around the plot. Once the leaves for *SLA* calculation have been removed, remaining fine litter should be removed into bags and dried at around 80°C to constant mass as soon as possible to prevent decomposition (within 48 h[107]). Once dried, litter should be separated into (**1**) leaves (including petioles, rachises and petiolules), (**2**) woody material, (**3**) fruits, (**4**) flowers, (**5**) seeds[108] and (**6**) fine debris (unidentifiable particles that pass through a 2 mm mesh) (separate epiphytes, if they occur, but do not divide into parts), HOWEVER if there are insufficient staff or time then the litter may be left unsorted at this point. Then, weigh (if separation takes more than a few minutes, re-dry in the oven and weigh immediately on removal) and place into appropriately named (site, plot, date, site, trap number, type of litter) paper bags for storage (old samples will need to be discarded regularly according to the availability of

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[105] If it is logistically impossible to maintain a regular schedule all year round, nominate regular periods of e.g. two weeks and at each period visit and empty all traps at the beginning (discarding all trap contents) and then at the end (recording trap contents as described above). If litterfall is high at certain sites, it may also be necessary to visit and empty the traps outwith these periods to avoid mesh breakages.

[106] Slightly higher than the 1 cm upper bound in Clark *et al.* (2001a) but lower than the 10 cm upper limit for 'fine litter' in the IPCC (2006).

[107] If this is logistically impossible, either keep refrigerated or air-dry the samples and oven-dry at first opportunity.

[108] Combine categories 3-5 into "reproductive parts" if it is unfeasible to separate them (e.g. crushed fruit containing many seeds).

storage space). Dry weight of the litter should be noted in an appropriate datasheet, multiplied by 10000/(area of trap in m^2) to produce a per ha figure (Clark *et al.* 2001b:Table3).

A critical consideration with litter separation is to calculate the amount of time available to separate all litter before even more is collected, and modify the separation rate accordingly[109]. Otherwise, the rate of litter separation may lag far behind litter collection, leading to accumulation of unprocessed samples and increased risks of sample loss and/or degradation.


Litter sorting and SLA scanning in Ghana (photos S. Moore).

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[109] e.g. nominate a subset of traps to be separated each time and separate all traps every few months to ensure that spatial variability is still being adequately sampled.

Specific Leaf Area (SLA)

Equipment: Digital camera and tripod or scanner (e.g. Canon LiDE 100 which can be powered via a USB connection to a laptop), transparent sheets (e.g. perspex, glass), ruler, drying oven, paper bags, weighing balance (0.01 g resolution).

Every three months, leaves should be collected from the 12 evenly numbered litter fall traps (out of the 25 litter traps in total per ha) and stored in plastic bags to avoid desiccation. These are for calculating specific leaf area (*SLA*) and if possible should be immediately separated in the field whilst still wet (if not possible, bag them wet and sort back at the lab, keeping unsorted samples in a freezer). Images of the leaves should be taken, either with a digital camera or scanner (the digital camera approach is more versatile in field situations, while images captured with a scanner do not have the uncertainty of lense differences and require much less time for image processing).
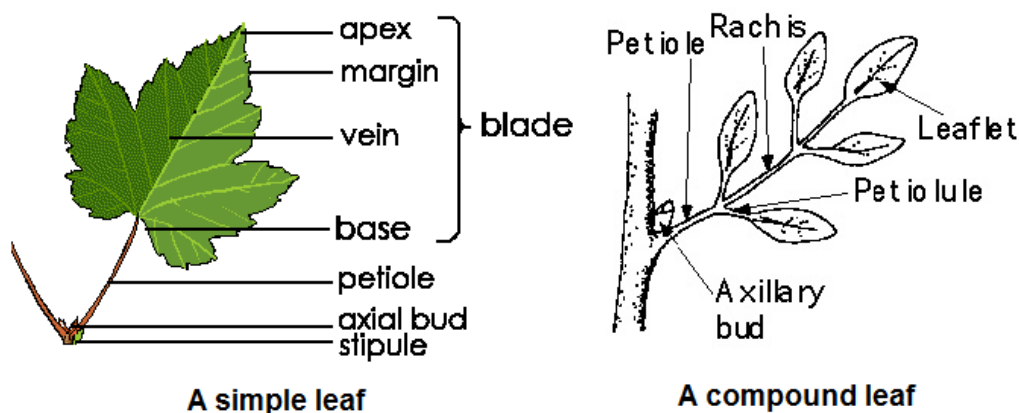
For SLA calculations the petiole *is* included with the other parts of the leaf (as are the rachis and petiolules within a pinnately compound leaf) (see the SLA section of Cornelissen *et al.* 2003 for some rare exceptions to this rule). Note: although not illustrated here, many topical plant species have petioles that are winged or wrapped or otherwise photosynthetically active (collenchyma tissue).

Separate leaves from the rest of the organic material collected in the litter traps. If using a scanner, scan the leaves (colour jpg, at least 300 dpi). If using a camera system, first place all the leaf material from each plastic bag onto a clear white paper sheet together with a label (site, plot, date, point) and a measuring ruler as a scale. Cover with a transparent sheet to flatten the leaves and position the camera above the leaves with a tripod (remember that "leaf area" usually means including petioles and petiolules; for greater comparability with other projects it is a good idea to scan each leaf with and without petioles to calculate SLA with and without too). Take a colour, high resolution picture of the leaves (from not more than 0.5 m distance), making sure that you include the label and ruler, and save the image in jpg file format. Save the scanned or photographed images with suitable names (site_plot_date_point) in a designated file. If several scans/photographs are required to capture all the leaves from a single point, these different images may be distinguished with letters after the measurement point number. Leaf area for each image may be calculated with image analysis software.

All the leaf samples from each point should now be transferred to a paper bag (one per point), dried at ~80°C to constant mass and weighed (along with all the other litter samples). Once dried, the dry mass of the leaf material collected at each of the 25 measurement points per ha should be entered into a datasheet. After weighing, all the dried leaf material should be placed back into their respective paper bags. Bags from a single sample period and plot should be placed into two plastic bags, compressed to remove excess air and sealed. The outer plastic bag should be marked with measurement type (e.g. "SLA") sample date and plot. After this the samples may be placed in a safe, cool, dry place for long term storage (in the case of e.g. later data loss, the possibility of chemical analysis of samples). *SLA* is calculated as total one-sided leaf area divided by total dry mass per point.

Image processing

Leaf area will be calculated with **ImageJ** (http://rsb.info.nih.gov/ij/). The images are stored in a more versatile colour jpg image format, but ImageJ only accepts greyscale images. So **IrfanView** (http://www.irfanview.com/) is required to create greyscale copies of the original colour images. First, open the Irfanview software and click on *File → Batch Conversion/Rename...*. Select the file which contains the colour images to be analyzed (top left of the Batch Conversion window), and click *Add all*. Then select the output file (left of the Batch Conversion window) in which to place the greyscale images. To specify the desired conversion type, select the *Use advanced options (for bulk resize...)* window then click *Advanced* to open the advanced conversion window and select *Convert to greyscale* and *OK*.

To begin image analysis with the greyscale images, open ImageJ and select *File → Open* to select the desired image. To highlight the desired portion of the selected image (the leaves) click on *Image → Adjust → Threshold*. Vary the two bars to accurately separate the leaves from the background, then press *Set* and *OK*. Now the scale of the image has to be set, to allow accurate area calculation. In the case of scanned images, the scale may be calculated directly from the dpi selected when scanning (dots or pixels per inch). For example, 200 dpi means that there are 200 pixels (dots) per inch in the image, or 78.74 pixels per cm. The relation between image pixels and real length is set in *Analyze → Set Scale*. For a 200 dpi scanned image put 200 for *Distance in Pixels* and 2.54 for *Known Distance*. For photographs the scale has to be re-set for every image (unless the camera is held static with a tripod, in which case the scale only has to be set the first time). To do this select the straight line drawing tool and trace a line along a known length of the measurement ruler in the image. Then navigate to the *Set Scale* window, the *Distance in Pixels* will already be set by the line, so you only have to enter the *Known Distance* in cm measured from the ruler in the image. This establishes a relation between the number of image pixels and the real length which enables accurate estimation of area in the image. To proceed with area measurement, use one of the selection tools (e.g. *Rectangular selection*) to surround the objects of interest (the leaves), then select *Analyze → Analyze particles → OK*. Several output windows will be generated: the important one is the *Summary* window from which you can note the *Total Area* of the objects of interest. The *Count* may often be greater than the number of leaves in the image, this is usually because the areas of very small objects in the image are also included, but these should make very little difference to the overall area estimate. You can confirm this in the detailed *Results* window which presents area for every object identified in the selected region.

Repeat this process for all of the images. It is not necessary to set the scale for subsequent analyses if using a scanner, or if the camera was kept stationary. Total leaf area for each measurement point should be entered directly into a datasheet (where dry mass for the same points has already been entered).

5. DENDROMETERS

Rather than using preassembled dendrometer bands, we recommend assembling dendrometer bands from basic parts in the field, which gives a more 'tailor-made' fit to each tree (and, in addition, represents a significant cost saving).

Dendrometers in Wytham Woods, UK (left; installed at POM+10 cm), and Vianet Mihindou at Lopé, Gabon (right; installed above 2 m to avoid elephant damage).

Equipment: For each installing team (ideally 2 per team; the more teams the better and the quicker the job will get done) you need a diameter tape, pencil, notebook, stanley knife, wire-cutters, protective glasses, pliers, strong scissors (e.g. kitchen scissors), strapping band (packing tape), a sealer, enough seals (aka. clips) for two per tree plus spares, enough dendrometer springs[110] for one per tree plus spares (see the film mentioned below for many of these items).

*** Our current standard is to use plastic band dendrometers at all sites, however in areas where low-intensity fires are common, metal band dendrometers may be more appropriate for which please see the *ForestGEO-CTFS* protocol at http://www.ctfs.si.edu/data///documents/Metal_Band_Dendrometer_Protocol_20100330.pdf ***

As soon as possible after the initial DPOM measurements, dendrometer bands should be installed in the plot. Ideally all tagged trees and lianas >10 cm DPOM[111] should have dendrometers installed (excluding palms,

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[110] n.b. we use only ONE size of spring (differing from *ForestGEO-CTFS* who recommend to use three different sizes of spring in http://www.ctfs.si.edu/data///documents/Metal_Band_Dendrometer_Protocol_20100330.pdf for their metal band dendrometers and two different sizes in http://www.ctfs.si.edu/data///documents/Plastic_Band_Dendrometer_Protocol_20091105.pdf for their plastic band dendrometers, although no reason for this is given there).

[111] This minimum was suggested by Jhon del Aguila Pasquel in Feb 2013 based on experience in Allpahuayo and Jenaro Herrera in Peru and Ducke in Brazil. Below this diameter, the band can't be wrapped properly around the stem and instead sits rather like a square with too little contact (this happens even if the stem is not irregular). Note that some tree species do not reach this minimum DPOM even as adults, therefore we cannot measure these species with dendrometers according to the current design (please make a

cycads, aloes and tree ferns which generally do not increase in diameter[112]), but logistical or time constraints may mean only a selection receive them (chosen either randomly or spaced uniformly across the plot[113]). As long as at least 200 tagged stems/ha have dendrometers then a reasonable estimate may be made. If possible, make sure that all the stems where CO_2 efflux collars were installed (§3.2) are included in the selection for dendrometer bands[114], which will allow correlations between CO_2 efflux and growth to be investigated.

At this point, every tagged stem will have a tag at 1.6 m and a POM red mark. Dendrometers should be installed 10 cm above the POM or, if there is a stem irregularity or other problem there, at the next available point above that [115] [116] (i.e. for the majority of stems at 1.4 m above the ground). See the Dec 2010 film "How to attach a plastic band dendrometer" by Terhi Riutta, Nathalie Butt, Toby Marthews and Leo Butt, downloadable from http://www.eci.ox.ac.uk/research/ecodynamics/downloads/pbd-wytham-dec2010.m4v, for how to install a dendrometer band in a temperate forest[117] [118].

Diameter increment should be recorded from all dendrometers at least every 3 months in order to record the seasonal cycle of the site (ideally, every month, but if not possible measure them every three months and a subset of the 50 fastest-growing trees every month). One measurement should ideally be towards the end of the dry season in order to record minimum growth. At every tree census, a selection of the new recruits should be fitted with dendrometer bands so as to maintain the same proportion of banded stems.

Before installation, clear loose bark and dirt, termite nests, bryophytes, moss, ant trails (etc.) from the stem but *** *DO NOT EVER* *** scrape the stem to make a smooth bark surface before installing the dendrometer bands (as done by some groups e.g. Keeland & Sharitz 1993, http://www.nwrc.usgs.gov/Dendrometer/index.htm#3). Pull lianas away from the bark at the dendrometer installation location. If a liana cannot be pulled away (partly inside the trunk) record this in the notes.

Timing: Installing dendrometer bands like this in a 2-person team, estimate 100 dendrometers/day.

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note of any such excluded species). Also note that if you are using less flexible tape and/or different clips then this 10 cm minimum may have to be replaced with 15 cm or 20 cm (if so, please make appropriate notes).

[112] (unless the intention is to measure 'diffuse secondary growth').

[113] Note that any selection method may mean that some rare species may remain unsampled, but these trees should only make a small difference to overall carbon dynamics (make a note of these excluded species).

[114] Very large trees and ones with stem irregularities or aerial roots may have to be excluded from this selection for practical reasons.

[115] POM+10 cm is not yet fully standardised across the RAINFOR network so if local circumstances dictate then go for POM+20 cm or another height as required (e.g. following the height used in a previous dendroband study). In areas where elephants occur, put dendrometer bands at >2.0 m height (record the height) following our current practice in Gabon.

[116] Why not simply at the POM height? Some people do indeed install the dendrometers at POM height but that means you can't measure DPOM directly any more. We put them at a different height so that we can measure the dendrometers every month or so *and* at every main census we measure the DPOM as well. This provides a very useful check because you can plot dendrometer diameter *vs.* DPOM and check that there is a tight relationship.

[117] It should also have been mentioned in that film that:

(i) You should be extra careful moving the spring out of the way to make measurements (as shown at one point in the film) because if the spring becomes stretched the growth increment will be over-estimated

(ii) You can save yourself a fair amount of time in the field if you clip all your dendrometer springs in camp beforehand and

(iii) Specifically in tropical forests, you should cut the strapping much longer, e.g. 1.5 * circumference rather than 1.0 * circumference as shown in the film (species that have rugged/spikey bark will snag the dendrometer springs during growth (e.g. Rosaceae, Burseraceae) so it becomes necessary to wrap extra strapping around the tree to allow for future growth and avoid letting the spring come in contact with the bark and, for consistency, this should be done for all species not just Rosaceae and Burseraceae).

[118] The method described in our film here is similar to the *ForestGEO-CTFS* method described at http://www.ctfs.si.edu/data///documents/Plastic_Band_Dendrometer_Protocol_20091105.pdf.

An Ecomatik automatic point dendrometer in plot AC1, Alerce Costero National Park, Chile.

For soft-barked species such as *Fitzroya cuppresoides*, plastic band dendrometers do not work well and point dendrometers may be necessary. For point dendrometers it is necessary to 'shave' the stem a little in a small, localised area close to the point of installation (be careful not to damage the cambium of the tree). Currently (2012), automated dendrometers (i.e. devices that automatically record the diameter at prespecified intervals, not necessarily connected to a data logger) are being trialled in the UK, and Chile. Because we are unable to measure daily changes in stem hydration and growth, these dendrometers are of great interest, however because of high cost these are not intended for wide use in *RAINFOR-GEM* and are not part of the standard protocols[119].

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[119] Automated dendrometers are also not yet in use in the *ForestGEO-CTFS* network.

6. FOLIAGE (LEAF AREA INDEX, *LAI*)

RAINFOR-GEM recommends measuring *LAI* using hemispherical photos because this method can be replicated across a large number of plots at low cost (see Jonckheere *et al.* 2004, Zhang *et al.* 2005 for a review of alternative methods). Note that in the following we estimate *LAI* directly from the hemispherical photos without attempting any subtraction of non-foliar parts of the canopy present in the photos (called the "effective *LAI*" or "Plant Area Index *PAI*" in CAN-EYE depending on version) therefore remember that *LAI* here will be slightly overestimated when non-foliar parts predominate.

A hemispherical camera lens 1 m high (left, from the 50 yr after logging plot at Bobiri, Ghana) and Rocío Urrutia with a camera (right, in plot AC2, Alerce Costero National Park, Chile; note the orange tube marking the position of the camera so that subsequent photos may be taken at the same spot).

Equipment: A digital camera with a hemispherical/fish-eye lens, robust tripod with a built-in spirit level, camera memory card reader. You also need to metre pole or tape measure to keep the lens 1 m above the ground, a compass to align the camera and perhaps also a separate spirit level that you can put on top of the lens cap (allows you to move the entire camera to level it in places with a pronounced slope).

LAI should be estimated by collecting colour images[120] of the canopy every month with a digital camera set to automatic connected to a hemispherical lens on a tripod close to the centre point of each subplot of the plot. At each subplot, locate an area close to the centre that is free of vines or branches at least 2 m above the camera lens (which should always be 1 m from the ground). Once a point has been chosen, mark it in the ground to enable the same point to be used for all subsequent photos. When taking the photographs it is important to ensure that:

- Direct radiation is low, so either when the sky is overcast with clouds or at sunrise or sunset when there is little visible reflection off leaves[121],

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[120] Colour is essential for bright reflections on leaves to be distinguished from sky.
[121] This requirement is the same as it is for *LAI* meters such as the LAI-2000 (see LI-COR 1992:4-10 or http://www.licor.com/env/newsline/2008/05/appropriate-sky-conditions-for-using-the-lai-2000/).

- The tripod is level so that the lens is pointing directly upwards at exactly 1 m height (*do not* tilt the camera to match the slope as is done for an LAI-2000, LI-COR 1992),
- All photos are taken at the same resolution (preferably the highest available) on the 'fisheye' setting and
- All photographs are taken in the same direction (e.g. directly north).

When downloaded, image files should be given an appropriate name (date, time, site, plot, point number) and rotated to have north at the top. Organise the images into directories (*CAN-EYE* will calculate an average *LAI* across all images in the directory so have e.g. a directory each for each plot and each month; up to 20 images in each directory) and process with *CAN-EYE* free software[122] (https://www4.paca.inra.fr/can-eye):

Instructions for *CAN-EYE* v6.2: Select the *CAN-EYE Hemispherical Images* option in the opening screen (RGB images, upward). Select the folder that contains the images to be analysed, click on "HELP" at the bottom to know what to enter on the page (you must specify a projection function) then press *SAVE*. The software now uploads the selected images: if they appear correct then press *OK* (otherwise you can select images to discard). On the next screen you can vary image contrast to optimum levels with the *Gamma* option and use *Select/Mask* to exclude parts of the image which should not be analyzed. When finished, press *Done*. You now have to select how to distinguish vegetation from sky: select the option *No mixed pixels→Sky* and continue.

The software now reduces the image colour range to the ones shown in the box on the right side of the classification screen. To start, click the circle to the left of the *Sky* box in the lower right of the screen. Click *Yes* for an automatic pre-selection of colours in the images which represent sky. All the classified pixels will move to the top of the right hand box. To continue the classification process, left-click in the circle to the left of the *Sky* box in the lower right of the screen, and left-click on any of the colours in the box which you think represent vegetation (rather than sky). To help you, colours which occur commonly and very commonly in the images are highlighted in the right-hand box with white and red dots respectively. When you have selected your chosen colours, *right*-click outside of the box to reclassify the images based upon your new selection[123]. Repeat this process until you are satisfied and then click *Done*. After some time, *CAN-EYE* will output your results to the nominated folder and you will be given the option to exit.

To check results of the analysis, open the "*CE...Report...html*" file in the output folder, which summarises key values and graphics from the analysis. The output presents three types of *LAI* estimate: (**1**) Straight *LAI* for the image, (**2**) *LAI* for when the camera is tilted at 57.5° from the vertical and (**3**) True *LAI* (incorporating an estimate of the degree of canopy clumping). Of these, choose (**3**). The *ALA* variable also gives an estimate of leaf inclination angles in the canopy (see https://www4.paca.inra.fr/can-eye/content/download/2939/29494/version/1/file/Variables_Meaning_CAN_EYE.pdf).

Repeated measurements

Take a hemiphoto at the central point of each subplot every month, as described above.

Finally, where topographic and canopy data is available (§9), correct for slope and canopy height using the methods of Walter & Torquebiau (2000) (especially important in steep terrain, e.g. mountains). Canopy productivity and *CLM* (*Canopy Leaf Mass*[124] in t/ha) may be deduced from the seasonal variation of *LAI* values (de Weirdt *et al.* 2012).

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[122] *CAN-EYE* is our current recommended package, although others do exist (e.g. *HSP*, http://to.ee/download/m535a917128f45/).

[123] It is well-known that this thresholding step is partially subjective. As Jonckheere *et al.* (2004) put it, "One of the main problems cited in the literature of hemispherical photography for determination of LAI is the selection of the optimal brightness threshold in order to distinguish leaf area from sky area thus producing a binary image". To minimise this, only allow one operator to process all the images in any particular batch (or cross-check between operators using a small set of 'calibration' photos).

[124] **Canopy Leaf Mass** (t/ha) (aka. **Canopy Foliar Mass**) may be calculated by multiplying *LAI* (m^2/m^2) by average Leaf dry Mass per unit Area *LMA* (g/m^2; n.b. *LMA*=1/*SLA* from §4.3) using

(CLM in t/ha) = (LAI in m^2/m^2) * (LMA in g/m^2) * (10000/1000000)

e.g. for a canopy with *LAI*=5 m^2/m^2 and average *LMA*=90 g/m^2, *CLM* is 4.5 t/ha, approximately half of which is carbon.

7. SMALL HERBS (GRASSLAND PROTOCOLS)

Currently (2012), this protocol has been implemented only at the Ghanaian and Gabonese sites within *GEM*, but will be increasingly important as *GEM* expands into other woodland, savanna and grassland sites. Note that currently the following protocol is designed to be flexible enough to measure carbon stocks and changes in a grassland environment where tree cover is minimal but also to measure the herb layer within a closed-canopy forest.

Equipment: measuring tape, clip plot frame (optional), clippers, paper bags, permanent markers, drying oven, weighing balance (0.01 g resolution)

Establish five 1 m × 1 m sampling areas ('clip plots') per plot (50 m separation distance), at ~5 m inside each subplot in the inner corner of each subplot (this location will avoid the disturbance due to plot establishment).

Sample small herb biomass once after plot establishment:
- At each sampling area, harvest all small herbs[125] <1 cm basal diameter (cut at ground level) into a separate (labelled) bag (do not include parts of herbaceous plants that reach outside the clip plot, even if rooted within the plot).
- Oven dry and weigh.

Measure small herb productivity every 4 months (including grass re-growth). During each time interval do two collections: one after 3.5 months, and then another after a further 2 weeks (to catch quick regrowth). Repeat this process on a 4 month cycle.
- At each sampling area, harvest all small herbs <1 cm basal diameter (cut at ground level) into a separate (labelled) bag.
- Vegetation is allowed to re-grow for 2 weeks and then harvest again in the same way.
- Oven dry and weigh.

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[125] Herbs are non-woody plants, including all grasses and forbs (Geldenhuys *et al.* 1988). Small herbs <1 cm DBH includes arrowroots (Marantaceae), gingers (Zingiberaceae), aroids (Araceae) and grasses/graminoids (Poaceae *** EXCEPT WOODY BAMBOOS CENSUSED IN §1 ***, Cyperaceae, Juncaceae and Restionaceae). Only include large monocots such as dracaenids, Strelitziaceae, Musaceae or Heliconiaceae if they are not woody (e.g. young individuals before they gain wood and 'move across' to being included in the tree census in §1). Herbaceous vegetation does not include lichens, ferns, bryophytes or other epiphytic/hemiepiphytic vegetation (Geldenhuys *et al.* 1988).

8. WEATHER AND CLIMATE

Local climate and weather are characterised by the energy available to, and used by, the land surface, and thus play a key role in carbon dynamics. To characterise this land surface energy exchange and its interplay with carbon dynamics, meteorological data are taken at all the *RAINFOR-GEM* plots (WMO 2008). We measure both **above-canopy meteorological variables** (either from a tower or from a MET station in as large a clearing as possible) and **understorey environmental variables** using a smaller MET station within the forest. These data add immeasurably to the value of the carbon monitoring data gathered through the above protocols and allow forest dynamical data to be related to changes in local weather and climate.

MET stations at Wayqecha, Peru (left) and Lopé, Gabon (right). Note the temperature and humidity sensors in both are housed within Stevenson screens in an unshaded position (which defines "screen height").

In its simplest form, the available energy (net radiation, R_n) and its exchange between the land surface and atmosphere is represented by (e.g. Oke 1987, Landsberg & Sands 2011):

$$R_n = (SW_{down} - SW_{up}) + (LW_{down} - LW_{up}) = LH + H + G$$

so the magnitude of water and heat transport (latent heat flux LH and sensible heat flux H, respectively) plus the ground heat flux (G) is equal to the net incoming shortwave and longwave radition (*SW* and *LW*, respectively). Heat and water transport is mediated by turbulent transport, which is essentially a function of wind speed (U) and pressure (P). As a result, above-canopy measurements of *LW*, *SW*, *LH*, *H*, *U*, *P* should be made as a bare minimum to characterise land surface energetics, including their use to parameterise land surface exchange models. Measurements of precipitation (*Precip*) and wind direction (V) are also essential to inform us of the general energy balance of the land surface.

Due to the inherent variability in these quantities, measurements should be made at least every 10 s for averages (typically 15 minutes) to be representative. As such, data should be logged automatically using

appropriate equipment. We recommend Campbell Scientific Automatic Weather Stations (http://www.campbellsci.co.uk/index.cfm?id=105: see TEAM 2011 for recommendations for all components), although other devices such as Skye MiniMET stations (http://www.skyeinstruments.com/category/products/minimet-weather-station/) are in use across the network.

Measurements in the clearing

In the absence of a tower, the primary MET station should be set up in a clearing as close as possible to the census plot. As emphasised in TEAM (2011), the clearing should be no smaller than 70 m^2 and wherever possible located *away from* sloping ground, hollows or valleys, permanently-shaded areas, swamps and low places that hold water after rain (however note the sloping ground around the MET station pictured above: these are guidelines and it is frequently not possible to avoid sloping ground). The implicit assumption in all meteorological measurements is that sensors are positioned at a height which is representative of a homogenous land cover below. Therefore, meteorological measurements are made using sensors mounted on a tower of sufficient height to make representative measurements of the land cover below.
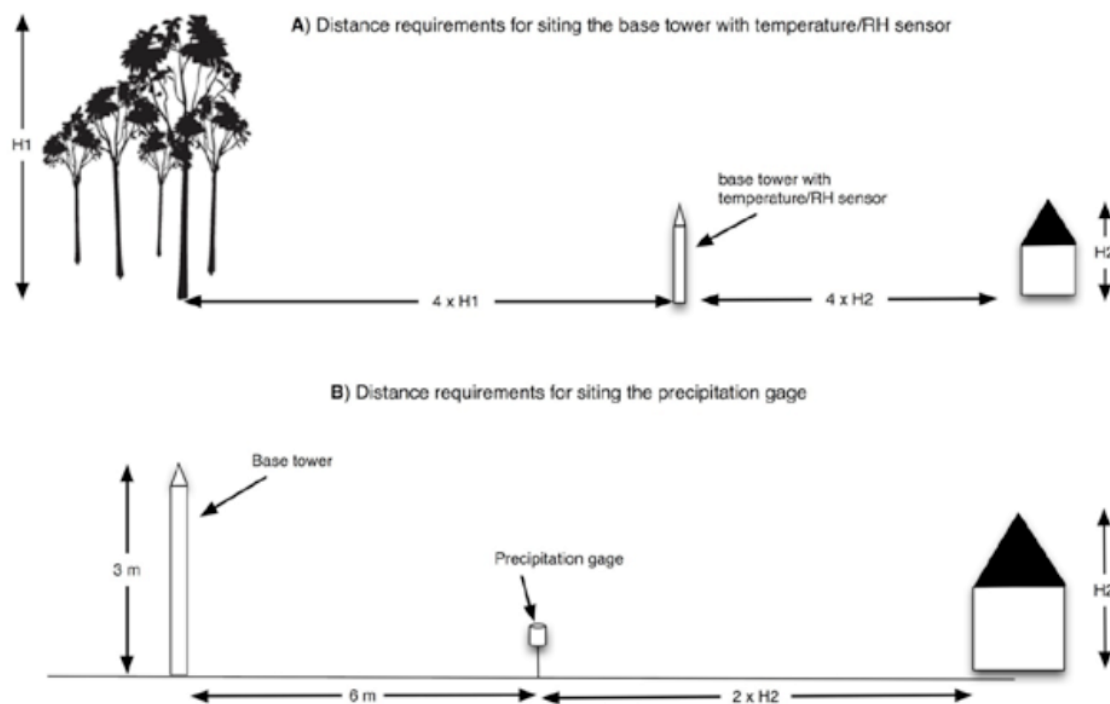
Figure 3. Siting requirements for a climatic base tower with a temperature/RH sensor (A) and a precipitation gauge (B). Distances are not shown to scale. In this example, the base tower with the temperature/RH sensor needs to be at a distance from the trees at least four times their height (4H1). It also needs to be at a distance from the house at least four times its height (4H2). The precipitation gauge needs to be 1) at least 30 cm from the ground, 2) at least 6 m away from the 3 m base tower, and 3) at a distance from the house twice its height (2H2) away from it. Guidelines from WMO (2008) and EPA (1995).

For areas of short grass/bare ground, position the MET station following the 'level 2' siting guidelines of TEAM (2011), i.e. following the 4*H* and 2*H* rules illustrated in the figure above (from TEAM 2011). The screen height of the MET station should be set at 2 m above the ground (WMO 2008) which gives an acceptable footprint for upwelling radiation measurements (with a sensor height of 2 m, 90% of the flux originates from a circle of diameter 12 m on the surface). Apart from the precipitation gauge, all equipment should be mounted as close as possible[126] to screen (reference) height on the station tower.

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[126] n.b. "close" is a relative term here: within a metre or so is fine for above-canopy measurement, e.g. photo above, although for below-canopy work they should be somewhat closer, e.g. photo below. Also, note that in TEAM (2011) the temperature and humidity sensors are at 2 m height but the radiation sensor is at 3 m.

Luiz Aragão repairing an understorey MET station in Tambopata, Peru.

In practice in many tropical forest research areas it is impossible to find a clearing of the size described above. If a tower is available then that should be used, however if not then use the largest available gap. Note that in small clearings there will be some turbulence effects from the surrounding canopy (i.e. wind speeds will be underestimated). Also, in small clearings low sun angles will be missed: for these points, mark them clearly as gaps in the time series and they will be filled at a later point when gap-filling algorithms are applied, e.g. see Marthews *et al.* 2012:AppxI).

Measurements within the forest

Within tall vegetation (e.g. forest), below-canopy environmental variables are measured using a met station situated either at ground level or at various heights within the canopy, depending on available equipment and vegetation complexity. About 90% of available energy from the sun is absorbed and used by the forest canopy. Hence, preference should be given to above canopy measurements for cost-effective station deployments: for example, in dense canopies the amount of light on the forest floor can be so low the soil heat flux term (G) is negligible and thus can be ignored if equipment/budget is limited.

The primary reason for within-forest measurements is to quantify soil moisture (and to a lesser extent soil temperature) in forest conditions. These cannot be measured in clearings, where soil conditions are greatly different, or from towers.

Which variables?

Strongly recommended variables are air temperature, air humidity, incoming solar (SW) radiation, precipitation and, within the forest, soil moisture and soil temperature. Desirable variables are reflected solar radiation (from tower), photosynthetically-active radiation (PAR), longwave radiation (both downward and upward), wind speed and direction, air pressure and the fraction of SW radiation that is diffuse.

Values should be recorded every 30 min (or 10 min if possible) and a schedule for regular downloading (e.g. weekly) should be formulated based on the memory capacity of the data logger and other logistical considerations.

9. TOPOGRAPHY, TREE MAPS, BOTANICAL DETERMINATION AND WOOD DENSITY

A **botanical survey** is necessary and should be carried out as soon as possible after the first census and after every recensus (Condit 1998 and see updated details in Condit *et al.* 2014), depending on the local availability of trained botanical personnel. Note that it may be necessary to make surveys both in the wet and dry seasons to increase the likelihood of collecting reproductive material for species identification.

A protocol has been formulated for measuring **wood densities** across a plot once botanical information is available   http://www.rainfor.org/upload/ManualsEnglish/wood_density_english%5B1%5D.pdf   (developed from the outline at Phillips *et al.* 2009:13). However, note that we *do not* allow tree cores to be taken within any permanent plot (the same species can be sampled outside the plots if necessary).

A **topographical survey** is carried out as standard in *ForestGEO-CTFS* (Condit 1998) and would be desirable, but is not currently required by *RAINFOR-GEM*.

A **tree map** (i.e. measuring the *x,y* coordinates of each stem within each subplot) is clearly desirable and essential for studies where precise distances to stems must be calculated (e.g. fine-scale seed dispersal studies; one is carried out as standard in *ForestGEO-CTFS*, Condit 1998). However, if all that is required is locating trees inside the plot for remeasurement, (i) the census itself shows which subplot contains each stem (even if *lx*, *ly* numbers are not recorded) and (ii) after the first census the trees are in sequence along the known tree tagging route (see figure in §1.3) and therefore easy to locate quickly even without a map. After several censuses, new recruits mean that the trees are no longer 'in order' so a tree map begins to become more useful (e.g. two examples are shown at the start of §1), however until that point this is desirable rather than essential.

10. DATA ENTRY AND SERVER UPLOAD

A crucial, although often thankless, task. It is probably fair to say that the most common error people make about data entry is not to allow enough time for it. Estimate one day per ha census and *do it in the field before you leave* so that errors can be checked, if necessary, by revisiting the plot straight away. Here is an example spreadsheet from the Fragment E plot in Sabah, Malaysia (note that the full names of *all personnel involved* should be recorded in each data sheet, as shown here):

	SUBPLOT	TREE NUM	DATE	D.POM (cm; or DV for lianas)	H.POM (m)	HEIGHT (m)	FLAG1	COMMENTS (tangga=ladder)	DH (cm)	DMAX (cm)	D.DEN (cm)	H.DEN (m)	Stem respiration tube?
424	23	421	28-May-11	12.4	1.30	7	a				11.2	1.40	n
425	23	422	28-May-11	33.7	2.10	15	ac						n
426	23	423	28-May-11	28.4	1.80	15	a	On boundary with subplot 22 but take as in 23; dendrometer is lower than POM			27.9	1.40	y
427	23	424	28-May-11	23.8	1.80	15	a				23.2	2.00	n
428	23	425	28-May-11	11.5	1.30	7	a						n
429	23	426	28-May-11	37.8	1.30	20	a				35.8	2.60	n
430	23	427	28-May-11	31.6	1.30	20	a				33.0	1.95	y
431	23	428	28-May-11	13.4	2.00	5	ac				13.1	2.15	n
432	22	429	28-Jun-11	20.6	3.20	15	a	OUT OF ORDER in subplot 22; tagged with flagging tape only; tangga					n
433	23	430	28-May-11	10.5	1.30	5	a				10.7	1.40	n
434	23	431	28-May-11	25.2	1.30	13	b				24.2	1.40	n
435	23	432	28-May-11	13.4	1.30	10	a				13.3	1.40	n
436	23	433	28-May-11	24.9	1.30	14	a				25.2	1.40	n
437	23	434	28-May-11	22.6	1.30	15	a						n
438	23	435	28-May-11	12.8	1.30	7	a						n
439	23	436	28-May-11	10.0	1.30	4	a				10.1	1.40	n
440	23	437	28-May-11	11.8	1.30	7	a				11.7	1.40	n
441	23	438	28-May-11	12.6	1.30	6	a				11.3	1.45	n

Condit (1998) gives some sample code that may be used to create automated 'sanity checking' scripts to check census data for various data entry errors (now included in the R package of tools at http://ctfs.arnarb.harvard.edu/Public/CTFSRPackage/) and it is strongly advised to carry out your own error-checks like these[127]. Finally, when you are satisfied that all is correct, prepare the data for upload:

- Send a copy of *all* data to *GEM* organisers in Oxford http://gem.tropicalforests.ox.ac.uk/ and
- Upload your *census* data[128] to the database at http://www.ForestPlots.net/ (Lopez-Gonzalez *et al.* 2011).

Worldwide census plot data from the *RAINFOR* network is managed online at *ForestPlots.net*. We encourage you to take advantage of this resource for census data management and analysis. If you are planning to use *ForestPlots.net* for storing and managing your plot data please email admin@forestplot.net beforehand to discuss your plot monitoring plans. Please provide information on how many plots you will be uploading and who will be the plot data manager (person responsible for the data uploaded, usually the project Principal Investigator). You will also have to budget time to format, upload and check the data uploaded into *ForestPlots.net*. The *ForestPlots.net* team can provide in-house training and will email you the latest field sheet templates upon request[129].

If you are intending to upload and manage existing plot data on behalf of the plot data manager, please request the plot data manger or project Principal Investigator to email admin@forestplots.net stating to which plots you will need edit access (see maps in the introduction above).

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[127] *ForestPlots.net* includes an 'upload wizard' which does some sanity checking for you (Lopez-Gonzalez *et al.* 2011) but server upload is usually done *after* you have come back from the field. It is essential to do as many data checks as possible *in the field* before you leave so that possible errors may be corrected by revisiting the plot.

[128] If possible, it is more ideal to use the standardised field sheet templates used by *ForestPlots.net* rather than a spreadsheet like the one illustrated here because they are preformatted for upload to the *ForestPlots.net* database. These are available to Principal Investigators and Field Leaders by emailing admin@forestplots.net.

[129] You can also download and print the field sheets using the file sheet downloadable files available in *ForestPlots.net* (not available on a public-access log-in: please email admin@forestplots.net if you would like copies of these spreadsheets).

APPENDIX I: *RAINFOR-GEM* Fieldwork codes for tree censuses

(also uploaded on http://www.rainfor.org/en/manuals)

FLAG 1: ALIVE STATUS (If a tree is dead, enter "0" in this column; n.b. not all these codes can be applied to liana stems)

a = Alive & normal (this code may only be used either alone or in combination with '**n**' (if a tree is a recruit, code '**an**') or '**h**' (a normal multiple stemmed tree, '**ah**')). "Normal" here means standing straight, trunk clear, healthy without damage, with leaves and without lianas.

b = Alive, broken stem/top with regrowth. If different from recorded height, write in the comments column at what height the stem is broken (e.g. if regrowth reaches 4 m but stem broken at 3 m).

c = Alive, leaning by ≥10°. The leaning code should not be used with the fallen code '**d**'. Do not use for lianas.

d = Alive, fallen (but still alive, e.g. on ground)

e = Alive, tree is fluted or fenestrated (i.e. irregularities all the way up to first major branching point). Do not apply this to liana stems.

f = Alive, stem hollow.

g = Alive, stem rotten

h = Multiple stemmed individual. Each stem ≥10 cm gets a different tag and all are coded '**h**' (put in Comments the tag number(s) of the stem(s) it is connected to). Should always be used with another code, e.g. if a tree is alive and with multiple stems use '**ah**'.

i = Alive, with no leaves or only a few leaves.

j = Alive, burnt stem

k = Alive, but <1.3 m high (e.g. stem has broken below 1.3 m since last census: record 0 cm diameter).

l = Alive, and has liana(s) ≥10 cm diameter on stem or in canopy.

m = Covered by lianas. Use only in case where canopy is at least 50% covered by lianas even when no individual liana is ≥10 cm diameter.

n = New recruit. Always use with another code- e.g. if a tree is normal and new then use the code '**an**', if a tree is broken and a new recruit the code is '**bn**'.

o = Alive with lightning damage

p = Alive despite having been cut or logged

q = Alive with peeling bark (bark loose/flaking either because the tree is dying (i.e. also coded '**z**') or for natural reasons (e.g. eucalypts))

s = Alive but has a strangler.

t = Tree is a strangler (whether free-standing or not). Also write "strangler" in the comments column.

z = Alive, but with little productivity (nearing death or diseased)

Note: Tree Alive Status Codes can be used together in any combination except codes '**a**', '**c**' and '**d**' (e.g. use '**if**' for a hollow tree that is alive with few leaves *not* '**aif**').

Optional life form codes (put in a different column: e.g. a leaning palm could be coded 'c' followed by '**Fp**'):

Fl = liana

Fp = palm or cycad

Ff = tree fern or aloe

Fh = large woody herb (inc. woody bamboos, dracaenids, Musaceae, Heliconiaceae, Strelitziaceae)

FLAG 2: MODE OF DEATH (Enter "1" in this column if the tree is alive)

1) Physical mechanism of mortality (How the tree died)

a =   Tree died standing
b =   Stem broken (snapped trunk; record height of break in Comments)
c =   Uprooted (root tip-up)
d =   Standing or broken, probably standing (not uprooted) (i.e. between **a** and **b** but closer to **a**)
e =   Standing or broken, probably broken (not uprooted) (i.e. between **a** and **b** but closer to **b**)
f =   Standing or broken (not uprooted) (i.e. between **a** and **b**)
g =   Broken or uprooted, probably uprooted (i.e. between **b** and **c** but closer to **c**)
h =   Broken or uprooted, probably broken (i.e. between **b** and **c** but closer to **b**)
i =   Broken or uprooted (not standing) (i.e. between **b** and **c**)
k =   Vanished (found location, searched for tree but could not find it)
l =   Presumed dead (location of tree not found e.g. impossible coordinates, poor maps)
m =   Unknown

2) Number of trees in Mortality event

p =   Died alone
q =   One of multiple deaths in a large event
r =   Unknown

3) Killed or killer

j =   Anthropogenic (human causes, e.g. logging)
n =   Fire/burnt
o =   Lightning
s =   Unknown whether killed or killer
t =   Killer
u =   Killed, no more information
v =   Killed by another dead tree with a broken stem
w =   Killed by another tree that was uprooted
x =   Killed by branches from a dead standing tree
y =   Killed by branches fallen from a living tree
z =   Killed by a strangler
2 =   Killed by a liana
3 =   Killed by strangler/liana weight (tree died broken or fallen; use with 'z' or '2')
4 =   Killed by strangler/liana competition (tree died standing; use with 'z' or '2')

Note: Select one code from each category. For example a dead tree that is standing, died alone and was killed by lighting would be '**apo**'.
For multiple deaths the numbers of trees that died should be recorded and written in the comments column.
Also see the Mode of Death manual at http://www.rainfor.org/en/manuals

FLAG 3: Measurement Technique

0 =   Normal/tape measurement
1 =   Relascope
2 =   Digital camera
3 =   Estimate
4 =   Ladder, with diameter tape
5 =   Unknown

FLAG 4: Data Manipulation

0 =   Normal measurement, no retrospective modification
1 =   Extrapolated from previous measurements forwards or final measurement backwards
2 =   Corrected expected typographical error
3 =   Interpolated (two good measurements either side of a problem measurement)
4 =   Estimated using median growth rates
6 =   The POM was changed because it had to be, good measurement before.
7 =   Zero growth rate assumed
8 =   Another transformation, see notes/ not clear what was done
R =   Correction using Ratio between non-affected and affected measurement (i.e. deformation, bark peeling)

Note: Only one measurement technique and one data manipulation code should be selected for each tree.
Comments: Everything else! If a tree is outside a plot, add to comments, but leave blank in census data.

APPENDIX II: How to use an EGM-4 Infra-Red Gas Analyser (IRGA)

An infra-red gas analyzer (IRGA) records the rate of CO_2 accumulation within a sealed chamber, to estimate CO_2 efflux from whatever is enclosed within the chamber (whether it is a live tree stem, dead wood or soil). There are several commercially available systems but here we use the PP-Systems EGM-4 and SRC-1 IRGA system because of its relative simplicity, portability and cheapness (see Pumpanen *et al.* 2009).

An EGM-4 portable Infra-Red Gas Analyser (IRGA) (right) and an SRC-1 Soil Respiration Chamber (left) for measurement of CO_2 efflux[130], which is the current system of choice for all *RAINFOR*, *AfriTRON* and *GEM* plots (see http://www.ppsystems.com/co2_gas_analyzers.htm for these and Pumpanen *et al.* 2009 for a discussion of alternatives).

*** PLEASE NOTE ***

*** BOTH THE IRGA (EGM) AND THE CHAMBER (SRC) ARE VERY FRAGILE ***

*** AND NEITHER OF THEM ARE WATERPROOF SO DRY THE SRC OUT THOROUGHLY (INCLUDING THE GAS-IN AND GAS-OUT TUBES) BEFORE ANY USE ***

*** IF THE SRC GETS WET THEN *DO NOT* CONNECT IT TO THE EGM BECAUSE WATER WILL ENTER THE EGM THROUGH THE GAS-IN TUBE AND CAUSE EXTENSIVE DAMAGE ***

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[130] When purchasing an EGM & SRC system, we recommend calibrating for the 0-2000 ppmv CO_2 range and, if possible, getting a NiMH battery (more expensive but lasts longer in the field). See http://www.ppsystems.com/Literature/EDSEGM4.pdf for details.
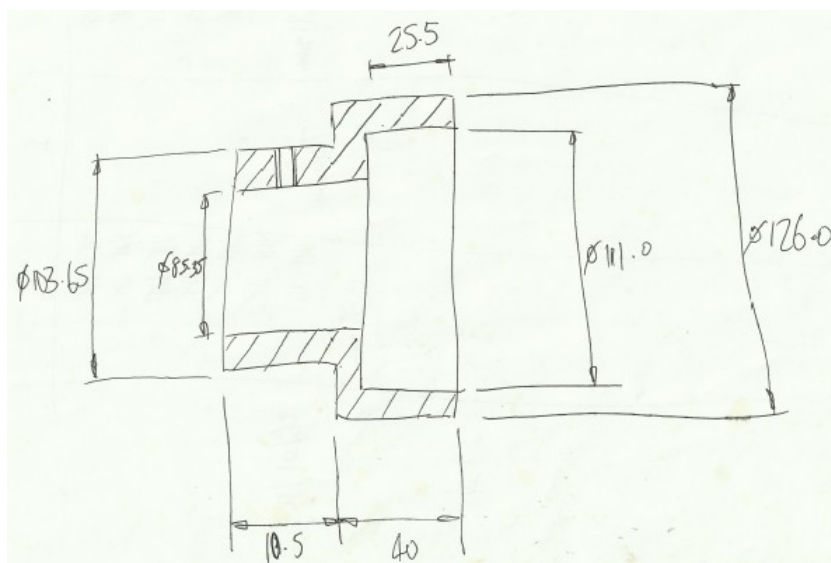
Stem CO_2 efflux measurements using an EGM-4 and SRC-1 (Maricarmen Ruiz Jaén and Kwame Sekyere in the 50 yrs after logging plot at Bobiri, Ghana). Note that the EGM is being held vertically by a strap during measurement. Note the purpose-built steel adapter ring at the base of the SRC-1 which allows it to be fitted more snugly onto the plastic collar attached to the tree stem (optional if the SRC and collar are close enough in size)[131].

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[131]

A drawing for this adapter ring is given right (to fit 110 mm piping) and is on file with the Thom Building Machine Shop (Room G.02) in Oxford, UK. The engineers there can have one made up on request if you are in Oxford (for £105 in 2012) or the engineers' drawing has been included here so that an adapter ring of the same spec can be made up in a workshop near you. The SRCs are a little bit variable because the outer part is made of plastic not steel (the grey outer casing) and the screw holes are sometimes no placed exactly regularly around the rim, so it's best to take your SRC down to your workshop along with the drawing so that they can check sizes and positions.

Finally, if possible add a plastic 'O' ring to the drawing where the adapter ring sits on the PVC collars (and glue it in using a solvent-free glue like Evo-Stik   Sticks   Like http://www.accessplastics.com/wp-content/uploads/pse-all-weath-evo-stik-sticks-like-ze0021.pdf   or   Simson   ISR   70-03 http://www.sheffins.co.uk/Literature/ISR%2070-03.pdf).

(Screwholes should be M3 size)

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Initial testing

Firstly, make sure you have a copy of the PP Systems EGM manual at http://www.vtpup.cz/common/manual/PrF_biofyz_PPSystems_EGM4_manual_EN.pdf.

- To set up, first make sure the SRC-1 came with a hydrophobic filter part 10045-1 already fitted (see manual 800611 EGM4_Operation_V418.pdf p. 6) and if the power fuse was removed for delivery, insert it into the black fuse-holder marked "1 AMP" at the back of the EGM-4. Charge the battery of the EGM-4 [132] which can take up to 12 hours (if the battery is not fully charged after 12 hours check the 1 A fuse: this protects the charging circuit - they can blow quite often so we recommend getting a bag of spare 1 A fuses). Next, connect the "in" and "out" rubber tubes of the SRC-1 chamber to the corresponding "GAS IN" and "GAS OUT" ports on the EGM-4. Connect the SRC-1 serial cable to either of the I/O ports on the EGM-4.

   Power options (NiMH battery EGMs only): If running the EGM-4 from battery, move the battery slide-switch to "Run" not "Charge". If running the EGM-4 from mains, move the battery slide-switch to "Charge" not "Run" and connect the mains supply to "12 V DC" (this does *not* mean the battery will charge from the mains supply: it will only charge from the battery charger). ** Do not move the battery slide-switch while either the battery charger or mains supply are connected **

   Do all of this *before* you switch on the EGM-4 (switch at back).

   Now do a test measurement (see field instructions below) and make sure you can transfer the result to your computer.

   To transfer saved measurements to a computer, you will need an RS232 to USB converter to connect the RS232 port on the EGM-4 to the computer. Be sure to install the necessary drivers for the converter first[133], and the EGM-4 Transfer software[134]. Then switch on the EGM-4 and open the Transfer software (see instructions below).

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[132] EGM-4 lights:
- 12V Power Supply/AC Adapter has a green LED indicating power (if you have a lead acid battery, this unit is both a power supply and charges the battery; if you have a NiMH battery, this unit is simply a power supply for the EGM)
- 12V NiMH Battery Charger (to charge a NiMH battery inside the EGM) which shows various lights: Orange = Battery not connected or Battery initialisation, Red = Fast charge, Green with intermittent orange flash = Top-off charge, Green = Trickle charge, Alternating Red-Green = Error.

[133] Thanks very much to Kathryn Jeffery in Oct 2012 who provided the following further details in a GEM post: "We've had some installation problems with the EGM-4: these have been linked to the IOGEAR USB serial adapter (GU232A) not installing properly. Note the installation disk with the USB to serial adapter is not compatible with 64 bit operating systems. If you're having problems installing the USB to serial adapter follow these instructions:
1.   Check your system software in System Properties; System type (32 or 64 bit) and operating system
2.   Go to this site: http://www.iogear.com/support/dm/driver/GUC232A
3.   Choose the correct driver software for your computer. Click download and when finished, unzip the zipped file and save to a known location.
4.   For Windows 7 64 bit; now plug in the USB to serial adapter to your computer (important: please remember which USB port you have used) and wait for several minutes- windows should automatically install the driver. To check, go to Computer, System Properties, Device Manager, Click next to Ports (COM and LPT) and you should see "ATEN USB to Serial Bridge". If nothing has happened after several minutes, look in Device Manager and you should see an exclamation mark next to "USB serial converter D"; right click on this and a window should appear with an option to select the driver manually; you can navigate to the folder where you saved the driver and install it. Once it is installed you should see the name has changed to "ATEN USB to Serial Bridge in Device Manager".
   However, Terhi Riutta also advises that sometimes USB-RS converters simply don't work with the EGM, no matter how many times you re-install the programs and drivers, so please *test this before you go to the field* and be prepared to buy more than one cable (from different manufacturers) if necessary.
[134]   This must be the most up to date version: a July 2012 version has been uploaded at http://gem.tropicalforests.ox.ac.uk/postings#cecilegirardin12101611031 for general use.

CO_2 flux measurements with EGM: A field guide

CO_2 measurements from soil and tree collars once a month

Equipment
EGM (CO_2 analyser) *charged up the night before and*

       with a clear memory (all previous data downloaded)
       and check the soda lime (replace if ⅔rds of it is brown)
SRC-1 Chamber
Computer
Waterproof bag
USB–RS port cable
Temperature sensor
Soil moisture sensor
Data sheets or notebook
Pencils
Plot map
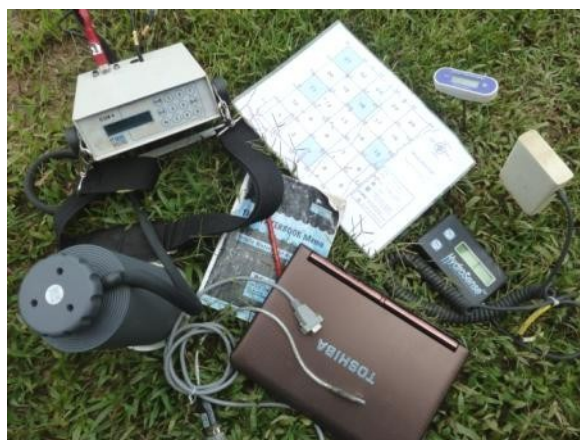
- Never measure in heavy rain (EGM may get wet)
- Transport the EGM and the computer in a waterproof bag

Starting

Connect the chamber to the EGM.

Turn the Charge / Run switch of the EGM to Run and
turn the On / Off switch to On.

Warm-up, wait 5 to 10 mins (EGM temperature will go up to ~50°C).

Main Menu[135]:

1REC 2SET 3CAL 4DMP 5CLR 6CLK	SOIL RESP. DATA RECORD 1ALL 2END
Press 1 (REC)	Press 1 (ALL)
DATA FITTING 1LINEAR 2QUAD.?	V: 1171 A: 78 V/A*100: 1491
Press 1 (LINEAR)	Press Y to accept default values (V=1171 cm^3, A=78 cm^2)
1DT:120 2DC: 50 3APPROX.TEMP. 25	
Press Y [136]	Press Y

---

[135] The 6 options here mean: 1 to begin taking a flux measurement, 2 changes the factory settings, 3 begins calibration, 4 transfers data to a computer, 5 deletes stored data and 6 sets the time and date.
[136] Measurements will continue until *DT* sec have passed or *DC* ppm of carbon has been registered so when the CO_2 flux is high the reading will stop short of the maximum time specified here.

Measurement

Type in a record number (start from 1) and press Y. Needs two digits (for example 1 = 01).

Hold the chamber in the air while it flushes, upwind and away from people. Don't breathe into the chamber or into the collar.
*** !! NO SMOKING !! ***

Place the SRC chamber on the collar and keep the EGM upright (use the hanging band). Press Y to start the measurement.

After equilibriating, measurement usually takes 80-124 s (shorter if CO_2 concentration goes up very fast).

When the measurement has ended press Y.

To save the measurement press Y. If the measurement was not good, don't save (press N) and repeat the measurement[137].

Take the chamber off the collar and press Y.

Type in a new record number and press Y.

Start a new measurement as before.

Note: CO_2 concentration in the beginning of the measurement should drop to <500 and then start to go up. If the CO_2 concentration is very high at the start of the measurement, start again. Press Y to end the measurement, don't record (press N) and do the measurement again with the same number.

If the measurement is very short (<30 seconds), repeat to make sure this is not happening because someone has stamped on the ground too much, however if the flux is high enough that a reading from >30 sec cannot be obtained, record the measurement with a note of the duration.

Measure soil temperature and moisture close to each collar (not for the tree collar measurements).

Measure air temperature at the chamber height for soil and tree measurements (for partitioned CO_2 efflux, one air temperature per group is ok).

PLOT NO = 1
Y OR NEW VALUE

CHAMBER FLUSHING
HOLD IN AIR   4

Time

PLACE ON SOIL
PRESS Y TO START

CO_2 concentration

C 405
A 0.313 Q0000 014

C 415
A 0.313 Q0000 END

RECORD Y/N

REMOVE FROM SOIL
THEN PRESS Y KEY

PLOT NO = 2
Y OR NEW VALUE

---

[137] For reference, CO_2 concentrations range over 350-450 ppmv in the field depending on location and time of day. In an office/urban setting, however, 500-1500 ppmv is usual depending on time of day and the number of people who are present.

Downloading data

EGM memory can store 36 measurements, after that the memory is
full so you will need to download data to computer.
Turn on the computer. Connect the USB-RS cable to the EGM and to COM port 3.
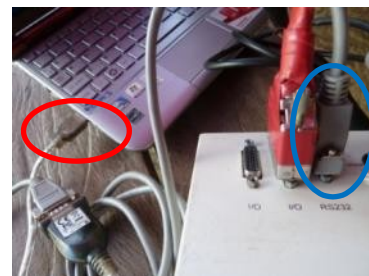
In the EGM press N until you are back to the Main Menu. Press 4 (DMP).

Press 2 (DATA DUMP).

> 1REC 2SET 3CAL
> 4DMP 5CLR 6CLK

> 1: SCREEN DISPLAY
> 2: DATA DUMP 1-2?

Double click Transfer to open the data program. Choose File → Preferences → Instrument Type → EGM-4 and
choose the correct Com Port where the USB converter is connected. Click Transfer → Start.
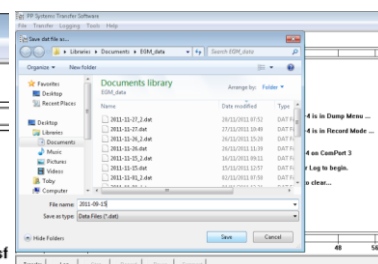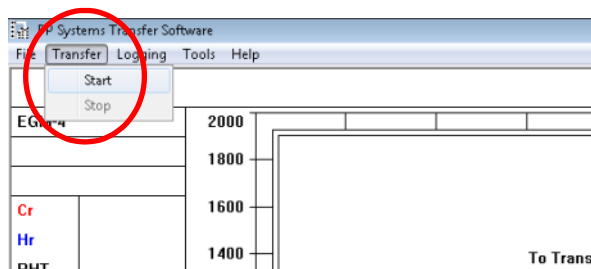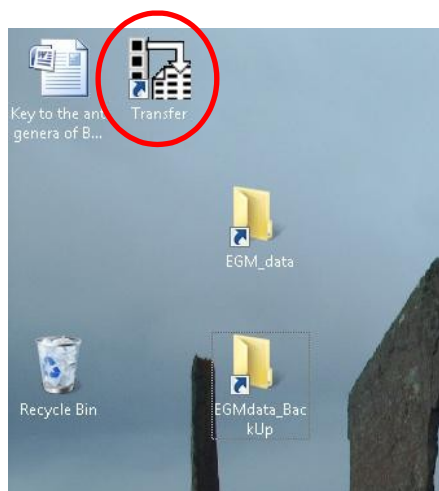
Type in the file name (YYYY-MM-DD), for example 15th of September 2011 is 2011-09-15. If
more than one file on the same day, name the second file 2011-09-15_2. Click Save.

> CONNECT TO PC
> ANY KEY TO SEND

Now press any button of the EGM to send data to the computer.

You should now see the data downloading. If the data transfer is not successful try again a few times, check
the USB to Serial connection, try other USB to Serial converter cables, and/or try other Com Ports (*File →
Preferences → Com Port*) [138]. Open the saved file in MS Excel to verify that all the data has been transferred
successfully. Remember to download data also at the end of the day.

The number of peaks in the green curve (CO_2 increase for every measurement) is equivalent to the number of
measurements made. Count them when you download the data and you will have the certainty that all the

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[138] Sometimes the computer is unable to open the Com Port because the port the program is using is not the same as the one the
computer has made available. If so, look in My Computer → Properties → Hardware → Device Manager, check Ports (COM & LPT)
and see if the number is different from that in the Transfer Program, then right click on the Port → Properties → Port Settings →
Advanced Options and change the number to the desired one and click OK.

measurements you made were safely downloaded to the computer. After the data has been downloaded successfully, quit the Transfer program.
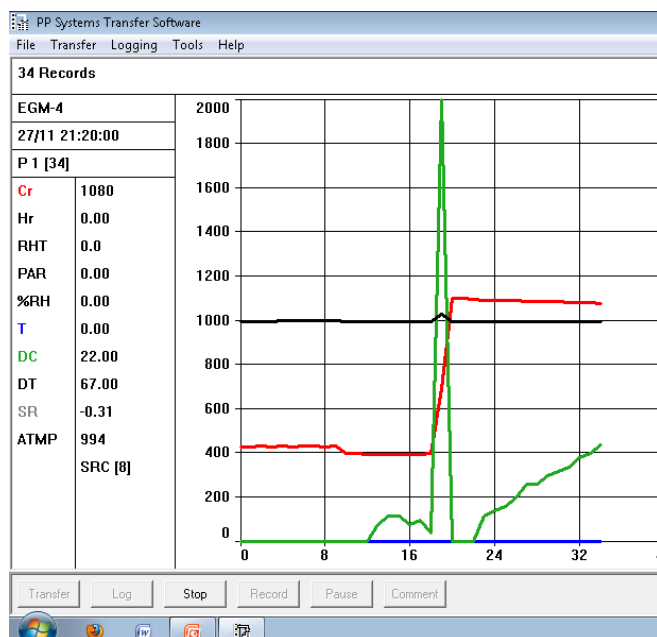
In the EGM, empty the memory. Press 5 (CLR).

| 1REC | 2SET | 3CAL |
| 4DMP | 5CLR | 6CLK |

Press Y to clear database.

CLEAR DATABASE (Y/N)

Press 0 to confirm.

PRESS 0 TO CONFIRM

Start the CO_2 measurements as before. Continue from the next record number (do not start again from 1 during the same day).

Finishing the measurements

Record the last measurement

For lead acid batteries

For NiMH batteries

Download data from the EGM to the computer in the forest or in the camp.

Switch the On/Off switch of the EGM to Off. Disconnect the chamber.

In the camp, charge the EGM.

Turn the Charge / Run switch to Charge. Put the computer to charge.

Problems

- I pressed something by mistake: Press N to go back.

- Something went wrong when I was measuring the flux: When it says RECORD?, press N and take the measurement again.

- EGM-4 displays CO_2 CONCENTRATION TOO LOW:
  Check if the chamber is sealed with the PVC ring
  Check if the fan is working properly by blowing close to the chamber
  Check the colour of the soda lime –it should be replace if ⅔rds of it is brown.

- EGM-4 displays NONLINEAR FLUX: Check you did choose "1:LINEAR".

- EGM-4 displays CHECKSUM ERROR: Firstly, try turning the EGM off, waiting a few seconds and then turning it on again.

  If that doesn't work, you'll have to reinitialise the system following the steps described in the EGM-4 Operator's Manual. VERY IMPORTANT: Before reinitialising the EGM-4, you must dump all stored records to your PC or they will be lost for good. All records will be cleared from system memory during initialisation.

  From the checksum error press any key to proceed to the Main Menu. From the Main Menu, press key 0 and then key 2 to re-initialise the system. After pressing key 2, you will be presented with ???? on the display. At this point, enter the code 0462. This will follow with a "Database Cleared" message before returning you to the Main Menu. At this point, your system will be returned to its original factory default conditions (i.e. calibration, etc.).

  If that doesn't clear the error, contact the Hansatech technical team (info@hansatech-instruments.com or +44 1760 338877).

CO$_2$ efflux calculations

While the EGM-calculated values are useful (and should not be discarded), it's preferable to directly calculate your fluxes from the raw data (the .dat file). To do so, take only the last 10 records of any individual measurement, verify that there is an approximately linear increase in CO$_2$ over time (open the .dat file in Excel and check by plotting CO$_2$ concentration ("CO2 Ref") against time ("Input E")), and use the following equation[139][140]:

$$R_{uc} = \frac{C_{10} - C_1}{t_{10} - t_1} \frac{P}{(T_a + 273.15)} \frac{V_d}{A} * \frac{44.01 * 0.36}{R_u} \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$$

Where R_{uc} is uncorrected CO$_2$ efflux (g CO$_2$ m^{-2} h^{-1}) [141], t_{10} and t_1 are the times (sec) since that measurement began at records 10 and 1 chosen respectively (e.g. record 1 could be 86 s after measurement began and record 10 the final record of this measurement after 124 s), C_{10} and C_1 are CO$_2$ concentrations (ppmv) at times t_{10} and t_1, P is ambient pressure at time t_{10} (mb as recorded by the EGM, e.g. ~1013.25 mb at sea level) and T_a is air temperature at time t_{10} (°C). The SRC-1 chamber volume V_d is 0.0012287 m^3 from measurement of the SRC-1

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[139] The Ideal Gas Law states that n moles of any gas at ambient pressure P mb and temperature T_a °C will occupy Vol m^3 where
$Vol = \frac{nR_u(T_a + 273.15)}{100P}$ (R_u=8.31432 J mol^{-1} K^{-1} is the Universal Gas Constant). Therefore, at time t_1 the CO$_2$ occupies a volume
$\frac{C_1}{1000000}V_d = \frac{n_1 R_u(T_a + 273.15)}{100P}$ m^3.

    Between times t_1 and t_{10} the CO$_2$ concentration rises from C_1 to C_{10} ppmv, so the number of moles CO$_2$ that enter the chamber during this time is $n_{10} - n_1 = \frac{C_{10} - C_1}{1000000}V_d \frac{100P}{R_u(T_a + 273.15)}$ mol CO$_2$. This equates to a molar flux of
$\frac{C_{10} - C_1}{1000000}V_d \frac{100P}{R_u(T_a + 273.15)} \frac{1}{(t_{10} - t_1)A}$ mol CO$_2$ m^{-2} s^{-1}. The molar mass of CO$_2$ is 44.01 g/mol so this is equivalent to
$\frac{C_{10} - C_1}{1000000}V_d \frac{100P}{R_u(T_a + 273.15)} \frac{1}{(t_{10} - t_1)A} * 44.01 * 3600$ g CO$_2$ m^{-2} h^{-1}.

[140] Several PP Systems manuals (e.g. http://katedry.czu.cz/storage/2673_2_SRC1_CIRAS-2_Operating_Instructions.pdf) and
Metcalfe *et al.* (2009:§4.2.2.2) had $R_{uc} = \frac{C_{10} - C_1}{t_{10} - t_1} * \frac{P}{1000} * \frac{273}{(T_a + 273)} * \frac{V_d}{A} * \frac{44.01 * 3.6}{22.41}$ g CO$_2$ m^{-2} h^{-1} but the 22.41 L there
was for old Standard Temperature and Pressure (0°C, 1013 mb) when it should have been for new STP (0°C, 1000 mb) which would have been 22.7 L. These forms are essentially equivalent through the value of $R_u \approx 100*22.41/273$.

[141] EGM-4 machines record CO$_2$ flux in units of g CO$_2$ m^{-2} h^{-1} and 1 g CO$_2$ m^{-2} h^{-1} is equivalent to (12.011/44.01=)0.27 g C m^{-2} h^{-1} or
(1000000/(44.01*60*60)=)6.31 µmol C m^{-2} s^{-1} =6.31 µmol CO$_2$ m^{-2} s^{-1}.

and the upper part of the adapter ring[142] (=1228.7 cm^3 or cc or mL; just the internal volume V_d in the diagram above, not V_d+V_t because the adapter ring contains the stem collar and this volume will be accounted for in the correction below). A is the area of exposed soil/tree stem against which the SRC+collar have been placed (for a collar of internal diameter 10.6 cm[143] A=3.1416*(0.106/2)^2 =0.00882 m^2 =88.2 cm^2). For example, at T_a=27°C the calculation might go something like:

$$R_{uc} = \frac{470 - 451}{124 - 86} \frac{982}{(27 + 273.15)} \frac{0.0012287}{0.00882} * \frac{44.01 * 0.36}{8.31432} = 0.434 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$$

(see this calculation on an Excel spreadsheet in the 'Respiration equations exercises' spreadsheet at www.rainfor.org/upload/ManualsEnglish/Respiration%20equations.xls). If you are measuring soil respiration on sandy soils then there is a possibility this method overestimates CO_2 efflux (see Pumpanen *et al.* 2004), but we currently do not correct for this.

Chamber volume correction: The uncorrected CO_2 efflux R_{uc} should be corrected when the SRC-1 is attached to any extension to the chamber (in these protocols there is always at least the adaptor ring). In the case of stem and soil CO_2 efflux the additional volume is the PVC collar sealing the chamber to the soil/stem. In the case of coarse litter CO_2 efflux, it is the same but the volume of the wood piece(s) inside the collar must be subtracted from the collar internal volume. The volumes of both the collar and the wood pieces may be calculated from the equation for the volume of a cylinder (3.1416 * (*diameter*/2)^2 * *length*) (or see Harmon *et al.* 1999 for more shapes). The equation to correct raw fluxes for changes in chamber volume is:

$$R_c = R_{uc} * \frac{V_d + V_{added}}{V_d}$$

where R_c is the actual soil/stem CO_2 efflux (what we want) and V_{added} is the additional volume (in m^3). If soil respiration is being measured, V_{added} will be the internal volume of the h cm of PVC collar protruding above the soil surface (the height h must include the width of the 'O' ring inserted into the adapter of the SRC, if there is one)[144]:

(V_{added} for soil CO_2 efflux in cm^3) = 3.1416 * (d_{collar}/2)^2 * h

(where d_{collar} is the internal diameter of the collar in cm). For example, for 110 mm tubing (external diameter) with wall thickness 2 mm, the internal diameter is d_{collar}=10.6 cm and for h=5 cm V_{added}=441.3 cm^3.

If the stem CO_2 efflux of a tree is being measured using a PVC collar of length h cm[145] affixed to the stem then we use a similar equation:

(V_{added} for stem CO_2 efflux in cm^3) = (3.1416 * (d_{collar}/2)^2 * h) + $V_{airspace}$

where the $V_{airspace}$ accounts for the possible curvature of the stem at the point of collar attachment. If a flat section of stem was selected for collar installation then $V_{airspace}$=0 cm^3, but if not the curvature of the stem surface will mean that there is some space between the collar and the stem surface.

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[142] From the measurements in the drawing in the footnote above this implies that the top ~4 cm of the grey part of the SRC-1 is occupied by the fan and other parts. Metcalfe *et al.* (2009) had 0.001208 m^3 here with a slightly different adapter ring design.
[143] Metcalfe *et al.* (2009) had slightly smaller collars of internal diameter 10.3 cm (area 0.0083 m^2).
[144] Measure d_{collar} and h in the field for *each collar* as there will be variation not only as a result of inconsistent manufacture and cutting but also because of the collar either settling into the soil or rising as a result of soil faunal activities since the previous measurement.
[145] Measure d_{collar} and h in the field for *each collar* as there will be variation as a result of inconsistent manufacture and cutting.
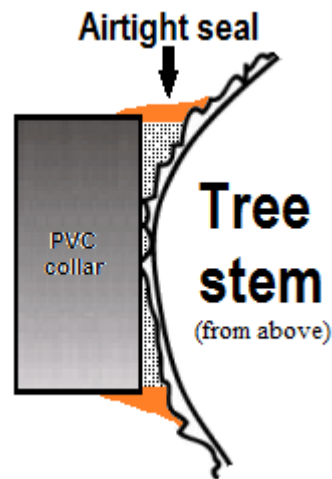
The volume $V_{airspace}$ (stippled) between the PVC collar and the stem surface (bark) between the airtight seals (e.g. sealant).

$V_{airspace}$ may be calculated from the formula for the intersection of two cylinders (Hubbell 1964, which assumes a tree of circular cross-section but should be approximately correct). If the collar above is attached to a $DPOM$=26.5 cm stem then $(d_{collar}/DPOM)$=0.4 (and therefore, from the table below, v=0.98477969)

$$V_{airspace} = ((3.1416 * (d_{collar}/2)^2 * DPOM) - ((DPOM/2)^3 * v)) / 2$$

So, $V_{airspace}$=24.03 cm^3 and $V_{added} = V_{collar} + V_{airspace} = 465.32$ cm^3.

Table giving the v factor for all values of $k=d_{collar}/DPOM$ between 0 and 1 (Hubbell 1964). However, for $k<0.36$ (i.e. for trees >30 cm diameter) slight stem irregularities will almost certainly mean that the collar has been installed on a flat area of bark and therefore no correction is necessary and $V_{airspace}$ may be taken as zero.

k	v	k	v	k	v	k	v	k	v
0.01	0.00062831	0.21	0.27555248	0.41	1.03351716	0.61	2.22346226	0.81	3.74831740
0.02	0.00251315	0.22	0.30225502	0.42	1.08334356	0.62	2.29281490	0.82	3.83063696
0.03	0.00565423	0.23	0.33016786	0.43	1.13425009	0.63	2.36300478	0.83	3.91333972
0.04	0.01005109	0.24	0.35928660	0.44	1.18622764	0.64	2.43401570	0.84	3.99639206
0.05	0.01570305	0.25	0.38960667	0.45	1.23926686	0.65	2.50583093	0.85	4.07975861
0.06	0.02260928	0.26	0.42112326	0.46	1.29335809	0.66	2.57843325	0.86	4.16340199
0.07	0.03076874	0.27	0.45383135	0.47	1.34849141	0.67	2.65180490	0.87	4.24728265
0.08	0.04018019	0.28	0.48772572	0.48	1.40465657	0.68	2.72592757	0.88	4.33135853
0.09	0.05084222	0.29	0.52280092	0.49	1.46184304	0.69	2.80078235	0.89	4.41558469
0.10	0.06275321	0.30	0.55905129	0.50	1.52003999	0.70	2.87634974	0.90	4.49991288
0.11	0.07591138	0.31	0.59647093	0.51	1.57923625	0.71	2.95260957	0.91	4.58429096
0.12	0.09031471	0.32	0.63505373	0.52	1.63942033	0.72	3.02954102	0.92	4.66866213
0.13	0.10596104	0.33	0.67479336	0.53	1.70058043	0.73	3.10712253	0.93	4.75296390
0.14	0.12284797	0.34	0.71568323	0.54	1.76270439	0.74	3.18533179	0.94	4.83712665
0.15	0.14097294	0.35	0.75771654	0.55	1.82577969	0.75	3.26414571	0.95	4.92107158
0.16	0.16033316	0.36	0.80088626	0.56	1.88979346	0.76	3.34354031	0.96	5.00470740
0.17	0.18092569	0.37	0.84518508	0.57	1.95473247	0.77	3.42349073	0.97	5.08792495
0.18	0.20274735	0.38	0.89060549	0.58	2.02058308	0.78	3.50397111	0.98	5.17058678
0.19	0.22579478	0.39	0.93713970	0.59	2.08733128	0.79	3.58495457	0.99	5.25250293
0.20	0.25006441	0.40	0.98477969	0.60	2.15496262	0.80	3.66641308	1.00	5.33333333

Coarse litter flux per unit mass/surface area calculation

For coarse litter CO_2 efflux measurements we need CO_2 efflux units per unit dry mass rather than per unit ground/stem. To calculate CO_2 efflux per unit litter dry mass (R_{lm}, g CO_2/h per kg litter dry mass) and surface area (R_{la}, g CO_2/h per m^2 litter surface area) use the following:

$$R_{lm} = R_c * \frac{1}{A} * L_m$$

$$R_{la} = R_c * \frac{1}{A} * L_a$$

Where L_m and L_a are litter dry mass (kg) and surface area (m^2) respectively. For equations for estimating R_{la} for decomposing litter of various shapes see Harmon *et al.* (1999).

APPENDIX III: Possible schedule of jobs for first 4 months

Monitoring	Month 1				Month 2				Month 3				Month 4			
In-growth cores every 3 months (§2.1)	■												■			
Rhizotrons every 1 month (§2.2)		■				■				■				■		
Soil respiration collars every 1 month (§3.1)	■				■				■				■			
Stem respiration collars every 1 month (§3.2)		■				■				■				■		
Partitioning experiment collars every 1 month including all control collars (§3.3)			■				■				■				■	
Ground fine litter every 3 months (§4.1)															■	
Litter traps every 2 weeks, separating leaves for SLA every 3 months (§4.3)	■		■		■		■		■		■		■		■	
CWD survey (for both biomass accumulation and CO_2 efflux) every 2-3 months (§3.4 and §4.2)				■								■				
Dendrometers at least every 3 months (ideally, every month) (§5)				■												■
Hemispherical photos in every subplot every month (§6)				■				■			■					■
Herb productivity on a 4 month cycle with measurements after 3.5 and 0.5 months (§7)															■	■
MET station readings are continuous, but downloaded every week both from the clearing and understorey stations (§8)	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦
Data entry (§10)	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦	▦

Recensus of plot every year, as close as possible to the same calendar date as the first census (§1.7)
One-off survey of soil carbon stocks (§2.4)
One-off botanical, topographical and other surveys (§9)

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