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**Project 2: Testing the current critical load maps of
acidity for coniferous and deciduous forests**

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1. Introduction

The report covers the period 1.06.99 to 30.06.99 of the collaborative study involving staff at the ITE Merlewood and Bangor Research Stations, the MLURI, SSLRC and Forest Research (FR). Much of the first year has been spent in developing data bases of appropriate sites and related environmental information, site selection and development and testing of sampling and analytical protocols. The field sampling is being divided between 1999 and 2000 and the 1999 campaign was begun in June.

2. Project team

Prof. M Hornung and Ms R Creamer (ITE Merlewood), Dr B Reynolds and Mrs S Bell (ITE Bangor), Dr S Langan (MLURI), Dr I Bradley (SSLRC), Dr F Kennedy (FR). Dr H Jones and Dr A F Harrison (ITE Merlewood) are providing expertise and assistance on the root bioassay while a number of FR and FC staff are providing specialist advice. Ms J Hall (ITE Monks Wood) provides deposition and critical load data from national datasets.

3. Overall objective

To examine the relationship between mapped exceedance of critical loads of acidity for woodlands, the Ca:Al ratio of soil solution and forest status as measured by canopy condition and foliar chemistry.

4. General approach

The approach is following closely that set out in the tender document. This aimed to use a sample of 30-40 forest sites in GB stratified in terms of forest type, soil type and magnitude of exceedance of the critical load. FC monitoring plots, or other sites where there is existing background data would be used as far as possible. The initial target set of parameters to be measured at each site included soil solution and soil extract chemistry (cations, sulphate and nitrate); root condition and chemistry, tree nutrient demand, foliar chemistry and canopy condition.

5. Site selection

Following an initial project meeting, members of the project team identified potential sites where there was existing background information and/or other research in progress and submitted these to a central database established by FK. Two further meetings of the project team were then held to discuss the possible sites, select the final target group, as well as considering sampling and analytical protocols. An initial list of some 78 possible sites was identified: 10 Scots pine, 4 Norway spruce, 37 oak and 27 Sitka spruce. These sites were then ranked in terms of the magnitude of exceedance of the critical loads of acidity as predicted from the national deposition data bases. 30 sites were eventually selected for sampling, comprising 10 Sitka spruce sites, 12 oak, 1 Norway spruce and 7 Scots pine. The list of sites identified to date is included as Tables 1 and 2 and Figure 1.

Table 1. List of sites identified to date, organisation responsible for sampling, critical loads and current exceedance

JH's no.	Site name	Level I/II no.	Grid ref.	Land owner	Phone number of Forest District	Scientific owner	Proposed sampler	SMB CLA keq/ha/a	Exceed. S + totN keq/ha/a	Ratio Exceed/CLA
	15/06/99									
OAK	4 Hafod	N/A	SH810578	Snowdonia NPK	-	ITE-B	ITE-B	1.82	1.32	0.73
	17 Rheidol (1)	N/A	SN712783	CC for Wales	-	ITE-B	ITE-B	1.34	1.14	0.85
	19 Oak plantation	N/A	SJ271086	Powys Estates	-	ITE-B	ITE-B	0.75	3.41	4.55
	21 Pendugwm wood	N/A	SJ108140	Montgom. Wildlife trust	-	ITE-B	ITE-B	1.31	2.43	1.85
	26 Mabie	OK007	NX935724	FC-Ae	01387 860247	FR	ITE-M	1.17	1.94	1.65
	28 Castle Howard	OK010	SE694712	FC-NYM	01751 472771	FR	ITE-M	0.35	3.99	11.4
	31 Cropton	OK020	SE805924	FC-NYM	01751 472771	FR	ITE-M	0.95	2.68	2.83
	37 Savernake (LII)		516 SU055888	FC-Wilts&Avon		FR	FR	1.75	1.83	1.04
	38 Grizedale (LII)		517 SD334915	FC-lakes	01229 860373	FR	ITE-M	1.52	1.9	1.25
	39 Alice Holt (LII)		512 SU795402	FC-SEEEng		FR	FR	1.84	1.01	0.55
	5 Bryn Brethynau	N/A	SH733676	SNP	-	ITE-B	ITE-B	2.65	0.2	0.07
	30 Torrachilly	OK019	NH115868	FC-Inverness	01463 791575	FR	MLURI	2.35	NONE	N/A
SCOTS										
	40 Dartmoor	SP014	SX682803	FC-Peninsular	01392 832262	FR	FR	1.51	2.16	1.43
	42 Cymer	SP022	SS904903	FC-Coed Y Cymoedd	01639 710221	FR	FR	1.53	2.13	1.39
	43 Clwyd	SP027	SJ078767	FC-Llanrwst	01492 640578	FR	ITE-B	0.91	2.64	2.9
	46 Thieves wood	SP067	SK543563	FC-Sherwood	01623 822447	FR	ITE-M	1.24	3.22	2.59
	48 Ladybower (LII)		716 SK163908	FC-Sherwood	01623 822447	FR	ITE-M	3.4	1.84	0.54
	41 Lossie	SP051	NJ296672	FC-Aberfoyle	01877 382383	FR	MLURI	4.14	NONE	N/A
	45 Torrachilly	SP056	NH427561	FC-Inverness	01463 791575	FR	MLURI	1.45	NONE	N/A
	47 Theiford (LII)		715 TL954832	FC-East Anglia	01842 810271	FR	FR	10.72	NONE	N/A
	80 Tanar	N/A	NO440930	Glen Tanar Est.	-	MLURI/AU	MLURI	1.07	0.07	0.06
	81 Mharcaidh	N/A	NH882044	SNH	-	MLURI/ITE	MLURI	1.17	NONE	N/A
SITKA										
	3 Aber (2)	N/A	SH672710	FC-Llanrwst	01492 640578	ITE-B	ITE-B	1.78	0.79	0.45
	52 Pelena	SS026	SS803984	FC-Coed Y Cymoedd	01639 710221	FR	FR	1.39	2.27	1.63
	55 Hamsterly	SS037	NZ043286	FC-NYM (if not Kielder)	01751 472771	FR	ITE-M	1.38	1.65	1.19
	60 Coalburn (LII)		919 NY693781	FC-Kielder	01434 220242	FR	ITE-M	0.1	2.25	22.48
	63 Eilbank (Memo)	N/A	NT399353	FC-Hawick	old name - ask M	MLURI	ITE-M	1.05	0.86	0.81

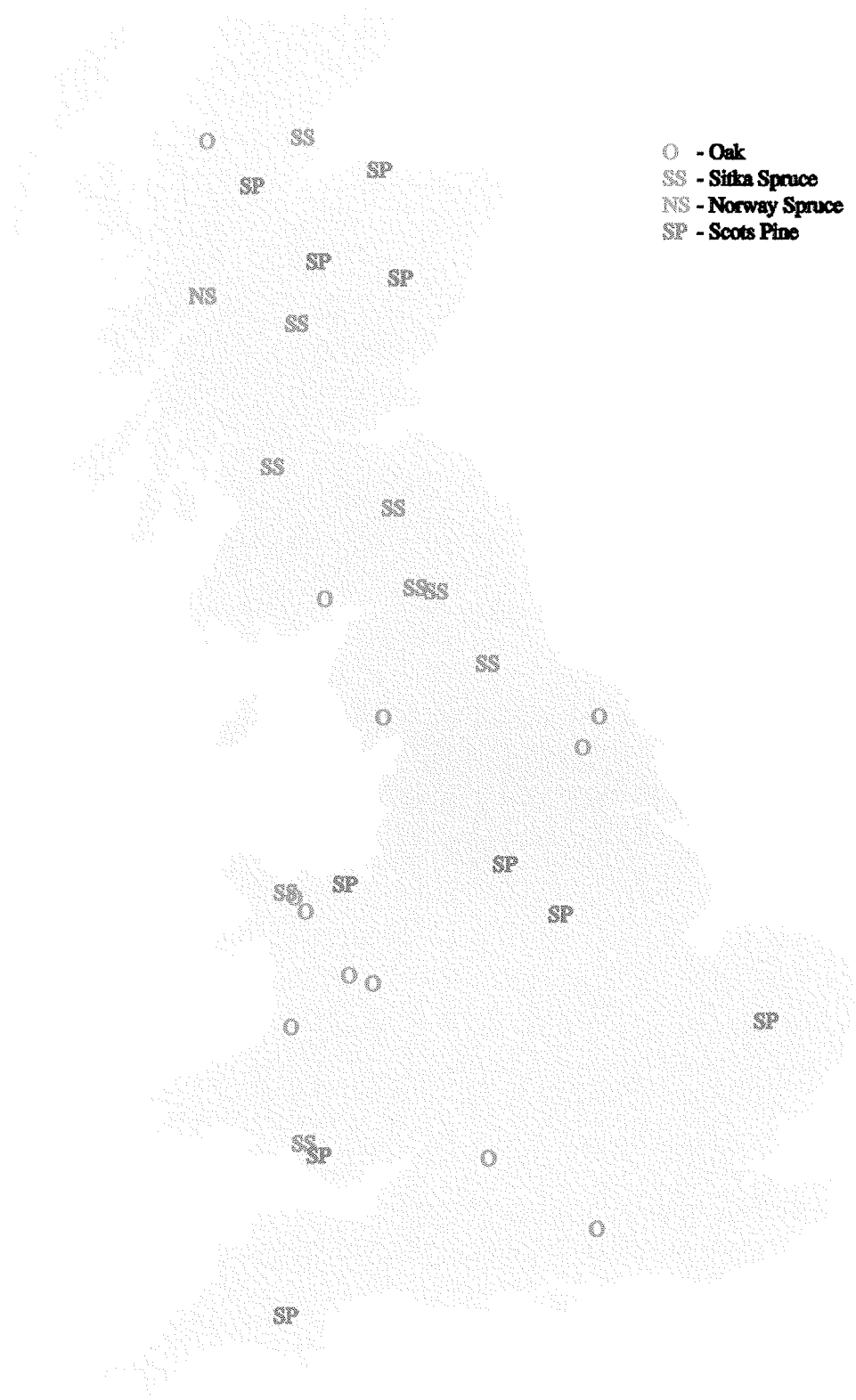


Figure 1. Distribution of sample sites

In the case of the Sitka, oak and pine sites the initial selection took the sites with the highest exceedance and then added 'controls' in non-exceeded areas. Additional Norway spruce and Scots pine sites are to be added and a meeting will be held in late 1999 to collate options identified in the meantime and make a selection ahead of the 2000 field season. The additional Scots pine sites will, as far as possible be taken from the native pine wood sites in Scotland surveyed and sampled by ITE in 1978. The possibility of adding a number of beech sites is also being explored.

The sites have been allocated between the research teams: ITE Merlewood – Northern England, the Midlands and Southern Scotland; ITE Bangor – Wales; FR – Southern England; MLURI – Central and Northern Scotland..

6. Sampling and analytical protocols

A series of standard protocols were developed for application by all participating groups/laboratories. These were largely developed in discussions at the two project team meetings referred to in paragraph 5 above. The recording and sampling protocols were then tested in a collaborative exercise, involving representatives from all sampling teams, in Cropton Forest, North Yorkshire. The protocols were re-evaluated and modified as necessary following this field test.

Lead groups have been identified for the various parameters and analyses. Soil analyses, vegetation recording and analysis, root bioassay – ITE Merlewood; Ca:Al ratio of soil solution, canopy condition, foliar chemistry and analysis of growth rings – FR; soil mineralogy – MLURI; soil classification and description - SSLRC. All root bioassay analysis are to be carried out ITE Merlewood (see section 6.2), all determinations of base cation:Al ratios and interpretation of tree growth data at FR Alice Holt; all canopy measurements by FC staff and all mineralogical analyses at MLURI. Soil and foliar analyses will be carried out by each of the participating laboratories but using standard methodologies. Available data on the soil series which occur at each of the sampling sites is being collated by SSLRC. Where existing data is considered inadequate to characterise the soils, SSLRC will visit the sites and provide full profile descriptions.

Soils from all the sites will be analysed for pH, loss on ignition, extractable Ca, Mg, K, Na and Al, and total N and P; foliar samples will be analysed for Ca, Mg, K, N, P and C; root bioassays will be carried out for N, P and K; soil slurry extracts will be analysed for base cations and Al.

6.1 Access, site recording and general sampling

The draft protocol was, as noted above, tested at the Cropton Forest site and modified in the light of those trials. The following version is being implemented at all sample sites.

6.1.1 Site location and access

The site should be located at the grid reference used to identify the site in the spreadsheet of sites. For FC sites, please refer to the 'ground rules' on access produced by FK, who will also provide maps for FC sites.

6.1.2 Site sketch plan

A sketch plan of the site should be drawn on the recording sheet. This should indicate the orientation of the sampling cross (see 3 below), the angle and direction of slope, the location and numbers of the vegetation plots, the rough position of the sample trees in relation to the arms of the sampling cross and their numbers. If the slope angle varies across the site, a section should be sketched to indicate the location of the change of slope.

6.1.3 Location of sample trees, soil and root sampling positions and site details

The recording and sampling is focussed on a cross with 30m arms and the square that encloses the cross; the cross should be laid out at the site using a tape rather than pacing. If possible the cross is laid out along north-south and east-west bearings. On steep slopes it may be laid out with one arm across and one up and down the slope. The bearings along each arm should be taken and recorded on the site diagram.

A 20 x 4 m 'corridor' is defined along each arm of the cross; the corridor should start at 10m from the centre of the cross and extend to the end of the arm, and stretch 2m either side of the respective arm of the cross. The trees within the corridor are numbered, starting from the inner, closest to the centre of the cross, end of the 'corridor' and three trees are then selected in the corridor along each arm of the cross using random numbers. The resultant 12 trees, three in the corridor along each arm of the cross, are the focus for recording of tree attributes and for soil sampling. The 12 trees should be numbered 1 to 12, with 1 to 3 on the northerly arm of the cross, 4 to 6 on the easterly arm etc. the tree number should be marked (discretely) on the stem using day-glow spray paint.

In deciduous woodland/plantations, there may be relatively few trees within the 20x4m corridors. As a fallback, the first three trees in the corridor should be recorded and used to locate the soil sampling position.

6.1.4 Tree density

The number of trees should be recorded in the 10x10m square around the centre of the cross and defined by diagonals joining the starting points of the 20x4m corridors, i.e. points 10m from the centre along each axis of the cross.

6.1.5 Tree recording and sampling

Breast height diameter of each of the 12 sample trees should be measured at a height of 1.3 m and recorded using the sample tree identifier number.

The canopy condition of the trees will be assessed and foliar samples collected by FC staff.

A stem core should be taken using the separate protocol from FK. Cores should NOT be taken from any trees that have a FC identification number marked on, or attached to them.

Tree height to be measured using a hypsometer.

6.1.6 Soil and root sampling

Soil samples are collected from a small pit located between 1 and 2 m, along a random co-ordinate, from the stem of each of the 12 sample trees. If the random co-ordinate falls on a path, rock etc move the position by 180°.

Samples are collected of recent litter, and from each horizon occurring within 20cm of the soil surface plus an additional sample for base cation:Al determinations. The latter is taken from the first horizon below the F (Of) or H (Oh). The thickness of the litter and each soil horizon is recorded. One soil sample per site should be collected from the base of one of the pits for mineralogical analysis. Roots for bioassay analysis are collected from the forest floor in the vicinity of the small soil sampling pit; separate root samples are needed for P and N determinations (see 6.2 for a more detailed protocol).

6.1.7 Vegetation recording

A general description of the site vegetation is recorded, e.g. canopy of oak with an under storey of Rowen and scattered holly, ground flora dominated by male fern, *D. flexuosa*, *Oxalis* and *Dicranum*, with significant areas of bare litter.

A 2x2m vegetation pot is recorded in each quarter of the 30x30 square enclosing the cross. The plots are positioned at random co-ordinates in each quarter. All species present are identified and cover assigned down to 1%, species with a lower cover than 1% are recorded as +. Cover can add to more than 100% if the vegetation is layered, eg ferns over *D. flexuosa*. Mosses and liverworts giving significant cover (1% or more) should be identified, or SMALL sub-samples collected for identification.

6.1.8 Sample numbering

Samples/records should be labelled as follows:

Site/tree number/sample type

Sample types are coded as follows:

D	breast height diameter
F	foliage
C	Stem cores
L	litter
R	roots
S	soil (+horizon identifier)
I	soil sample for base cation:Al determinations

For example: Cropton/4/L (litter sample from above soil pit adjacent to sample tree 4 at Cropton) or for soil samples Cropton/4/SA (A horizon soil sample from soil pit adjacent to tree 4 at Cropton).

Vegetation plot data labelled as follows:

Site name/V1 to V4.

Plot 1 is normally in the north easterly quarter of the square, plot 2 in the south easterly quarter etc.

6.1.9 Sample handling/storage

Root samples must be packed immediately between layers of damp tissues. They must be dispatched to ITE Merlewood on the day of collection (see 6.2).

Soil samples should be stored at 4°C prior to pre-treatment and analysis.

The soil sample for base cation: Al determination (I samples) must be placed in a 'cool' box containing cold freezer blocks immediately after sampling. On return to the 'home' laboratory they should be stored at 4°C prior to analysis.

6.2 Root sampling for bioassay and 1999 timetable.

The root bioassay (Jones et al 1994) has been shown to be a sensitive indicator of the nutrient demand of trees. Studies using the approach (Carreira et al. 1997, Harrison et al. In press) have also shown an increased demand for P at sites where the critical load for acidity is calculated to be exceeded. It was decided early in the development of the project that the assay should be applied to samples from all sites sampled.

Radioisotopes have to be specially ordered for the analyses and, in some cases used within a limited time frame. A large team also has to be assembled to carry out the root washing prior to carrying out the assays. For these reasons it is most efficient if the collection of the root samples for the assays is co-ordinated, allowing the analyses to be carried out in relatively large batches. We plan to carry out all the 1999 bioassays on two days in July. The following protocol covers sample collection and the delivery to Merlewood for the two days scheduled for analysis.

Roots are to be sampled from 3 trees in the 'plot' along each arm of the cross laid out at each site.

1 set of roots to be collected from each tree, for each bioassay - three root samples from each tree.

For P and K bioassays, 0-10cm of fine root material are needed for each.
For N bioassay, 0-20cm of fine root material is needed.

It is important to collect one piece of root, with abundant fine roots attached, for each of the bioassays.

For each site there will therefore be 36 roots, separated into 3 packages of 12 roots (one package each for N, P and K)

In 1999, the sample processing and analysis at Merlewood has been programmed as follows:

Tuesday 13th July, 8 sites = 288 roots,
Thursday 15th July, 8 sites = 288 roots

The sampling should take place on the Monday (12th) and Wednesday (14th), of July 1999 with 2 sites being sampled by each institute on each day.

Equipment needed: 3 trays labelled N, P & K.
Paper tissue, - layers.
Kitchen Foil,
Water Spray bottle,
Labels (label bioassay type first – example. N-CROPTON/5/R)
P-CROPTON/5/R
K-CROPTON/5/R

Root samples must be dispatched to arrive at ITE Merlewood by 9am on Tuesday and Thursday the 13th and 15th July.

6.3 Protocol for the extraction of soil solution for the determination of base cation:Al ratio using the slurry or equilibrium soil solution method (F Kennedy)

The ratio of base cations or calcium to aluminium in soil solution is widely used in the calculation of critical loads and in the assessment of the likelihood of root damage at given sites. A number of approaches have been used to extract or collect the solution for determination of the base cation and aluminium content, for example centrifuge extraction from a soil slurry, tension lysimeters, laboratory leaching of soils. Dr Fiona Kennedy has carried out an extensive review of the various approaches and presented the results at one of the project team meetings. Following discussion it was decided that a slurry based approach with centrifuge extraction would be used in the project and Dr Kennedy was asked to produce a detailed protocol for the production of the slurry and extraction of the solution. The protocol follows.

	LABORATORY	REQUIREMENTS
DAY 1		
1	Label a foil tray for each soil sample	Foil trays
2	Weigh each empty tray and record its weight	Balance, Record sheet
3	Weigh approx. 20g of field wet soil into each corresponding tray (record exact amount added)	Balance
4	Spread soil evenly in tray and air dry at approx. 30°C	Oven - 30°C /Drying room
5	Seal the remaining soil samples and store at < 5°C	Fridge space
6	Place the same number of ceramic crucibles as there are soil samples in an oven at 105°C	Crucibles, Oven - 105°C, Heat proof gloves
DAY 2		
7	Remove crucibles from oven and cool in a desiccator – leave oven on.	Desiccator
8	Cool, reweigh and record weight of each foil tray plus its air dry soil	Balance
9	Label and record weight of each empty crucible	Balance
10	If possible crush any large aggregates of air dry soil with the back of a spatula, then mix and weigh approx. 10g of air dry soil into each crucible (record exact weight added)	Spatula
11	Place crucibles, with air dry soil in them, back in the oven at 105°C	
DAY 3		
12	Remove the crucibles containing oven dry soil from the oven and cool in a desiccator	Desiccator
13	Weigh and record weight of each crucible plus its contents	Balance
14	Calculate the required amount of ultrapure water to make the slurry as shown on attached sheet	Spread sheet/Calculator
15	Weigh 100g of each field moist soil sample into a 250 ml glass beaker	250ml glass beakers, Balance
16	Add to each the calculated volume of ultrapure water. Note that depending on their organic matter content the samples may vary considerably in consistency.	
17	Mix each sample with a glass rod to form a slurry. NOTE remove excess soil from the rod by brushing it against the inside of the beaker and then remove it dirty. DO NOT rinse the soil on the rod back in to the beaker using more water.	Glass rod
18	Cover beakers and leave at < 5°C for 24 hrs	Fridge space, Film
DAY 4		
19	Extract solution from slurry using a centrifuge at 5000g for up to 1 hour.	Centrifuge and accessories
20	Filter the extract first through a 20µm pre filter and then through a Whatman 0.45 µm nylon syringe filter under a vacuum. I found that a pressure of 5 in. Hg was sufficient.	Prefilters, Syringe filters, Vacuum pump, Vac Master + accessories. (Exact details below)

Pre filters

These are 20µm polyethene 'frits' bought from IST (International Sorbent Technology). Their Customer Services Department address is as follows :

Customer Services Department,
International Sorbent Technology Ltd.,
IST House,
Duffryn Industrial Estate,
Hengoed,
Mid Glamorgan,
UK
CF82 7RJ

Phone : 01443 816656
Fax: 01443 816657
E-mail :info@ist-spe.com

The same firm also supplies Vac Master boxes and accessories.

Syringe Filters

Whatman 0.45µm nylon disposable filters. Cat. No. 402/0956/72 supplied by BDH (Merck).

To determine how much ultrapure water to add to each sample:

First determine how much water there is in each field wet subsample of approximately 20g :

Water in field wet subsample = that lost in air drying + that lost in oven drying

That lost in air drying (X) = weight of wet soil - [(weight of air dry soil + foil tray) - weight of foil tray]

That lost in oven drying (Y) = [weight of wet soil /weight of air dry soil] x {weight of air dry soil - [(weight of oven dry soil + crucible) -weight of crucible]}

Therefore in 1g of field wet soil there is Z mls where

$Z = (X + Y)/\text{weight of wet soil}$

The samples have to be brought up to 0.8 ml water per gram of field wet soil so the amount of water required per gram of wet soil is :

$0.8 - Z$ mls

and the amount of water added to exactly 100g of soil would be :

$100 \times (0.8 - Z)$ mls

Note. Should the result be negative then there is already more than 0.8 ml water/g in the field wet soil. I therefore suggest that the sample undergoes the same treatment as all the other samples but has NO water added.

6.4 Soil Analyses

It was decided that all the participating laboratories would follow the methods detailed in the volume of protocols for the terrestrial sites of the Environmental Change Network (ECN) (Sykes and Lane 1996).

6.5 Vegetation analysis

The plant species records from each plot will be used to classify the vegetation in terms of the National Vegetation Classification (NVC) (e.g. Rodwell 1992) and the Countryside Vegetation System (CVS) (Bunce et al 1999). Ellenberg scores for nutrient status will be calculated for all plots using the Ellenberg indices as modified for the UK by Hill et al (Hill et al 1999).

6.6 Mycorrhizas

Mycorrhizal status is being assessed on roots from a subset of sites sampled in 1999. A decision on whether to extend this work will be taken after the 1999 field season.

7. Future schedule

The 1999 field recording and sampling is programmed to take place in the period June to August. All site records for 1999 will be submitted to ITE Merlewood by the end of October 1999. The bioassay sampling and analyses will be carried out in June 1999. The work on the base cation contents of the soil slurry extracts from the 1999 soils samples will be completed by the end of 1999. The remaining soil and foliar analyses will be started in August 1999 but the precise schedule will be determined by each of the participating laboratories, ensuring completion of all analyses, from 1999 and 2000 by the end of the year 2000. Vegetation data from the 1999 sites will be submitted to ITE Merlewood by October 1999. Canopy condition surveys, to be carried out by FC staff, are scheduled for August 1999.

Project team meetings are scheduled for October 1999 and January 2000. The latter will assess available results, finalise the sites to be sampled in 2000 and agree the timetable for the bioassay sampling and analysis. The year 2000 field campaign will take place between June and the end of August.

All sample analysis is planned for completion by the end of the year 2000 with interpretation, analysis and preparation of the final report between January and May 2000.

8. References

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