Bridging restoration and multi-functionality in degraded forest landscapes of Eastern Africa and Indian Ocean islands

FOREAIM

Partner 3 - CEH

Report on Activities

Second reporting period: 1st June 2006 - 31st May 2007

Personnel:

Stephen Cavers,
Julia Wilson,
Jan Dick,
Kevin Ingleby,
Robert Munro

Summary

CEH activities in the second period have been concentrated in WP 3 and 4. Significant field visits have been undertaken: in WP3, a population of *Prunus africana* in Kenya was mapped, sampled and marked for subsequent seed collection; in WP4 a training workshop was held in Nairobi to introduce partners to methods for assessment of mycorrhizal diversity. The molecular analysis of the Prunus africana population is now well advanced and should result in significant outputs by the end of period 3. In WP4 assessments of mycorrhizal spore diversity have been completed for Kedowa and differences in soil mycorrhizal diversity tested through trap culture using Pinus patula and Albizia gummifera seedlings. In WP2 acitvities are largely complete, although plans were put in place for a student visit in the third period and in WP5 CEH's supervisory role continued.

WP2 - Impact of degradation and rehabilitation on vegetation

Summary of progress

Following the presentation by Jan Dick of a CD to all the participants at the annual meeting in Kenya - January 2007 - no further work has been undertaken in WP2.

Planned Activities in next 12 months

Jan Dick will supervise the coming visit of Katumba Muthalib Balikitenda (MSc Student, Makerere University, Uganda), for which CEH has funded the airfare. Muthalib is scheduled to visit CEH 18 Jul to 7 Aug 2007 to learn about data analysis and access library facilities. He has made a good start to his masters studies checking and updating the CEH trait database for Ugandan trees and will bring with him specific leaf area data which he has collected.

WP3 - Rehabilitation of forest ecosystems and incomes through planting

CEH Staff involved: Stephen Cavers, Robert Munro, Kevin Ingleby

Summary of progress

CEH activities in WP3 in the second year have been to map and sample a population of *Prunus africana* in Kenya for gene flow studies, for the purposes of examining the impacts of plantation on mating systems and investigate genetic component of seed sourcing for plantation in the species. An experiment using *Albizia gummifera* was also devised and initiated at the coordination meeting. Collections have been made and an exchange organised (CEH - University of Makerere) to facilitate completion of the genetic analysis.

Activities

Prunus africana

A site in Kenya was identified - Kibiri - at the southern end of the greater Kakamega Forest. Here, a natural population of *P. africana* and a mature plantations are found within potential reproductive contact. A total of 74 trees were sampled in a transect across the plantation and a further 203 trees sampled as an exhaustive mapped block from the natural forest (Fig 1, 2). All trees were sampled by removal of two 1 cm^2 cambium disks (Colpaert et al, 2005) which were preserved by drying on indicating silica gel.

DNA was extracted from all samples using QIAGEN DNEasy 96 well plate extraction kits. DNA extracts were visualised and quantified by electrophoresis on 1% agarose gel. A large number of microsatellite primers are available in the genus *Prunus* and a series were tested - first targeting those known to have amplified in a previous study of *P. africana* (Farwig, 2005): U1-5, P1,2 and then a range known to have cross-amplified in other tropical Prunus species EMPA001-018 (Clarke & Tobutt, 2003). Microsatellite fragments were amplified by PCR on a Hybaid MBS 96-well thermocycler following the PCR reaction mixes and PCR protocols given in the papers. Some optimisation of running conditions was carried out. Loci were screened for polymorphism against a panel of 20 samples from Kibiri. Following screening a set of optimal loci was selected and genotyping of the full collection using these markers has begun. Preliminary "full-collection" results are available for two (U3, U5), and are given in Table 1.

Screening will continue, using alternative primers from other species in the genus, to prepare a dataset including 10 loci for all of the 274 mature trees sampled. Then, in late 2007, collections of seed will be made from trees in the plantation and the natural forest and, during 2007/08, DNA extraction, genotyping and analysis of the progeny arrays will be carried out.

Table 1: Screening results for *Prunus africana* for 14 microsatellite loci transferred from other species in genus Prunus.

Locus name	Amplification	Na	Ho (plantation)	Ho (forest)
U1 (UDP96-001)[1]	fair	monomorphic	-	-
U2 (UDP98-406)[1]	fair	monomorphic	-	-
U3 (UDP97-403)[1]	good	2	0.85	0.94
U5 (UDP96-018)[1]	good	10	0.50	0.70
P1 (Pchcms5)[2]	good	multiple	-	-
P2 (PS12A02)[2]	good	multiple	-	-
EMPA001[3]	good	multiple	-	-
EMPA005[3]	fair	monomorphic	-	-
EMPA009[3]	fair	monomorphic	-	-
EMPA010[3]	fair	monomorphic	-	-
EMPA014[3]	fair	monomorphic	-	-
EMPA015[3]	fair	monomorphic	-	-
EMPA016[3]	good	multiple	-	-
EMPA018[3]	fair	monomorphic	-	-

1- Cipriani et al (1999): *Prunus persica* , 2 - Sosinski et al (2000): *Prunus persica*, 3 - Clarke & Tobutt 2003: *Prunus avium*

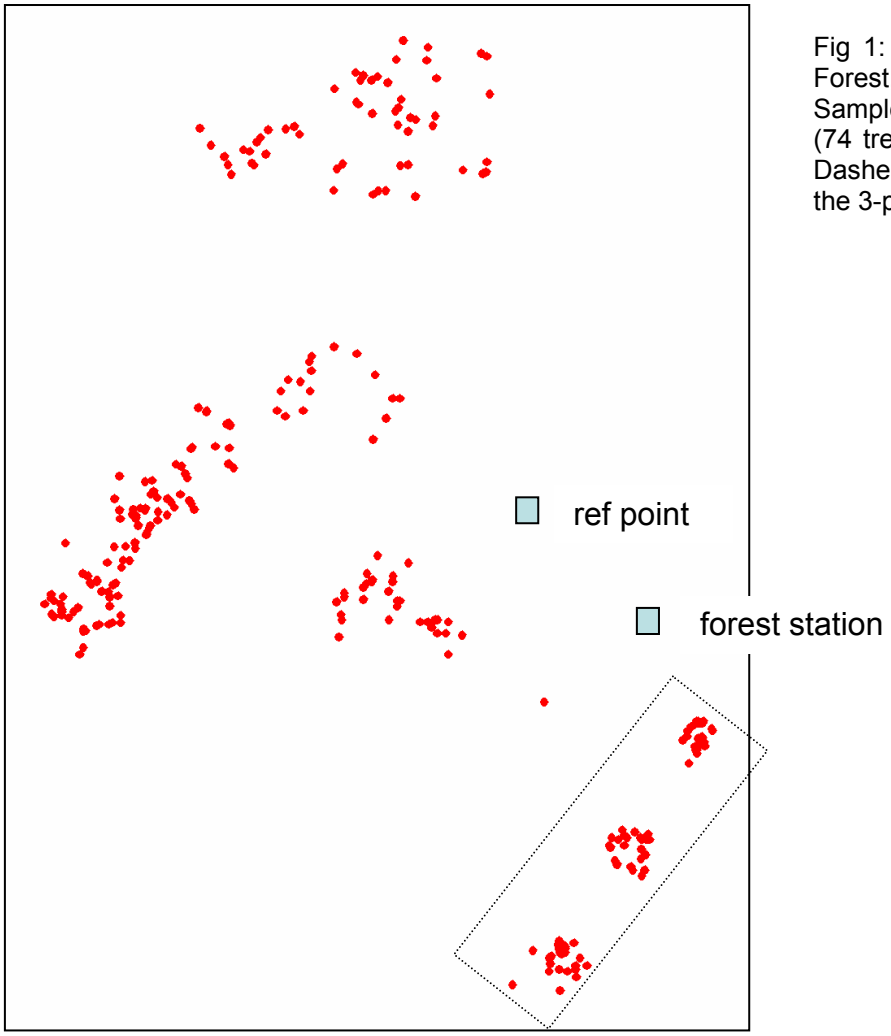

Fig 1: Map of sampled trees from Kibiri Forest, Kakamega, Western Kenya. Samples were collected from plantation (74 trees) and natural forest (203 trees). Dashed box in lower right corner shows the 3-plot transect through the plantation.

Figure 2: (see attached photos)
a. Kibiri Prunus africana plantation
b. Kibiri forest
c. Sampling team (KEFRI, CEH). Inset: cambium sampling.

Albizia gummifera
A proposal for an experiment exploring the linkage between provenance differentiation and soil microbiology, using the widespread pioneer species *Albizia gummifera,* was presented at the second coordination meeting in Kenya. As *A. gummifera* is a pioneer, a good timber producer, forms mycorrhizal associations and is nitrogen fixing, it has clear potential as a reforestation species. The study will comprise a randomised block growth trial with seedlings of provenances from each of the three field sites in Kenya, Uganda and Madagascar grown in bulked soils sampled from each of the sites. Linked molecular analyses (AFLP, tRFLP) will evaluate the degree of differentiation between populations of the species at each field site and examine soil microbiological variation. The experiment will provide valuable insight into the necessity for planting of local provenances and useful quantifiable assessments of the relative consequences of planting local versus exotic genotypes, as well as initial exploration of the mechanisms controlling local adaptation in trees.

At the coordination meeting, it was agreed that parallel experiments will be established at CEH, Edinburgh and at KEFRI. In the UK the focus will be on establishing the genetic basis of variation in the provenances, and mycorrhizal species assays, whilst in Kenya the focus will be on rhizobial differentiation. Both experiments will collect growth performance data from the seedlings.

A sampling strategy for plant and soils from each of the three field sites was determined:

1. 30 *Albizia gummifera* trees sampled by collection of leaf (5 leaflets) dried on silica gel (or at least thoroughly dried and bagged). The 30 trees should each be separated by around 100m. If possible collect GPS coordinates of each tree.
2. A sample of around 100 seeds from 5 of the trees (total 500)
3. 10KG of soil: a bulked sample from across the site from which the trees are sampled. 2KG to be sampled below each of the 5 seed trees, sampled as 0.5KG from points distributed north, south, east and west below the tree. All soil from the site to be bulked.

Collections of leaf material from Madagascar and Kenya have now been prepared and material from Uganda will arrive in July 2007. Soils from Madagascar and Kenya have been collected, however some concerns have been raised regarding the decline in rhizobial and mycorrhizal activity in the time between collection and planting of seedlings - therefore new soil collections may be required at the time of seed harvest, which is expected in mid-2007. A Masters student from the University of Makerere - Judith Nantongo - will travel to CEH for 6 months to undertake genetic analysis of tree populations, soil fungal populations and growth measurements of the seedling trial as her research project. She will commence her studies in July 2007.

Planned CEH WP3 Activities in next 12 months

1. Prepare collections of *Prunus africana* seed
2. Complete genotyping of *Prunus africana* collection adults and seed
3. Data analysis for estimation of genetic structure, gene flow and mating system
4. Initiation of *Albizia* / myco experiment
5. Visit of Judith Nantongo (MSc student, University of Makerere) to CEH
6. Analysis of genetic diversity and differentiation in *Albizia gummifera* tree collection
7. 6-month growth performance results for *Albizia gummifera* provenance*soil experiment.

WP4 - Impact of degradation and rehabilitation on soil functioning

Summary of progress

- Assessments of mycorrhizal spore populations were made in soils from the experimental plots at Kedowa in Kenya (Task 4.2a, Deliverable 4.3). Spores attributable to an E-strain fungus (an ectendomycorrhizal associate of pine) were abundant in soil from the pine plantation and crop-pine regeneration sites, but absent from the other sites. Arbuscular mycorrhizal spores were most abundant in soils from regeneration sites and the dominance of several species was strongly affected by treatment plots

- Trap cultures were set up in CEH glasshouse using soil from Kenyan plots at Kedowa and seed of *Pinus patula, Albizia gummifera*, and 2 crop plants. Assessments of seedling growth and root infection (ecto, ectendo and arbuscular mycorrhizas) were made (Task 4.2a, Deliverable 4.4). Good growth of the *Pinus patula* seedlings was linked to early infection by the E-strain fungus in soils from the pine plantation and crop-pine regeneration sites. No differences in growth of *Albizia gummifera* and 2 crop plants was observed between the soils, although levels of infection in the crop plants were associated with high numbers of 'live' spores in the soils.

- A mycorrhizal training workshop was conducted at KEFRI in March 2007. The workshop provided training in the assessment of mycorrhizal diversity (WP4,Task 4.2) and nursery inoculation techniques (WP3, Task 3.2). An 87 page manual was prepared to accompany the workshop. Following the workshop, planning of bioassay and field experiments in Uganda and Madagascar were discussed (Deliverable 4.1).

Activities

- Assessment of mycorrhizal spore populations in Kedowa soils

Spore populations were assessed in duplicate soil samples to those taken by KEFRI and CIRAD. 54 soil samples from the 0-10 cm horizon were examined. There were 6 replicate samples from the natural forest, cypress plantation and pine plantation sites and 18 replicate samples from the pine-crop and cypress-crop regeneration sites (6 from each upper/middle/lower slopes). Spores were extracted from 50 g sub-samples of soil and numbers of total and 'live' spores were assessed with 'live' spores separated into species or types based on spore morphology.

Pine-crop and cypress-crop regeneration sites had more total spores, more 'live' spores, a greater % of 'live' spores and more diversity (species/types present) than the natural forest, cypress plantation and pine plantation (P<0.001). 'Live' spores were also more numerous on the upper slopes of the regeneration sites than the middle and lower slopes (P=0.015). There were also differences in the species/types of spores in the different soils: spores attributable to an 'E-strain' fungus (a group of Ascomycete fungi in the order Pezizales which form mycorrhizal associations with Pinaceae) were more numerous in the pine plantation soil than in the pine-crop regeneration soils and virtually absent in soil from the other sites (P<0.001). The arbuscular mycorrhizal (AM) spores *Glomus etunicatum* and *Scutellospora calospora* were most numerous (both P<0.001) in the cypress-crop regeneration soil, while *Acaulospora scrobiculata* (P<0.001), *Pacispora scintillans* and *Entrophospora infrequens* were most numerous in the pine-crop regeneration soil.

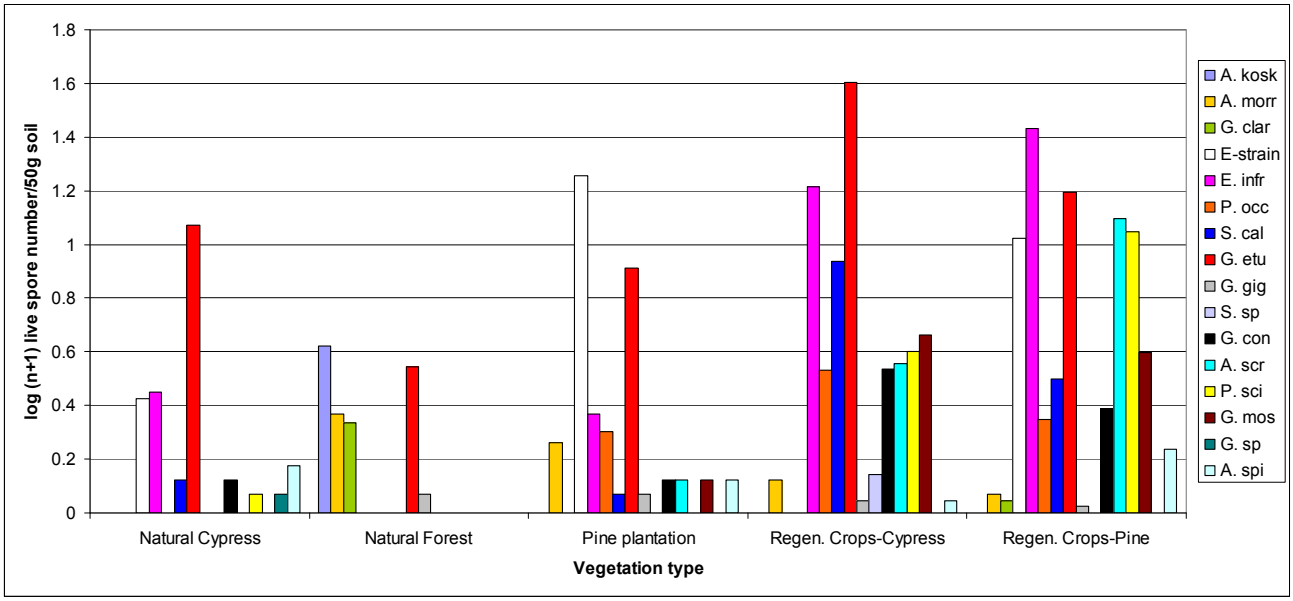

Figure 1. Populations of 'live' spores in Kedowa soils

- Growth and mycorrhizal infection of *Pinus patula*, *Albizia gummifera* and crop seedlings in trap cultures grown in Kedowa soils in CEH glasshouse

A mycorrhizal bioassay of the Kedowa soils was set up in the CEH glasshouse in order to examine the mycorrhizal activity in the soils, the effect on seedling establishment and growth, and to provide fresh spore and root material for taxonomic determinations and isolation of fungal material for molecular work. Seeds of *Pinus patula* (an ectomycorrhizal and ectendomycorrhizal host plant), *Albizia gummifera* (an arbuscular mycorrhizal host plant) and the crop plants millet and sorghum (fast-growing, arbuscular mycorrhizal plants) were sown in 1 litre pots containing approximately 100 g of test soil and a sterilised soil mixture. Mycorrhizal activity in the soils was determined by assessing root infection (ecto and arbuscular mycorrhizas) on the seedling roots. As there were only 2 replicate pots for each soil/host plant species, significant differences between test soils were often difficult to detect.

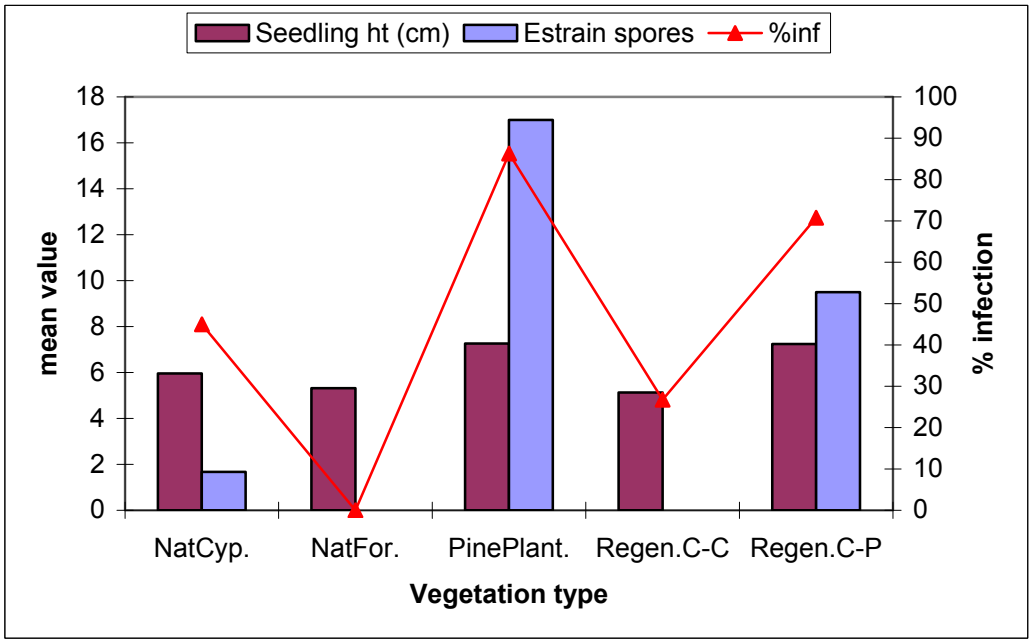

Figure 2. Growth and mycorrhizal infection of pine seedlings in Kedowa soils, and numbers of E-strain spores present in original soils.

Height of the pine seedlings was greatest in soils from pine plantation and pine-crop regeneration sites (P=0.026). Although not significant, % infection was also greater in these soils, with the dominant mycorrhizal morphotype present on the roots attributable to an E-strain fungus. Other morphotypes present were attributed to *Rhizopogon* sp. and *Tuber* sp.. Seedling height was positively correlated with % mycorrhizal infection (P<0.05), numbers of mycorrhizal tips (P<0.05), numbers of E-strain mycorrhizal tips (P<0.01), and numbers of E-strain spores in the original soil (P<0.001).

These results indicate that soils from sites where ectomycorrhizal tree species have not been recently grown lack the ectomycorrhizal propagules necessary for the establishment and growth of ectomycorrhizal tree species. This conclusion has been more dramatically demonstrated in the KEFRI bioassay of the same soils, where pine seedlings were grown in soil from the site (i.e. the soils were not diluted with a sterilized nursery compost containing soluble nutrients). In the KEFRI bioassay, pine seedlings growing in soil from the pine plantation or in soil from plots regenerating after pine plantation survived and were growing well, whereas those growing in all the other soils died. Following the workshop in Nairobi, roots of surplus pine seedlings from the KEFRI bioassay experiment were examined and the E-strain morphotypes was found to be dominant on the root systems. Subsequently, fruitbodies of a Pezizalean fungus have appeared on the pots: this means that the causal fungus (or one of the causal fungi) can now be identified.

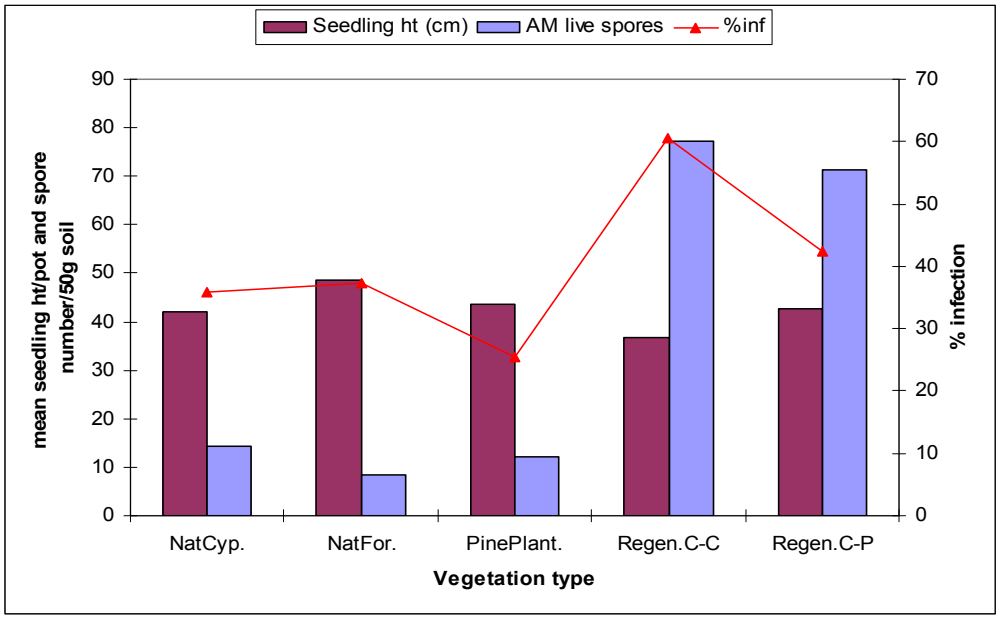

Figure 3. Growth and mycorrhizal infection of crop seedlings in Kedowa soils, and numbers of 'live' AM spores present in original soils.

No differences were observed in height or mycorrhizal infection of the *Albizia gummifera* or crop seedlings growing is soil from the different sites. However, seedling height was negatively correlated to % infection (P<0.05), while % infection was positively correlated with the number of 'live' spores in the soil. This suggests that greater AM activity at the regeneration sites resulted in higher levels of infection, and that high levels of soluble nutrients in the sterilised compost enabled plants with less AM infection to allocate more C for shoot growth.

Mycorrhizal training workshop at KEFRI, 14-20 March 2007

The workshop was designed to provide basic training in the assessment of mycorrhizal diversity in field soils and root samples. The workshop aimed to familiarise participants with the general features of arbuscular mycorrhizas (AM) and ectomycorrhizas (ECM) dealt specifically with the laboratory, nursery and field methods relevant to mycorrhizal work in the project.

An 87 page manual was prepared to accompany and supplement the training provided by the workshop. The manual includes a brief introduction to arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) associations and describes in detail (with illustrations) methods relevant to project work. The manual is available in pdf format to all project members.

The workshop was attended by Dr Isabelle Ratsimiala Ramonta (UNIV AN) and Ms Hanitra Andriamampandry (FOFIFA) from Madagascar, and by Dr Gilbert Majaliwa Mwanjalolo (MU), Mr Charles Kizza Luswata (MU) and Ms Esther Sebuliwa (MU) from Uganda. Several members of staff and students from University of Nairobi, Jomo Kenyatta University, TSBF-CIAT and KEFRI also attended.

A more detailed report on the workshop and planning discussions is appended.

Planned activities in the next 12 months

- The molecular work at CEH will focus on developing TRFLP methods for assessing mycorrhizal diversity (Task 4.2c).
- The trap cultures will be maintained in the glasshouse and provide fresh material for taxonomic determinations, isolation of key species and the molecular work (Task 4.2b).
- CEH will also assist in ECM identification of roots and fruitbodies from bioassay and field experiments in Kenya as required (Task 4.2b).

WP5 - Impact of degradation and rehabilitation on erosion

Summary
CEH activities under this work package have been purely in the role of WP coordinator and a full description of partner progress is given in the WP5 report.

Table 1: Deliverables List

List all deliverables, giving date of submission and any proposed revision to plans.

Del. no.	Deliverable name	Workpackage no.	Date due	Actual/Forecast delivery date	Estimated indicative person-months *)	Used indicative person-months *)	Lead contractor
D2.2	data base on vegetation composition and selected variables characterising degraded areas	2				0.5	4
D2.3	data base on vegetation composition and selected variables characterising natural succession areas – plantations and fallow	2				0.5	4
D3.4	molecular markers for economically important traits	3				3.50	6
D4.1	experimental design, network of plots in the main site of each country	4				1.00	2
D4.4	Data base on the capacity of the native tree species (legumes and non-leguminous) to form efficient symbiosis with endogenous rhizobial and mycorrhizal populations	4				1.50	2
D5.1	experimental design, network of plots in the main site of each country	5				0.5	3
D5.3	On-site demonstration and dissemination Workshops to inform local stakeholders GOs and NGOs etc of the extent of erosion from differing landuses and landscapes and the effectiveness of anti-erosion measures.	5					3

Table 2: Milestones List

List all milestones, giving date of achievement and any proposed revision to plans.

Milestone no.	Milestone name	Workpackage no.	Date due	Actual/Forecast delivery date	Lead contractor
M2.2	general analysis of the state of degradation of the target sites	2			
M2.3	identification of species suitable for ecological restoration	2			
M3.4	genetic diversity and variation of economical products are completed	3			
M 4.1	Site stratification established ; temporary plots established and characterized ; corresponding soil samples collected and soil analyses initiated ; collecting nodules, fungi and roots, prior to isolation of rhizobial and mycorrhizal strains; collecting tree samples for determination of natural abundance in ^{15}N	4			
M 5.1	Selection of experimental sites, month 10,	5			
M 5.2	Installation of erosion control measures started, month 12,	5			
M 5.3	Purchase of Micro-sensors and installation of catchment pits, month 14	5			
M 5.5	First on-site dissemination Workshops, month 30	5			

Plan for using and disseminating the knowledge

Dissemination of knowledge

The dissemination activities section should include past and future activities and will normally be in the form of a table**.** Entries in the table should be accompanied by a short description for each major activity (conference, exhibition, etc.) having taken place or planned since the last report. Relevant details, such as references of journal publications and conferences, website addresses, dates, quantitative data, etc. should be explicitly mentioned. Completed as well as future activities should be mentioned with their actual or planned date. (examples in table in green)

Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
14-20/03/2007	*Mycorrhizal Workshop*	*Research*	Kenya, Uganda, Madagascar	small	3,5,6,7,8

Appendix 1
Report of FOREAIM mycorrhizal workshop held at KEFRI, 14-20 March 2007

1. Participants and contributors

Ms Hanitra Andriamampandry (FOFIFA)
Mr Milton Obote Esitubi (KEFRI)
Mr Kevin Ingleby (CEH)
Dr Joyce Jefwa (TSBF-CIAT)
Ms Susan Njuguini Kabacia (TSBF-CIAT)
Mr Isaiah Ndaburu Kiteto (JKUAT)
Dr Didier Lesueur (CIRAD)
Mr Charles Kizza Luswata (MU)
Mr Emmanuel Tendwa Makatiani (KEFRI)
Dr Gilbert Majaliwa Mwanjalolo (MU)
Ms Joyce Njore (KEFRI)
Mr John Ochieng (KEFRI)
Dr David ODee (KEFRI)
Dr Shiella Okoth (UON)
Mr James Were Otieno (KEFRI)
Dr Isabelle Ratsimiala Ramonta (UNIV AN)
Mr Juma Robinson (JKUAT)
Ms Esther Sebuliwa (MU)

2. Programme of activities

Although the programme was re-scheduled to accommodate participants travel arrangements, all of the topics outlined in the manual were covered during the course of the workshop.

Wednesday 14 March
pm: Welcome, introductory talk on mycorrhizas, workshop objectives. Field visit - sampling of soil and roots from KARI plantation.
Thursday 15 March
am: Extraction of AM spores and roots from the soil samples. Clearing and staining of roots for AM assessment.
pm: Examination and identification of AM spores.
Friday 16 March
am: AM spore identification. Finish staining of roots for AM assessment.
pm: Examination and assessment of AM infection in roots.
Saturday 17 March
am: Assessment of AM infection.
pm: Isolation and culture of AM fungi and nursery inoculation.
Monday 19 March
am: Field visit - sampling of ECM fruitbodies and roots from a pine plantation.
pm: Description and identification of ECM fruitbodies. Examination, assessment and characterisation of ECM roots.
Tuesday 20 March
am: Isolation and culture of ECM fungi and nursery inoculation.
pm: Nursery facilities and set up of experiments. Discussion and experimental plans for mycorrhizal studies in WP4.

Numbers of AM spores in soils from Uganda and Madagascar were very low with the majority being parasitized and dead. These soil extracts also contained nematodes and mites. In contrast, numerous viable spores (and no nematodes or mites) were found in soil taken from pot cultures of *G. etunicatum* and *Gi. Albida,* growing in the KEFRI nursery. This demonstrated the importance of trap culturing and the establishment of pot cultures for producing effective AM inoculum. Spores of *G. etunicatum*, *S. calospora, Gi. albida* and *E. infrequens* spores were mounted on slides in PVLG and Melzers' PVLG, examined and their identification features discussed.

Root samples of *Sorghum, Calliandra, Makhamia, Musa* and local grasses were stained for AM examination. *Calliandra* roots were included as *Albizia* roots were not available. AM infection was observed in roots of all the plant species sampled. It was possible to assess AM infection in all of these roots under the dissecting microscope using the gridline intersect method. However, sub-samples of *Calliandra* roots were also mounted in PVLG on slides so that the method used for assessment of AM infection under the compound microscope could be demonstrated.

Although still early in the rainy season, ECM fruitbodies of *Scleroderma* sp. and *Russula* sp. were collected from the pine plantation. Field notes and photographs were recorded. Spore-bearing tissue and collections of mature spores were mounted on slides in PVLG and Melzers' PVLG for examination. The *Scleroderma* sp. collected was thick-stalked with a thin, fine-scaly peridium and possessed distinctly reticulate-spiny spores which indicated *S. bovista*. The *Russula* sp. was characterised but could not be identified.

Mature fruitbodies of *S. bovista* were dried and a concentrated spore suspension prepared in a kitchen blender. This concentrate was diluted in a watering can and used to demonstrate a method for inoculation of seedlings in the nursery.

Pine roots were also collected from mature pine trees and from under the fruitbodies. ECM roots were examined under the dissecting microscope and selected root tips were dissected, mounted on slides and characterised under the compound microscope. ECM attributable to *S. bovista*, *Russula* sp., *Tuber* sp. and an ectendomycorrhizal type were examined.

During the workshop it was also noted that survival of pine seedlings, in the KEFRI bioassay of soils from the Kedowa site, was closely linked to soil type. In fact, those growing in soil from the pine plantation or in soil from plots regenerating after pine plantation had survived and were growing well, whereas those growing in all the other soils had died. Similar, although less dramatic, effects on growth of pine seedlings in these Kedowa soils were also seen in the CEH bioassay experiment in Edinburgh, where soils had been mixed with a sterilized nursery compost. On Wednesday, after the conclusion of the workshop, surplus pine seedlings from the KEFRI bioassay experiment were washed out and ECM roots were characterised and identified so that root assessments could be made. As found in the CEH bioassay, an ectendomycorrhizal morphotype dominated the seedling roots.

3. Discussions

Albizia gummifera experiments at KEFRI and CEH.

These experiments, proposed by Stephen Cavers, will test 3 seedlots x 3 soils from *A. gummifera* sites in Kenya, Uganda and Madagascar.
Soil collected from all 3 countries was brought to the workshop, divided between KEFRI and CEH and half brought back to CEH. Leaf material collected from the sites in Madagascar and Kenya was also divided and half brought back to CEH. Full details of the collections were supplied by Madagascar and Kenya, but are still to be supplied by Uganda.
However, seed from trees of *A. gummifera* at all 3 sites/countries was not ready for collection and it may be another 1-2 months before it is ready. This raised the issue of the survival of rhizobia in soil stored at 4^{o}C during this delay. As a result, it was proposed that KEFRI set up a preliminary experiment to look at effect of storage time on viability of rhizobial propagules e.g. remove soil from storage after 2, 4, 6, 8, 10,12 weeks and bioassay with test plants. Later in the year, when seed is collected, we can set up the 3 seedlot x 3 soils experiments as planned using fresh soil (although soil will still need be stored and shipped to UK). Alternatively, if the KEFRI experiment shows rhizobial viability is not greatly reduced by storage, we can proceed with the original stored soil.

Bioassay experiment in Uganda.

Soils (6): a chronosequence of site regeneration from 0-3, 4-10, 11-20, 21-30, 40-50 years and natural forest.
Tree species (2): *Albizia gummifera* + at least one other arbuscular mycorrhizal host.
Replication (6): a total of 72 pots with soil treatments randomised in 6 blocks and split plots for the different tree species, plants harvested after approx. 4 months.

Bioassay experiment in Madagascar.

Soils (3): primary forest, secondary forest and fallow (4-7 years).
Plots (2): for each soil type
Tree species (2): *Albizia gummifera* (AM), *Uapaca* sp. (ECM).
Replication (6): a total of 72 pots with soil and plot treatments randomised in 6 blocks and split plots for the different tree species, plants harvested after approx. 4 months.

The exact harvest period will depend on growth of the different tree species, but all plants of the same species should be harvested at the same time.

Assessments:

These will depend on staff time, experience and the facilities available, but could include:
Baseline studies – numbers and species of AM spores in test soils, soil characteristics, numbers and species of ECM fruitbodies produced in the forest.

Non-destructive measurements during the experiment – e.g. weekly measurements of stem diameter and seedling height.

Final harvest measurements – stem & leaf DW, nutrient content.
A. gummifera and other AM legumes - % AM infection, root and nodule DW.
Uapaca and other ECM plants - % ECM tips and morphotypes, root DW.

4. Acknowledgements

The workshop was indebted to the National Museums of Kenya for the loan of several dissecting and compound microscopes which ensured that all the participants had easy access to a microscope. We were also extremely indebted to the University of Nairobi for the loan of a compound microscope with video monitor and image capture facility which proved to be an invaluable teaching aid, and also meant that we were able to record many (maybe too many!) microscope images of AM spores, AM root infection, ECM spores and ECM root features during the course of the workshop. Lastly but not least, we thank the many people at KEFRI for the warm welcome and friendly co-operation and assistance provided throughoout.