

SuperSites Vegetation Monitoring Protocols

TERN Australian SuperSite Network

This documents outlines a cut back version of the draft Australian SuperSite Networks vegetation monitoring protocols, a subset of which, is to be implemented during the NCRIS-2013 contract period.

Please send any comments, corrections and suggestions to the TERN Australian SuperSite Network Coordinator (mirko.karan@jcu.edu.au)



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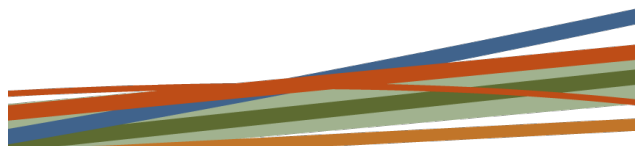


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Vegetation Monitoring Protocol Document Version Control

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Change Record

REVISION	Version #	DATE	DESCRIPTION OF CHANGE
A_DRAFT	1.0	2014-01-21	Initial draft for Internal release
A_DRAFT	1.1-1.1.7	2015-02-27	minor edits, Vegetation Monitoring Protocols compiled from SuperSites 2014 NCRI2013 Annexure 1 document
B	1.2	2015-02-28	Initial Release
	1.21	2015-03-04	minor edits
	1.22	2015-03-30	code change from "fnqr_cape_trib" to "fnqr_daintree" and added code for Daintree Discovery Centre "fnqr_ddc"

1 Milestone deliverables for the NCRIS2013 (Jul 2014- Jun 2015) contract period

ACTIVITY	PROTOCOLS	DELIVERABLES
Establish Core 1 ha (if required)	Section 4.1	
Vegetation Monitoring at the Core 1 ha		
• Complete Vascular Plant Species List	Section 7.1	Data
• Plant Vouchering	Section 7.2	Vouchers
• General Structure Description	Section 8.1	Data
• Abundance, Cover and Structure	Section 8.2-8.3	Data
• Above Ground Biomass	Section 8.4-9.1	Data
• Photopoints	Section 11	Images
• Leaf Area Index (Canopy)	Section 12	Data/Images
• Phenocameras	Section 17	Images
• Coarse Woody Debris	Section 9.2	Protocol
• Plant Functional Traits - Recruitment	Section 10.3	Protocol
(each SuperSite to develop a suitable protocol for Coarse Woody Debris and Recruitment)		
Isotope and Plant Genetic Sampling		
• Sampling for Stable Carbon Isotope Analysis	Section 19.1, 19.2	Samples
• Plant Genetic Sampling	Section 19.3	Samples

Preliminary draft protocols are italicized in the text. Where appropriate, protocols specific to vegetation types are indicated for forested areas, rangeland areas, grasslands, mallee and mulga

2 Quality Control of Data Input to the SuperSites Database

Quality Control and Quality Assurance measures to be used in the collection of field data will be outlined in the associated metadata entry.

Field data is submitted by SuperSite Principal Investigators or their representatives to the SuperSites Data Librarian (Data deposition through Morpho software will be disabled). Where possible, data is to be submitted in the recommended format (in some cases using standard template spread sheets).

The Data Librarian will prepare the dataset and metadata for publication through the SuperSite Database. This process may require the assistance of the SuperSite PI or representatives. The final metadata and data files are made available to the SuperSite PI for final checking. The SuperSite PI will acknowledge the correctness of the data and metadata entries by the prescribed mechanism. Acknowledgment of data ownership and final QA/QC checks are currently sent to the Data Librarian by email. An automated web based process is in development and will allow the SuperSite PI to access, check and give the final permission to publish via the click of a button.

3 Background

The vegetation monitoring protocol aims to provide generalized data that will be useful for describing each ecosystem, for detecting future change, and to contribute towards addressing cross-SuperSite level questions. These are currently listed as:

What are the current stocks and fluxes of energy, carbon, water and nutrients between the terrestrial (and aquatic) ecosystem components and the atmosphere/hydrosphere/geosphere.

- 1a. How are stocks/fluxes conditioned by management/disturbance/inter-annual variability?
- 1b. What are the key processes that determine ecosystem / non-biosphere exchanges?
- 1c. How are the key processes expected to respond to future environmental change?
- 1d. Are there general trends in changes in inter-annual stocks and fluxes across the network?

What are the current patterns and dynamics of terrestrial biodiversity (and aquatic).

- 2a. How is terrestrial biodiversity impacted by management/disturbance/inter-annual variability
- 2b. How will biodiversity be expected to respond future environmental change?
- 2c. Are there general patterns in changing abundance and/or biodiversity indices across the network?

4 Sampling Strategy

A two-tiered sampling strategy will be established at each supersite involving a core 1 ha plot and a set of additional plots that will be used to assess biodiversity and biomass. Currently we are specifying the nature of only the core 1 ha plot monitoring.

4.1 Core 1 ha plots

At least one core 1 ha plot will be established at the flux tower location to represent the vegetation in the tower footprint. This plot will include intensive measurement of soil, hydrological, vegetation, faunal and physiology variables as described herein. One hectare was chosen because it is the plot size already associated with many currently operating flux towers and other TERN facilities. Sub-sampling may be needed at some sites for intensive measurements. Measurements at these plots will provide descriptive data characterizing the functioning and dynamics of each ecosystem, contribute to questions 1a-d, and feed into AusCover and modelling projects such as CABLE.

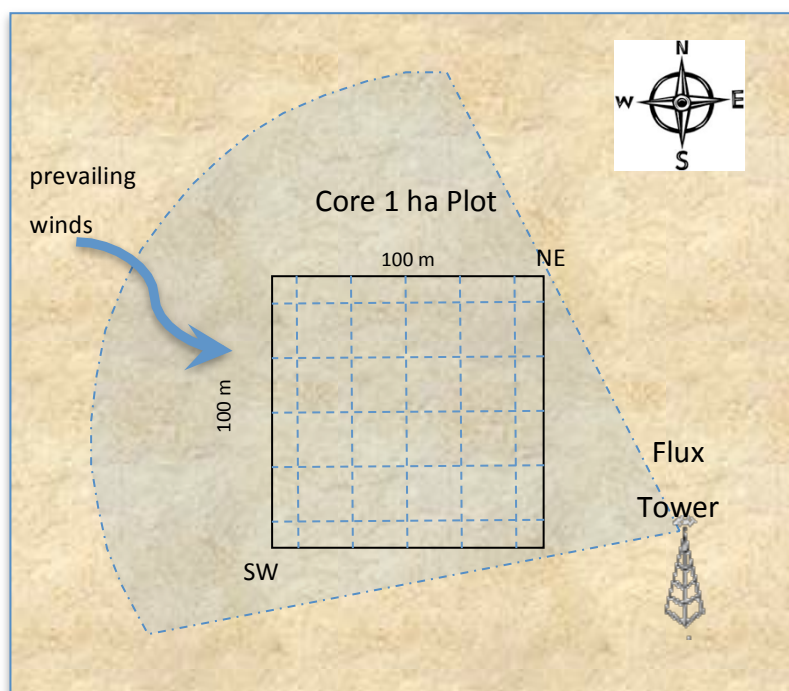


Figure 1: Schematic layout of SuperSite core 1 ha vegetation plot.

5 Plot Alignment and Marking

Standard plot alignment will be grid N-S, E-W with corners located using a Geographical Positioning System (GPS). Plot configuration and alignment can be modified if necessary (due to topography or limited area of land-type), ensuring a consistent area is assessed and plot dimensions and corners are recorded.

It will be important to notify data-users when plots are not of standard alignment and shape.

All plots will be permanently marked in at least two opposing corners (SW and NE) of each plot using steel star posts or similar and labelled with durable metal tags. For 1 ha plots, it is likely that additional permanent markers every 10 m along each edge and in the centre of the hectare, will facilitate regular data collection. Where coordinates are used to identify locations within the core 1 ha plot the 0,0 coordinate will be located at the SW corner.

6 Summary of Measurements for Core 1 ha Plots

Initial measurements at core 1 ha plots will be followed by annual monitoring for a subset of variables. Frequency of sampling for some measures might be increased after key disturbance or other events.

Essential components of this for the SuperSite core 1 ha plot include assessments of vegetation Biodiversity; Abundance; Cover and Structure; Biomass; Plant Functional Traits and Photopoints.

- Full vascular plant composition will be recorded for each plot. As references for taxon identity, at least one voucher specimen will be lodged at the relevant state herbarium for each species that is recorded in the survey as a whole. Additional specimens should be collected for taxa with uncertain identity or of other specific interest.
- Abundance cover and structure are measured using different methods for rangelands and forests. A once-off general structural description will be undertaken. Additionally the three most dominant species per strata (ground, mid and upper) will be recorded, along with an estimate of cover for each stratum, a dominant growth form per species, and an average height class for each stratum. In forested areas, measures will include direct measurement of all stems ≥ 10 cm DBH, RAINFOR Gentry transects for stems < 10 cm DBH and Seedling transects for stems < 1.5 m and > 0.1 m in height. In rangelands, measures will include the direct measurement of stems ≥ 10 cm diameter at DBH + basal wedge measurements, Point intercept method and a Structural summary as described in the [AusPlots Rangeland protocols](#) (White *et al.* 2012).
- Height, diameter at breast height (DBH), and species for all live and standing dead woody plants ≥ 10 cm DBH will be measured across whole core 1 ha plots. In plots where small trees dominate, stems ≥ 5 cm DBH should be measured. Live or dead status will be recorded. Laser range finders may assist with measuring height. It is recommended (subject to funding) that this be carried out at the core 1 ha plot on all stems > 1 cm DBH. Recommendations for mallee and mulga can be found at 4.4.1.
- Coarse woody debris for the whole core 1 ha plot will be measured at the same time as floristic composition. Scores will include a class for diameter and for degree of decay. This measure is considered important for completing above-ground biomass estimates (in conjunction with basal area, height, and wood density data), and is also an indicator of faunal habitat.

Table 1. Summary table of essential measurements for Vegetation Protocol

Measure	Priority & frequency in core 1 ha plot
Biodiversity Vascular plant list, missing stems Voucher specimens	Essential, annual Essential, at least one per species, once only Build up local voucher collection for SuperSite
Abundance, Cover and Structure General structure description Direct measure stems ≥ 10 cm and height Direct measure stems < 10 cm (forest/rangeland methods)	Essential, once only Essential, every 5 years Essential, every 5 years
Above Ground Biomass Standing AGB <i>Coarse Woody Debris</i>	Essential, every 5 years <i>Protocol development for each SuperSite</i>
Plant Functional Traits (PFT) <i>Recruitment - woody plants</i>	<i>Evaluation of how to do this at each SuperSite. Where possible field trials.</i>
Photopoints	Essential, annual (AusPlots)
Leaf Area Index (Canopy)	Essential, biannual (peak season/low season)
Phenocameras	Essential, continuous (hardware supplied by AusCover)

7 Vegetation Biodiversity

7.1 Complete Vascular Plant Species List

A complete vascular plant species list should be taken for each plot. The plot should be systematically searched and the presence of every vascular plant recorded. Stems missing from earlier surveys to be noted.

7.2 Vouchering Protocols

The identification, investigation and description of species can inform our understanding of their roles in ecosystem function. The identification of species-rich areas can be helpful to determine priority areas for conservation, particularly those areas acting as refugia in arid zone ecosystems. The decline in species richness in a plot or across similar plots is an indicator of pressures on the

environment and can help to define conservation measures or understand ecosystem processes. See Dugan *et al.* (2007) for a detailed method for surveying and collecting plant vouchers for morphological and DNA analysis for a large sampling area.

Guidelines for vouchering are available in the AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012). Considerations should be made for the implementation of a sample bar coding system and the collection of additional vegetative samples for DNA and isotope analyses where required. Fertile specimens should be collected where possible.

8 Abundance, Cover and Structure

8.1 General Structural Description

A once-off general structural description according to the National Vegetation System (NVIS) level 5 (Australian Vegetation Attribute Manual, 2003), (<http://www.environment.gov.au/topics/science-and-research/databases-and-maps/national-vegetation-information-system>) will be undertaken. The dominant growth form, cover, height and species (3 species) per traditional strata (ground, mid and upper) will be recorded. Refer to <http://www.environment.gov.au/node/18931>; Table 4, for listings of cover estimations and growth form. Further structural data may be collected via the plant physiology protocol.

8.2 Abundance, Cover and Structure - Forest Areas

Forested areas are defined as having a closed canopy, complex vegetation structure, and a light or non-existent grass/sedge ground layer. Rangelands are defined as having an open or semi-open canopy, simple vegetation structure and a grass/sedge or open ground layer. Plots may fall between these two definitions and in this case a single method should be chosen or both forest and rangeland methods will need to be combined.

In **forested areas**, vegetation abundance, cover and structure are derived from:

- The direct measurement of all stems ≥ 10 cm diameter at breast height (DBH)
- RAINFOR Gentry transects for stems < 10 cm DBH
- Seedling transects for stems < 1.5 m and > 0.1 m in height

8.2.1 Direct Measure of Stems

The direct measurement of stems ≥ 10 cm diameter at DBH (see below)

8.2.2 RAINFOR Gentry Transects

While the direct measurement of trees ≥ 10 cm DBH captures the abundance, cover and abundance of the upper stratum, the Gentry transects (Torello-Raventos *et al.* 2013; Fig 2) measure:

Woody stems with DBH ranging from 1.0 cm to 10 cm (Mid-stratum).

All other plants < 1.5 m but > 10 cm in height, and < 1.0 cm DBH. (i.e. treelets, small shrubs and seedlings) (Subordinate).

It is recommended that at least six and up to 10, 50 x 2 m transects are established per hectare (Gentry, 1988; Fig 2A). No stems are marked, although re-assessment of the mid-stratum and subordinate layers are done on the same transects.

a) *Mid stratum assessment using Gentry transects*

For all woody species > 1.5 m high and DBH > 1.0 cm (but less than 10 cm DBH) and within 1 m either side of transect line measure the DBH, height and estimate crown area.

Crown area is measured as the crown radii from the centre of the stem to each of the four cardinal points at the distance furthest from the stem. Identify all individuals to species level where possible.

b) *Subordinate stratum assessment using Gentry quadrats*

For plants < 1.5 m but > 10 cm in height, and < 1.0 cm DBH.

At each 5 m interval of each transect, a 1 x 1 m quadrat is positioned (10 quadrats per transect, Fig 2B) to assess the subordinate stratum, Visually estimate 1) the total projected cover of both grasses/herbs and woody stems separately and 2) the projective cover of the dominant grass/herb species and woody species recording the species of the dominant grass/herb and woody plant in each case.

At completion of all 10 transects an area of $50 \times 2 \times 10 = 100 \text{ m}^2$ should have been sampled.

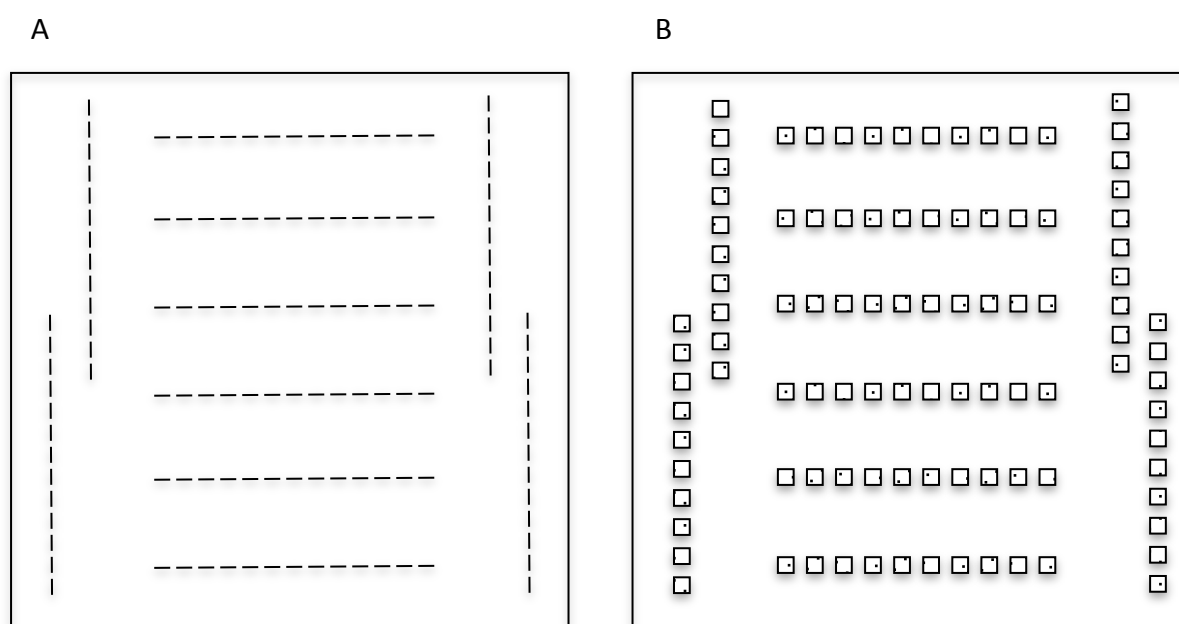


Figure 2: A recommended distribution of the sampling transects/quadrats within the plot. A) Each line represents a "Gentry transect". B) Each square represents a "Gentry quadrat".

8.2.3 Seedling Transects

Regeneration and turnover of stems < 1.5 m but greater than 0.1 m in height is to be monitored by establishing seedling transects.

A minimum of 6 transects of 20 m x 1 m are to be established on the plot in the layout represented in Fig. 2.

All stems < 1.5 m but > 0.1 m in height are to be marked with an aluminium tag and attached securely by copper wire. The position of each stem is recorded with a length and width coordinate along the transect. The species and height (length) of each stem to the top leaf is recorded.

8.3 Abundance, Cover and Structure - Rangelands

In **rangelands**, measures of abundance, cover and structure are derived from:

- The direct measurement of stems and basal wedge measurements
- Point intercept method
- Structural summary
- Plot homogeneity

8.3.1 Direct Measure of Stems and Basal Wedge Measurements

The direct measurement of stems ≥ 10 cm diameter at DBH (or diameter at 10 cm or ground level) (see 8.4 below) and basal wedge measurements as per AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012).

8.3.2 Point Intercept Method

Point Intercept survey carried out as per AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012) using 1010 points in year 1 at least.

8.3.3 Structural Summary

A structural summary and assessment of homogeneity is carried out as described in the AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012) to provide an NVIS level 5 (association level) vegetation structural description.

For each of the three vegetation strata below, nominate in descending order the three most dominant species in each stratum.

Upper Stratum - trees, tree mallee

Mid-Stratum - shrubs, low trees, mallee shrubs, tall grasses, grass trees

Subordinate Stratum (ground) - grasses, forbs, rushes, sedges, lichens, low shrubs, ferns, grass trees.

8.3.4 Plot Homogeneity - Rangelands

A “homogeneity measure” is recorded for each site. The homogeneity measure is defined as “a record of the visual estimate (in metres) of the shortest distance from the plot centre to a vegetation community different to the one that you are sampling in”. Where there is uncertainty, it is recommended that this measure be an under-estimate, so be conservative in estimating the distance. Where it is not possible to directly observe the distance to a different community, then estimate the furthest distance that you are sure is the same community as the one you are sampling within.

8.4 Direct Measurement of All Stems ≥ 10 cm DBH - Forested Areas and Rangelands

All plots should conduct a direct measurement of diameter and height for all stems ≥ 10 cm DBH. For lightly forested areas, stems to ≥ 5 cm DBH should be measured to gain an accurate measurement of biomass, structure and cover. Stems to be surveyed are: trees, shrubs, palms, vines, ferns, strangler figs and standing dead stems ($>45^\circ$). In mallee and mulga sites, stems >5 cm diameter measured a height of 10 cm (D10) are surveyed, tagged and mapped.

8.4.1 Stem Mapping

For ease of mapping and to ensure no stems are missed, stems should be surveyed within 20 x 20 m subplots starting at the south-west corner of the hectare.

It is recommended that each measured stem is permanently marked for re-measurement. Stems ≥ 10 to ≤ 30 cm at DBH are to be marked with a tag attached to stainless steel wire encircling the stem allowing enough wire for growth. An example of suitable numbered tree tags (1.2 mm thick tags are more weather/fire resistant) can be found here: <http://www.forestrytools.com.au/index.php?id=32>

A line completely encircling the stem is to be painted at the point of measurement (POM) and the POM noted. Stems > 30 cm can either have the tree number painted on the stem or a tag and stainless steel wire (or nail) tapped into the stem allowing for tree growth. Avoid using nails as they often cause abnormal swelling of the stem. When the stem is extremely large the POM can be marked with a line painted partly around the stem. Regular household exterior acrylic paint has been found to be last longer as a POM marker. In cases where bark is likely to be regularly shed, simply note the POM. In later years where significant movement of the POM has been noted, due to stem growth or soil erosion, the team leader may decide to create a new POM but must record the change in POM in the data and mark the new POM.

Stems are to be mapped within each 20 m x 20 m subplot to an accuracy of 0.5 m and then converted to geographic coordinates. In more open forests a high sensitivity GPS unit can be used to map the trees but only if this method is proven to be more accurate than by sighting or measuring off a post. Where Differential GPS can be used, it is recommended that the tree position is recorded from a consistent side (N, S, E or W) of the tree. This position should be recorded in the Metadata. Having precise geographical locations will aid analysis and interpretation of detailed imagery.

In mallee and mulga sites stems > 5 cm measured at ground level (D0) or a height of 10 cm (D10) are to be mapped and tagged for stand dynamic reasons.

8.4.2 Measuring Heights

Heights are to be recorded to the nearest 1 m (for trees) for each measured stem and are recorded as the length of the stem from ground to highest leaf, not height above ground level. Heights for easily visible and larger stems are measured using a laser range finder or measuring pole. The heights of all other stems are estimated by comparing to the measured stems.

8.4.3 Rules for measuring

1. Stems are measured in centimetres diameter at 1.3 m (breast height) from the ground on the uphill side of the stem.
2. Stems leaning $> 45^\circ$ are measured 1.3 m along the stem along the underside of the stem.
3. Vines are to be pulled away from the stem before measuring where possible. Where a vine cannot be pulled away from a stem or the tape cannot be slipped under the vine, the stem is to be measured with callipers.
4. Stems with a swelling or deformity that precludes taking a normal DBH measurement at 1.3 m will be measured above or below the deformity. If the deformity continues to < 1 m above the ground then the measurement is to be taken above the deformity. The point of measurement (POM) is noted and painted.
5. Stems with buttresses are to be measured at least 1 m above the highest buttress. Smaller specimens of particular species that are known to exhibit buttressing when they grow larger are

measured at a higher POM to account for future buttressing. In this case the POM is decided on by the team leader.

6. Where the trunk is irregular, deformed or fluted at all heights, the POM should be at 1.3 m.
7. If there is a reason why no measurement can be made, a DBH must be estimated and noted. This occurs when stems have large and high buttresses and when large stems have many vines or strangler figs around them.
8. Stems that fork above 1.3 m will be measured below the fork where the stem is not swollen or deformed.
9. Stems that fork below 1.3 m will be treated as separate stems, at or close to 1.3 m. Order of measurement will be from largest to smallest stem.
10. Palms, ferns and grass trees are included if the stem is ≥ 10 cm DBH (at 1.3 m) below the lowest living leaf base.
11. Dead stems are to be measured and mapped but not painted and tagged.
12. Lianas/vines are measured 1.3 m along the stem after they leave the ground. The height of a liana will be the estimated length of the stem and will generally be higher than the host stem. They are mapped where they are considered to originate.
13. The total DBH of strangler figs with more than one stem (the usual case) will be estimated and the POM noted and painted.
14. Codes are recorded for unusual measurements: leaning $> 45^\circ$ (L), snapped above POM (S), near dead or sick (N), rough hollow or dead side at POM (R), multi-stemmed (M), estimated DBH (E), callipers used for DBH (C).

8.4.4 Rules for measuring mallee and mulga

Stem diameters are measured in centimetres at 10 cm (D10) from the ground on the uphill side of the stem with a diameter tape or calliper accurate to 0.1 cm. Stems with diameter < 5 cm are not included.

Stems leaning > 45 degrees are measured 10 cm along the stem along the underside of the stem.

Where there is a reason why no measurement can be made, a visual estimation of the D10 is made.

Stems with a swelling or deformity that precludes taking a normal D10 measurement are measured above or below the deformity. If the deformity continues to the ground then the measurement is taken above the deformity.

Where the trunk is irregular, damaged, deformed or fluted at all heights, the diameter is taken at 10 cm.

Stems that fork above 10 cm are measured below the fork where the stem is not swollen or deformed. Stems that fork below 10 cm are measured as separate stems, at or close to 10 cm.

Vines are measured 10 cm along the stem after they left the ground. The height of a vine is recorded as the estimated length of the stem and was generally higher than the host stem. Vines are mapped where they are considered to originate.

9 Above Ground Biomass

9.1 Standing AGB

Each 1 ha plot should use the most appropriate method of estimating above ground biomass and should attempt to incorporate DBH, height and wood density values and clip plots. For example, currently the most reliable estimates of AGB for tropical forests are given by Chave, *et al.* (2014).

9.2 Coarse Woody Debris

This measure is considered important for completing above-ground biomass estimates (in conjunction with basal area, height, and wood density data), and is also an indicator of fauna habitat.

Both plot-based and line intercept methods are available for the measurement of Coarse Woody Debris (CWD). Plot-based methods are useful for studies that compare stocks of CWD with inputs from tree mortality within existing one hectare permanent plots. Line intercept methods are useful for assessing questions about the stocks of CWD at larger scales – for example, for assessing the degree to which the plots are representative of the wider landscape. The efficacy and accuracy of the line intercept method has been favorably assessed in savanna against a plot based method (Rose, 2006).

9.2.1 CWD Line/Point Intercept Measurements

CWD can be measured at the same time as abundance or floristic composition via the line intercepts method using the AusPlots Rangelands protocol using at least 1010 intercepts per plot. Scores will include a class for diameter and for degree of decay (e.g. Grove et al. 2011).

9.2.2 CWD Plot-Based Measurements

Fallen dead trees and branches

The length and diameter at both ends of all pieces of fallen wood, including lianas, with diameter larger than 10 cm within the 1 ha plot should be measured. Note that:

- For logs tapering to less than 10 cm diameter, measurements are made only up to the point where the log tapers to less than 10 cm diameter.
- Branches greater than 10 cm diameter attached to fallen trunks should be measured separately, in approximately linear segments.
- Measurements of length are made to the plot border, regardless of whether the tree is rooted inside or outside the plot.
- For measurements at the bases of fallen, buttressed trunks, diameters are measured above the buttress.

Standing dead stems

The diameter of standing dead stems is measured at 1.3 m height, or the lowest part of the trunk without buttress roots. Note that:

- The height of the standing trunk should be measured with a laser range finder.
- If the trunk decreases in size to below 10 cm, the height should be measured to the point where it is 10 cm diameter.
- The small-end diameter of broken standing trunks can be measured from the log formed by the fallen top.

- The dimensions of major branches > 10 cm diameter still attached to a standing dead tree should be visually estimated.
- For stumps less than 1.3 m tall, the height and top diameter should be measured.

CWD Decomposition Classes

The state of decomposition should be classified in the field into one of 5 classes, based on simple characteristics of the dead wood. These classes are based on the standard five point decay class system used in Tasmanian lowland wet eucalypt forest (Meggs 1996) and incorporating the RAINFOR five point system for closed forest (Baker and Chao 2001).

- Class 1: Recently fallen. Structurally intact or almost so; bark or small branches still attached; few signs of wood decay; wood mostly retains original colour
- Class 2: Structurally less intact but still hard when kicked; small branches absent; little or no bark present; early signs of wood decay, bark loss or discoloration
- Class 3: Clearly decaying but still supports its own weight; may be slightly soft when kicked; may be hollow in places; no bark; moss and fungi may be prominent
- Class 4: Cannot support its own weight; soft to kick (but may still be hard in places; in which case may be extensively hollow); moss, fungi and invading roots likely
- Class 5: No longer retains original shape; wood very soft or largely disintegrated; sometimes only outline visible beneath moss, invading roots.

Calculation of CWD Volume

The volume of dead palm trees and stumps should be calculated as a cylinder.

For other pieces, CWD volume, V (m³) should be calculated using Smalian's formula as:

$$V = L \left[\frac{\pi (D_1/2)^2 + \pi (D_2/2)^2}{2} \right]$$

Where L (m) is the length of the piece of CWD and D is the diameter (m) at either end. Smalian's formula gives the correct volume if each piece of CWD is a frustum of a quadratic paraboloid or a cylinder.

Density of Decomposition Classes and Measurement of Void Space (Optional)

A range of studies have calibrated the subjective decomposition classes in different forests in Amazonia (Chao *et al.* 2008). The density values obtained for different decomposition classes are site specific but can be estimated from the species composition of the surrounding living trees (Chao *et al.* 2008). In humid, lowland neotropical forests, density of each CWD decay class (ρ_d , g cm⁻³) is closely related to the plot-level living wood density (Chao *et al.* 2008). Thus, ρ_d was estimated as a function of the plot-average wood density of live trees. For estimating three decay class:

$$\rho_{d=1} = 1.17 [\rho_{BAj}] - 0.21 \quad \text{and} \quad \rho_{d=2} = 1.17 [\rho_{BAj}] - 0.31$$

where $\rho_{d=1}$ and $\rho_{d=2}$ represent the CWD densities in decay class (d) one and two, respectively, and ρ_{BAj} (g cm⁻³) is the wood density of living trees of plot j, weighted by their basal area. For CWD in decay class three, the average value of density for debris in 'decay class three' from published

studies of humid, lowland neotropical forests (0.29 g cm^{-3}) can be used, as suggested by Chao *et al.* (2008).

Calculation of CWD biomass

a) Calculation of decomposed wood density

Chao *et al.*, (2008) gives estimated wood density values for different decomposition classes in tropical rainforests based on the wood density of surrounding forests. For most forests, however, site specific density for each decomposition class should be determined.

For the site-specific wood density calibration of the decomposition classes, samples are taken at 5 cm length intervals from the middle of each chosen piece of CWD (Fig. 3). At each interval, a wood sample at least 30 x 30 x 30 mm in one of four randomly chosen cross sectional directions (top, bottom, left, right; Fig. 4) is taken.

The volume of these samples is calculated by direct measurement using Vernier calipers in three length dimensions for a rectangular solid shape, or radius and length for a cylindrical sample. Volumes of irregular shape samples are determined by the water displacement measurement, to the nearest 0.5 mm, using a graduated plastic cylinder. For very decomposed CWD (class 4 or 5) sampling is performed in situ with a sampling container of known volume. The samples are dried at 65°C , weighed, and density calculated as dry mass divided by fresh volume.



Figure 3: Sampling CWD for calibrating the density of different decomposition classes

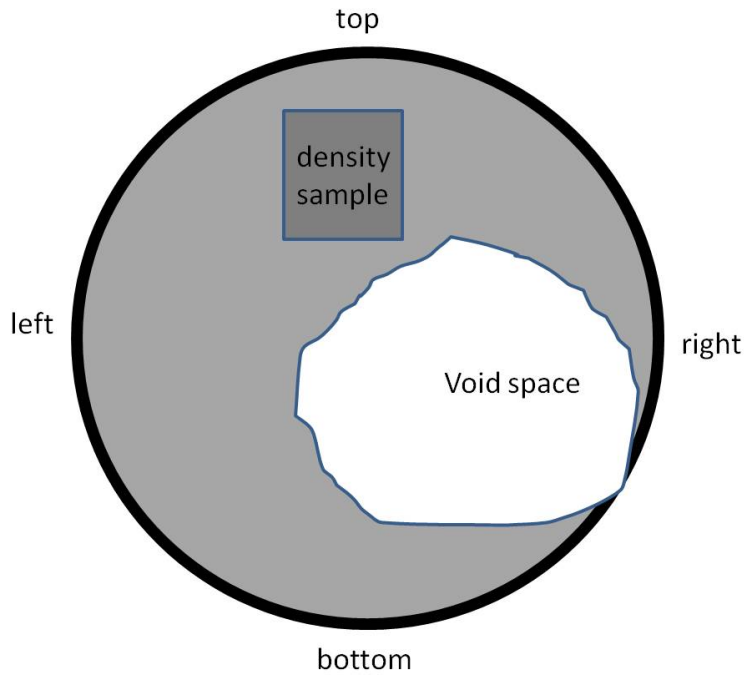


Figure 4: Position of samples for calibrating density of decomposition classes and definition of void space (dots)

b) Void Space

The proportion of void space of the different decomposition classes can be measured by taking cross-sectional pieces of a random sample of CWD pieces stratified by decomposition class.

Void space, defined as a region enclosed by more than 180 degrees of solid CWD, is measured as the proportion of the total area occupied by void space. This can be estimated or calculated using ImageJ software (available at <http://rsb.info.nih.gov/ij/>). Note that void space may extend to the exterior of the piece of CWD, for incompletely circular pieces of CWD.

Calculating the Mass of CWD

To estimate the mean mass of CWD, M (Mg ha^{-1}), in each decomposition class, n , external CWD volume (V , m^3) is multiplied by the mean proportion of solid wood ($S = 1 - \text{proportion void space}$) and by the wood density values (ρ , $\text{g cm}^{-3} \equiv \text{Mg m}^{-3}$) of the different decomposition classes. If void space, wood density and volume measurements for each plot or transect are not correlated (Baker *et al.* 2007), the standard error for M can be calculated assuming independent random errors (Taylor 1997), as:

$$SE(M)_n = (M)_n \left(\sqrt{\left(\frac{(SE_V)_n}{V_n} \right)^2 + \left(\frac{(SE_S)_n}{S_n} \right)^2 + \left(\frac{(SE_\rho)_n}{\rho_n} \right)^2} \right)$$

The mean total mass of CWD is calculated by summing the mass of each decomposition class, and the standard error calculated conservatively as the sum of errors of the constituent classes (Taylor 1997).

10 Plant Functional Traits

10.1 Site List of Plant Functional Traits

A site list of plant functional traits will be established; these will supplement those required by the plant physiology protocol, and are based on Cornelissen *et al.* (2003). Key whole plant traits include growth form, life form, plant height, clonality, spinescence and flammability; leaf traits include Specific Leaf Area (SLA), leaf size, leaf dry matter content, photosynthetic pathway; stem and below ground traits include wood density, bark thickness, diameter of fine roots and nutrient uptake strategy, and regenerative traits include dispersal mode, fruit shape, size and type, and resprouting capacity. Optional measures include leaf life span, diameter of fine roots, seed dry mass and nutrient uptake strategy. The scope of work at each SuperSite will be determined by local scientific questions being investigated and funding levels received.

10.2 Semi-Quantitative Estimates of Flowering and Fruiting

Semi-quantitative estimates of flowering and fruiting will be made monthly along selected, permanently marked transects for relating to recruitment dynamics, faunal abundances and seasonal climatic conditions.

For all vegetation types, a minimum of three 100 x 5 m transects should be established in the hectare. For each individual plant, reproductive effort is recorded within the vertical confines of the transect as numbers of ripe fruit and flowers on the plant and on the ground. In the case of a ground layer or shrub species dominating a transect, fruiting and flower effort can be estimated for the species as a whole at resolution of 1 m. Ideally fruit and flower numbers will be counted, however for expediency, a simple logarithmic estimation can be used: 1 = 0-10 fruit/flowers, 2 = 10-100, 3 = 100-1000, 4 = 1000-10000, 5 = 10000-100000. For final calculations, a midpoint of these ranges can be taken.

10.3 Recruitment of Woody Plants

Each SuperSite will develop a suitable protocol to measure recruitment. This requirement is met for forested areas that employ Seedling Transects (8.2.3 above) in Abundance, Cover, Structure assessments, where stems < 1.5 m but greater than 0.1 m in height are monitored.

Recruitment of woody plants will be scored annually in appropriate subsamples of the 1 ha plot, preferably matching transects, measuring fruiting and flowering. The initial survey will include all stems within the chosen height range. Recruits are defined as all stems that have reached the minimum height since the last survey. All recruits of woody plants will be counted, using appropriately designed classes if numbers are high (e.g. using a log scale). Individuals surviving to more than a specified height (site dependent) will be permanently tagged, mapped and measured for height each year. These data will provide an understanding of the demography of the dominant woody species and will link with fruiting, flowering and seasonal conditions.

This survey monitors regeneration and also allows the capture of individuals missed in the Gentry or >10 cm DBH survey. In forested areas the height range monitored is < 1.5 m, > 0.1 m. However, the maximum height for the recruitment survey may be reduced if a plant group (eg shrubs) is adequately described in another survey type.

The minimum height for seedling transects in forested areas was selected to allow accurate plant identification and to take into account the high seedling mortality rate below this height. If there is sufficient confidence in plant species identification of seedlings then it may be appropriate to reduce the minimum height.

11 Photopoints

Photopoints will be taken annually using the five point photopoint method to create reference images for the core 1 ha. The panoramic photopoint method (18) is to be used where possible (eg. rangelands) to supply images for analysis at the University of Adelaide that produces 3D reconstructions to monitor change over time and provide an estimate of basal area and biomass.

11.1 Five Photopoint Method

Four photographs are to be taken at each of five photopoints on the hectare as shown in Figure 5. All photographic sequences to be preceded by an identifier photograph that includes location, date and photopoint number. Camera is to be mounted on a suitable tripod with the central part of the camera lens at 1.3 m. The photographic sequence should be taken between 10 am and 4 pm (where possible) to minimise sun and shadow effects.

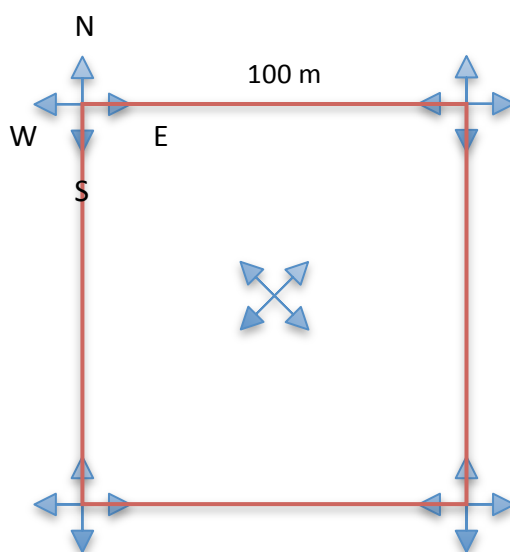


Figure 5: Photopoints for closed forest SuperSites

The photos at each corner are to be taken in the direction of the four cardinal compass points and the photos at the centre of the plot are to be taken in the direction of each corner. Vegetation within 1 m of the photopoint is to be removed or pushed aside.

11.2 Panoramic Photopoint Method

The AusPlots Rangelands Protocols Manual (White *et al.* 2012; <http://www.tern.org.au/AusPlots-Rangelands-Survey-Protocols-Manual-pg23944.html>) describes the panoramic photopoint method in detail. This method may be the most informative in open forests/woodlands and rangelands. In summary, three photopoints are to be established configured in an equilateral triangle (2.5 m sides) with the centre marked with a star dropper and the location recorded with DGPS (Fig. 6).

11.2.1 Camera Specifications

Use a digital SLR with minimum 12 megapixel resolution, ability to export raw images and a 24 mm focal length lens (AusPlots uses a Canon EOS 550D camera with a variable lens set at 24 mm). Record all camera details.

At each photopoint take photographic sequences in a 360° panorama, with up to 40 photographs with a minimum 50% overlap between consecutive photographs. All photographic sequences to be preceded by an identifier photograph that includes location, date and photopoint number. Photographic sequences are to commence with an image of the central dropper (height 1.3 m) with a line marked 25 cm from the top. Photographic sequence to end at the central dropper. Camera is to be mounted on a suitable tripod with the central part of the camera lens at 1.3 m. The photographic sequence should be taken between 10 am and 4 pm (where possible) to minimise sun and shadow effects.

In some sites where the vegetation is very dense it may not be possible to photograph the vegetation using photo-panoramas.

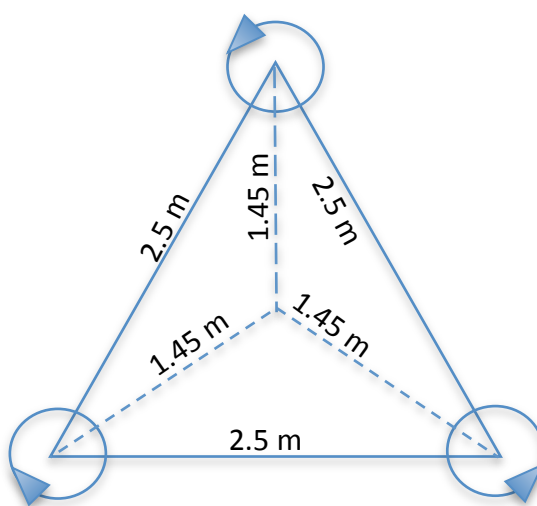


Figure 6: Photopoints for photo-panoramas in open forest/rangelands SuperSites

12 Canopy Cover / Understory Cover / Leaf Area Index

LAI vs Canopy Cover

Leaf area index (LAI) can be defined as the total one sided area of leaf tissue per unit area of ground and is a key derived parameter that is associated with water and light interception, radiation transfer, water and carbon exchange (Bréda, 2003). Canopy cover can be defined as the fraction of ground shaded by the vertical projection of tree crowns (Walker *et al.* 1981). In this document woody understory cover relates to canopy cover of vegetation **below 1.5 m in height**, ground cover relates to the canopy cover of non-woody vegetation (such as grasses, forbs and herbs) These measures may be used as proxies for actual canopy leaf area and actual understory, ground cover leaf biomass.

Canopy LAI

Leaf area index is the preferred measure of cover for vegetation and as a key variable used in total biomass estimation and in carbon cycling prediction models.

Leaf area index can be estimated using "direct" or "indirect" methods.

LAI Direct Measures

Direct methods include clip harvests in grasslands and litter fall traps for deciduous species as well as labour intensive destructive measures from trees and shrubs. One-off direct assessment methods are encouraged at all sites where possible, as they can be used for calibration of indirect measures. Direct measures can be conducted at a range of scales down to individual branches and used to create allometric equations that relate DBH or branch diameter to LAI (Bréda, 2003).

LAI Indirect Measures

Indirect measures of LAI include digital photographic methods using flat or hemispherical images, referred to respectively as DCP (digital cover photography) and DHP (digital hemispheric photography). A range of alternative technologies are also widely used such as the LI-COR LAI-2200 and the TRAC sensor system.

It is recommended that LAI measurements be carried out at each SuperSite using the most appropriate method for the vegetation type present as indicated in Table 2 below.

AusCover campaigns at most SuperSites have collected ground based LAI measurements using a range of different methods (DHP, CID Bio-Science CI-110 instruments and LI-COR LAI-2200). These LAI measures have been collected to validate aerial LiDAR datasets.

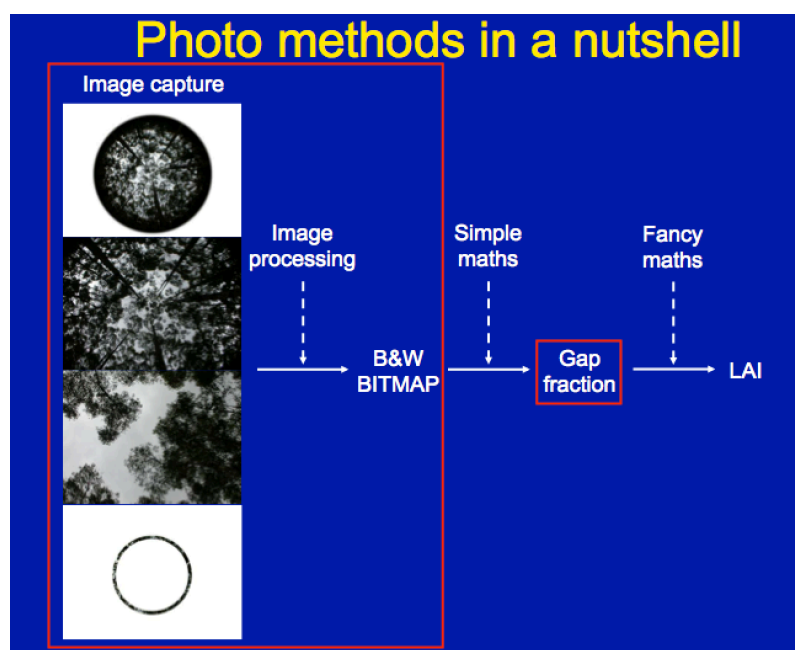


Figure 7: Photo methods in a nutshell

12.1 Recommended Methods for Different Vegetation Types

Table 2: Spatial and temporal sampling

Spatial sampling	Temporal sampling
100 m x 100 m core 1 ha plot, grid established in this 1ha plot depending on the method (DCP or DHP).	Initial measurements: Four times throughout the year to capture seasonal variation and to determine when to carry out the subsequent on-going sampling. On-going sampling: Twice a year at the times of peak and minimum biomass / leaf area.

Table 3: Recommendations of LAI and Cover methods for different vegetation

Vegetation	Method	References
Low sparse vegetation e.g. spinifex grasslands	Point Intercept Method (PIM). Direct measures of LAI using Clip Plot harvests and destructive harvesting will be used to calibrate and validate PIM	AusCover Star Transect Protocol; AusPlots Rangelands Survey Protocols Manual (White et al. 2012); ABARES ground cover monitoring field manual (Muir et al. 2011)
Short vegetation (4-8 m) e.g. low banksia woodlands	Digital Hemispheric Photography (DHP)	
Medium stature (10-40 m) vegetation with simple structure (single stratum) e.g. broadleaf eucalypt forest	Digital Cover Photography (DCP)	Pekin and MacFarlane, 2009
Medium stature (10-30 m) vegetation with dual structure (two strata) e.g. savanna	Digital Cover Photography (DCP) Point Intercept Method (PIM) for understory cover. Direct measures of LAI using Clip Plot harvests for grasses will be used to calibrate and validate PIM Where there is dense grass cover (e.g. savanna) the PIM method becomes impractical for the grass component. An alternative procedure is used to measure the LAI of the grasses based on the LI-COR LAI-2200 (or LAI-2000) instrument.	Pekin and MacFarlane, 2009 AusCover Star Transect Protocol; AusPlots Rangelands Survey Protocols Manual (White et al. 2012); ABARES ground cover monitoring field manual (Muir et al. 2011)
Medium - Tall vegetation with complex structure (multiple strata) e.g. rainforests	Digital Hemispheric Photography (DHP)	
Mulga and Spinifex. Dual Structure 3-6 m.	Digital Cover Photography (DCP) Procedure in development at Alice Mulga SuperSite	

Image Data storage

Raw image files will be stored on a dedicated SuperSite image database with appropriate contextual and metadata. Image archiving instructions are detailed further below at 18.

12.2 Digital Cover Photography

Digital Cover Photography (DCP) is recommended for medium stature (10-40 m) vegetation with simple structure. DCP was originally developed for sparse to moderately dense broadleaf forest and has also been tested in sparse savanna woodland. DCP is recommended for these vegetation types and has also been suggested for more dense forests (Pekin and MacFarlane 2009).

12.2.1 Advantages of DCP

1. DCP can be carried out during daylight hours, not just dawn and dusk, especially in dense vegetation and at higher latitudes.
There can be problems with excessive sunlit foliage in sparse vegetation at low latitudes, however, this can be dealt with by switching from a single-channel, grayscale-based analysis to a three-channel, colour-based analysis. This gives you more useful working hours in a day and has significant safety implications as well, as pre-dawn starts are not necessary.
2. The analysis and hardware are simple and cheap. MATLAB code for doing the analysis based on MacFarlane *et al.* (2007) is available, DCP software version 3.12 (Macfarlane 2011; Macfarlane and Ogden, 2012).

DCP provides estimates of foliage projective cover and crown cover that are very accurate (perhaps more so than LAI estimates). DHP doesn't provide this data.

12.2.2 DCP Site Details

Photographs are taken along nine 100 m transects (10 m spacing) at 10 m intervals in the core 1 ha. Photographs are taken at the intercepts along the ten transects, totaling 81 positions (see Figure 8).

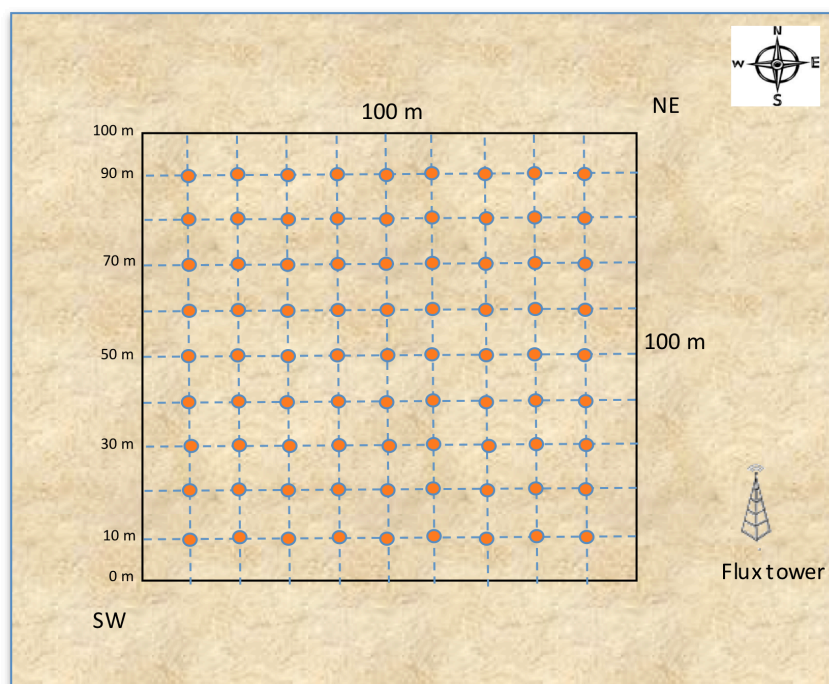


Figure 8: Positions for DCP photography within the core 1 ha plot.

12.2.3 Suggested Equipment for DCP

Lens - 50 mm prime lens (assuming 24 mm CCD/CMOS with 1.5/1.6 multiplication factor). RRP \$150-\$250. Alternatively, most DSLR cameras will come with a ~18-55 mm kit lens that will do fine.

Camera - DSLR camera with > 10 Megapixel sensor of 24 mm size. Use 'native ISO' where possible (100 or 200) or use ISO400 (even ISO800 if you really have to) in dark and windy conditions to increase shutter speed by one stop. Aim for aperture at least f8.0 to get good depth of field.

For the fixed angle (prime) lens an internal motor is needed to drive the focus of the lens if it doesn't already have one; Nikon's original 50 mm prime lens was a 'D' lens that had no motor but they've since released a 'DX' 50 mm prime that does. The kit lenses are DX and can be used with cheaper cameras.

12.2.4 DCP Protocol for Determining Leaf Area Index

Protocol adapted from Nicolas Boulain and Derek Eamus, University of Technology, Sydney

12.2.4.1 Image Acquisition

Photographs are taken along nine 100 m transects (10 m spacing) at 10 m intervals in the core 1 ha.

Images are acquired with the camera looking towards the sky at the nadir (0°) using a tripod mount and level (**ensure images are recorded in raw format**).

12.2.4.2 Basic Data Required

The following data **must** be recorded for data management purposes:

Geographic coordinates (longitude, latitude) at the beginning and end of each Transect

Operators: who collected the data

Date: consistent format (dd/mm/yyyy)

Start Time: hh:mm when transect started

End Time: hh:mm when transect completed

Plot name/Transect number (starting from SW corner):

Photo number for last photo at each point:

Azimuth of camera top (0 for top of camera at north):

Camera make:

Camera model:

Lens details:

Height photos taken at (in cm):

Before starting each transect, take a picture of a white paper sheet with the date, the location and the transect number.

12.2.4.3 Field Equipment Checklist

100 m tape measures (ideally x 3)

Camera

Tripod or monopod
GPS – preferably differential, or GPS accessory interfaced camera.
Vertical camera mount
2 axis bubble levels
Field sheet
Compass

12.2.5 Data Collection Process

1. Lay out plot

This protocol is designed for data to be collected in conjunction with fixed marked 100 m transects in the core 1 ha.

2. Take a GPS reading at the beginning and end of each transect.

It is best to use survey-grade differentially corrected GPS if possible. The horizontal error from a handheld GPS can be in the range of 2 m to 10 m and can cause problems when validating high spatial resolution products, such as airborne LiDAR.

3. Record site details on field sheet

Record the following details on the field sheet:

Plot name/Transect number (starting from SW corner):

Field operator names/initials

Date

Start time

Camera and lens details

Height photos taken at

Orientation of camera to north

Photo format (**raw**)

4. Take photos

Ensure photos are stored in the raw format. Take photos at 10 m intervals along each transect. **Make sure all required photo details (photo numbers, height and time) are recorded on the field sheet in the appropriate location.**

12.2.6 DCP Camera setup

Ensure the following settings are used for the photographs:

Exposure - Exposure set at -1 (1 f-stop below automatic exposure and at automatic exposure).

Format - Set the camera to take the photos in **raw format**.

Image Resolution - ensure the total number of pixels in the image is as high as possible (e.g. 12 MP). Take photos in raw format as this will default to the cameras maximum resolution.

Level camera - Make sure that the camera is reasonably level. Ideally the camera should be within 10 degrees of horizontal. Slope doesn't matter for DCP because of the narrow FOV lens. The camera doesn't 'see' very far uphill or downhill. Don't work on steep slopes ($> 15^\circ$). If available, use a tripod with a bubble to level the camera. If the terrain is lumpy use a monopod.

Alignment - Align the camera so that magnetic north is aligned with the top of the photograph.

Camera height - Images should be taken at a standard reference height that is appropriate for the site. Typically this is at breast height (1.3 m above the height of the ground surface). However, if there is an understory at this height, it is good to have photos above the understory and below it (e.g. at 1.4 m - if 1.3 m is the height of the understory, and 0.5 m). Also, if branches extend to the ground (like at a jack pine sites), then it is good to take the photo from as close to the ground as possible to capture most of the leaf area.

12.2.7 DCP Recording of Data in the Field and Ordering the Files Subsequently

The photos taken in the field will initially be stored on the memory card and downloaded to a computer each day after the fieldwork has been completed. Create a folder for each date of measurements. In each date folder, create sub-folders by transects. Once collected sort the images and place them in the appropriate folder. Back up the files as soon as they are off the camera.

12.2.8 LAI Computation Using DCP

It is recommended to analyse images with DCP software version 3.12, written by Craig MacFarlane (CSIRO) or to use the DCP batch processing (requires Matlab®) by Nicolas Boulain (University of Technology Sydney). To compute the canopy LAI, images are analysed with a MATLAB program based on algorithms obtained from MacFarlane *et al.* 2007. The program computes the fraction of cover porosity based on the fraction of foliage protective cover and the fraction of crown cover for each image.

The LAI is then calculated from the fraction of cover porosity.

The fraction of foliage protective cover is calculated as: $ff = 1 - (\text{totalgaps}/\text{totalpix})$

The fraction of crown cover is calculated as: $fc = 1 - (\text{bigaps}/\text{totalpix})$

The fraction of cover porosity is calculated as: $\Phi = 1 - (ff/fc)$

Finally the LAI is calculated as: $LAI = -fc * \ln(\Phi)/k$

with k the beer-lambert extinction coefficient, $k = G(\theta)/\cos(\theta)$

Choose a process method (convenience)

12.2.9 LAI Analysis Process using McFarlane Interactive DCP-313 GUI

As a single (blue) channel of the RGB image is processed to create a grey-scale, contrast stretching of the blue channel is recommended (Macfarlane *et al.*, 2014) and can be achieved using the RAW2JPG MATLAB script (available at <http://www.tern-supersites.net.au/index.php/publications-and-resources/resources-for-supersiteusers>) before saving in JPG format prior to analysis.

12.2.9.1 DCP LAI software download

RAW2JPG and "Leaf Area Index DCP-313 Image Analysis Software" are executables compiled using the 64-bit version of Windows OS and the MATLAB 2014a. They require a 64-bit Windows OS and 2014a version of the MATLAB Compile Runtime (MCR.exe available from mathworks.com.au) to use.

Download and install the RAW2JPG and DCP-313 executables from the SuperSites Resources web page (<http://www.tern-supersites.net.au/index.php/publications-and-resources/resources-for-supersiteusers>). This may take a while if you are also downloading the MATLAB Component Runtime (MCR.exe) file.

To install RAW2JPG download the RAW2JPG_Installer.exe found in the folder: COMPILE_64bit, execute and follow the instructions. This will install the application in the following location: C:\Program Files\RAW2JPG. The application itself (RAW2JPG.exe) along with a file named 'dcraw.exe' will be installed at C:\Program Files\RAW2JPG\application. Move the dcraw.exe file to "C:\dcraw\".

For DCP-313, download the Leaf Area Index DCP-313 Image Analysis Software files from the SuperSites portal (<http://www.tern-supersites.net.au/index.php/publications-and-resources/resources-for-supersiteusers>) and follow instruction in readme.txt file.

12.2.9.2 DCP LAI image pre-processing for analysis using RAW2JPG

It is important to make sure there are no spaces (including "-" in the path to where the image files are stored on the computer, including the name of the image file. The user can alter the fraction of pixels to saturate at each end of the histogram; this could be used to overexpose clouds and increase the heterogeneity of the background sky. The default is to saturate 1% of pixels at both ends of the brightness histogram.

The RAW2JPG application will use the functionality of dcraw to convert the raw DCP images to 'pgm' format which can be opened by MATLAB. The application will then contrast stretch (Macfarlane *et al.*, 2014) the blue channel of the raw image only. The resulting image is saved in JPG format ready for analysis by DCP-313. RAW2JPG can also apply an optional gamma correction (typically 2.2) to improve later pixel classification.

Detailed Instructions:

- Open the **RAW2JPG** program

- Select **Image type** as "Cover"

- Click on **Adjust gamma** (this improves pixel classification, especially of imperfect images)

- Leave the **Stretch limit** on the default setting

- Click on **Run** and select the raw DCP images using the shift key to select multiple files.

This results in the JPG output files (using same names as originals - if camera JPG images are stored in the same folder then they will be overwritten) deposited in the same folder, ready to be used with the **DCP-313** software.

The RAW2JPG converts a raw image to a JPG format canopy image via the following workflow:

1. Read metadata from raw file using DCRaw functionality.
2. Convert raw image to 16 bit pgm format using DCRaw functionality.
3. Select the blue channel of the image.
4. Contrast stretch the image (or mask) such that 1% of pixels are saturated at each end of the brightness histogram (i.e. 1% of pixels are pure black and 1% of pixels are pure white).
5. Apply a gamma adjustment if selected.
6. Save the resulting image as an 8 bit JPG file.

12.2.9.3 Quick Guide to DCP Version 3.12.

The software is configured and run via a GUI that looks like this:

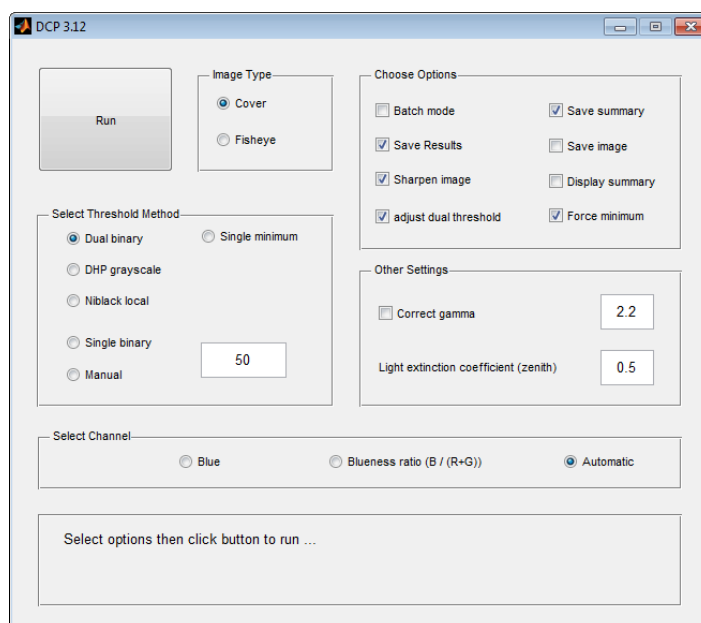


Figure 9: Guide to DCP Version 3.12

When the 'Run' button is pressed most options will be greyed out and the current file being analysed, as well as the analysis time remaining, will be displayed in the text box at the bottom.

Image type: Cover or fisheye. Select 'cover' for non-fisheye images taken with a 70 mm equivalent (or similar) lens, and select 'fisheye' for fisheye images. The 'fisheye' routine is a bit of an afterthought. It does not analyse the images - it only classifies the pixels and saves a bitmap file. The user also needs to alter the settings for the fisheye lens within the code to use this properly.

Select Channel: 'Traditional' analysis of canopy images uses the blue channel of the RGB image. Images that are badly affected (more than a couple of percent) by reflected sunlight from the canopy will often give better results if the 'blueness index' is used instead. This is calculated as $B/(R+G)$ then rescaled to 0-255. The Automatic channel selection will distinguish between images taken in overcast and clear sky based on the blueness index value of the open sky sections of the image and automatically use the blue channel for overcast images and the blueness index for clear sky images. I don't recommend the blueness index unless images are badly affected by reflected sunlight.

Select Threshold Method: There are six threshold methods. Read Macfarlane (2011) for more details of the threshold methods.

1. The Dual binary method applies a different threshold to small and large gaps within the image (see Options to alter the choice of threshold that the Dual binary method uses for small gaps).
2. The Single binary method applies a single threshold to the whole image. The user can specify the threshold as a percentage of the distance between the two corners. 50% is the default. 0% will select the lower corner as a threshold and 100% will select the upper corner as a threshold.
3. The DHP grayscale method is the method described by Leblanc *et al.* (2005) and used in the DHP software. The gap fraction of mixed pixels is based on their linear distance between the lower and upper threshold values (25% and 75% of the distance between the lower and upper corners).
4. The Niblack local method applies a local threshold (Niblack 1986) to the mixed pixels. Pixels whose mean brightness is above the local mean (5×5 square neighbourhood) are classified as sky and other pixels are classified as canopy. My experience is that this method introduces odd textural variations within the canopy region, and I wouldn't recommend its use.
5. The Manual method fixes the threshold at a specified digital number, which is entered in the same box used for the Single binary method just to confuse you. That is, if Single binary is selected then the number in the box is a percentage (0-100) but if Manual is selected then it's an absolute grayscale value (0-255). The Manual method can be used to analyse tricky images if the optimal threshold value has already been determined, or for analysing many images with the same threshold. For dealing with tricky images it's usually easier to use the interactive version of DCP.
6. The minimum method uses the least frequent grey level between the two corners as a threshold. It is particularly well suited to classifying pixels using the 'blueness' index.

Select Options:

1. Batch mode. The name is a bit misleading. DCP can analyse batches of images regardless of whether you select this or not. If it is not selected then DCP will prompt you to select one or more images within a folder. If it is selected then it will prompt you to select a single folder, and then analyse all images in all the immediate sub-folders of the selected folder.
2. Save results. Saves an Excel file (results.xls) containing the results for all images. Note that a comma-delimited text file (results.txt) is written during processing regardless of whether this option is selected. Don't forget to rename them before starting a new batch analysis or they could be over-written. The files are saved in a sub-folder of the folder where the images are contained called 'done', unless 'batch' mode is selected in which case they are saved in the original folder selected.
3. Sharpen image. Applies a sharpening filter to the blue channel or blueness index to improve contrast between foliage and sky. Sharpened images tend to have a larger gap fraction, but fewer mixed pixels, than unsharpened images. Purists don't like it – I've always used it because it improves image analysis. The relevant code is: `b=imfilter(b,fspecial('unsharp',0.1),'symmetric')`.
4. Adjust dual threshold. The published version of the dual method uses the 25% and 75% thresholds, and these are the defaults in DCP. If this option is selected then the software will automatically detect whether images are taken in clear sky or cloudy sky conditions, and adjust the threshold applied in small gaps from 25% to 50% for clear sky images. It doesn't affect the processing of images taken in overcast conditions. I recommend using this.
5. Save summary. Saves a PNG file for each image that displays the blue channel (or blueness index) of the original image, the classified image (foliage black, small gaps white and large gaps mid-grey) and the image histogram. On the histogram the peaks are indicated by green asterisks and the

corners by black asterisks. Depending on the threshold method selected, red asterisks indicate either the mid-point between the corners, the manual threshold, the minimum between the corners, or the 25% (50%) and 75% thresholds. The summary sheets are saved in a sub-folder called 'summary'.

6. Save image. Saves the classified image as a black and white bitmap. They take a lot of disk space so only select this if you really want them. I use this to create classified images for analysis in other software. You would need to use it for fisheye images.
7. Display summary. If selected, the analysis will pause after each image is processed and display the summary sheet (Option 1 above). Analysis will not continue until the user presses a key. I almost never use this anymore. It was useful during development and before I wrote the manual version of DCP.
8. Force minimum. Forces the threshold method to be 'Binary minimum' if the blueness index is used for analysis rather than the blue channel. I find that the binary minimum method is the most reliable when the blueness index is used, and I use this option together with the automatic selection of blue or blueness. Does not affect threshold method of images analysed using blue channel.

Other Settings:

1. Gamma. This only applies to fisheye images. It can be used to back-correct the gamma function of images so that the digital numbers linearly approximate actual radiances.
2. Light extinction coefficient. This only applies to cover images. It is used to calculate LAI from crown cover and crown porosity.

12.3 Digital Hemispheric Photography

Digital Hemispheric Photography (DHP) is recommended for short vegetation (4-8 m) e.g. low banksia woodland, complex (multi strata) and tall vegetation (> 40+ m) using images taken 20 m apart (MacFarlane *et al.* 2007).

DCP analysis of these types of vegetation areas can be problematic. DHP has been used to measure canopy structure in boreal forests (Fournier *et al.* 1997) and light environment in temperate rainforest (Weiss 2000) and is used by AusCover for calibration and validation of LiDAR derived products (<http://data.auscover.org.au/xwiki/bin/view/Field+Sites/Data+Collection+Resources>).

12.3.1 Advantages of DHP

1. Fewer images required to sample a plot e.g. for a 40 m x 40 m plot 25-30 DCP images are required compared to 9 - 16 DHP images.
2. May provide leaf angle distribution function (LADF). DHP theoretically provides both LADF as well as LAI using measurements at multiple angles. Calibration will be required as LADF is highly sensitive to stand structure (location of large gaps in the image) and correction for clumping can produce results (near spherical LADF) that do not correspond to actual leaf angles.
3. This method is very well represented in the literature indicating its wide acceptance even with its well documented limitations.

12.3.2 Disadvantages of DHP

1. The requirement to work in diffuse light of dawn or dusk and the added risks involved with travel to and from the site.
2. Expensive lens required.
3. Greater sky luminance heterogeneity and greater gap fraction variability within an image.
4. Doesn't provide an estimate of vertical cover.
5. Need to consider slope – don't work on steep slopes with large LAI.

12.3.3 DHP Site Details

Photographs are taken along six 100 m transects (20 m spacing) at 20 m intervals in the core 1 ha. Photographs are taken at the intercepts along the five transects, totaling 36 positions (Figure 10).

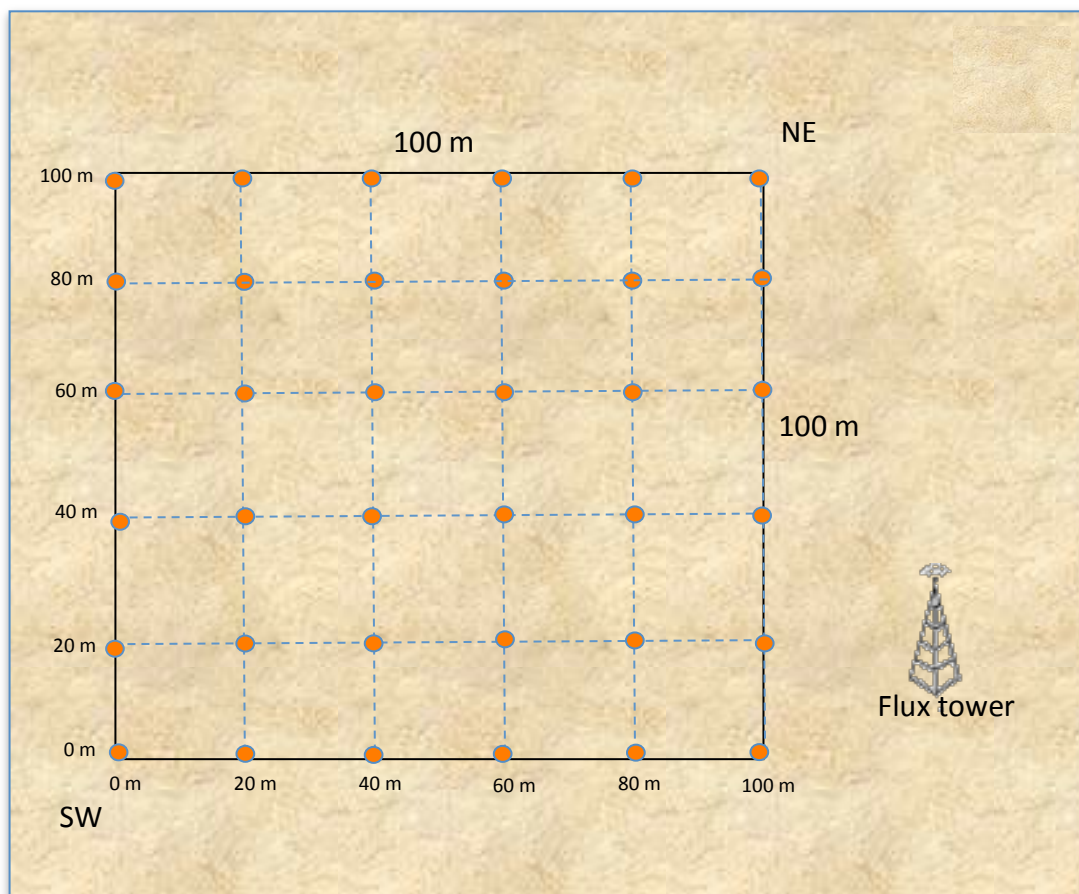


Figure 10: Positions for DHP photography within the core 1 ha plot.

12.3.4 Suggested Equipment for DHP

Lens - Sigma 4.5mm F2.8 EX DC HSM Circular Fisheye. RRP approx. \$1250 but may be available for less than \$700. Mounts available for Nikon, Canon, Sigma, Sony/Minolta

Camera - DSLR camera with > 10 Megapixel sensor of 24 mm size. Use 'native ISO' where possible (100 or 200) or use ISO400 (even ISO800 if you really have to) in dark and windy conditions to

increase shutter speed by one stop. Fisheye lenses have nearly infinite depth of field so aperture is less critical but don't go too wide or vignetting will occur – once again f8.0 or narrower is advised. Sigma's 4.5 mm lens is an 'EX' lens same as Nikon's DX so no camera focus motor is needed.

The AusCover protocol document outlines the process for the collection of hemispherical canopy photography used at the validation SuperSites (AusCover Digital Hemispheric Photography Protocol).

12.3.5 DHP Protocol for determining Leaf Area Index

Protocol adapted from the AusCover protocol for determining LAI. [http://data.auscover.org.au/xwiki/bin/view/Field+Sites/Hemispheric_Protocol Matt Paget 2013/04/30]

12.3.5.1 Image Acquisition

Photographs are taken along six 100 m transects (20 m spacing) at 20 m intervals in the core 1 ha. Images are acquired with the camera looking towards the sky at the nadir (0°) using a tripod mount and level.

IMPORTANT NOTE: Hemispherical canopy photographs are best collected in diffuse lighting conditions, when the sky is uniformly grey (e.g. around dawn and dusk when the sun is below the horizon, or on days with 100 % cloud cover). Images cannot be acquired when there is significant moisture in the air; condensation, mist/fog and rain affect light and therefore image quality.

The quality of DHP images for analysis purposes is compromised if taken at other times, particularly around solar noon. If it is only possible to take pictures during sunlight conditions, it is possible to mask out the influence of the sun, but this is not ideal and will bias the gap fraction estimates. Try not to have fluffy clouds in the images either, as the brightness of clouds can reduce leaf cover at the edges of the leaves. In addition, hemispheric photos should not be taken in windy conditions.

12.3.5.2 Basic Data Required

The following data **must** be recorded for data management purposes:

Geographic coordinates (longitude, latitude) at the beginning and end of each Transect

Operators: who collected the data

Date: consistent format (dd/mm/yyyy)

Start Time: hh:mm when transect started

End Time: hh:mm when transect completed

Plot name/ Transect number (starting from SW corner):

Azimuth of camera top (0 for top of camera at north):

Camera make:

Camera model:

Lens details:

Height photos taken at (in cm):

Before starting each transect, take a picture of a white paper sheet with the date, the location and the transect number. Commence each transect running South-North from the South-West corner of the plot.

12.3.5.3 Field Equipment Checklist

100 m tape measures (ideally x 3)

Camera with hemispheric lens, preferably a circular fisheye lens

Tripod or monopod

GPS – preferably differential

Field sheet

Compass

12.3.5.4 Data Collection Process

1. Lay out plot

This protocol is designed for data to be collected in conjunction with fixed marked 100 m transects in the core 1 ha.

2. Take a GPS reading at the beginning and end of each transect.

It is best to use survey-grade differentially corrected GPS if possible because it is important that the gaps within and between trees lines up with the fractional cover estimated from LiDAR. The horizontal error from a handheld GPS can be in the range of 2 m to 10 m and can cause problems when validating high spatial resolution products, such as airborne LiDAR.

3. Record site details on field sheet

Record the following details on the field sheet:

Plot name/Transect number (starting from SW corner):

Field operator names/initials

Date

Start time

Camera and lens details

Height photos taken at

Orientation of camera to north

Photo format (**raw**)

4. Take photos

Ensure photos are stored in the raw format. Take photos at 20 m intervals along each transect. **Make sure all required photo details (photo numbers, height and time) are recorded on the field sheet in the appropriate location.**

12.3.6 Camera Setup

Ensure the following settings are used for the photographs:

Exposure - Exposure set at -1 (1 f-stop below automatic exposure)).

Format - Set the camera to take the photos in **raw** format

Image Resolution - ensure the total number of pixels in the image is as high as possible (e.g. 12 MP). Take photos in raw format as this will default to the cameras maximum resolution.

Level camera - Make sure that the camera is level. Ideally the camera should be within 10 degrees of horizontal. This becomes particularly important with large LAI (> 4) and steep slopes (> 15°) and as a result don't work on slopes > 15° (you would either have a shorter path length downhill and a longer path length uphill, assuming that your canopy height is constant and uniform as you move upslope. Or if you put the camera at an angle to the slope you violate the assumption that the foliage angle has a random azimuth distribution. The more you tilt the camera the worse you violate that assumption). If available, use a tripod with a bubble to level the camera. If the terrain is lumpy use a monopod.

Alignment - Align the camera so that magnetic north is aligned with the top of the photograph. Make a note of the alignment if there is any variation to this.

Camera height - Images should be taken at a standard reference height that is appropriate for the site. Typically this is at breast height (1.3 m above the height of the ground surface). However, if there is an understory at this height, it is good to have photos above the understory and below it (e.g. at 1.4 m - if 1.3m is the height of the understory, and 0.5 m). Also, if branches extend to the ground (like at jack pine sites), then it is good to take the photo from as close to the ground as possible to capture most of the leaf area.

12.3.7 Recording of Data in the Field and Ordering the Files Subsequently

The hemispheric photos taken in the field will initially be stored on the memory card and downloaded to a computer each day after the fieldwork has been completed.

Create a folder for each date of measurements.

In each date folder, create sub-folders by transects. Once collected sort the images and place them in the appropriate folder. Back up the files as soon as they are off the camera.

Hemispheric Photography Data Entry Form			
Date (dd/mm/yyyy):			
Time (hh:mm):			
Plot Name:			
Easting:			
Northing:			
Zone:			
OR			
Latitude:			
Longitude:			
Camera Make:			
Camera Model:			
Lens Details:			
Lens Type (Infrared/Simple):			
DPI (MP):			

Point	Photo No f-2 exposure	Photo No f-1 exposure	Photo No automatic exposure
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			

Figure 11: Field data to be recorded

12.3.8 DHP Image Analysis - RAW2JPG and MagicFish

12.3.8.1 DHP LAI software download

RAW2JPG and MagicFish are executables compiled using the 64-bit version of Windows OS and the MATLAB 2014a. They require a 64-bit Windows OS and 2014a version of the MATLAB Compile Runtime (MCR.exe available from mathworks.com.au) to use.

Download and install the RAW2JPG and MagicFish executables from the SuperSites Resources web page (<http://www.tern-supersites.net.au/index.php/publications-and-resources/resources-for-supersiteusers>). This may take a while if you are also downloading the MATLAB Component Runtime (MCR.exe) file.

RAW2JPG Download and Installation

To install RAW2JPG download the RAW2JPG_Installer.exe found in the folder: COMPILE_64bit, execute and follow the instructions. This will install the application in the following location: C:\Program Files\RAW2JPG. The application itself (RAW2JPG.exe) along with a file named 'dcraw.exe' will be installed at C:\Program Files\RAW2JPG\application. Move the dcraw.exe file to "C:\dcraw\".

MagicFish Download and Installation

For MagicFish, download MagicFish_Installer.exe found in the folder: COMPILE_64bit. Initial unpacking of the software takes some time as the MATLAB components are installed. During the program unpacking process you may get repeated "InternetReadFile Failed" error messages. Continue to press OK and persevere and there should be no problems.

12.3.9 DHP LAI image pre-processing for analysis using RAW2JPG

It is important to make sure there are no spaces (including "-" in the path to where the image files are stored on the computer, including the name of the image file.

To process fisheye images the user must specify (in pixels) the centre co-ordinates and diameter of the circular mask. Alternatively, the 'Create fisheye image mask' function will process a template image taken with the same camera and lens as the canopy images and store the centroid and diameter of the hemisphere to the Options panel, ready for analysis of the canopy images. The easiest way to get a good fisheye template is to put the camera, with fisheye lens equipped, inside a white bucket and take a photo. Ensure the image mask file is saved to the same folder as the images to be processed.

The user can alter the fraction of pixels to saturate at each end of the histogram; this could be used to overexpose clouds and increase the heterogeneity of the background sky. The default is to saturate 1% of pixels at both ends of the brightness histogram.

The RAW2JPG application will use the functionality of dcraw to convert the raw DHP images to 'pgm' format which can be opened by MATLAB. The application will then contrast stretch (Macfarlane *et al.*, 2014) the blue channel of the raw image only, after first applying a circular mask to eliminate pixels outside the circular field of view of the fisheye lens. The resulting image is saved in JPG format ready for analysis by MagicFish or other software. RAW2JPG can also apply an optional gamma correction (typically 2.2) to improve later pixel classification.

12.3.10 RAW2JPG Detailed Instructions

Open the **RAW2JPG** program

Select **Image type** as "Fisheye"

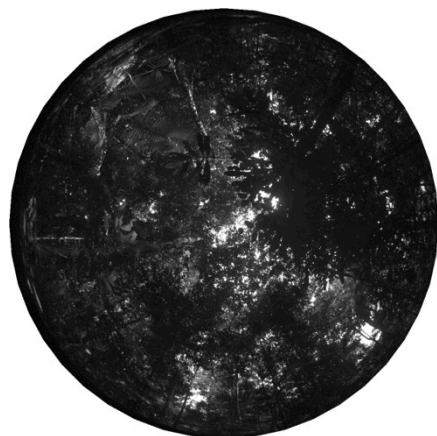
Click on **Adjust gamma** (this improves pixel classification, especially of imperfect images)

Leave the **Stretch limit** on the default setting

Fisheye settings can be typed in manually, but can be ignored if you use a "white bucket photo" with the **Create fisheye image mask** which will populate these field as it create the mask.

Click on **Run** and select the raw DHP images using the shift key to select multiple files.

This results in the JPG output files (using same names as originals - if camera JPG images are stored in the same folder then they will be overwritten) deposited in the same folder, ready to be used with the **MagicFish** software. They should look like this:



DHP LAI analysis using MagicFish

For long term monitoring purposes the DHP LAI is calculated and reported using MagicFish software (C. Macfarlane) which analyses LAI in a zenith ring section of the image between the default angles of 52.5 and 62.5. At the zenith angle of 57.5° the light extinction coefficient is known and equals 0.91 (Bonhomme and Chartier 1972).

12.3.11 MagicFish Detailed Instructions

Open **MagicFish** software

Select the **Fisheye image type** as **Circular**

Select **Threshold** (will not make much difference, especially with good quality images)

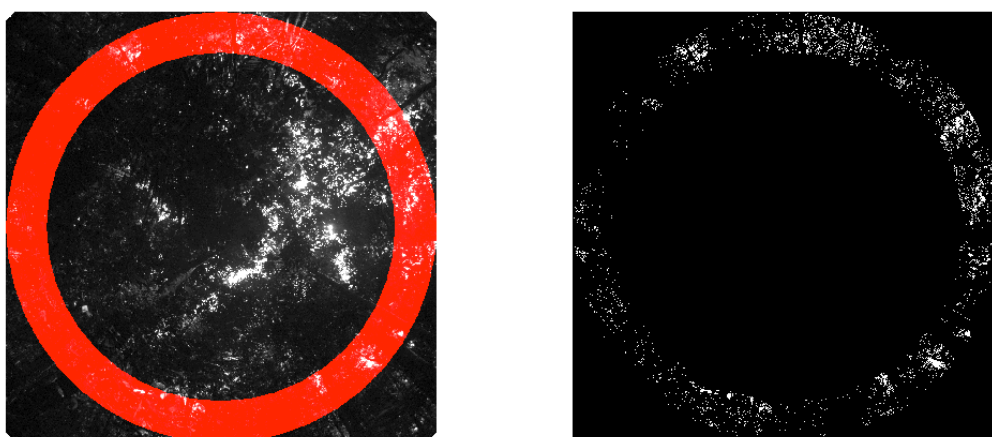
Select all **options**: **Sharpen image**, **Save Summary**, **Display segments**, **Save Detailed Results**

Leave the **Settings** as default values

Manually fill in **Camera Settings: X centre, Y centre** and **diameter** (of the JPG images created by RAW2JPG)- get these numbers by looking at Properties / Details information for the JPG files to be analysed. ie an image of 1224 x 1224 pixels has an X and Y centre of 613 and a diameter of 1224. Note that the images are one-quarter the size of the camera JPEG because only the blue pixels are used. This means that mask coordinates calculated from a camera JPEG will be incorrect.

Click Run and select the DHP images (JPEG format) using the shift key to select multiple files.

The summary images that result should look like this:



Results file:

The results file is also saved in a sub-folder called 'summary'. It contains the following columns:

Filename	Name of the file
Single/Manual Threshold	The threshold value you specified for the single or manual method. Will be zero if you used the dual classification technique.
Low Thd	Low corner detected using the Rosin method (see Macfarlane <i>et al.</i> 2007).
High Thd	Upper corner detected using the Rosin method (see Macfarlane <i>et al.</i> 2007).
GF	Gap fraction.
CP	Crown porosity (see Macfarlane <i>et al.</i> 2007).
FC	Foliage cover (see Macfarlane <i>et al.</i> 2007).
CC	Crown cover (see Macfarlane <i>et al.</i> 2007).
LAI_CC	LAI from gap size analysis (see Macfarlane <i>et al.</i> 2007).
LAI_Lin	LAI uncorrected for foliage clumping.
Log-Ave GF	Log-averaged gap fraction (used for Lang-Xiang method).
LAI_LX	LAI from the Lang-Xiang method.
No Azimuth segments	Number of Azimuth segments used to calculate LAI_LX (CM to add in program)

13 Understory LAI - Point Intercept Method

The components of the Understory will generally be measured by the Point Intercept Method (PIM) and this will include woody plants < 1.5m (Woody understory) and sparse grasses, forbs and herbs (Ground cover). From this an LAI Understory (PIM) is obtained.

$$\text{LAI Understory (PIM)} = \text{LAI Woody understory (PIM)} + \text{LAI Ground cover (PIM)}$$

Where there is dense grass cover (e.g. savanna) the method becomes impractical for the grass component and an alternative procedure is used to measure the LAI of the grasses based on the LICOR LAI-2200 (or LAI-2000) instrument (see below).

LAI Undercover (LAI-2200)

LAI Undercover (LAI-2200) is used instead of the LAI (PIM) then in vegetation types with dense grass understory vegetation.

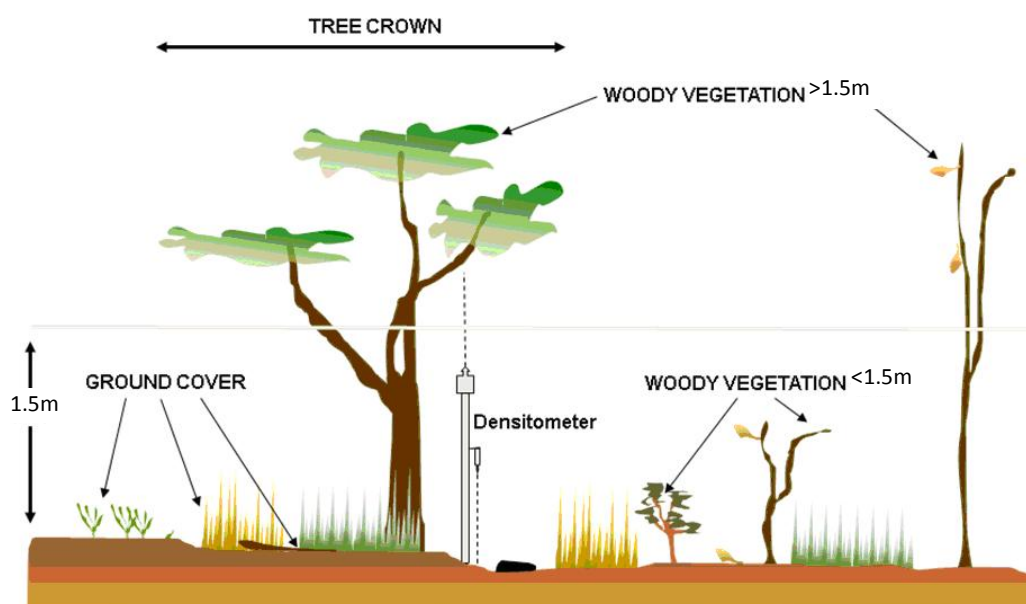


Figure 12: Vegetation categories measured (Adapted from AusCover SLATS STAR Transect protocol)

The PIM method is adapted from AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012) and additional sources including

http://www.webpages.uidaho.edu/veg_measure/Modules/Lessons/Module%207%28Biomass&Utilization%29/7_3_Direct%20Methods.htm).

The Point Intercept Method (PIM) is recommended in low sparse vegetation with hit/miss of crown cover. This method will be calibrated with direct measures from clip harvests. Point Intercepts and clip harvests will be collected every 6 months including the period of maximal leaf coverage for the initial 5 years to establish the basic relationships.

It is important to measure foliar cover at the same phenological or plant growth stage each year. Because plant growth stages are reached at slightly different times, due to variations in the weather each year, it is more accurate to monitor at the same plant growth stage each year than on the same date. It is expected that frequency and methods will be modified when clear relationships are derived from the direct (Clip Harvest) and indirect measures (Point Intercepts) of LAI.

The point intercept method is a rapid, repeatable and accurate method of quantifying canopy cover of individual plant species and total vegetation in sparse vegetation. With this method, cover is measured along linear transects and is based on the number of "hits" for each plant species from the total number of points measured along all transects.

13.1 Point Intercept Site Details

Three internal transects are located in the core 1 ha plot with records collected every 1 m giving a total of 303 points. These transects are permanently marked to allow recurrent monitoring every 6 months, including periods of greatest foliar coverage.

Attributes of point intercepts were based on the Australian Bureau of Agricultural and Resource Economics and Sciences 'Ground Cover monitoring for Australia' project 2010-2013 (Rickards *et al*, 2012) to quantify total vegetation canopy/crown cover and understory ground cover.

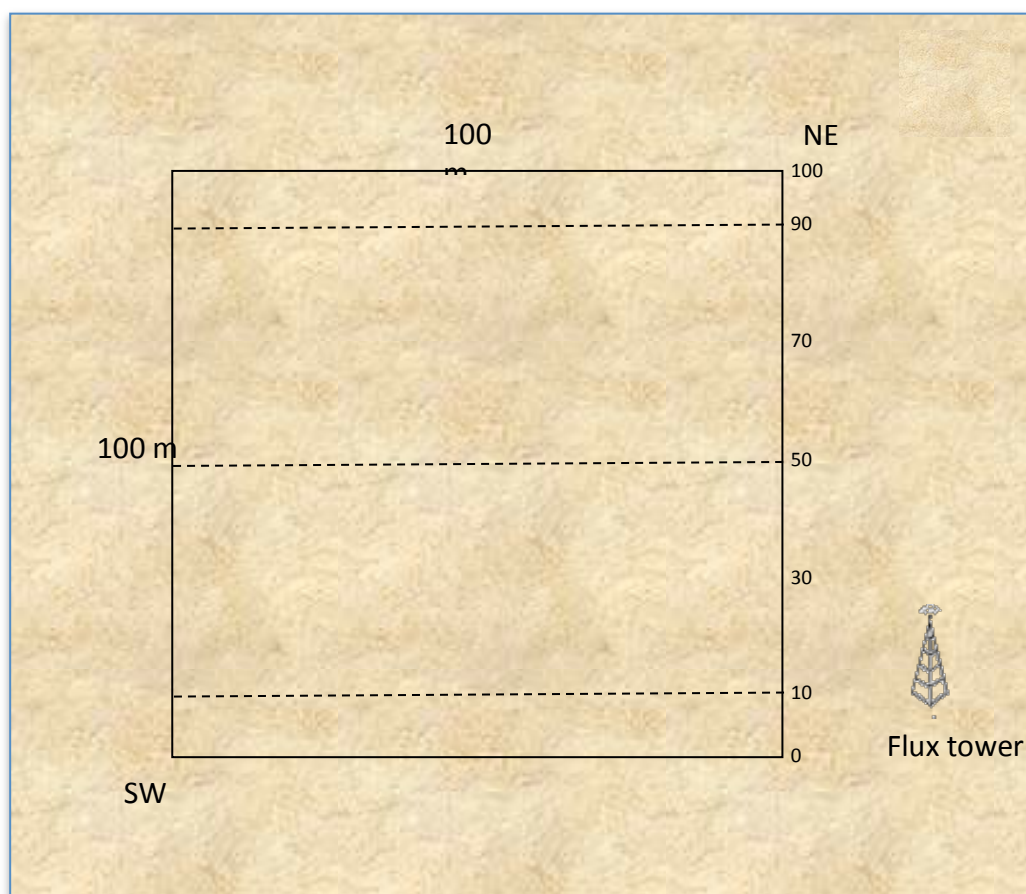


Figure 13: Layout of Point Intercept transects within core 1 ha plot.

13.2 Point Intercept Procedure

1. For each transect lay out a 100 m tape or graduated cord between the start and end points/pegs.

Ensure Tape is:

- orientated to align with the grid,
- straight, and
- on the ground (where possible) and not draped over shrubs.

2. Using an extendable pole (preferably with a red or green laser pointer mounted at 1.5 m), start at the 0 m mark of the first transect. Place the bottom of the staff at the 0 m mark, ensure the staff is vertical.

Collect intercept data for ground and woody understory strata as described below.

Continue recording the same information at each 1 m interval along each of the 3 transects, laying out the tape afresh for each new transect.

This will give a total of 303 points for each plot.

Measuring ground cover LAI and woody understory LAI will be done at each measurement point at the same time. The hits recorded will be allocated into ground cover or understory depending on the classification of the hit (does it hit woody vegetation or not - see Table 4 for details).

3. Measuring Ground Cover LAI

This category includes **non-woody vegetation** (such as sparse grasses, forbs and herbs), litter, cryptogams, soil and rock. **There is no height restriction for the non-woody vegetation.**

- Position the pole (if it has a laser pointer point this down) vertically immediately adjacent to the metre mark on the measuring tape. Use a level to ensure the pole is vertical.
- Record the first intercept of the pole (or laser beam) with leaf branches etc when looking downwards, as you work your way down you may need to move the upper vegetation back. Place the intercepts (hits) in the appropriate category from the list below:

Mineral Crust - the hard surface layer of soil (field name cr, Table 4).

Disturbed soil - cracks in a soil crust, ant nests or other disturbances in the natural surface e.g. by animal hoof prints. In ploughed agricultural sites most soil recordings will be disturbed (field name ds, Table 4).

Rock - rock includes all stones and rock material greater than 2 cm (field name rk, Table 4).

Cryptogam - a biological crust composed of lichen, moss and algae (field name cy, Table 4).

Green leaf - a leaf with green pigmentation (one that is actively photosynthesising) attached to the plant. Sometimes the leaf in this state may appear more yellow than green. In this case a judgment call must be made as to whether it is placed in the green or dry category (field name gr, Table 4).

Dry leaf - a leaf with non-green pigmentation (one that is not actively photosynthesising). This can include senescent (alive) vegetation as well as dead vegetation. It must be attached to the ground or plant (field name dr, Table 4).

Litter - dead plant material that is not attached to the ground. Includes branches, leaves or fallen tree trunks (field name li, Table 4).



4. Measuring Woody Understory LAI

This category includes all **vegetation with a woody component** and a **height < 1.5 m**. These are generally shrubs and small trees.

- Maintain the pole in the same position as where the ground cover measurement was taken.
- Determine if there is an intercept of woody vegetation <1.5 m with the pole directly above the point recorded for the ground cover. Cover is determined by recording the number of “hits”. To be counted as a “hit”, a portion of the vegetation must cross the transect tape’s interval number line e.g., 1m, 2m, 3m.... nth point. If a portion of the vegetation does not break the vertical plane at the interval number line, it is reported as a miss
- Record the intercept hit in the appropriate category—green leaf, dry leaf, branch.

Green leaf - a green leaf attached to a plant (field name mg, Table 4).

Dry leaf - a dead or dry leaf attached to a plant (field name md, Table 4).

Branch - woody component of the plant (branch or trunk) (field name mb, Table 4).

13.3 Equipment

- 100 m tape(s) or non-stretch cord marked with 1 m graduations
- Graduated staff (extendable pole that can be adjusted to eye height for different operators).
Ideally equipped with Laser pointer (taped to staff at approx. 1.5 m and pointing downwards)
- Pegs to mark ends of transects
- Star droppers to mark plot corners

Flagging tape (bright coloured to make sighting transect ends easier)

13.4 Data Collection

Raw point intercept data is collected on printed sheets or directly into a spread sheet with attributes and values as outlined in Table 4 (based on Rickards et al. 2012) with understory height set to 1.5 m.

Table 4: Attributes of point intercept data

Field name	Description	Data type	Values allowed	Strata
point	Transect observation	Integer	1–300	All
cr	Soil crust	Integer	0, 1	Ground
ds	Disturbed soil	Integer	0, 1	Ground
rk	Rock	Integer	0, 1	Ground
gr	Green leaf non-woody vegetation	Integer	0, 1	Ground
dr	Dry leaf non-woody vegetation	Integer	0, 1	Ground
li	Litter	Integer	0, 1	Ground
cy	Cryptogam	Integer	0, 1	Ground
mg	Green leaf woody vegetation <1.5 m	Integer	0, 1	Under
md	Dry leaf woody vegetation <1.5 m	Integer	0, 1	Under
mb	Branch <1.5 m	Integer	0, 1	Under
oic	In crown for live woody vegetation >1.5 m	Integer	0, 1	Over
og	Green leaf woody vegetation >1.5 m	Integer	0, 1	Over
od	Dry leaf woody vegetation >1.5 m	Integer	0, 1	Over
ob	Branch >1.5 m	Integer	0, 1	Over
unique_obs	Site identifier (longitude_latitude_date)	Text	00000000_0000000_yyyymmdd 32 characters	All
g_total	Only one observation for ground cover (cr+ds+rk+gr+dr+li+cy) is permitted at each point	Integer	1	Ground
m_total	Zero or one observations of woody vegetation <1.5 m (mg+md+mb) are permitted at each point	Integer	0, 1	Under
o_total	Zero, one or two observations of woody vegetation >1.5 m (oic+og+od+ob) are permitted at each point.	Integer	0, 1, 2	Over
all_total	Number of strata for each transect point (g_total+m_total+o_total)	Integer	1, 2, 3, 4	All

Note: Primary key = unique_obs, point. Non-woody vegetative cover, such as grasses, has no height restrictions; Dry leaf is senescent or dead vegetation attached to a plant or the ground; Litter is unattached dead vegetation; Cryptogam is a biological crust on the soil surface; In crown is the vertically projected perimeter of all foliage and branches of the plant and is recorded for live trees only. Latitude and longitude are given in decimal degrees. Each site visit is assigned a 'unique_obs' code of 'longitude_latitude_date' to join or relate all tables.

13.5 Calculating LAI from the Point Intercept Method

Leaf area index (expressed as units of **green** foliage surface area per unit of ground surface area) is determined by dividing the number of foliage hits by the total number of points in a given area.

LAI is calculated from point intercept data by summing the number of green hits per sample transect and dividing by the total number of sample points along the transect.

e.g. there are 101 sample points per transect; if there were a total of 120 green hits along a transect the LAI would be 1.025 (i.e. $120/101 = 1.188$). LAI (PIM) = 1.188

One then regresses a relationship between the direct (harvest) LAI value and the PIM LAI.

e.g. LAI (harvest) = $0.011 + 1.1 \text{ LAI (PIM)}$ $R^2 = 0.941$

We next assume the LAI (harvest) is the true value for LAI and from this comes.

LAI (PIM-corrected) = $0.011 + 1.1 \text{ LAI (PIM)}$

LAI (PIM-corrected) is obtained from the regression and is used in subsequent calculations.

14 Understory LAI using the LAI-2000/2200

Note this method is to be used for ecosystems with dense grass understories, it is not a convenience method. It would be quite inappropriate for sparse understories - i.e. most ecosystems.

Step 1: read the LAI-2000/2200 manual to make sure you understand basically what you are doing. Referring to Figure 8 (DCP layout) you will record LAI values at two heights at each position along each transect at 10 m intervals.

Step 2: attach a 10° view cap restrictor to the LAI-2000/2200.

Step 3: Place a 5 cm block of wood (level on the ground) adjacent to the Tape measurement position.

Place the LAI-2000/2200 sensor on the block and ensure that there is no vegetation within 5 cm of the sensor – duck down low. Take a measurement and calculate an LAI – store the value against the position. Raise the sensor above the grass to a set position ideally either 1.5 m or 2.0 m (note the value used and use for all locations along all transects), level take a measurement and store the value against the position.

Step 4: Calculate the difference between measurements at a position to generate an LAI Undercover for that position.

e.g. LAI Understory $(\text{Transect 1Position 5.0m}) = \text{LAI}[0.05\text{m}](\text{Transect 1Position 5.0m}) - \text{LAI}[1.5\text{m}](\text{Transect 1Position 5.0m})$

Step 5: Average the LAI Understory values across all transects (k) and all positions (n) to generate a clumping-corrected LAI for the 1 ha. This is LAI Understory (LAI-2200)

$$\text{LAI Understory (LAI-2200)} = \sum_{k=1}^{n=1-9} \text{LAI Understory}$$

This is the uncalibrated LAI understory which subsequently needs calibration against clip harvests.

14.1 Calculating LAI from the LAI-2200/2000 Method

When using the LAI-2000/2200 instrument it is important to remember that the conversion from light transmission (or gap fraction) to LAI depends on crop/vegetation structure and leaf angle (and the relative contribution of wood to plant area index). The LAI-2000/2200 (used as specified in the manual) makes no attempt to correct for vegetation structure at all and guesses the leaf angle: the calculated LAI is a mathematical transformation of the gap fraction that requires calibration against reality. For example in blue gum plantations a few studies have come up with a rule of thumb that the correct answer is about 1.5 times the apparent answer from the instrument i.e. the error is not small.

One then establishes a relationship between the direct (harvest) LAI value and the LAI-2200 LAI.

$$\text{e.g.: LAI (harvest)} = 0.011 + 1.1(\text{LAI-2200 LAI}) \quad R^2 = 0.941$$

We next assume the LAI (harvest) is the true value for LAI and from this comes.

$$\text{LAI (LAI-2200-corrected)} = 0.011 + 1.1(\text{LAI-2200 LAI})$$

LAI (LAI-2200-corrected) is obtained then from the regression and used in subsequent calculations.

15 Total Leaf Area Index

The total LAI is calculated as the sum of canopy and understory LAI.

Total LAI = Canopy LAI + Woody Understory LAI (PIM-corrected) + Ground cover LAI (PIM-corrected)

or for ecosystems with a dense grass understory

Total LAI = Canopy LAI + LAI (LAI-2200-corrected)

The total LAI may be compared to the product MOD15A2 centred on the coordinates of a flux tower (8-Day Composite [Collection 5], 1 km Wide x 1 km High) from the MODIS Land Product Subsets project (<http://daac.ornl.gov/MODIS/>).

16 Clip Plot Harvests for LAI Calibration

Clip plot harvests will provide direct measures of cover for low sparse vegetation that will be used to calibrate and validate the indirect Point Intercept Method and to calibrate the LAI-2200 measurements in productive grasslands. Clip plots will be established outside of the core 1 ha in comparable vegetation immediately adjacent to the plot.

Leaf area is measured on a sub-sample of leaves and related to dry mass (e.g. via specific leaf area, SLA, $\text{cm}^2 \text{g}^{-1}$). The total dry mass of leaves collected within a known ground-surface area is converted into LAI by multiplying by the SLA. Direct methods provide the reference for the calibration or evaluation of indirect methods (Bréda 2003).

16.1 Clip Harvest Plots

Individual SuperSite data managers must determine the most suitable area for clip harvests that take into account the size of the vegetation (e.g. tall stature vs short stature grasses, the former will require larger areas; sparse vegetation will require correspondingly larger areas to be sampled). As

an indication of how to proceed for a short stature grass (procedures adapted from NEON and He *et al.* (2007). A 2 m × 3 m subplot should be designated for destructive clip harvest sampling, the location should be external but in close proximity to the core plot and in representative vegetation. This subplot is divided into thirty 0.1 m × 2 m strips. A given strip will not be harvested more than once every 10 years, thus mitigating effects of harvest on the biomass estimate of any given year.

16.2 Clip Harvest Procedure

Two strips are harvested each period. When strips are harvested, the area should be temporarily delineated with tent stakes and nylon cord pre-marked in 10 cm increments, and all biomass rooted within the strip should be clipped as close to the ground as possible, taking care not to damage perennial crowns associated with certain graminoid species. With a pair of clippers, remove all vegetation in a three dimensional area above the area enclosed by the marked strip. Clip to ground level. Grass shears, powered shears, sickles etc. can be used to accomplish clipping. Clip harvest strips should be moved in a given year if atypical obstacles are encountered – e.g. ant mounds – and the location of each year’s harvest should be recorded in order to prevent repeat sampling of a given area at less than a 10 year return interval.

Clip harvest from each strip is placed in a sealed bag, and stored in a cooled esky for transport to the laboratory. In the laboratory the leaves in each sealed bag are sorted into green and dead fractions for each species and the leaves of the green fraction are used to measure leaf area index. Ten percent of sampled leaves by weight are taken as a subsample. (the subsample is chosen to be representative across the size classes of leaves). The subsampled leaf area is measured using a commercial planimeter e.g. Li-3000 Portable Area Meter with transparent belt conveyor Li-3050. The rest of the green leaves are dried separately in an oven for 48 hours at 60 °C and weighed. The green leaves must be measured using the planimeter shortly after collection – **maximum 4 hours**.

Total leaf area (LA; m²) per strip is calculated by multiplying the total weight (W_t; g) of all leaves from a strip by the Specific Leaf Area (SLA; m²g⁻¹) of the subsample (10% by weight of green fraction).

$$LA = W_t \times SLA$$

SLA is calculated by dividing Leaf area of the subsample (LA_s; m²) by the weight of the subsampled leaves (W_s; g)

$$SLA = LA_s / W_s$$

The site mean destructive LAI (DLAI) is calculated by dividing the total LA (m²) for the 2 strips by 0.2 m² (He 2007)

$$DLAI = \frac{1}{4} \sum_{i=1}^4 \frac{LA_{hi}}{0.2} = \frac{1}{4} \sum_{i=1}^4 \frac{W_{ti} \times (LA_{si} / W_{si})}{0.2}$$

As indicated the samples should also be weighed in concert with the leaf area measurements. The weight of freshly harvested plant material is highly variable and depends on recent weather, atmospheric conditions, and the water status of the plant. Plant biomass is typically expressed on an oven-dried basis. All samples are collected and brought back to the laboratory, weighed and then dried. Once a sample is dried the biomass is expressed as % dry matter = (Dry Mass/Fresh Mass) *100 where the “Dry Mass” is the mass of the sample after oven drying and “Fresh Mass.” is the mass of the sample recorded in the field.

16.3 Recommended Drying Procedure

- (1) Dry sample within 24 hours of clipping.
- (2) Place samples (in paper bags) in a forced-air oven 60 °C
- (3) Dry to constant weight. Most samples will take 24-48 hours to dry.

To determine if a sample is dry, a few bags can be removed from the oven, weighed and then returned to the oven. A few hours later, (4-8 hours) the bags can be removed again and weighed. Samples are dry when no changes in weight occur between reweighing. Record these final stable values.

17 Phenology camera (Phenocam) Monitoring

17.1 AusCover Phenocams

Extracted from the AusCover Good Practice Guidelines (A technical handbook supporting calibration and validation activities of remotely sensed data products). Version 05, 25/03/2014. Natalia Restrepo-Coupe, Alfredo Huete and Kevin Davies. Plant Functional Biology and Climate Change Cluster, University of Technology, Sydney

<http://data.auscover.org.au/xwiki/bin/view/Good+Practice+Handbook/PhenologyValidation>

17.1.1 Phenocam Introduction

Automated cameras have been installed across the SuperSite network with the aim of recording hourly and daily changes in vegetation. The cameras are permanently placed and will provide hourly daytime near-surface remote sensing data of the forest canopy (from the top of an eddy flux or fire observation tower) and/or understory phenology (3 cameras at each study site). In addition several of the SuperSites have installed StarDot IP cameras that are providing live digital image data streams.

17.1.1.1 RGB and Spectral Cameras

In Australia, efforts to instrument flux tower sites with RGB cameras for phenology validation started in early 2000. Their value as a recording tool of the different phenological changes (visual or more complex analysis) has been proved in different applications (Crimmins and Crimmins 2008; Huemmrich *et al.* 1999). The selection between multispectral, hyperspectral and RGB cameras is generally made based on the cost and available technical resources. AusCover uses the WingScapes® RGB programmable camera which collects images to an SD card.

17.1.2 Camera Inclination and Azimuth

The positioning of the camera may have direct consequences on the data analysis. A combination of oblique and nadir cameras can therefore be used. The nadir looking (straight down) cameras capture an image where issues related to backscatter (sun behind observer) and forward-scatter (sun opposite observer) can be minimized. An oblique camera will capture a wider portion of the ecosystem and specifically focus on some key elements of the site. However, given that specular reflection of leaves can occur for camera inclination angles > 30° from the vertical, it is suggested to work at < 30° angles.

In the southern hemisphere, primarily in summer months (Figure 14), orienting the camera to face towards the south results in backscattering, and the image will show a bright region where all shadows are hidden (hotspot; see Figure 14). By contrast, forwardscattering (sun opposite to the observer) will result in mirror-like reflection from the leaves and bright object edges. Interesting, at many sites it is common to have both scenarios (forwardscattering / backscattering) as the solar azimuth will change during the year from N to S and vice versa.

It is preferred to seasonally maintain backscatter conditions, and limit the analysis to images collected when the sun is close to local noon (11:00 - 12:00 am), even if this configuration results in the greatest variation in the solar zenith angle (SZA). Having the sun facing into the camera is less desirable as it is difficult to separate the different vegetation components (wood, leaves, and shadows among others) (B Nelson personal communication). In summary, the camera azimuth position is a compromise for each individual flux-tower site as it is necessary to balance the needs of the cameras with the EC, which usually has priority.

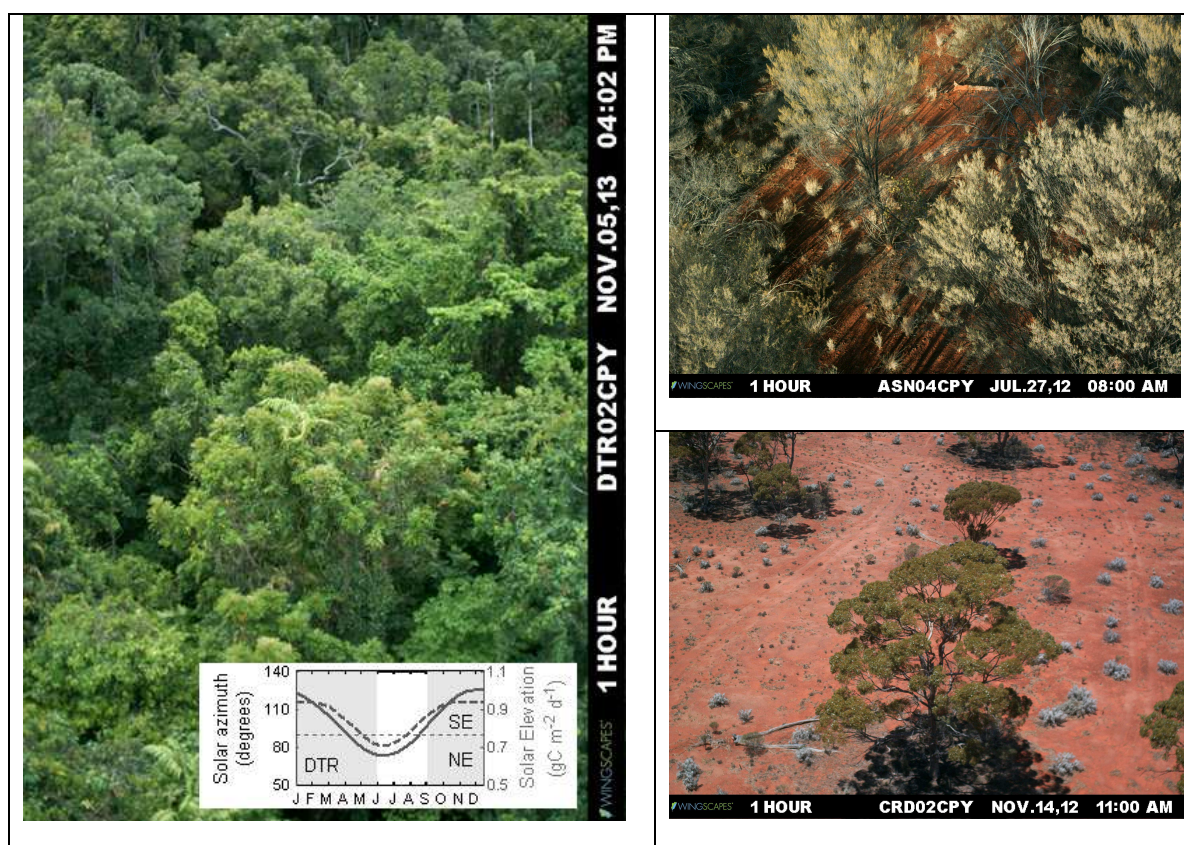


Figure 14: Camera azimuth and inclination.

Left panel: Daintree/Cape Tribulation flux tower phenocam, sun behind the observer (backscatter) and the presence of a hot spot at the center of the image (special thanks to M. Liddell and N. Weigand). Left side inset: seasonal cycle solar elevation at 11:00 am (right axis, grey), and azimuth (left axis, black), azimuth values >90 indicate sun at the southeast (SE) and <90 at the northeast (NE). Right top panel: Sun at low angle and mirror like effect on leaves. Sun behind the observer at the Alice Springs Mulga flux tower phenocam (special thanks to D. Eamus and J. Cleverly). Right low panel: Credo flux tower site phenocams images showing forwardscatter (Special thanks to C Macfarlane). Both right panels show issues posed by the shadows at arid and semi-arid environments. For all camera orientations, shadows from the vegetation and existing structures (e.g. from the

flux tower) can increase the difficulty of processing the images. Using Green/Red band ratio and other ratios will decrease, but not completely remove, the influence of dark or bright areas across the image (see section on the computation of Red/Green (RGB) and NIR/Red ratios).

17.1.3 Over- and Understory orientations

For Australian multi-functional and multi-strata canopy types, two sets of cameras are needed to adequately characterise landscape phenologies, including an overstorey and understory camera (or herbaceous and woody layer camera). The tree layer needs an oblique view (30-60° from zenith) to capture sufficient number of trees and sampling of landscape cover while the understory should be nadir view or slightly oblique (0-30°). Azimuthal orientation should be as described in section above (camera inclination and azimuth). For the understory cameras, key species or the location of the soil moisture/temperature array will dictate the location of the camera.

17.1.4 Diurnal, Daily, and Seasonal Settings (including frequency of observations)

Most phenocams record images every 30 – 60 minutes. Our experience in very wet environments (e.g. Amazon basin, see Figure 15) shows that a high frequency of images captures allows us to choose a time of day to be used when calculating the time series and to avoid using a “fixed” capture time where rain or fog may affect the quality of the images. Some researchers do select images captured during cloudy periods (under diffuse radiation) in order to avoid saturation, stray light or to correct for a seasonal changing SZA, in particular at those locations where the camera alternative captures images in forwardscatter and backscatter conditions (see the works of B. Nelson and previous section “Camera inclination and azimuth”). This approach, however, is known to introduce significant noise, thereby increasing the uncertainty of the observations, as the light environment is difficult to characterise. Arid and semi-arid sites (~75% of Australia) are cloudless for long periods of time (weeks to months), and this may translate into an incomplete time series if only images during diffuse radiation periods are used, although they will correspond to the dry/dormant season.

Some ecosystem components like the soil biological crusts respond after rain (greenup) at a faster rate (< 30 minutes). Even if the satellite will not capture these biological pulses, the phenocam can inform the flux tower measured C-fluxes about the length and spatial extent of the response - an interesting result by itself.

17.1.5 Camera Settings (integration times, F- stop, etc.)

Some hyperspectral cameras (e.g. SOC710 Surface Optics) allow the user to change the camera settings to obtain good quality images (spectral range, no saturation, etc.) under different light environments (e.g. outdoor or indoor locations). The f-stop regulates the aperture of the lens, a value of 2.8 or 5.6 means more light inside the camera compared to 11 or 22. Closing the lens (move the f-stop to higher values) translates in improving the depth of field and focus at the extremes of the spectrum and it can help in outdoor conditions by avoiding saturation. However, it is best to try to fix the f-stop and get more or less light into the camera via changing other parameters. Similar results can be obtained by modifying the integration time. In general, radiance and spectral factory calibrations are done using a fixed f-stop (e.g. 5.6) as it provides a good trade-off between speed and quality. If we assume that each increment in the f-stop (e.g. 5.6 to 8), cuts the illumination in half, the integration time can be doubled in order to obtain similar results (e.g. 10 milliseconds integration time at f5.6 vs 20 milliseconds at f-stop 8). It is always good practice to obtain the highest number of digital counts as possible.

Some cameras offer the possibility to modify the electronic gain as an alternative to integration times, as increasing integration times in windy conditions can be problematic. However, since the gain is electronic, noise in the image will also increase correspondingly. It is suggested to use a gain value of '1' or unity (no gain).

Very simple cameras (e.g. WingScapes®) do not offer any of the above- mentioned settings. However, light settings can be set to auto, sunshine, fluorescent and other light environments. As we want to capture each object reflective properties rather than changes to the camera settings, we fixed the light setting to sunshine (outdoor conditions). Moreover, if the camera allows the user to obtain RAW files rather than JPEG, it is recommended the use of RAW as Gamma correction and other image enhancement filters are usually applied to the RAW data in order to generate JPG extension files.

17.1.6 Use of White/Grey References

In order to calculate reflectance, measures of incoming light are necessary. For this purpose, a reference plate (TEFLON or Spectralon) is installed in front of the camera so the image captures all or part of the plate. If the plate is going to be used as a reference it needs to be installed horizontally (see Figure 15b). Our experience has shown us that the plate can be under a different light environment than the rest of the canopy (e.g. patchy clouds) and not represent the light environment of the canopy (see Figure 15a). The area immediately around the plate should not be included on the analysis. Moreover, the spectral range of the camera is generally stretched. For example, vegetation is “dark” on the red region of the spectra (0.63 - 0.69 nm) and the plate will be highly reflective. Thus to avoid saturation, the aperture will need to be closed. However, this would not be able to capture subtle changes in the canopy due to the strong absorption in the red region by vegetation (see Figure 15c). Grey standards have been suggested to bypass issues related to spectral range and saturation but they can easily degrade (e.g. due to dirt) in outdoor conditions. References therefore seem impractical and not required if working with ratios.

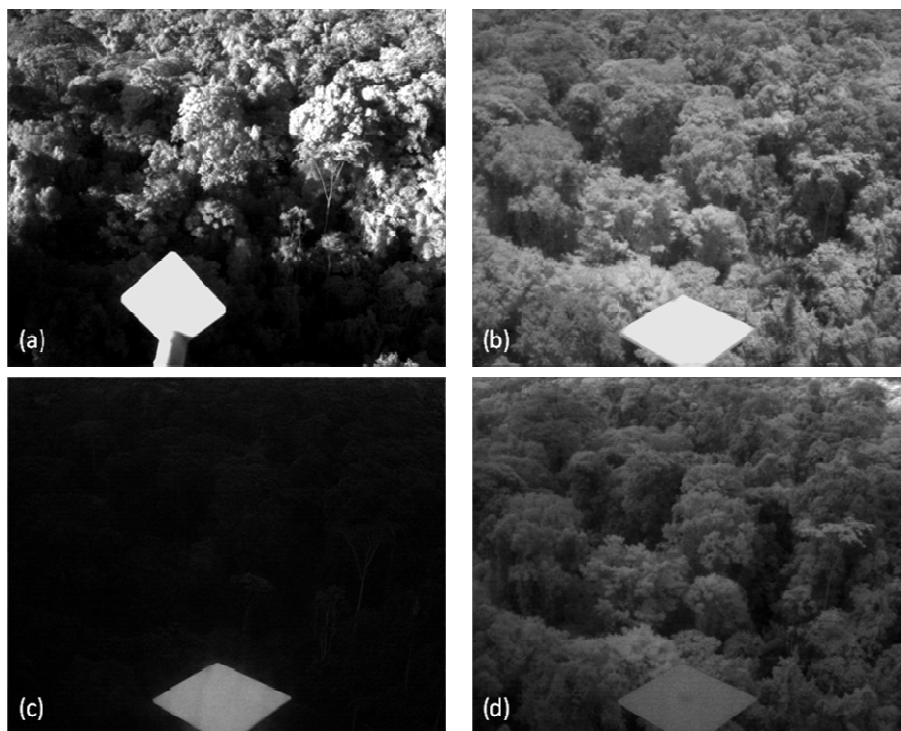


Figure 15: TETRACAM 3-band camera (NIR, Red, and Green) installed at the Amazon basin K67 eddy flux tower.

With (a) NIR band showing the TEFLON panel set vertically (check camera consistency, no use as reference); (b) NIR band showing the TEFLON plate set horizontally to be used as reference standard (note observable glare around the plate); (c) Red band; and (d) Green band. All images as Digital Counts 0-255. Acknowledgments to S. Saleska and A. Huete

17.1.7 AusCover WingScapes RGB Digital Camera Set Up

1. Three cameras per site
2. The cameras and solar panels are small (12.5 x 17 cm and 15 x 15 cm, respectively). However, we need to minimize any light obstruction.
3. Camera ecosystem 1 and 2: install one oblique, one nadir at the top of the tower
4. Camera understory 3: Install at 3 m oblique looking oblique towards the south towards the South with the sun behind the "observer" (less shadows and glare).

17.1.8 Materials

WS camera (3)

Solar panel (3)

Straps and Velcro

Camera mounting bracket (3)

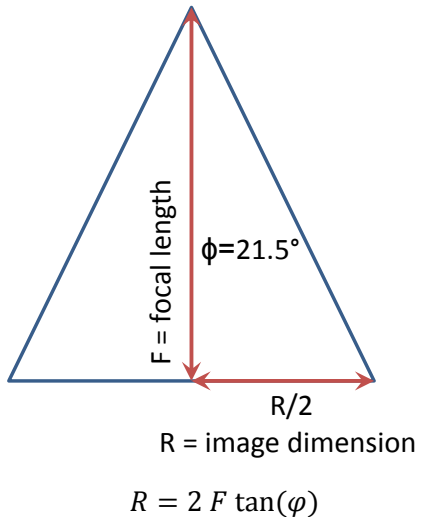
Batteries (3x6 C-batteries)

SD card (6). Therefore you are able to swap the cards.

Compass + clinometer

17.1.9 Camera properties

Lens Field of View Low, High, Max: 43 Degrees

 <p> $F = \text{focal length}$ $\phi = 21.5^\circ$ $R/2$ $R = \text{image dimension}$ $R = 2 F \tan(\phi)$ </p>	Total Image Dimension (m)	Focal length (m)
	1	1.27
	2	2.54
	3	3.8
	4	5.0-8
	5	6.35
	6.54	8.3
	8	10.15
	10	12.69
	12	15.23
	15	19.04
	20	25.38
	25	31.73
	30	38.08

17.1.10 Camera “program”

Time lapse interval: 1 hour

Photo or video: Photo

Photo Quality: High 2592x1944

Multi-program: 1-program

Program 1 Wakeup: 6 am; Sleep: 6 pm

Imprint Info: Yes

Date/Time: Local time Alice Springs UTC/GMT + 9:30 hours

Local time Sydney UTC/GMT + 10 hours (no savings time)

Camera Name: ASP-01UNDERSTORY (ASP = Alice Springs; 01: Camera 1; US: understory)

ASP-02UNDERSTORY (ASP = Alice Springs; 01: Camera 1; US: understory)

ROB-01UNDERSTORY (ROB = Robson Creek; 01: Camera 1; NADIR: top of the canopy nadir camera)

White Balance: Sunlight

17.1.11 Radiation Footprint

Understory camera should be placed outside the footprint of the radiation sensor. LWup fov 180 deg and SWup 150 deg. Instrument installed 20 m at height.

$$R = 2 \times 20 \times \tan(75) = 149 \text{ m}$$

$$R = 2 \times 20 \times \tan(88) = 1145.5 \text{ m}$$

$$R = 2 \times 20 \times \tan(89) = 2291.6 \text{ m}$$

17.2 StarDot IP Digital Camera

In a future iteration of funding there will be a roll out across the SuperSite network of StarDot 5 megapixel (MP) IP cameras across the SuperSite network as these have been chosen by both the US PhenoCam network and NEON networks due to its resilience to extreme environments (this will be tested by the conditions in the Daintree rainforest. The SuperSites will standardise on the IR enabled version of this camera with a 4.5-10 mm IR lens for improved image quality compared to the stock 4 mm lens. Currently two SuperSites have deployed these cameras and the installation details are as below:

17.2.1 Camera Details

Tropical Sites (wet):

- DOM-SEC5IR NetCam Dome 5MP IR. 4-12mm Lens, Day/Night

Non-tropical sites:

- CAM-SEC5IR-B – StarDot NetCam SC5 IR ^{1,2} (standard IP camera)
- 4.5-10mm IR/megapixel lens ³
- Heavy Duty Outdoor enclosure (ENC-OUTD)

Installation of the camera needs to ensure that an appropriate water proof enclosure and water proof cabling is supplied with the camera. For dome cameras, we recommend adding significant quantities of waterproof silicon sealant between the dome mount and the camera, both above and below the waterproof mounting gasket as well as covering the mounting screws on the top of the camera mount with sealant. The back of the mount where it attaches to the pole is closed as per normal mounting instructions and the cable pass-through holes are not sealed. Fill the cavity at the top of the camera mount and the space between the cable pass-through panel and the inside of the camera mount with 2 pieces of foam from the camera packaging to provide an additional barrier to water entry into the camera. This provides a solid waterproof seal for the main part of the camera while permitting moisture to escape the housing and reducing water ingress points from the back of the camera where the wires come out.

For non-tropical installations a standard box camera can be used with the StarDot heavy duty housing. Note that this housing is not environmentally sealed but has been widely tested in extreme (non-tropical) environments. For locations that require a locking housing an alternative housing must be used.

17.2.2 StarDot Camera Overstorey Orientation

The camera orientation is as for the WingScapes set up – canopy mount.

17.2.3 StarDot Camera Settings

The timelapse images are collected as follows:

Capture rate	15-min interval*
Start Time (AM)	06:00
Stop Time (PM)	18:30
Image resolution	2592x1944 (5MP)
Avg. image size	850KB (avg of 29,000 files captured from the Daintree camera)

Camera is configured to auto-ftp an image over an Ethernet connection to a central server through modem connectivity.

Camera requires 12v nominal input (~0.5A/4w power usage)

- Capture interval can be lengthened or image size can be reduced if cellular data requirements limit amount of data that can be transferred.

18 Archiving Photographs in the SuperSites Image Database

Archiving of photopoint, photopoint-panorama and LAI photographs in the SuperSites Image Database is carried out manually by FTP using the instructions below.

As proprietary raw image file formats vary and the specifications are not publically available, there is a risk that the use of these formats for long term archiving may become problematic for analysis software. Images should be uploaded to the SuperSites image database in the raw format (/raw directory) as well as a JPG format versions used in analysis (/jpg directory).

18.1 Upload of Image Data to the SuperSites Image Database

Instructions for image file uploads are available to Principal Investigators in a separate document. Files are uploaded to a staging area where the file names are checked before transferring images to the database.

Photopoint and LAI images are stored in the raw format and as JPG files. Images are uploaded using the file names downloaded from cameras. The data ingestion process includes renaming the files to include location, date and other descriptors. A Read_me.txt file is included with images that are uploaded to ensure the correct renaming and storage of the data in the database directory structure.

The photopoints and LAI images are stored in folders that have the date of image capture included in the name. A Field_Notes.txt file is included with the stored Five Point Photopoint and LAI images which contains details of the camera, field position and other notes from the field campaign.

Phenocam images are stored with a Camera_Config.txt file that contains details of the individual phenocameras.

18.2 SuperSite Codes Used in Directory Structures

FNQ Rainforest SuperSite - Daintree	fnqr_daintree
FNQ Rainforest SuperSite - Daintree Discovery Centre	fnqr_ddc
FNQ Rainforest SuperSite - Robson Creek	fnqr_robson
SEQ Peri-urban SuperSite - Samford	seqp_samford
SEQ Peri-urban SuperSite - Karawatha forest	seqp_karawatha
Tumbarumba Wet Eucalypt SuperSite	tumb
Cumberland Plain SuperSite	cblp
Warra Tall Eucalypt SuperSite	wrra
Victorian Dry Eucalypt SuperSite - Whroo	vicd_whroo
Victorian Dry Eucalypt SuperSite - Wombat forest	vicd_wombat
Calperum Mallee SuperSite	clpm
Alice Mulga SuperSite	alic
Great Western Woodland SuperSite	gwwl
Litchfield Savanna SuperSites	lfld

plot name:

core_1ha

or user defined name eg. blackbutt, floodplain etc.

19 Sampling for Stable Isotope and Plant Genetic Analysis

19.1 Plant Isotope Sampling Procedure - Leaves/Needles

Leaf samples will be collected from at least 20 plant species (trees, shrubs, grasses, forbes) at each core 1 ha plot and processed as follows to facilitate ¹³C analyses.

1. Leaf/needles samples should be collected from significant functional types and dominant species based on basal area. Five trees (ideally) of each species to be sampled, where possible. If not five, then three or four, where possible.
2. Sampling the crown:
 - a. For short (< 2 m tall) trees/shrubs: Leaves/needles should be sampled from branches that are of similar length from the upper third of the crown.
 - b. Sample from the north facing aspect of the crown.
 - c. For taller trees (> 2 m) samples should include bottom, middle and the top thirds of the crown, where possible. If this isn't possible, the upper third of the crown is requested. Within each third of the crown, (i.e. upper, middle, lower) try to sample branches of (approximately) the same length. Sample from the outer third of each branch.

- d. Keep samples from each tree and from each part of the crown separate from each other, and labelled (**Species, Date, Time, Location** (geographic), **Tree Number, Leaf Number** (e.g. Tree 1 Leaf 1, Tree 1 Leaf 2 etc.). **Part of Tree** (crown position) the sample was taken from.
 - e. Sample mature fully expanded leaves, preferably not diseased.
 - f. Collect two leaves from each of the five replicate trees, leaves should **not** be collected from the same branch, if you can avoid it.
3. Samples should be collected once, in March/April or October/November, to coincide with ant monitoring (however, further sampling in an opposing season would be scientifically very useful, but not a milestone requirement for this funding period).
 4. Individual sample weight should be *around* 1 g (fresh weight).
 5. Collected leaves can be stored in plastic zip-locked bags. Alternatively 50 mL centrifuge tubes (capped) may be used.
 6. Samples can be dried at 80° C for at least 48 h. If you can't get to an oven in the field you can air dry the samples in the sun. It is important to minimise respiration during storage, so either dry them quickly in the field in direct sunlight, and then store in a fridge, or if the weather doesn't allow quick drying in the field, then frozen in the field until drying in the lab is a good alternative, as freezing them will stop the respiratory losses during storage in the field. To keep leaf samples useful for DNA extraction leaves can be dried in paper bags with sachets of silica gel for drying, or use a teabag in a sealable lunch box with 1 cm of silica granules (10% self-indicating mixed with 90% standard non-indicating granules) and seal.
 7. Once samples are dried, they can be kept dried, double plastic bagged in a freezer until posting.
 8. Dry samples can be sent to University of Technology Sydney (address below), or you can grind them to a fine powder in a ball mill grinder prior to sending in microcentrifuge tubes. A minimum of 150 mg dry weight is required per sample.
 9. Each sample should be labelled. The label should include: **Species, Date, Time, Location** (geographic), **Tree Number, Leaf Number** (e.g. Tree 1 Leaf 1, Tree 1 Leaf 2 etc.). **Part of Tree** (crown position) the sample was taken from.
 10. Record tree dimension (**DBH** and **Height**). Tree height can be estimated or categorized in height class 0 – 2 m; 2 – 5 m; 5 – 10 m; etc.

Post samples to:

James Cleverly

University of Technology Sydney,

UTS Science Store,

Building 1, Level 2, Room 40C,

Thomas St,

Ultimo 2007 NSW

and send an advisory email James Cleverly <James.Cleverly@uts.edu.au>

19.2 Wood Sampling for Isotope Analysis (OPTIONAL)

Take cores (at least five or six centimeters long) from the trunk at 1 m height above the ground. UTS can assist with lending people / core samplers if required.

It is important to keep the spatial relationship along the core intact, even if the core itself breaks into 2 or more pieces, so that we know that as we sample the core along its length, we are moving backwards in time. Wrapping the core in cling-film and binding with tape along its entire length can assist in this. Packing several (labelled) cores into lengths of small diameter (1 cm) plastic water pipe inside a jiffy bag can be used for posting.

Storing in the cold before sending is preferred as this minimizes fungal/bacterial growth during storage.

Sample one core from 3 – 5 replicate trees per species. Label cores with:

1. Species ID
2. Tree size (DBH, H)
3. Date of Sampling.

19.3 Plant Genetic Sampling

Protocol abstracted from the AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012).

Leaf samples will be collected from at least 20 plant species (preferably more) at each core 1 ha plot and dried to enable subsequent genetic analyses. By collecting these samples in synthetic tea bags and gathering a sufficiently large volume of material, one field sample can be sub-sampled to enable both genetic and isotope analyses to be undertaken.

1. Collections may be made during voucher specimen collection or specifically for genetic/isotope studies. If collecting a voucher specimen for identification and lodging in state herbaria (as per the vouchering protocol), take a small sub- sample (equivalent to around 10 cm² or five eucalypt leaves) of green leaves and ensure labelling of sub-sample indicates the link to the vouchered specimen.
2. The collected material (around 10 cm²) should be young and free from disease, insect or fungal contamination wherever possible.

Handle the sample to minimise skin contact and hence contact with other sources of organic carbon (important for the isotope analysis). For broad-leaf plants, hold the leaves by the petiole (leaf stem) or by the base of the leaves for grasses where possible. This sample should then be carefully placed into a synthetic teabag and sealed.

3. Label the teabag with an adhesive voucher label.
4. Place the teabag in a sealable lunch box with 1 cm of silica granules (10% self-indicating mixed with 90% standard non-indicating granules) and seal. If possible a single lunchbox, clearly labelled with the plot identifier should be used for each plot.
5. Over the duration of a sampling trip replace the silica granules when necessary. When the self-indicating granules change colour from blue to pink their moisture absorbing capacity has been reached and the granule mix should be replaced with fresh silica mix. Do not discard the used silica, as it can be oven dried and re-used.

6. Store the container in a cool location out of direct light and then send all samples in a batch to the SuperSite Central:

**M. Liddell
James Cook University,
Building E1 Room 102B,
McGregor Rd.
Smithfield Qld 4878**

Plant genetic samples will be collated and barcode identified to allow integration into the University of Adelaide / AusPlots Rangelands database and repository.

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<http://data.auscover.org.au/xwiki/bin/view/Good+Practice+Handbook/PhenologyValidation>
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22 Appendix

22.1 AusPlots Rangelands Survey Protocols Manual

A number of protocols have been derived or abstracted from the AusPlots Rangelands Survey Protocols Manual (White *et al.* 2012) that can be downloaded from the TERN website at <http://www.tern.org.au/AusPlots-Rangelands-Survey-Protocols-Manual-pg23944.html> with updates periodically available at www.tern.org.au/ausplots-pg17871.html