

An aerial photograph of a vast, dense tropical forest. The trees are lush green and cover a large area. In the background, a range of mountains is visible under a clear sky. The overall scene is a natural, undisturbed landscape.

APPENDIX 2: EXPERIMENTAL DESIGN AND STATISTICAL TESTS

A2.1 Randomised complete block design experiments

All ecological experiments generate highly variable results. Therefore, experiments must be repeated or 'replicated' several times, and the results must be presented as mean values followed by a measure of variation among replicates that are subjected to the same treatment (e.g. variance or standard deviation). Luckily, most of the experiments required for forest restoration research (e.g. germination tests, seedling growth experiments and field trials) can all be set up using the same basic experimental design and the same method of statistical analysis: a 'randomised complete block design' (RCBD), with the results analysed by a two-way analysis of variance (ANOVA) followed by pair-wise comparisons.

What is a randomised complete block design?

Each of the replicated 'blocks' within an RCBD consisting of one replicate of the control, plus one replicate of each of the treatments being tested. Each treatment and the control are represented equally in every block (i.e. by using the same number of seeds, plants etc.). In each block, the positions of the control and the treatments are allocated randomly. The replicate blocks are placed randomly across the study area (or nursery).

Why use RCBD?

An RCBD separates the effects that are due to environmental variability from those of the treatments being tested. Each block may be exposed to slightly different environmental conditions (light, temperature, moisture etc.). This creates variability in the data that can obscure the effects of applied treatments; but as a control replicate and treatment replicates are grouped together in each block, all germination trays or plots within a block are exposed to similar conditions. Consequently, the effects of variable external conditions can be accounted for and the effects of the treatments applied (or the absence of effects) revealed by a two-way ANOVA (see **Section A2.2**).

How many blocks and treatments?

Ideally, the combined number of blocks and treatments used should result in at least 12 'residual degrees of freedom' (rdf) according to the equation below...

$$\text{rdf} = (t-1) \times (b-1)$$

...where t is the number of treatments (including the control) and b is the number of blocks. In reality, it is often very difficult to achieve an rdf of more than 12 in nursery or field experiments because of shortages in the availability of seeds, trees, land or labour. An rdf of <12 can still yield robust results if you ensure as much uniformity among the blocks as possible. Otherwise, you could use a simpler experimental design (e.g. paired experiments, which compare a single treatment with a control) and simpler analytical methods (e.g. Chi-square for germination or survival data (see **Section 7.4**)).

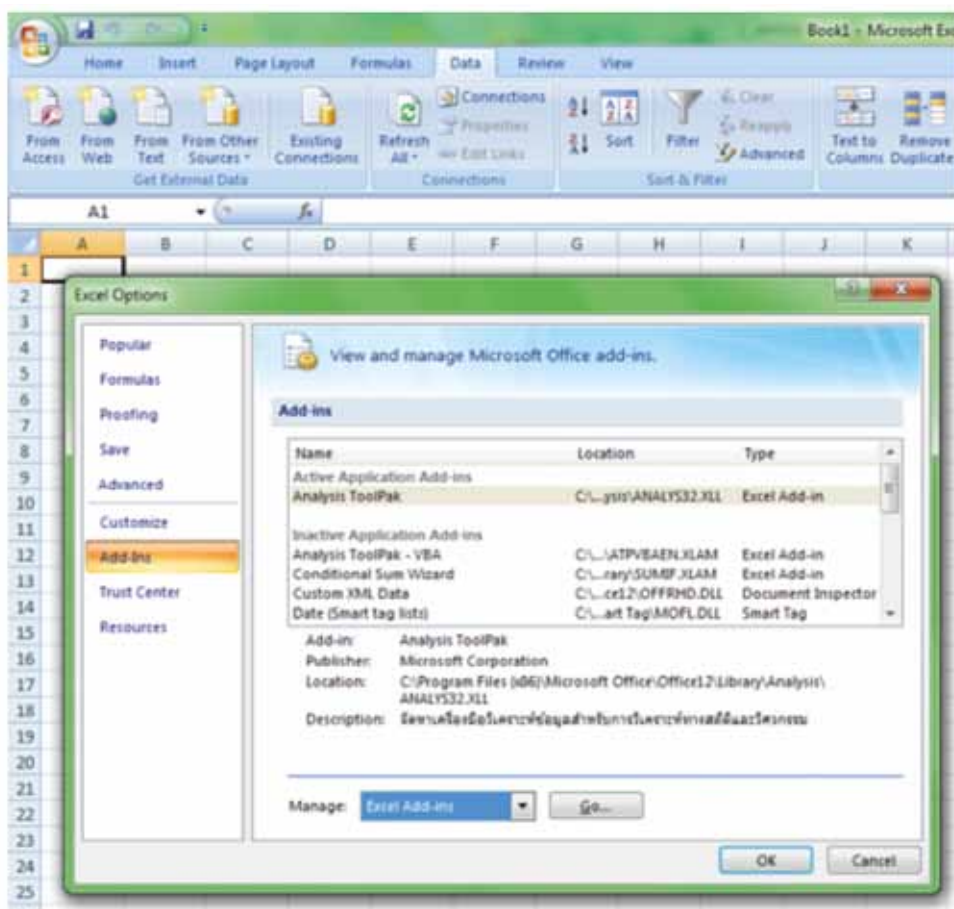
A2.2 Analysis of variance (ANOVA)

Data from RCBD experiments can be analysed by a rigorous standard statistical test called analysis of variance (ANOVA). There are several forms of this test. The one used to analyse RCBD experiments is a 'two-way ANOVA (without replication)'. The 'without replication' part is confusing because treatments are replicated across the blocks, but in statistical jargon, it means that there is only one value for each treatment in each block; for example, for germination experiments, there is one value for the number of seeds germinating in each replicate germination tray.

The simplest way to perform an ANOVA is to use the Analysis ToolPak that comes bundled with Microsoft Excel, so first make sure that you have the Analysis ToolPak installed on your computer.

If you are using Windows XP, open Excel and click on 'Tools' in the toolbar and then click on 'Add-Ins...'. Make sure that the box next to 'Analysis ToolPak' has a tick in it. If the tick box does not appear, you must re-run Excel set-up and install the Analysis ToolPak add-in.

If using Vista or Windows 7, click on the Microsoft Office button (top left), then on the Excel Options button (bottom right of the dropdown menu), then on 'Add Ins' and finally on the 'Go' button next to 'Manage Excel Add Ins'. Tick the box labelled 'Analysis ToolPak'.



APPENDIX 2 – EXPERIMENTAL DESIGN AND STATISTICAL TESTS

The experiments described in **Chapters 6** and **7** generate two kinds of data: i) binomial data, which describe variables that have only two states, e.g. germination (i.e. germinated or not germinated) and survival (i.e. alive or dead); and ii) continuous data (which can have any value), e.g. seedling height, root collar diameter, crown width or relative growth rate. If you are analysing binomial data, you should first arcsine transform the data, for statistical reasons, before carrying out the analysis of variance. If you are analysing continuous data, you can skip the next section and move straight to ANOVA.

Preparing binomial data for ANOVA

Enter your data (e.g. number of germinated seeds or number of surviving trees) in a table as shown below (original data), with blocks as rows and treatments as columns.

The screenshot shows an Excel spreadsheet with the following data:

	A	B	C	D	E	F	G	H	I
1		Control	T1	T2	T3	T4	Original data		
2	Block 1	24	26	30	23	25			
3	Block 2	22	26	31	21	26			
4	Block 3	26	26	35	22	27			
5	Block 4	29	32	30	23	35			
6									
7		Control	T1	T2	T3	T4	Data as percentage		
8	Block 1	48	52	60	46	50			
9	Block 2	44	52	62	42	52			
10	Block 3	52	52	70	44	54			
11	Block 4	58	64	60	46	70			
12									
13		Control	T1	T2	T3	T4	Data arcsine transformed		
14	Block 1	43.85	46.15	50.77	42.71	45.00			
15	Block 2	41.55	46.15	51.94	40.40	46.15			
16	Block 3	46.15	46.15	56.79	41.55	47.29			
17	Block 4	49.60	53.13	50.77	42.71	56.79			
18									

In this example, the original data are the number of seeds germinated (out of 50) in each of 4 blocks for each of 5 pre-sowing treatments: for example, T1 = soaking in hot water for 1 hour, T2 = scarification with sand paper, T3 = soaking in acid for 1 minute, and T4 = soaking in cold water overnight.

Next, construct another table to calculate percentage values: e.g. for the control in block 1, 24 seeds germinated out of 50 sown, so the percentage germinating = $24/50 \times 100 = 48\%$.

Then set up a third table below, to calculate the arcsine-transformed percentages; for example, for the control in block 1 (located in cell B8), type the following formula into the third table:

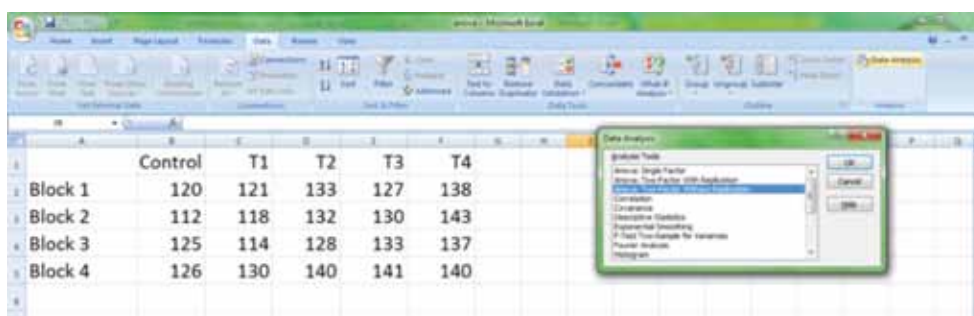
$$=ASIN(\text{SQRT}(\mathbf{B8}/100))*180/\text{PI}()$$

Then, copy the formula into the other cells of the third table. To make sure you have entered the formula correctly, entering 90 in the percentage table. An arcsine-transformed value of 71.57 should be returned in the third table.

Now carry out the ANOVA as described below, using the arcsine-transformed percentages.

ANOVA

In this example, we are using the mean height of trees (cm) 18 months after planting in a field trial plot system (see **Section 7.5**), subjected to different fertiliser treatments. Open a new spreadsheet and type in your data with blocks as rows and treatments as columns, as shown below.



	Control	T1	T2	T3	T4
Block 1	120	121	133	127	138
Block 2	112	118	132	130	143
Block 3	125	114	128	133	137
Block 4	126	130	140	141	140

In this example, the data show tree height (cm). Different fertiliser doses were applied to the trees at planting time and three times in the rainy season: T1 = 25g fertiliser, T2 = 50g, T3 = 75g and T4 = 100g.

Next, if using Windows XP, click on 'Tools' and then on 'Data Analysis...'. With Vista or Windows 7 click on the 'Data' tab at the top of the screen and then on 'Data Analysis' (top right). A dialogue box, containing a list of various statistical tests, will appear. Click on 'ANOVA: Two-Factor Without Replication' and then click 'OK'.

Another dialogue box will appear. Click on the square button to the right of the 'Input Data' box ('Input Range' in Windows 7). Then, using the mouse, drag the cursor across the data table to select the entire data set, including column and row headings. Click on the square button again to get back to the dialogue box, then make sure there is a tick in the 'Labels' box and that the value in the 'Alpha' box is 0.05. Click on the circular button, 'Output Range:' and then on the square button to the right of the output

APPENDIX 2 – EXPERIMENTAL DESIGN AND STATISTICAL TESTS

range box. In the spreadsheet, move the cursor to a cell immediately below your data table and click. Then go back to the dialogue box and click 'OK'. Two tables of output results will appear below your data table. The upper one summarises mean values for each treatment and for each block, along with a measure of variability (i.e. variance). The lower one will tell you if there are significant differences among the treatments.

	Control	T1	T2	T3	T4
Block 1	120	121	133	127	138
Block 2	112	118	132	130	143
Block 3	125	114	128	133	137
Block 4	126	130	140	141	140

	Control	T4
Mean	120.75	139.5
Variance	40.916667	7
Observations	4	4
Pearson Correlation	-0.738603	
Hypothesized Mean	0	
df	3	
t Stat	-4.39155	
P(T<t) one-tail	0.0109377	
t Critical one-tail	2.3533634	
P(T<t) two-tail	0.0218754	
t Critical two-tail	3.1824463	

Full results from the upper table of output results are as follows:

ANOVA: Two-Factor Without Replication.

Summary	Count	Sum	Average	Variance
Block 1	5	639	127.8	59.7
Block 2	5	635	127.0	149.0
Block 3	5	637	127.4	77.3
Block 4	5	677	135.4	47.8
Control	4	483	120.75	40.92
T1	4	483	120.75	46.25
T2	4	533	133.25	24.92
T3	4	531	132.75	36.25
T4	4	558	139.50	7.00

In this example, variances within blocks (among treatments) are generally higher than variances within treatments (among blocks), suggesting that the effects of the treatments are stronger than random variations resulting from differences in conditions among the blocks. It looks like treatments 2, 3 and 4 increase germination compared with the control, whereas treatment 1 has no effect. But are these results significant? The lower table answers this question.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	241.6	3	80.5333	4.3066	0.02799	3.49029
Columns	1110.8	4	277.7	14.8503	0.00014	3.25917
Error	224.4	12	18.7			
Total	1576.8	19				

In this table, ‘rows’ refers to blocks and ‘columns’ refers to treatments. ANOVA tests the ‘null hypothesis’ that there are no real differences among the control and the treatments tested and that any variation among the mean values is just due to chance. Consequently, if large differences among the mean values for treatments and blocks are found, then the assumption will be false, and at least one of the treatments has had a significant effect. The important values to look at are the P-values, which quantify the probability that the null hypothesis (i.e. no differences) is valid. The table, therefore, shows that there is only a 0.00014 in 1, or 0.014% probability that differences among treatments do not exist (and hence a 99.986% probability that they do). Similarly, real differences among the blocks are highly probable (97.2% likely). The significant differences among blocks show that a randomised block design was necessary in order to remove a substantial amount of variation associated with differences in the micro-environments that affect each block. Although this ANOVA shows significant differences among treatments, it does not say which of the differences are significant. In order to determine that, it is necessary to perform a pair-wise comparison. For further information about ANOVA and for a wider choice of analytical techniques, please refer to Dytham (2011) and Bailey (1995).

A2.3 Paired t-tests

If significant differences among mean values are confirmed by ANOVA, pair-wise comparisons are needed to determine which differences are significant. Statistical tests that determine whether the difference between two means is significant include Fisher’s Least Significant Difference (LSD) test, Tukey’s Honestly Significant Difference (HSD) test and the Newman Keuls test. These tests can be performed using statistical software, such as Minitab or SPSS, trial versions of which can be downloaded from the internet¹.

¹ spss.en.softonic.com

APPENDIX 2 – EXPERIMENTAL DESIGN AND STATISTICAL TESTS

In Excel, you can perform a paired t-test using the analysis ToolPak. It is not statistically valid to use this test to compare all means with all other means automatically. Adopt the so-called *a priori* approach, i.e. decide on the questions you want to answer beforehand and only carry out only those tests that answer those questions. In this case, the main question is “do treatments significantly increase or reduce performance compared with the control?”

In ‘Data Analysis’, click on ‘t-test: Paired Two Sample for Means’ and then click ‘OK’. In the dialogue box, click on the square button, to the right of the ‘Variable 1 Range’ box. Then, using the mouse, drag the cursor down the table to select the data set for ‘control’, including the column heading. Repeat for ‘Variable 2 Range’ by selecting the data set for whichever treatment you have decided to test (the screen print below shows the results for ‘control’ compared with ‘T4’). Back in the dialogue box, select a ‘Hypothesized Mean Difference’ of ‘0’ (the null hypothesis being that there is no significant difference between the treatment data). Make sure there is a tick in the ‘Labels’ box and that the value in the ‘Alpha’ box is 0.05. Click on the circular radio button, ‘Output Range:’ and then on the square button to the right of the output range box. In the spreadsheet, move the cursor to a cell immediately adjacent to your data table and click. Then go back to the dialogue box and click ‘OK’. A table of output results will appear adjacent to your data table. Repeat the process for all pair-wise comparisons that you decide will be useful.

	Control	T1	T2	T3	T4
Block 1	120	121	133	127	138
Block 2	112	118	132	130	143
Block 3	125	114	128	133	137
Block 4	126	130	140	141	140

	Control	T4
Mean	120.75	139.5
Variance	40.916667	7
Observations	4	4
Pearson Correlation	-0.738603	
Hypothesized Mean	0	
df	3	
t Stat	-4.39155	
P(T<t) one-tail	0.0109377	
t Critical one-tail	2.3533634	
P(T<t) two-tail	0.0218754	
t Critical two-tail	3.1824463	

t-test: Paired Two Sample for Means.

	Control	T2	Control	T3	Control	T4
Mean	120.75	133.25	120.75	132.75	120.75	139.5
Variance	40.91667	24.91667	40.91667	36.25	40.91667	7
Observations	4	4	4	4	4	4
Pearson Correlation	0.25316		0.629662		-0.7386	
Hypothesized Mean Difference	0		0		0	
df	3		3		3	
t Stat	-3.54738		-4.48252		-4.39155	
P(T<=t) one-tail	0.019081		0.010353		0.010938	
t Critical one-tail	2.353363		2.353363		2.353363	
P(T<=t) two-tail	0.038162		0.020706	sig	0.021875	sig
t Critical two-tail	3.182446		3.182446		3.182446	

The ANOVA results table for tree height in **Section A2.2** above showed higher mean values for treatments 2, 3 and 4 and a similar mean value for treatment 1. For these differences to be significant, the value of 't Stat' must be greater than a critical value determined from the number of degrees of freedom and the acceptable value of P (usually 5%). The significance of the differences is therefore determined by looking at the value for 'P(T<=t) two-tail'. If the value is **0.05 or less**, the difference is significant. It means that there is only a **5% or lower** probability that the null hypothesis (i.e. that the difference between the means is zero) is correct. In the t-test example above, treatments T2, T3 and T4 all satisfy this condition. So the result is that applying 50–100g fertiliser most probably increased tree height compared with the control from around 121 cm to around 133 to 140 cm, depending on the amount of fertiliser used. Applying 25g fertiliser most probably had no effect. You can ignore the other data shown in the t-test table, such as the values for one-tail tests, unless you are confident about interpreting them.

GLOSSARY

Agro-forestry: a plantation design that increases and diversifies the economic benefits from forestry by adding crops and/or livestock to the system.

Accelerated (assisted) natural regeneration (ANR): management actions to enhance the natural processes of forest restoration, focusing on encouraging the natural establishment and subsequent growth of indigenous forest trees, while preventing any factors that might harm them.

Analogue forestry: forestry that uses a combination of domesticated and indigenous forest tree species and other plants to re-establish a forest structure similar to that of climax forest.

Biodiversity: the variety of life encompassing genes, species and ecosystems.

Biodiversity offset: payments made by agencies whose actions destroy or diminish biodiversity in one place that are used to restore biodiversity in another place, thereby achieving no net loss of biodiversity.

Candidate framework species: local tree species undergoing nursery and field performance testing to determine their suitability as framework species.

Carbon credits: payments by carbon emitters (companies, governments or individuals) that are used to finance projects that aim to absorb carbon dioxide from the atmosphere, leading to zero net increase in atmospheric carbon dioxide.

Climax forest: the final stage of forest succession, a relatively stable forest ecosystem having attained the maximum development in terms of biomass, structural complexity and biodiversity that can be sustained within the limits imposed by the soil and prevailing climatic conditions.

Climax tree species: tree species that comprise climax forest.

Community forest: a forest that is managed collectively by local people, usually with the extraction of timber and non-timber forest products.

Conservation: the preservation, management, and care of natural and cultural resources.

Damping off: fungal diseases that attack the stems of young seedlings.

DBH (diameter at breast height): diameter of the tree trunk at 1.3 m above ground level.

Deciduous: shedding leaves annually or periodically; not evergreen.

Deforestation: conversion of forest into other land uses with less than 10% tree cover, e.g. arable land, pasture, urban uses, logged areas, or wasteland.

Degradation: disturbance leading to decrease forest quality and impeded ecological functioning of the forest ecosystem.

Direct seeding: the establishment of trees on deforested sites by sowing seeds rather than by planting nursery-raised saplings.

Dormancy: a period during which viable seeds delay germination, despite having conditions (moisture, light, temperature etc.) that are normally favourable for the later stages of germination and seedling establishment.

Ecotourism: low impact, nature-based tourism that produces positive benefits for the conservation of biodiversity.

Ectomycorrhiza: an association between vascular plant roots and fungi that forms a fungal sheath on root surfaces and between root cortical cells.

Endemic: indigenous to and confined to a particular area.

Enrichment planting: planting trees to i) increase the population density of existing tree species or ii) increase tree species richness by adding tree species to degraded forest; also used to mean restocking logged-over or otherwise degraded forest with economic species.

Epiphyte: a plant growing on (but not penetrating) another plant, e.g. orchids growing on the branch of a tree.

Evergreen: a plant that retains green foliage throughout the year.

Exotic: of species – introduced, not native.

Extinction: the complete loss of a species globally; when no more individuals of a species exist.

Extirpation: the disappearance of a species from a particular area, while it survives elsewhere.

Extractive reserve: designated conservation areas in which natural-resource extraction is carried out complementary to the objective of conserving biological diversity and the natural resource base.

Forest landscape restoration (FLR): integrated management of all landscape functions in deforested or degraded areas to regain ecological integrity and enhance human well-being; usually including some forest restoration.

Forest restoration: actions to re-instate ecological processes that accelerate recovery of forest structure, ecological functioning and biodiversity levels towards those typical of climax forest.

Forest Restoration Research Unit (FORRU): established to develop methods to harness and accelerate the natural processes of forest regeneration, so that biodiversity-rich forest ecosystems, similar to climax forest, can be re-established.

Foster ecosystem: tree plantations of not necessarily indigenous species used to facilitate the natural regeneration of native species.

Framework species method (or Framework forestry): planting the minimum number of indigenous tree species required to re-instate the natural processes of forest regeneration and recover biodiversity. It combines the planting of 20–30 key tree species with various ANR techniques to enhance natural regeneration, creating a self-sustained forest ecosystem from a single planting event.

Framework tree species: indigenous, non-domesticated, forest tree species, which, when planted on deforested sites, rapidly re-establish forest structure and ecological functioning, while attracting seed-dispersing wildlife.

Frugivorous: fruit-eating.

FTPS (field trial plot system): a set of small plots, each one planted with a mixture of different tree species and/or silvicultural treatments using the randomised complete block design (RCBD).

GBH (girth at breast height): circumference of the tree trunk at 1.3 m above ground level.

Gross domestic product (GDP): the total value of all goods and services bought or sold in an economy.

Genetic diversity: diversity within a species.

Geographic positioning system (GPS): a handheld or vehicle-mounted system that uses satellite communications to determine geographical position and other navigational information.

Germination: the growth of seeds or spores after a period of dormancy; emergence of an embryonic root through the seed coverings.

Geographical information system (GIS): Computerised manipulation of maps and other geographical information, useful for the planning of forest restoration projects.

Herbarium: a repository for easy accessible collections of dried, preserved and well-labelled specimens of plants and fungi.

Hypha: a long, branching filamentous cell of a fungus; the main mode of vegetative growth of a fungus; collectively called 'mycelium'.

Indigenous: native to an area, not introduced; the opposite of exotic.

Intermediate seeds: seeds that can be dried to low moisture contents, approaching those of orthodox seed, but are sensitive to chilling when dried.

Keystone tree species: species that flower or fruit at times when other food resources for animals are in short supply.

Maximum diversity/Miyawaki methods of forest restoration: restoring as much of the tree species richness of the original forest as possible without relying on natural seed dispersal.

MLD (median length of dormancy): the time taken from seed sowing of a batch of seeds to germination of half of the seeds that finally germinate; for example, if 10 seeds germinate out of a batch of 100 sown, it is the time to germination of the 5th seed.

Mycorrhiza: symbiotic (occasionally weakly pathogenic) association between a fungus and the roots of a plant.

Natural regeneration: the recovery of forest following disturbance in the absence of human intervention, resulting in increasing ecosystem functionality, vegetation species diversity, structural complexity, habitat availability and so on.

Non-governmental organisation (NGO): a legally constituted organisation created by private persons or organisations with no participation or representation of any government.

Non-timber forest products (NTFPs): broadly includes all non-timber vegetation in forests and agro-forestry environments that have commercial value. They include plants, parts of plants, fungi and other biological materials harvested from natural, manipulated, or disturbed forests. NTFPs can be classified into four major product categories: culinary, floral and decorative, wood-based, and medicinal and dietary supplements.

Nurse tree species: extremely hardy, usually fast-growing pioneer tree species planted specifically to restore environmental and soil conditions that are favourable for the establishment of a broader range of indigenous forest tree species.

Orthodox seeds: seeds that are easy to store for many months or even years.

Payments for environmental services (PES): compensating those involved in forest restoration or conservation for carbon storage, watershed protection, conservation of biodiversity and all the other environmental services provided by restored or conserved forest.

Phenology: the study of the responses of living organisms to seasonal cycles in environmental conditions, e.g. the periodic flowering and fruiting of trees.

Pioneer tree species: early-successional species that germinate only in full sun or the largest gaps. They exhibit high photosynthetic and growth rates, have simple branching patterns, and require high temperature and/or high light intensity for germination. These species are usually short-lived and are characteristic of pioneer forest.

Primary forest: climax forest that has not been substantially disturbed in recent history.

Production schedule: a concise description of the procedures for producing planting stock of optimum size and quality from seed (or wildlings) by the optimum planting out time. This timetable combines all available knowledge about the reproductive ecology and cultivation of a species.

Protected area: an area of land and/or sea that is especially dedicated to the protection and maintenance of biological diversity and of natural and associated cultural resources that is managed through legal or other effective means.

Rainforestation: a forest restoration technique, developed in the Philippines, that uses indigenous tree species to restore ecological integrity and biodiversity while also producing a diverse range of timbers and other forest products for local people.

RCBD (randomised complete block design): experimental design where single replicates of each treatment and the control are randomly positioned within a 'block', with each block being replicated in at least 3 locations across the study site.

RCD (root collar diameter): diameter of the plant stem at the root collar, just above the level of the soil.

Recalcitrant seeds: seeds that are sensitive to drying and chilling.

Recruit species: additional (non-planted) tree species that establish naturally in forest restoration sites.

Reforestation: planting trees to re-establish tree cover of any kind; includes plantation forestry, agro-forestry, community forestry and forest restoration.

Remnant forest: small areas of forest that survive in a landscape following large-scale deforestation.

RGR (relative growth rate): a measurement of plant growth rate that takes into account the plant's initial size.

Root collar: the point at which the above-ground parts of a plant meet the tap-root.

Secondary forest: a forest or woodland area that has re-grown after a major disturbance but is not yet at the end point of succession (climax forest), usually distinguished by differences in ecosystem functionality, vegetation species diversity, structural complexity and so on.

Seed bank: all of the seeds, often in a dormant state, that are stored within the soil of many terrestrial ecosystems. A seed bank can also refer to the storage of collected seeds as a source for forest restoration activities.

Seed rain: the movement of seed into an area through natural processes. This can occur through various mechanisms of dispersal, including wind and animal dispersal.

Senescent leaves: leaves that are losing their chlorophyll (and hence green colour) just before leaf fall.

Silviculture: controlling the establishment, growth, composition, health, and quality of forests to meet the diverse needs and values of landowners.

Site recapture: elimination of herbaceous vegetation by the shading effects of planted trees or by ANR.

Stakeholder: anyone affected by or involved in a forest restoration project.

Target forest: a forest ecosystem that defines the goals of a forest restoration program in terms of tree species composition, structure, and biodiversity levels and so on; usually the nearest surviving patch of climax forest that remains in the landscape at a similar elevation, slope, aspect etc. to those of the restoration site.

Vesicular arbuscular mycorrhizas (VAM): mycorrhizal fungi that grow into the root cortex of the host plant and penetrate root cells, forming two kinds of specialised structures: arbuscules and vesicles. Also known as arbuscular mycorrhizas.

Voucher specimens: dried specimens of tree leaves, flowers and fruits etc. that are kept for confirmation of species names (from phenology-study trees, seed-collection trees etc.)

Wildings: seedlings or saplings growing naturally in native forest that are dug up to be grown on in a nursery.

Wildlife: all non-domesticated plant and animal species living in natural habitats.

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INDEX

4 o'clock moth caterpillar, 81

A

above-ground carbon (AGC), 238
 abscisic acid, 168
Acacia, 38, 46
 mangium, 84, 144
 polyphylla, 22
 accelerated natural regeneration, see 'ANR'
Acer, 161
 Acre, 114
Acrocarpus fraxinifolius, 195
Acronychia, 46
 adaptive management, 91, **286**
 aerial seeding, 134
 agro-ecosystems, 136
 agro-forestry, 21, 91, 135, 214
 aid agencies, 275
 air pruning, 172, 180, 187
 air quality, 19
Alagoas curassow, 9
 alleles, 159
 allelopathy, 56–7
 alley cropping, 21
 allometric equations, 238
Alstonia, 46, 47
 alternate stable states, 48
 Amazon, 3, 20, 21, 24, 43, 59, 62, 93, 114, 138
 analogue forestry, 69–70
 analysis of variance (ANOVA), 202, 209, 246–8, **309–13**
 Andes, 39, 40
Andropogon, 57
 animal diversity, 126
Annona reticulata, 156
 ANR (accelerated natural regeneration), 12, 13, 68–70, 77–8, 106, **118–123**
 costs, 147
 limitations, 123
 origins, 119
 techniques, 121–3
 ants, 37, 52
 Apocynaceae, 28, 37
 arthropods, 6
Artocarpus heterophyllus, 156
 Asteraceae, 56–7
 Atherton Tablelands, 96–7
 Australia, 13, 14, 95, 96–7, 124, 129, 134, 137, 144, 146

B

Baccaurea ramiflora, 30
 badgers, 51
 Bagong Pagasa Foundation, 119, 147
 bamboo, 15, 33, 35, 36
 Ban Mae Sa Mai, 118, **272–4**, 294
 bare-rooted trees, 171
 bees, 37
 beetles, 6
 Belize, 57
 benefit sharing, 91, 101, **102**
Bhesa ceylanica, 47
 Bignoniaceae, 22
 binomial data, 310–1
 bio-banking, 16
 biodiversity (see also 'genetic diversity'), 32
 conservation, 13, 136, 139, **267–8**
 estimating, 6
 loss, 5–10
 market value, 15
 monitoring wildlife, 252–7
 of animals, 126
 recovery, 12, 76, 118, 120, 124, 126, 130, 134, 295
 research, 251–9, 269
 biodiversity credits, 16
 biodiversity offsets, 15–7
 biomass (above-ground), 239
 bird population monitoring and analysis, **253–5**, 295, 306
 bird perches, 123
 blight (disease), 183
 Borneo, 59
Brachiaria grass, 21
 bracken fern, 56–7
 Braun–Blanquet scale, 258
 Brazil, 2, 3, 4, 5, 9, 10, 20, 32, 93, 113–4, 140–1, 143, 147
 bromeliads, 28
 budgets, **105**, 107
 buffer zones, 21, 82, 93, **98–9**, 136
 bulbs, 50, 118, 255
 Burma, 9
 burning, recovery from, **116**, 124
 buttresses, 28

C

Calotes liocephalus, 8
 Cambodia, 2

- camera trapping, 256
 Cameroon, 5, 30, 59
 canker (disease), 183
 canopy, 29
 closure, 126, 138, 140, 144, 235, 294
 width, 282–5
Capparis tomentosa, 261
 Captan, 169–70, 178, 184
 carbon,
 carbon dioxide, 10, 17, 61
 carbon standards organisations, 237–8
 credits, **17–8**, 108, 238–9
 compliance credits, 17
 markets, 17–8, 238–9
 REDD+, 18, 101, 231, 268
 trading, 17, 91, 102, 147, 216
 voluntary credits, 17, 108
 emissions, 17–8
 foot prints, 17
 monitoring carbon accumulation, 237–9
 offset schemes, 231, 237, 268
 sinks, 17, 108
 storage, 10, 13, **17–8**, 42, 62, 108, 146, 237–9, 239, 247
 carbon-based global economy, 17
 CarbonFix, 238
 cardamon, 8
 cardboard (see also ‘mulch’), 224–6, 230
Cardwellia sublimis, 80
Casuarium casuarium johnsonii, 96
 cattle, 3, 10, 20, 50, 58, **117**, 149
 domestic, 58, **117**, 149
 ranching, 3, 10, 20, 117, 149
 wild, 50, 58
 CBD (Convention on Biological Diversity), 109, **267–8**
 CDM (Clean Development Mechanism), 17, 268
Cecropia, 22, 46
Ceiba, 46
Cerasus cerasoides, 213
Cercopithecus diana, 8
 Chairuangsri, Sutthathorn, 81
 charcoal,
 deposits in soil profile, 59
 for seed drying, 165
 making, 4, 260
 Chiang Mai University, 14, 293
 Chico Mendes Reserve, 114
 children (involving), 214–5
 China, 36, 134
 Chi-square test, 236, 237
Chromolaena, 57
 odorata, 56
 civets, 51, 256
 Clean Development Mechanism (CDM), 17, 268
 climate change, 2, 7, 10, 33, 59, 60, **61–3**
 adaptation to, 90, 159
 mitigation of, 17–18
 climate regulation, 19
 Climate, Community & Biodiversity (CCB) standard, 238
 climates,
 everwet, 25, 54, 63
 seasonally dry, 25, 54, 63, 124, 157, 212
 climax forest, 12–3, **45–8**, 63, 88–9, 123
 climax species, **45–7**, 49, 53, 60, 119–20, 125–7, 131, 135, 138, 285
 cloud stripping, 40
 CO₂, 10, 17, 61
 coconut husk (see also ‘growth media’), 166, 204
 Collaborative Partnership of Forests (CPF), 109, 275
 Columbia, 117
 communication strategy, 290–2
 community forestry, 112, 272–4, 293
 Compositae (Asteraceae), 56–7
 composite provenancing, 159–60
 conflict resolution, 91
 Congo Basin, 24, 43
 conifers, 182
 connectivity, 90
 conservation (see also ‘biodiversity’), 136, 139, **267–8**
 containers, 171, 176, 204
 coppicing, **49**, 116, 122, 124
 Corporate Social Responsibility, see ‘CSR’
 corporate sponsorship, 108, 141
 corridors, 90, **95–8**, 100, 136
 Costa Rica, 18, 95, 117, 146, 149–51
 costs and benefits of forest restoration (see also ‘forest restoration’), **146–8**, 273–4
 cotyledons, 156
 Cristalino Ecological Foundation (FEC), 20
 Cristalino State Park, 20–2
 cross-pollination, 158
 crown density method, see ‘phenology, scoring’
 crows, 50
 CSR (Corporate Social Responsibility) schemes, 108, 237
 cultivation, 45
 cultural services, 19
 cut and carry, 117
 cuttings, 170, 178–9

INDEX

D

damping-off diseases, 167, **169**, 170, 183–4
Darwin Initiative, 275, 295
data collection sheets, 158, 168, 197, 200, 211, 245, 254, **297–306**
databases, **276–81**, 286
decision support tools, 92, 277
deep ripping, see ‘soil ripping’
deer, 50
deforestation, **1–11**, 15, 20, 48, 61, 114, 293
degradation, 4, 10, 18, 20, 26, 37, 59, 65
 stages, **67–79**, 147–8
 Stage 1, **67, 76**, 147
 Stage 2, **68, 76**, 147
 Stage 3, **69, 77**, 147
 Stage 4, **70, 78**, 147
 Stage 5, **71, 78**, 147
Denaghy’s corridor, 96–7
Dennstaedtiaceae, 57
Dialium guianense, 22
Dicranopteris linearis, 57
Dipterocarpaceae, 29, 50, 37, 135, 182
Dipterocarpus, 47, 161
Dipteryx odorata, 22
direct seeding, 12, **130–3**, 203, **227**, 261
 cost, 251
 research, 248–51
 techniques, 228
Dischidia major, 37
Dischidia nummularia, 37
diseases (in nursery), 169, **183–4**, 186
 blight, 183
 canker, 183
 controlling, 184
 damping-off, 167, **169**, 170, 183–4
 Fusarium, 169
 leaf spot, 183
 Phytophthora, 169
 Pythium, 169
 Rhizoctonia, 169
 root rot, 183
 rust, 183
disturbance thresholds,
 threshold dynamics, 48
 site- and landscape-critical thresholds, **66**, 67–71, 75
diversity, see ‘biodiversity’ and ‘genetic diversity’
Doi Mae Salong, 214
Doi Suthep-Pui National Park, Thailand, 14, 272, 293
Domin scale, 258
dormancy, see ‘seed(s), dormancy’
doves, 50

drainage, 142
Dugan, Patrick, 119

E

East Africa, 32, 262
ecological adaptability, 89–90
ecological services, 12, 29, 124
economic species, 16, 146
ecosystem,
 composition, 45
 functioning, 14, 124
 services, 19, 91
 payment for (PES), 147
 structure, 45, 124
ecotourism (see also ‘tourist industry’), 13, 16, 91, 102, 118, 147, **273**
Ecuador, 28
Eden Project, 118
edge effects, 93, 138
education,
 campaigns, 118, 214–5, 274, **286–9**
 materials, 288–9
elephants, 32, 50, 58, 90
embryo (of seed), **156**, 157, 160–1, 166, 168
emergent trees, 47, 50
encroachment prevention, 111
endangered species (see also ‘extinction’ and ‘extirpation’), 8, 126, 139
endocarp, 156, 160, 198
endosperm, 156–8
enrichment planting, 70–1
Environmental Leadership & Training Initiative, 137
environmental protection, 12–3, 91, 136
epiphytes, 33, 37, 39–40, 257
 epiphytic ferns, 28
equilibrium relative humidity (eRH), 163–4
erosion prevention, 19, 41, 100
essential oils, 15
Eubenangee Swamp National Park, 80
Eucalyptus,
 camaldulensis, 146
 robusta, 84
Eupatorium, see ‘*Chromoleana*’
European Union (EU), 275
evapotranspiration, 24
exotic species, 84–5, 87, **143–4**
experiment plans (field), 244
experimental design,
 for direct seeding, 248–51
 for germination trials, 198–9
 for tree performance trials, 240–4

- experiments (see also 'research', 'Field Trial Plot System' and 'randomised complete block design'),
 analysis of, 309–15
 blocks, 198–9, 243, 308
 controls, 198–9, 203, 243, 259, 308
 plots, 241–3, 261
 treatments, 198–9, 202, 244, 248, 308
- extension services, 286–9
- extinction, 10
 extinct species, 6, 7
 rates of, 6, 7, 10
 threatened species, 8
- extirpation, 94–5
 of plant species, 67–8, 278
 of seed dispersers, 32, 41, 58, 76–7, 90, 117, 123, 137
- extractive reserves, 113–4
- F**
- Fabaceae, see 'Leguminosae'
- FAO (United Nations Food and Agriculture Organisation), 2, 118–9
- FEC (Cristalino Ecological Foundation), 20
- fences, 117
- fertilisation of flower, 156
- fertiliser application,
 in the field, 104, 106, 122, 130, 134, 142, **224–6**, 230–1
 in the nursery, 166, 170, **181–2**, 204, 212
- fertilisers, 170, 225
 green manure, 38, 142
 Osmocote, 170
- Ficus*, 28, 29, 31, 129, 139, 144–5, 166, 191, 220
congesta, 97
 propagation of, 170
- Field Trial Plot System (FTPS), **240–5**, 252, 254
 data analysis, 246–8
- fig, see '*Ficus*'
- fig wasps, 31
- fire, 35, 38, 40, 45, 48, 54–6, **59–61**
 causes, 60
 control, 150–1
 effect on forest regeneration, 60, 66
 effect on germination, 60–1
 effect on seedlings, 60–1
 for site clearance, 219
 prevention, 105–7, 112, **116**, 130
 fire breaks, 104, 112, **115**
 risk, **66–71**, 75
- fire-climax grasslands, 41, 60
- firewood, 15, 40
- flat-headed cat, 8
- Fleroya rubrostipulata*, 261
- flooding (see also 'water-logging' and 'drainage'), 11
- flowering phenology, see 'phenology'
- flower-peckers, 37
- fog drip, 40
- food plants, 19, 21
- foreign organisations, 271
- forest classification systems, 25–6
- forest cover, 2, 7, 18
 in landscape, **66–71**, 75
- forest degradation, see 'degradation'
- forest destruction, see 'deforestation'
- forest fragmentation, **93–4**, 159
- Forest Landscape Restoration (FLR), 13, **90–4**
- forest products, 11–3, **15–6**, 91, 102, 113, 147, 273
- forest remnants, 2, 12, 41, 48–9, 64, 66–70, 75, 77, 81, 88–9, **93–9**, 124, 134, 149, 241
- Forest Restoration Research Unit, see 'FORRU'
- forest restoration, 10, **12–9**
 aims, 74, **88–90**
 benefits, **15–9**, 67–71, 95, 147–8, 273
 ecological, 95, 273
 economic, **15–9**, 67–71, 147–8, 273
 intangible, 273
 sharing, 91, 101, **102**
- challenges facing, 32, 35, 37, 41
- costs, 107–8, 146–8
- definition, 12
- for carbon storage, 17, 18
- for local communities, 16, 260–2, **270–4**
- labour requirements, see main 'labour' entry
- measuring success, 12
- need for intervention, 63–4
- strategy, 67–71
- tasks, 106
- where is it appropriate? 13
- forest succession, see 'succession'
- forest types, 24–45
Albizia–Combretum woodlands, 260
 boreal, 26
 caatinga, 37
 cerrado, 32
 chaco, 37
 cloud forest, 25, 39, 40, 41, 149
 coastal dry, 37
 deciduous, 23, 25, 26, 53, 239
 dipterocarp, 37
 degraded (see also main 'degradation' entry), 26
 disturbed, 24, 34

INDEX

- dry, 34, 37, 61, 149, 239
 - sclerophyllous, 26
 - elfin, 39
 - evergreen, 20, 23–5, 26, 28, 29, 32, 53, 59, 134, 214
 - heath, 25, 41
 - Jaragua*, 151
 - limestone, 25, 42
 - lowland, 25
 - dry forest, 20
 - evergreen broadleaf forest, 26
 - evergreen rain forest, 20, 25
 - mangrove, 25, 26, 43
 - marsh, 43
 - miombo woodland, 37, 146
 - mixed needleleaf–broadleaf forest, 26
 - modified natural, 26
 - monsoon, 25, 32
 - montane, 5, 25, 39–41, 53, 117
 - lower montane rain forest, 25, 26, 34, 39
 - upper montane, 25, 26, 34, 39
 - moor, 42
 - mossy woodland, 39
 - needleleaf, 26
 - plantations, 24, 26
 - exotic species, 26, 144
 - native species, 26
 - primary, 2, 7, 10, 14, 26
 - managed, 26
 - rain forest, **23–30**, 149, 239
 - savanna, 53
 - seasonally dry, 32–6, 50, 59, 104, 157, 214, 239
 - seasonally flooded, 20, 21
 - secondary, 3, 7, 10, 26, 120, 123, 150
 - semi-deciduous/semi-evergreen, 22, **25–7**, 32–5, 53
 - sparse trees (parkland), 26
 - subalpine, 25
 - Sudanian woodland, 37
 - swamp, 25, 26, 43
 - freshwater, 25
 - peat swamp, 25, 42
 - periodic swamp, 25
 - sago swamp, 43
 - temperate, 26
 - thorn, 26
 - white sand formations, 20
 - Forestry Research Institute of Beipiao, 134
 - Forestry Research Institute of Guangdong Province, 134
 - FORRU (Forest Restoration Research Unit),
 - facilities, 267
 - organisation, 264–7
 - setting up, 263–95
 - staffing, 264–6
 - FORRU-CMU, 137, 147, 174, 178, 214, 272–4, 287, 293–5
 - fossil fuels, 10, 17, 61
 - foster ecosystems, 78, 142
 - fragmentation, see ‘forest fragmentation’
 - framework species method, 13, 21, 69–70, 77–8, **124–37**
 - costs, 147
 - direct seeding, 131–4
 - for corridors, 96–7
 - for stepping stones, 98
 - in Uganda, 260–2
 - limitations, 134, 138
 - origins, 80–1
 - framework species,
 - as nurse trees, 145
 - characteristics, 90, **124–5**
 - lists of species, 129, 294
 - pioneer or climax species? 156–6
 - selection of/candidate species, **126–9**, 191, 282–6
 - use of, 214–5, 295
 - Frankia* bacteria, 143
 - fruit(s),
 - ripeness, 157
 - set index, 196
 - structure, 156
 - types,
 - dehiscent, 157, 161
 - indehiscent, 161
 - nuts, 15, 161
 - samaras, 161
 - fruiting phenology, see ‘phenology’
 - funding (see also ‘grants’),
 - aid agencies, 275
 - foreign government funding, 275
 - fundraising, **108–9**, 271, 275
 - fungal diseases, see ‘diseases’
 - fungicides, 169–70, 170, 184
 - Captan, 169–70, 184
 - Thiram, 178
 - Fusarium*, 169
- ## G
- Gadgarra State Forest, 96–7
 - gaps, 44–5
 - Garcinia hermonii*, 47
 - GDP (gross domestic product), 11, 15
 - genetics
 - gene flow, 159–60
 - gene mutation, 159
 - genetic diversity, 89–90, 156, 158, 174, 178
 - genetic isolation, 94

- genetic resources, 19
- Geographical Information System (GIS), 111
- geology, 92
- germination (see also 'seed treatments'),
curves, 201
data collection sheets, 168, 200, **300**
data management, 200, 279
conditions necessary for, 54
effect of animal digestion, 55
effect of fire, 60–1
in micro-sites, 54
in the nursery, 156, **166–8**
medium, see 'growth media'
monitoring, 168
natural 53, 56, 63, 126,
nursery area, 153
process, 168
research, 196–203, 213
timing, 54
- Gleicheniaceae, 57
- global warming, see 'climate change'
- glyphosate, 36, **217–9**
- Gmelina arborea*, 144, 146, **149–51**
- goats, 117
- golden lion tamarind, 9
- governance, 111
- Gramineae (Poaceae), 36, 55–7
- grants,
aid agencies, 275
private sector, 108
- grassland, 45–6, 48, 120
- green image, 141
- green manure, 38, 142
- groundwater, 11
- growth curves, 207
- growth media,
for germination, 166–7
for potting, **172–3**, 176, 204
for rooting, 179
organic matter, 166, 172–3, 204
sand, coarse, 166, 172–3
soil, 143, 166, 172–3, 205, 228
- Guanacaste, Area de Conservacion (ACG), 117, 142,
144, **149–51**
- Gurney's Pitta, 9
- Gymnosporia senegalensis*, 261
- H**
- habitat destruction, 8
- Hacienda Santa Rosa, 149
- Hallwachs, Winnie, 149–51
- handouts, 288, 291
- harapan, 16
- hardening off, 104, 174, **186**, 213
- Hawk-moths, 37
- herbarium,
facilities, 264
specimens, 158, 190–1, 210
techniques, 190
- herbicides, 122, 134, **217–9**
glyphosate (Roundup), 36, **217–9**
- herbivores, 58
- highways, 3, 11, 40, 93–4, 111
- Himmapaan Project, 16
- Hmong, 272
- hog badger, 256
- Homalanthus novoguineensis*, 80–1, 146
- hormones, 168, 170, 178
abscisic acid, 168
indole-3-butyric acid (IBA), 178
naphthalene-1-acetic acid (NAA), 178
- hornbills, 50
- horticulture, 40
- Hovenia dulcis*, 50
- human population, 4, 7, 61
in landscape, 92
- hummingbirds, 37
- hunting, 7, 32, 40, 90, 118, 130, 150, 256
- hygrometers, 163–4
- Hyparrhenia rufa*, 149
- I**
- IGES-Japanese Centre for International Studies in
Ecology (JISE), 140
- Imperata*, 57, 119, 121, 135
- inbreeding, 7, 94, 158
- income, from restoration projects, 273
- India, 32
- indigenous knowledge, 128–9, 270, 277, 294
- indole-3-butyric acid (IBA), 178
- Indonesia, 2, 10, 16, 24
- industrial agriculture, 4, 10
- information management, 276–86
- Inga*, 21
vera, 143
- insecticides,
Pyrethrin, 184
- insects, 58
- invasive plants, 35, 144
- lprodione, 184
- Irian Jaya, 42, 43
- irrigation, see 'watering'

INDEX

ITTO (International Tropical Timber Organisation), 26, 92, 268, 275

IUCN (World Conservation Union), 92, 114, 214

J

Janzen, Daniel, 149–51

Japan Overseas Forestry Consultants Association (JOFCA), 119

Japan, 140

jays, 50

K

Kaliro District, 260–2

Kenya, 38, 140

Kew (Royal Botanic Gardens), 20–1, 161, 165, 190, 266

keystone species, 12, 31, 139, 191

Kuaraksa, Cherdsak, 170

Kyoto Protocol, 17

L

labour, 105–8

costs, 105, 107–8

requirements, 105–8

Lake Barrine National Park, 96–7

Lake Eacham National Park, 80–1, 96–7

land ownership, 101

landscape character (see also 'Forest landscape restoration'), 92–3

landslides, 41, 99

prevention of, 136

Lantana camara, 57

late successional species, 47

laws, 111

leaf spot (disease), 183

leafing phenology, see 'phenology'

Leguminosae (alternative name Fabaceae), 21, 22, 56–7, 61, 117, 129, 131, 142, 144, 157, 160, 182, 220

Leontopithecus rosalia, 9

leopard cat, 256

Leucaena leucocephala, 57, 144

livelihoods, 112–3

Livelihoods and Landscapes Strategy (IUCN Initiative), 214

livestock, 8, 11, 35, 58, **117**, 150

local communities, 16, 91, 105, 112–4, 124, 128–9, 215, 261, 270–4, 294

lodging (weed pressing), 119, **121–2**, 217, 230

logging, 4, 8, 40, 45, 150, 293

logos, 291

Luehea seemannii, 6

M

Macaranga, 46–7

macaws, 50

MacKinnon list method, 255

Madagascar, 82–5

Malawi, 2, 146

Malay Peninsula, 24

Malaysia, 8, 140

Maliau Basin, 24

mammal populations, monitoring, 256–7

Mangifera indica, 47

manuals, 288–9

Margraf, Josef, 135

masting, 190

Mato Grosso, 3, 20

maximum diversity methods, 70–1, 78–9, 98, **137–41**

costs, 147

limitations, 138

techniques, 137–40

maximum diversity patches, 99

media (for communication), 292

medicinal resources, 19, 261–2

medium, see 'growth media'

Mekong, 43

Melochia, 46

Mendes, Chico, 113–4

methane, 10, 61

methods of restoration, 103, **110–51**

Mexico, 117

microbial inoculation, 134, **143**

micro-climate, 142–3

Microsoft Access, 277

Mikania micrantha, 57

Milan, Paciencia, 135

Milliken, William, 22

Mimosa pigra, 57

minimum standards, 282–6

mining, 40, 79, 82–5, **138–9, 142–5**

Mitsubishi, 141

Mitu mitu, 9

Miyawaki method, 139, **140–1**

costs, 147

Miyawaki, Akira, 140

monitoring, 104–6, 237

costs, 107

of bird populations, **253–5**, 295, 306

of carbon accumulation, 237–9

of germination, 168

of mammal populations, 256–7

of recruitment, 257–9

of tree growth and performance, 216, **231–9**, 244

of weed control, 235
 of wildlife, 252–7
 use of photography in, 231
 mulch, 38, 121, 137, 142–3, **226**
 cardboard mats, 224–6, 230
 multipurpose trees, 261
Musanga, 46
 mycorrhizal fungi, 143, 166, 172, **182**, 187, 205
 ectomycorrhiza, 84–5, **182**
 inoculation, 143, **205**, 228
 vesicular arbuscular (VAM), 182

N

naphthalene-1-acetic acid (NAA), 178
 National Biodiversity Strategy & Action Plan, 267
 national parks, 269
 natural colonisation, 16
 natural regeneration, 12, **45–61**, 118, 150
 by coppicing, 49
 from seed, 49, 117
 limits to, 48
 of large areas, 48
 on-site sources, **66–71**, 73–5
 natural resource management, 91
 natural selection, 159
Nauclea orientalis, 80
Neolamarckia, 46
 Neotropics, 37
 Nepal, 57, 117
 New Guinea, 24
 newsletters, 290, 292
 Nigeria, 2, 28
 nitrogen fixation, 21, 84–5, 129, 131, 144, 187
 Rhizobium bacteria, 143, 172
 non-timber forest products (NTFPs), see ‘forest products’
 nurse trees/plantations, 71, 78–9, **139**, **142–6**
 costs, 147–8
 in Costa Rica, 149–51
 nursery (see also ‘research’),
 design, 154
 disease control, see ‘diseases’
 essential features, 153
 establishment of, 104, **153–5**
 location, 153
 pest control, 184–5
 production (see also ‘propagation’), 107
 costs of, 107
 schedules for, 212–3, 289
 quality control, 185–6
 record keeping, **188**, 212, 279–80, 302

tools, 155
 total nursery time (TNT), 280, 284
 watering, **180–1**, 188
 nuts, 15, 161

O

objectives, defining, 88, 102
Ochroma, 46
Octomeles, 46
 oil palms, 4, 8, 9, 19
 old growth forest, 47
 Old world tropics, 37
 Open Office, 277
 Orchidaceae, 28
 orchids, see ‘Orchidaceae’
 organic matter, see ‘growth media’ and ‘soils’
 orthodox seeds, **161–3**, 203
 Osmocote, 170
 ovary (of flower), 156
 overgrazing, 11

P

paired t-tests, 313–5
 Palawan Island, 119
 palm oil, 4, 8, 9, 19
 pamphlets, 288, 291
Panicum, 57
 parent trees, 49, **158–60**, 178
 parrots, 50
 Double-eyed fig parrot, 29
 pasture, 149–51
 pathogens, see ‘diseases’
 patrols, 111
Pennisetum, 57
 pericarp, 156, 168
 PES, (payments for ecosystem/environmental services),
 147
 pests, see ‘nursery, pest control’
 pharmaceuticals, 15
 phenology, 89, 157, **190–6**
 data collection sheet, 193–4, **298**
 data management, **193–6**, 213, 278
 of leafing, 39, **193**
 of flowering and fruiting, 157, **192**
 profile graphs, 195
 research, 196, **252–3**
 scoring, 191–3
 trails, **191**, 267
 Philippine Forestry Education Network, 137
 Philippines Department of Environment & Natural
 Resources (DENR), 119, 137

INDEX

Philippines tarsier, 135
Philippines, 16, 118–9, 135–7, 147, 277
photography,
 in communication, 291
 in monitoring, 231
photosynthesis, 17
Phragmites, 57, 121
Phytophthora, 169
pigeons, 50
 Wedge-tailed green pigeon, 50
pigs, wild, 51
pioneer species, **45–7**, 49, 60, 119–20, 125–7, 131,
 135, 144, 285
Pitta gurneyi, 9
plagioclimax, 48
Plan Vivo, 238
planning forest restoration, see ‘project plan’
Plant a Tree Today (PATT), 214
plant communities, 11
plant grading (in nursery), 185–6
plant growth hormones, see ‘hormones’
plantation forests, 4, 10, 12, 17, 24, 87, 144
 as catalysts, 78, **142–6**
planting (trees), 13, 104–6, 120, **216–27**
 density (see also ‘tree planting, spacing’), 219–20
 equipment, 221
 events, 104, **222**
 method, 223–5
 number of species, 220
 quality control, **227**, 283
 spacing, 219, **223**
 timing of, 212, **217**
 trials, 240–8
 watering, 225
plumule, 156
Poaceae, 36, 55–7
point-count method, 255
policy, national forest, 267–8
pollen dispersal, 159
 pollinators, 7, 29, 37, 54
pollination, 156, 191
polymer gel, see ‘water absorbing gel’
potential natural vegetation (PNV), 140
potting, **171–3**, **176–7**
poverty reduction, 13, 214, 273
pricking out, 166, **176**, 180, 212
primary forests, 2, 7, 10, 14, 26
primary forest species, 7
primates, 29, 51
Prionailurus planiceps, 8
Project Design Document (PDD), 237

project plan, 86–109, **101**, 104
Projecto Parque Nacional Guanacaste (PPNG), 149
propagation, **166–89**, 279–80
 data sheets, 211, 300–2
protected areas, 13, 112, **269–1**
protection of site, 67–71, 76, **111–8**, 118, 130, 147
provisioning services, 19
pruning, 174, **187–8**, 204, 211
Psorospermum febrifugum, 261
Psychotria, 47
Pteridium aquilinum, 56–7
Public Utilities Company of Heredia, 18
publications, 288–90
pyrenes, 156, 160, 198, 213
Pyrethrin, 184
Pythium, 169

Q

QIT Madagascar Minerals (QMM), 82–5
Queensland Parks and Wildlife Service, 96
Queensland Wet Tropics World Heritage Area, 80, 124
Queensland, 13, 80–1, 96–7, 137

R

R8 (seed predator repellent), 134
Rabenantoandro, Johny, 85
radicle, 156
rainforestation, 16, **135–7**
randomised complete block design (RCBD), 198–9, 204,
 243, **308**
 data analysis, 246–8, **309–15**
rapid site assessment, **72–9**, 297
rare species, see ‘endangered species’
rats, 52
rattan, 15
recalcitrant seeds, **161**, 165, 203
recovery chamber, 175
recreation and tourism, 19
recruitment, 123–42
 monitoring, 257–9
REDD+ (Reducing Emissions from Deforestation and
 forest Degradation), **18**, 101, 231, 268
Reduced Impact Logging (RIL), 145
reference site surveys, **88–9**, 128–9, 257–8
regenerants, 66–78, 118–25, 127
regulating services, 19
Rehdera trinervis, 151
reintroduction, 9, 67–8, 76
relative growth rate (RGR), **208**, 247
relocation, enforced, 112

- remnants, see 'forest remnants'
- research (see also 'germination trials', 'phenology', 'tree performance'), 126, 128, databases, 276–81
 in the field, 189–196, 240–259
 in the nursery, 196–213
 programme (see also 'FORRU'), 264–95
 publications, 290
 selection of species for study, 126–9, 189, 261
- restoration, see 'forest restoration'
- rhinoceros 32, 50, 57, 58, 90
- Rhizobium* bacteria, 143, 172
- Rhizoctonia*, 169
- rice farming, 10
- Rio de Janeiro, 9
- Rio Tinto, 82–5,
- riparian corridors, 95, 99
- roads, 3, 11, 40, 93–4, 111
- rodents, 29, 52
- Roloway monkey, 8
- root(s), 54, 187
 collar, 177
 pruning, 179, 186–7
 rot (disease), 183
 spiralling, 172
 trainers, 171–2
- root:shoot ratio, 207, 209
- Roundup, 36, **217–9**
- Royal Botanic Gardens, Kew, 20–1, 161, 165, 190, 266
- rubber plantations, 4, 9
- Rubiaceae, 28
- rural appraisal, 91
- rust (disease), 183
- S**
- Sabah, 24
- Saccharum*, 57, 121
- salt test, 164
- sampling units (SUs), 258
- sand traps, 257
- sand, coarse, 166, 172–3
- sapling production (see also 'propagation'), 104
- Sarcocephalus latifolius*, 261
- Sarcosperma arboreum*, 251
- schools (involving), 214–5
- Schumacheria*, 47
- secondary forests, 3, 7, 10, 26, 120, 123, 150
- Securidaca longipedunculata*, 261
- seed(s),
 acid treatments, 198
 banks, see 'soil seed banks' and 'seed(s), storage'
 cleaning, 160–1
 coat, 52, 156, 166, 198
 collection, 89, 104, 140, **156–60**, 214
 data collection sheets, 158, 197, **299**
 data management, 197, 279
 desiccation, 161–5
 development, 162
 dispersal, 7, 22, 29, 31, 48–51, 53–4, 90, 118, 124, 145, 159, **160**
 by animals, 123, 134, 256
 by bats, 22, 29, 51
 by birds, 29, 50
 by cattle, 58, **117**
 by non-flying mammals, 29, 51
 by water, 43
 by wind, 50, 135
 endozoochorous, 50
 human-assisted, 51
 in landscape, **66–71**, 75, 93
 lack of, 137
 types (or patterns), 33, 46, 50–1, 53, 75, 88, 191, 259, 278
 dispersers, protection of, 118
 dormancy (see also 'seeds(s), treatments'), 48, 52–4, 61, 156, 165–6, **168**, 191, 197–8
 mean and median duration, 202
 measuring, 202
 physical, 53, 168
 physiological, 53, 168
 drying, 161–5
 effect of fire, 60–1
 germination, see main 'germination' entry
 handling, 160–71
 intermediate, 161, 165, 203
 moisture content, 162–5
 orthodox, **161–3**, 203
 post-harvest technology, 160–71
 predators, 48, **51–3**, 123, 134, 248, 256
 repellents for, 134, 250
 quality, 161–2
 rain, **49**, 60, 122–3, 160
 recalcitrant, **161**, 165, 203
 research, 196–203
 ripeness, 157, 162
 scarification, 166, 198
 seed trees, 49, **158–60**, 178
 size, 52
 soaking, 198
 sources, 48, 134
 sowing, 166–7, 212
 seeding spots, 228

INDEX

- storage, 98, **161–5**, 203, 212–3
 - chilling, 161, 165
 - desiccants, 165
- structure, 156
- survival, 162–3
- treatments, 166, **198**, 202, 212, 228
- viability, 162–3
- seedling(s) (see also 'tree planting'),
 - effect of fire, 60–1
 - growth, **203–9**, 213, 247
 - data collection sheet, 301
 - data management, 280
 - in disturbed sites (natural establishment), **55–8**, 66–78, 118–25
 - in forests (see also 'wildings'), 44–9, 93
 - in nurseries, 38, 169, **177–88**, 203–13
 - morphology, 209–10
 - mortality, 200
 - predation, 58
 - production, see 'propagation'
 - shade tolerance, 46–7
 - storage, **177**, **179–80**, 212
 - survival, 203–6, **209**, 246
- selective logging, 32
- Semecarpus*, 47
- seral stages, 45, 46, 64
- sexual reproduction, 156
- sheep, 117
- shifting agriculture, 4, 20
- Shorea*, 47
 - roxburghii*, 37
- shrub-land, 45–6
- silvicultural treatments, 240, **244**, 283, 294
- similarity index, 259
- site,
 - amelioration, **139–46**, 147–8
 - description, 103
 - maintenance, 104, 139
 - management, 130
 - preparation, 104, 106, 139–40, 217
 - recapture, 126, 235
 - selection, 95–101
 - surveys, **72–9**, 88–9, 103, 120, 140
- slash and burn agriculture, 4, 20
- smart patrols, 111
- social media, 292
- soil(s),
 - aeration, 142
 - amelioration, 38, 71, 78, 129, 140, **142–3**, 224–7
 - bacteria, 143
 - beds, 171
 - compaction, 10, 139
 - cultivation, 139
 - degradation, **66–71**, 75, 143–5
 - demineralisation, 84
 - effect of fire, 60
 - erosion, 10, 11, 41, 99, 100, 139
 - fauna, 142–3
 - forest soil, 10, 38, 39–43, 54
 - as growth medium, 143, 166, 172–3, 205, 228
 - mounding, 142
 - nutrients, 54, 60, 142–3
 - organic matter addition, 35, 38, 140, 142, 226
 - ripping, 137, 139, 142
 - seed banks, 48, 56, 60
 - topsoil translocation, 84–5, 140
 - water-storage potential, 10, 18, 40, 60, 148
- Sorensen's Index, 259
- South America, 144
- Southern cassowary, 96
- species area curve, 6
- species area relationships, 7
- species reintroduction, 9, 67–8, 76
- species selection, 282–6
- Spineless forest lizard, 8
- Sponsor-a-tree campaigns, 109
- spreadsheets, 193–5, 245–8, 252, 258, 276, 282–4, 310–2
- Sri Lanka, 2, 8
- stakeholders, 86, **87**, 88, 92, 101–2, 104–5, 286
- stand development, 47
- standing down, **177**, **179–80**, 212
- Stangeland, Torunn, 260–2
- State University of Mato Grosso (UNEMAT), 20
- statistics,
 - ANOVA, 202, 209, 246–8, **309–13**
 - binomial data, 310–1
 - Chi-square test, 236, 237
 - sturdiness quotient, 209
 - t-test, 313–5
- Stelis macrophylla*, 47
- stepping stones, 90, 98
- structural complexity, 32
- student thesis projects, 287
- sturdiness quotient, 209
- subsistence farming, 40
- succession, 12, 26, **44–9**, 58, 64–5, 125
 - primary and secondary, 45
- suitability index, 282–6
- Sumatra, 3, 16
- sunbirds, 37
- swidden agriculture, 4, 20
- syconia, 31, 156

- T**
- Tabebuia*, 22
 - tapir, 32, 50
 - target ecosystems, 12–3, **88–9**, 120
 - task schedule, 104–5
 - taxonomy, 209–10, 271
 - Tectona grandis*, 61
 - TEEB (The Economics of Ecosystems and Biodiversity study), 19, 147
 - Terminalia*, 46
 - sericocarpa*, 80
 - testa, 156, 168
 - Tetragastris altissima*, 21
 - Thailand, 9, 14, 16, 33, 37, 39, 42, 118, 120, 129, 134, 147, 214, 272–4, 293–5
 - Theobroma cacao*, 29
 - thinning, 71, 122, 145
 - self-thinning, 122
 - Thiram, 178
 - thresholds, see ‘disturbance thresholds’
 - Thysanolaena*, 121
 - tigers, 57
 - timber, 21, 136
 - Tityras, 50
 - Toohey Creek, 96–7
 - topography, 92
 - total nursery time (TNT), 280, 284
 - tourist industry (see also ‘ecotourism’), 108
 - Toyota, 141, 147
 - training, 266, 286–7
 - transplantation shock, 171, 227
 - transportation of trees or saplings, 220–1
 - Treasure Tree Clubs, 214–5
 - TREAT (Trees for the Evelyn & Atherton Tablelands), 96
 - tree(s),
 - crowns, 6
 - growth, 234–5, 282–5
 - monitoring (see also ‘tree performance’), 216, **231–9**, 244
 - guilds, 47
 - identification, 189–90, 209–10
 - maintenance of planted trees, 216, **229–31**
 - costs, 107
 - data analysis, 236–7
 - labelling, 232
 - measurements, 234–5, 245
 - sampling, 232
 - timing, 234
 - parent trees, 49, **158–60**, 178
 - performance,
 - data collection sheets, 245, 303
 - data management, 246–8, 281–6
 - research, 240–8
 - planting, see main ‘planting’ entry
 - populations, 156
 - stumps, 49, 122
 - survival, 234, 236, 282–5
 - tree-fall gaps, 44–5
 - Trema*, 46–7
 - tropical forest loss, 3
 - t-test, 313–5
 - Tucker, Nigel, 80, 97
- U**
- Uganda, 260–2
 - UN Framework Convention on Climate Change (UNFCCC), 18
 - UNEP-WCMP forest classification system, 26–7
 - ungulates, 29, 57
 - United Nations Food and Agriculture Organisation, see ‘FAO’
 - Universiti Putra Malaysia (UPM), 141
 - university researchers, 104, 271
 - urban sites, 136, 139
 - Urticaceae, 22
 - USA, 134
- V**
- value,
 - of restored forests, 147–8
 - of tropical forests, 19
 - vegetation,
 - cover, **66–71**, 75
 - sampling methods, 258
 - survey (data collection sheets), 304–5
 - vegetative propagation, see ‘cuttings’
 - Verbenaceae, 57
 - Verified Carbon Standard (VCS), 238
 - Vernier-scale callipers, 206
 - Vietnam, 2
 - village committees, 105, **112**, 272
 - viruses, see ‘diseases (in nursery)’
 - Visaya State University (VSU), 135
 - volunteers, 107
 - voucher specimens, 158, **189–90**, 210
- W**
- Waorani Indians, 28
 - water-absorbing gel, 38, 142–3, 227
 - water,
 - catchments, 18, 99

INDEX

- infrastructures, 18
 - purification, 19, 273
 - regulation of seasonal flow, **10–1**, 19, 92, **99**, 272–3
 - resources, 10, **18–9**, 91
 - watering,
 - at restoration site (see also 'planting'), 38, 137, **225**
 - in the nursery, 167, **180–1**, 188
 - water-logging, 142
 - watersheds, 13, 18, 64, **99–100**, 146, 214, 272
 - classification of, 100
 - degradation of, 64
 - protection of, 13, 18, 146, 272
 - restoration of, 99, 214
 - weaning seedlings, 104, 174, **186**, 213
 - websites, 292
 - weed control, 32, 35, 104–8, 117
 - frequency, 229
 - in ANR, **120–2**, 123
 - in direct seeding, 131, 228
 - in framework species restoration, 130
 - in maximum diversity restoration, 138, 142
 - in nurse plantations, 144
 - in the nursery, 183
 - monitoring, 235
 - techniques, 230
 - herbicides, 122, 134, **217–9**
 - ring weeding, 121
 - root removal, 217
 - weed mats, 179
 - weed pressing, 119, **121–2**, 217, 230
 - weed whacking, 230
 - weeds (see also 'weed control'), 66
 - canopy, 54, 55
 - competition from, 55–6, **121**, 229
 - response to fire, 60, 219
 - species, 56, **57**
 - Whitmore forest classification system, 24–5
 - wildings, **174–5**, 210–1
 - wildlife, see 'biodiversity' and 'seed(s), dispersers'
 - Wildlife Conservation Society, 111
 - wilting, see 'diseases'
 - windbreaks, 98
 - workshops, 286–7, 291
 - WWF (World Wide Fund for Nature), 92
- Y**
- Yokohama, 141
- Z**
- Zaire Basin, 32, 39

NOTES

NOTES

NOTES