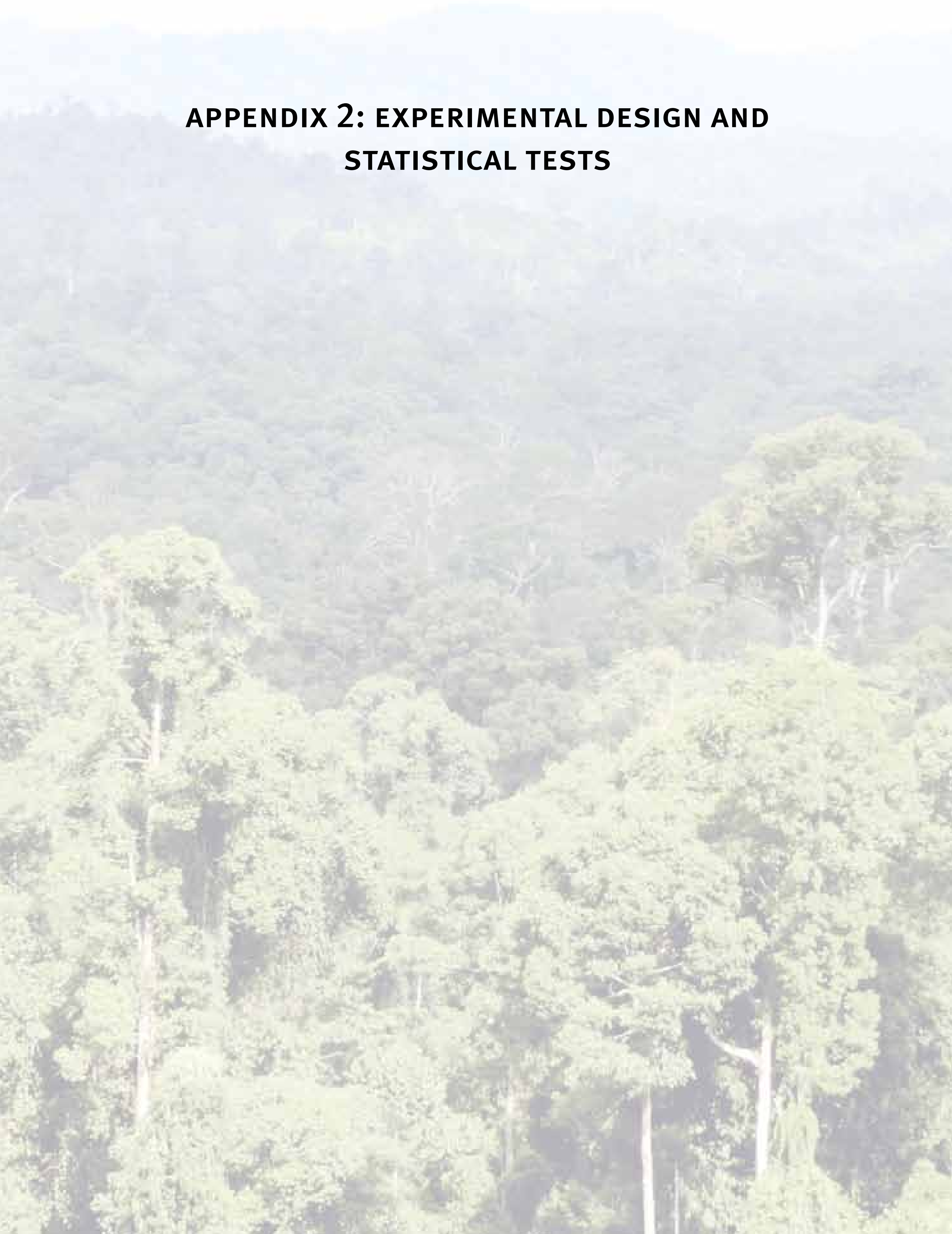
APPENDIX 2: EXPERIMENTAL DESIGN AND STATISTICAL TESTS



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…

$$rdf = (t-1) \times (b-1)$$

…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**)).

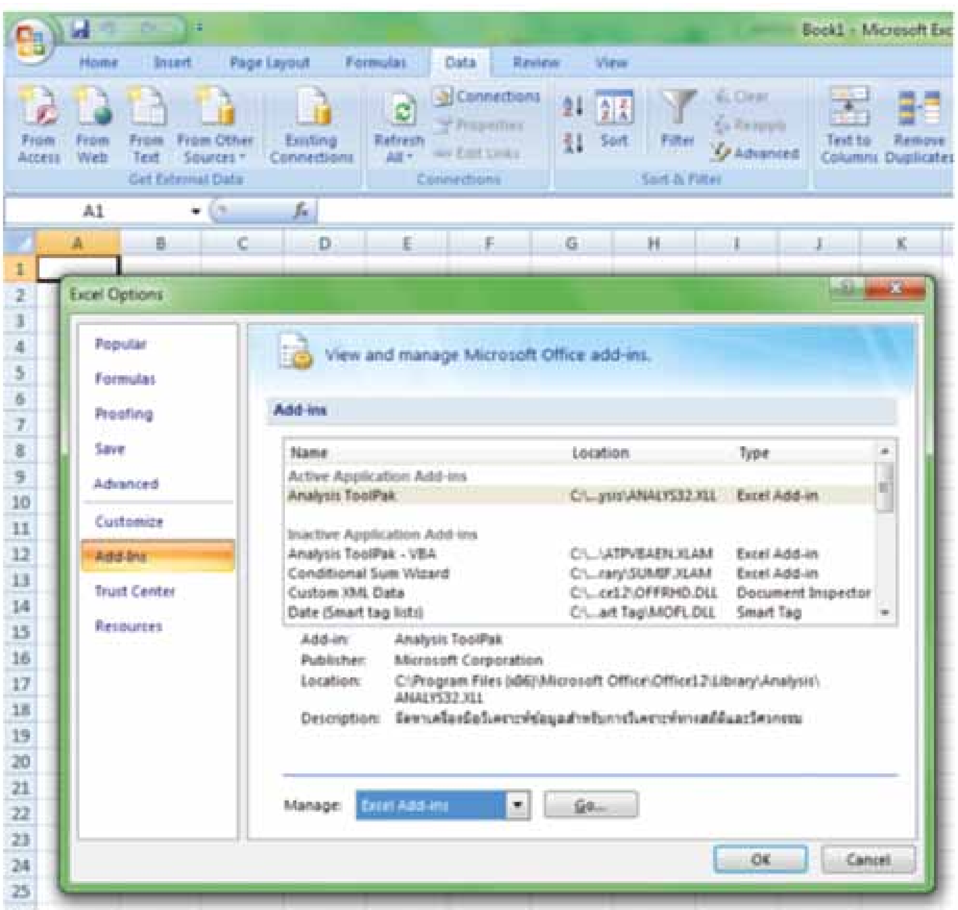
A2.2 Analysis of variance (ANOVA)

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.

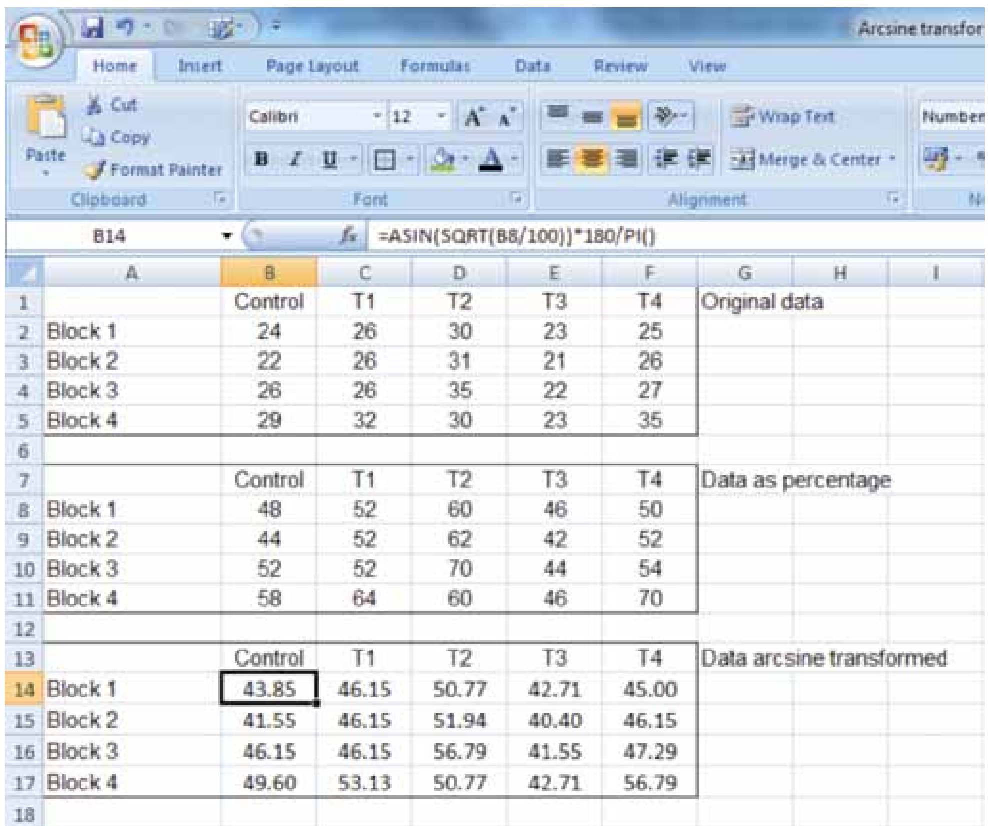
If using Vista or Windows 7, click on the Microsoft Office button (top left), then on the Excel Options button (bottom right of the dropdown menu), then on 'Add Ins' and finally on the 'Go' button next to 'Manage Excel Add Ins'. Tick the box labelled 'Analysis ToolPak'.



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 binomal 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.



	A	B	C	D	E	F	G
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 × 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:

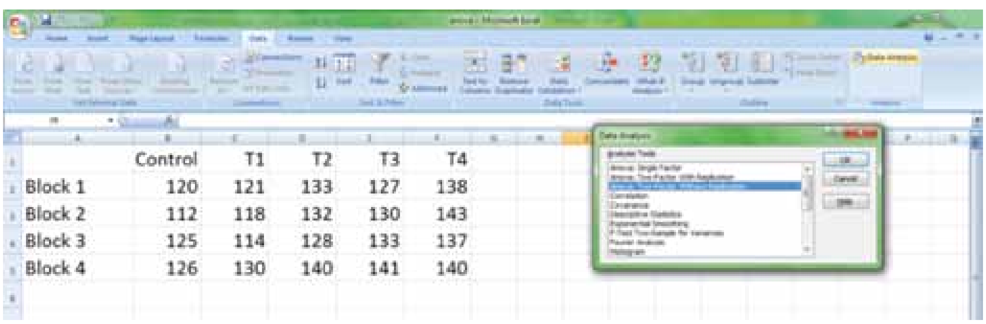
=ASIN(SQRT(**B8/100**))*180/PI().

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

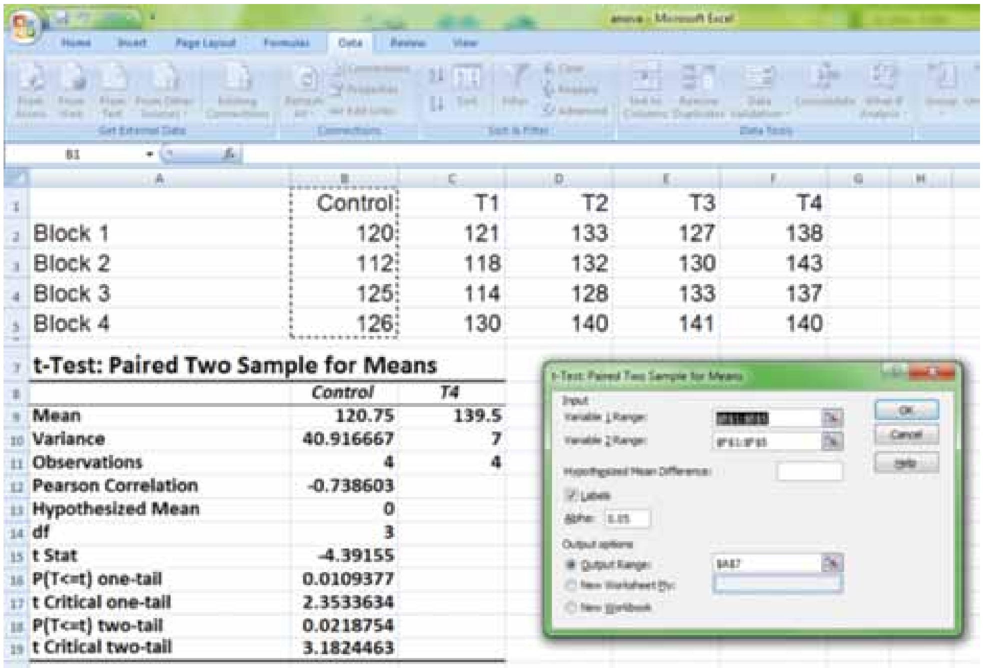
In this example, we are using the mean height of trees (cm) 18 months after planting in a field trial plot system (see **Section 7.5**), subjected to different fertiliser treatments. Open a new spreadsheet and type in your data with blocks as rows and treatments as columns, as shown below.



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 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

t-Test: Paired Two Sample for Means

	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).

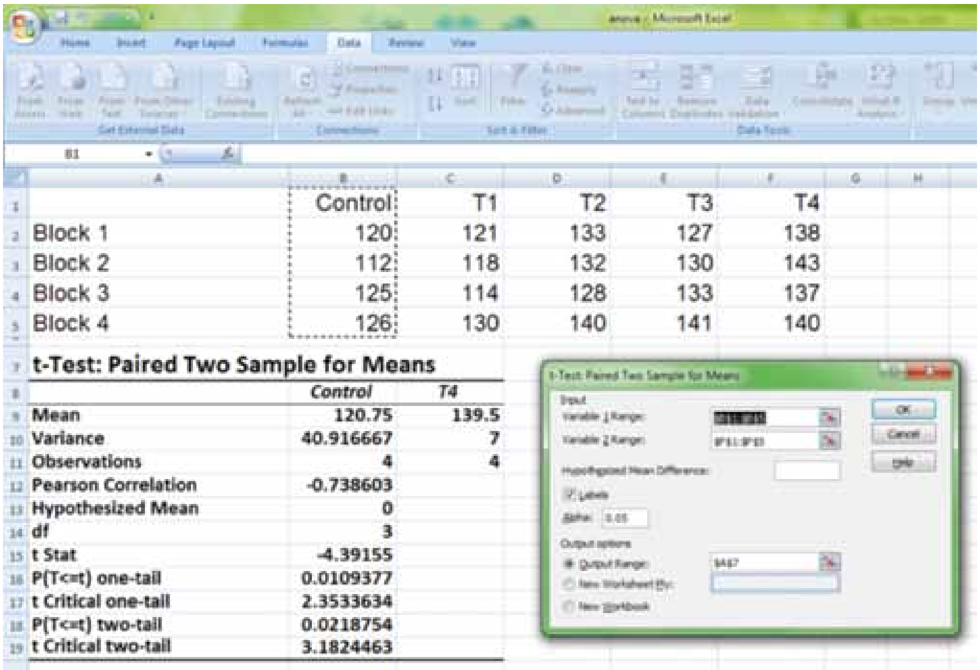
A2.3 Paired t-tests

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 internet[1].

[1] spss.en.softonic.com

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.



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 the 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 5^{th} 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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