CS Technical Report No.3/07

Soils Manual

BA Emmett, ZL Frogbrook, PM Chamberlain, R Giffiths, R Pickup, J Poskitt, B Reynolds, E Rowe, D Spurgeon, P Rowland, J Wilson, CM Wood

Centre for Ecology and Hydrology
(Natural Environment Research Council)

November 2008

Citation as: Emmett, BA, ZL Frogbrook, PM Chamberlain, R Griffiths, R Pickup, J Poskitt, B Reynolds, E Rowe, P Rowland, D Spurgeon, J Wilson, CM Wood. *Countryside Survey Technical Report No.03/07.*

Soil Manual : Contents

1. Introduction to CS soils

The overall purpose of the CS Soils (WP4) is to assess the status and changes in key soil properties, identify linkages between soil properties, attribute changes to different drivers, interpret possible effects on soil function, and help identify linkages to linked vegetation (WP1) and water (WP3) properties.

As part of the 1978 Countryside Survey, soil samples from the top 0-15cm were collected 15cm south of the southern corner of the five Main Plots in a sample of 256 x 1km squares (see *Table 1.3*). Samples from later Countryside Surveys were collected from the other corners resulting in soil sample locations approximately 2 to 3m apart between Countryside Surveys. These plots were re-sampled in 1998, whilst in 2007 soils were collected in all 591 x 1km squares, from the western corner of Main Plots. The soil (0-15cm) samples enable changes in several key soil characteristics to be studied, including pH, soil (0-15cm) carbon and nitrogen concentration, a measure of available phosphorus, heavy metal concentrations and soil biota. In addition, measurements of potentially mineralisable nitrogen and bulk density were made for the first time.

2. Sampling procedure

Task leader: Paul Chamberlain

Following is as handed to all field teams in 2007 survey

2.1. Equipment

Electric Cold Box, which should be kept cool by charging whenever the vehicle is being driven (connected to the vehicle lighter socket). An electrical mains charger is provided for use in accommodation. Additionally:

1 knife	1 plastic plate
Hammer	1 pair of pliers
Mallet	Regular trowel
Notebook & pen	Long thin trowel
Parcel tape	Spare cores

1 pack for the appropriate square containing:

- 5 X-plot packs with cores, end caps & labelled bags
- Stamped & addressed mailbags for 5 X-plots

2.2. Soil core samples

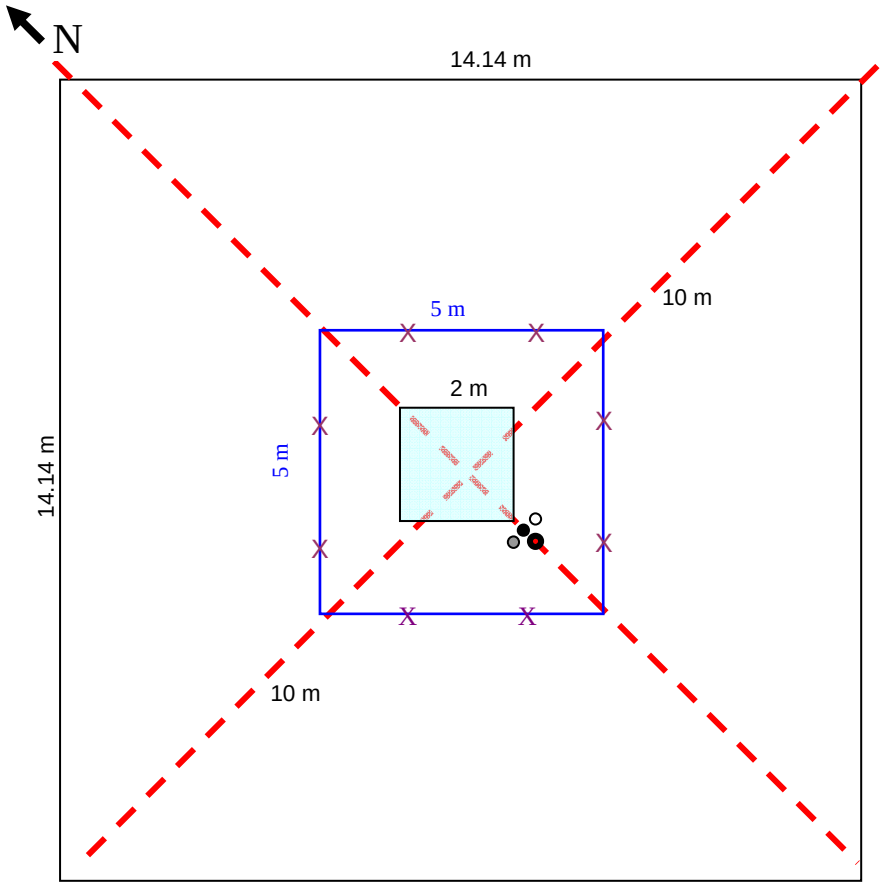
The cores will be taken approximately 15 cm S of the south corner of the centre quadrat in each X-plot of every square. Sampling procedures for each core are detailed below. If there are problems taking any of the soil samples or a specific comment needs to be made regarding the sampling then a note must be placed in the envelope (e.g. “large tree roots - 1st soil core taken 1 m E of centre quadrat”). If there is unusual vegetation, cow pat, boulder etc move minimum distance to get more homogenous sensible location and record problem.

Taking the cores

Take the appropriate labelled bag for this X-plot from the pack and find the 4 cores and 4 sample bags. For each core:

- Ensure that correct core is used in the correct position (see below)
- **All cores except short white**: move vegetation and loose litter to gain access to the soil surface
- **Short white core**: move vegetation, leaving the litter layer intact.
- Take the pipe and hold it upright with the bevelled end on the soil surface, while you cut round the bottom edge with the knife; cut vertically down into the soil through any roots. If the ground is very stony move the sampling point and record as above.
- Push pipe firmly into the ground until it stands upright.
- Hammer the pipe into the soil until the core is level with the soil surface.

- If there is not enough depth of soil, or the soil core is less than ¾ full when extracted from the ground, move the sampling point and start again. Record as above.
- If pipe breaks or distorts significantly, use one of the spare pipes provided.
- Use pliers to slowly twist and pull the pipe free from the ground, being careful not to lose soil from either end of the pipe (especially in dry/sandy soils). The trowel can be used to dig the pipe out or to stop soil falling from the bottom.
- Carefully scrape/remove any lumps of soil from the exterior of the pipe
- If needed, cut the bottom end of the core until it is level with the end of the plastic pipe
- See below for storage requirements for different cores
- Repeat for each centre quadrat in each X-plot (giving a total of five soil sampling locations in each square).



Soil sampling points 2007, 4 cores

X SQID Sub-sample, 8-10 samples to form one bag sample

● Core C: BLACK 15cm long x 5cm diameter

Locate the point 15cm south of the corner of the centre quadrat (USEFUL TIP: use the black core for distance as it is 15 cm long).
Once collected place into plastic bag and seal

○ Core F: SHORT WHITE 8cm long x 4cm diameter

NOTE: Remember to leave the litter layer intact for this core
Core F is located 15cm to the east of the black core
When the sample is obtained, push the caps over each end of the pipe.

Carefully seal the sample in its bag and return to the plastic bag

Core N: LONG WHITE 15cm long x 4cm diameter

Core 4 is located 15 cm to the south of the black core (30cm from the south corner of the centre quadrat). When sample is obtained, push the caps over each end of the pipe. Carefully seal the sample in its bag and return to the plastic bag

Core P: LONG GREY 15cm long x 4 cm diameter

Core P is located 15 cm to the west of the black core
It is vital that this core is the right way up, with the bevelled end placed on the soil surface. When sample is obtained, push the caps over each end of the pipe. Carefully seal the sample in its bag and return to the plastic bag.

2.3. Bag sample: SQID

These samples are to be taken in 120 squares only. A labelled sealable bag and mail bag will be provided in the pack for X-plots where these samples are to be collected.

One composite soil sample which fills the plastic bag up to the top of the top white panel. The sample consists of 8-10 soil sub-samples taken using the long thin trowel to a depth of 15 cm. The sub-samples will be taken along the boundary of the 5m quadrat, spaced evenly around the sides of the square.

If there are problems taking any of the samples or a specific comment needs to be made regarding the sampling then a note must be placed in the envelope. If there is unusual vegetation, cow pat, boulder etc move minimum distance to get more homogenous sensible location and record problem.

Sampling procedures for each SQID sample are detailed below.

- Move vegetation, as required, to gain access to the soil surface
- Insert the long thin trowel into the soil four times up to the end of the trowel blade (15 cm) to form a square the width of the trowel
- Lever the soil out of the ground and place into the labelled bag
- If the ground is very stony move the sampling point and record as above.
- If there is not enough depth of soil, move the sampling point and start again. Record as above.
- Repeat 8-10 times with at least 2 on each side of the quadrat, placing the soil into the same bag
- The soil should now fill the bag to the top of the top white panel. If it does not, take more samples (as above) until the level is reached.
- When the bag is full to the top of the top white panel, seal bag and enclose this bag in another bag and seal this one
- See below for storage requirements

2.4. Soil sample storage and dispatch

Take all 4 cores back to the vehicle and store:

Core C: BLACK

Store this core in its sample bag. Once all black cores for a square have been collected, store together in a spare plastic bag. Store in a cardboard box in the vehicle; keep out of direct sunlight. Return to your regional base.

Core F: SHORT WHITE; Core P: GREY; Core N: WHITE

Store these cores in their plastic bag in the coolbox immediately. When all 5 samples have been taken, place them in a spare larger plastic bag (short white, long white and grey cores separately) and put them in the appropriate mailbag, seal and post as soon as possible.

Soil Sample Bag

Place this sample in the cool box immediately. As soon as possible place in mailbag labelled for CEH Lancaster, seal and post.

Posting

Post samples as soon as possible. If samples cannot be posted by last post on Thursday, place them in the cool box over the weekend and post on Monday. Do not post any samples on a Friday. If the nearest post boxes will not take these packages please find a convenient Post Office. Check the OS map data for this square or Road Atlas for Post Offices.

3. Bulk Density and Hand Texture

Task leader: Paul Chamberlain

3.1. Key Question

What is carbon stock in soil 0-15cm and how does this vary between habitats?
How does soil texture vary between Broad Habitats?

3.2. Key products

- National and country-level assessments of topsoil bulk density (g cm^{-3}) in 2007, and change from CS2000
- Assessment of drivers of bulk density changes from CS2000
- Bulk density values will enable a more accurate determination of soil carbon stock (Section 4 Soil organic matter & carbon)
- Basic data on variability in hand texture between Broad Habitats

3.3. Policy background

Soil bulk density (BD) is the single most useful parameter of soil physical structure. It is a direct measure of soil compaction (or loosening) and is essential to assess total available pore space within a soil (that is, total porosity). Soil pore space occupies roughly half of the soils volume, and is essential for the sustainable use of soils since the pores hold air, water and soil biodiversity. Bulk density is an excellent measure of a most important contemporary form of soil degradation: that which occurs due to ill-timed cultivation, trafficking and stocking, and also affects soil biodiversity since increased BD means reduced macropore volume which is associated with decreases in microbial biomass and activity. In the USA, BD has been recommended as a key soil quality parameter because of its sensitivity to soil type and management and also its environmental relevance (Wander and Bollero, 1999).

The BD of a soil is also essential in the estimation of soil carbon (C) stocks, where it is necessary to convert from % soil organic carbon (SOC) to SOC per unit volume (e.g. g cm^{-3}). Changes to soil C content represent a major component of UK greenhouse gas emissions and under the Kyoto Protocol the UK is required to make estimates of net carbon emissions to the atmosphere. Changes in soil C content will need to be included in inventories and reporting if these estimates are to be meaningful. To date, our knowledge of soil C stocks and changes is limited; recent work by NSRI (Bellamy et al 2005) indicates that large changes have occurred recently, but these changes can only be related to broad habitat types.

Determinations of soil BD in CS2007 will allow the calculation of topsoil C stocks and change from CS2000, whilst relating these observed changes to potential pressures and drivers such as land use change, climate change and atmospheric deposition.

Soil texture is also a critical measure of soil physical characteristics. Unfortunately, resources do not allow for assessment beyond a simple hand texture methodology which is described in Figuire G-7 of ANNEX G.

3.4. Rationale for measurement

Table 3-1 Bulk density: rationale for measurement

	Facts	**Comments**
History in CS	All necessary measurements made in CS2000 with the exception of stone density and core depth.	Using stone density data from CS2007, can back-calculate to get topsoil bulk density in CS2000 (256 squares) This will be expanded to 629 CS squares in 2007, providing better spatial coverage and a time series
Links and compatibility to other monitoring programmes	*National Soil Inventory- England & Wales* Bulk density estimated from original survey measurements. *National Soil Inventory - Scotland* Some bulk density values available. *Environmental Change Network* Sample soils at 12 terrestrial sites every 5- and 20- years. Bulk density measured every 20 years to depth of 120cm or bedrock	Obvious links to NSI E&W and NSI Scotland, particularly in the 2007 Scottish resurvey, which will happen at the same time as CS2007. However, BD estimation methods differ and will need to be compared with caution Only one 20-year sampling has been made – next due in 2013. High intensity measurements on limited number of sites will complement CS data
Uniqueness of CS	At present, only national dataset to estimate bulk density on every soil sample measured for %C First national survey to re-measure bulk density	
Value for money (Policy priority or interpretative value X cost)	High	High policy and interpretative value, low cost. Cheap, reliable way of assessing soil physical structure. Expansion to 629 CS squares will allow change in SOC content to be measured accurately in future Surveys

3.5. Proof of concept

Bulk density is a measure of the amount of soil per unit volume. It is therefore an excellent measure of available pore space in a soil, and gives information on the physical status of the soil. BD values are also essential when estimating soil C stocks, as they allow a conversion from %C to C per unit volume, although BD values are often calculated based on C content rather than measured (e.g. Bellamy et al, 2005. Nature 437: 245-248). It is preferable, however, to measure bulk density and C content on the same sample.

Bulk density is calculated using the following equation:

$$\text{Bulk density (g cm}^{-3}\text{)} = \frac{\text{(Dry weight core (105℃) (g) - stone weight (g))}}{\text{(Core volume (cm}^{-3}\text{) - stone volume (cm}^{-3}\text{)}}$$

All necessary measurements for bulk density determinations will be made in CS2007, enabling the estimation of topsoil C stocks on a g cm^{-3} basis. In CS2000, all the measurements, with the exception of stone volume and core depth, were made, however stone volume estimates for CS2000 will be calculated from CS2007 data in the following way:

- In CS2007, samples will be taken from within 2m of the CS2000 locations; stone characteristics within such a close proximity are similar, and stone weight, density and volume will be measured.
- Since stone density (not the density of stones in the soil, but the physical density of the extracted stones) will be consistent between the two samples, stone weight (from CS2000) and stone density (from CS2007) will be used to calculate CS2000 stone volume using the equation: volume = mass / density.
- This information will allow the back-calculation of topsoil bulk density in CS2000.

The CS2007 Preparation Phase included a study of differing methods of extracting soil from the ground for BD determination, including coring and digging a soil pit (see ANNEX D for full details). Five different cores were tested: 10 cm long x 5 cm diameter round core, 15 cm x 6.4 cm round core, 10 cm long x 5 cm square metal core, 10 cm long x 8 cm square metal core, 8 cm long x 4 cm diameter round core. The soil pit method involved digging a pit, then filling the resulting hole with a plastic bag and using water to measure the volume.

Five field sites were visited, to allow the comparison of 5 different soil types. Soils tested were: clayey soil, sandy soil, peaty soil, stony soil and a woodland loam. Three replicates per extraction method (both coring and pit extraction), per soil type, were taken (excepting sand and clayey sand, as it was decided that the soils were uniform enough for just one sample to be taken). In the laboratory, samples were weighed, separated out onto a tray and dried at 105℃. Once dry, the soils were sieved and stones and soil separated. All components were weighed and the BD calculated.

Overall, the different core types tested gave fairly consistent BD values across soil types and no one core type gave consistently higher/lower BD values. The values of BD estimated from cores and pits were similar, and were within the ranges of typical values expected for each of the soil types. However, any methods used for sampling in CS must take into account the time available in the field, the capacity of the field

team to carry equipment, and the logistics of the survey. The recommendations for BD measurements in CS were as follows:

1. The pit extraction method is not feasible due to the amount of heavy equipment needed and the complexity of the task.

2. Although the metal square cores tended to hammer in more easily, they tended to distort easily in difficult soils, making their use limited. In 'sticky' soils, some of the sample was left in the corners of the core. They are also heavier to transport by post.

3. Taking the above into account, the coring method is the only method which can be realistically carried out in a consistent manner on such a large scale.

4. The black 15 cm cores used in the CS2000 are acceptable to use for measuring bulk density in the majority of soils, on the condition that surveyors/lab. personnel follow careful instructions.

Thus in CS2007 soils will be sampled using a 15 cm long x 5 cm diameter core, which will be hammered into the ground, and removed using pliers. This same core will be used for BD, soil C, total N and pH determinations.

3.6. Key models which require analyte data

Some soil models require BD measurements as input variables e.g. Profile, a steady state soil chemistry model, and SAFE, a dynamic soil acidification model. The Soil C model RothC and University of Aberdeen model ECOSSEalso uses BD as an input variable.

3.7. QA

There is an ISO standard for determination of dry bulk density (ISO 11272:1998); however the structure of CS does not lend itself to the recommended method, and an alternative method has been devised. See Proof of Concept (Section 4.2.5).

3.8. Field protocol

See Section 2 for full details. Samples for measured for bulk density were collected using a 15 cm long by 5 cm diameter black plastic core following the detailed field protocol described in Annex 1. Using the black core, a sample was collected from a point 15 cm to the south of the southern corner of the centre quadrat in each X plot in each 1 km square, giving 5 samples per 1 km square. The surface vegetation was removed to reveal the soil surface and the core was inserted to the full 15 cm depth. In stoney or shallow soils, the sampling point was moved if a full core depth could not be obtained. Any such variations in the protocol were recorded. On removal from the ground, the outside of the core was cleaned and any excess soil was trimmed from the bottom of the core. The core was then placed in a labelled plastic bag which was sealed and stored in the surveyor's vehicle pending delivery to Lancaster.

3.9. Laboratory protocol

All 3145 black cores were analysed for bulk density and hand texture. For standard operating procedure see Annex G. BD determinations require the exact dimensions and weight of the soil core to be recorded at first, as sub-samples of soil are taken for fresh pH measurements and these removals must be accounted for in the final BD calculation. BD determinations use the same material as LOI measurements, and therefore follow the LOI protocol to obtain moisture content: after initial measurements the soil is dried, weighed and sieved, after which the separated soil and stones are reweighed. A sub-sample of soil is then dried at 105℃ for 16 hours, cooled and weighed. The mass and volume of the stones are also determined, and the stone density (density = mass / volume) calculated and used to estimate CS2000 topsoil bulk densities. From the measurements of soil and stones, CS2007 BD is calculated using the equation in Section 3.13

3.10. Methods for sample storage and archiving

Bulk density determinations must be performed on fresh soil. Once the soil has been processed, it is not possible to measure bulk density subsequently. Air dried soils will be stored as detailed in Section 4.

3.11. Future use of material

All future use to be approved by CS topic group or steering group. Once processed, no future use of soils in bulk density determinations is possible. Other possibilities for use of archived soils include analysis for other chemical methods e.g. metabolomics, NMR etc. Biological methods unlikely to be appropriate.

3.12. QC

QC of CS BD estimates are limited due to the absence of a control soil which can be processed at the same time as the CS2007 samples. However, other QC proxies are possible and will be used to confirm the reliability of the data:

1. BD estimates will be checked against expected values for that soil type

2. BD estimates for CS2000 and CS2007 will be compared to establish whether there are abnormally large differences between the two samples, and work to establish the causes for any differences will be carried out

3.13. Calculations/Units

Topsoil bulk density will be reported as g cm^{-3}, calculated using the equation:

$$\text{Bulk density } (g\ cm^{-3}) = \frac{\text{(Dry weight soil (g) - stone weight (g))}}{\text{(Core volume } (cm^{-3}) - \text{stone volume } (cm^{-3})}$$

3.14. Data storage

Table 3-2 Bulk density data storage

Field Name	Description
SQUARE_NUM	CS Square number
PLOT_TYPE	Plot type
REP_NUM	Replicate number
BD_2007	Bulk density g cm^3
COMMENTS	Any additional information

Field Name	Description
SQUARE_NUM	CS Square number
PLOT_TYPE	Plot type
REP_NUM	Replicate number
Est_BD_2000	Estimated CS2000 bulk density g cm^3 based on 2007 stone volumes
COMMENTS	Any additional information

3.15. Statistical analysis

No statistical analysis has been made to determine the number of bulk density measurements in CS2007, since bulk density determinations will be made on all cores taken for C analysis (Section 4). See Section 4 and ANNEX C for power analysis of the number of topsoil C samples needed in CS2007 and ANNEX F for background to statistical approach for estimating stock and change.

3.16. Linkages to other tasks and work packages

- Link to soil C; will enable estimation of soil C in g cm^{-3}
- Links to contaminants and nitrogen determinations, as enables a total stock in g cm^{-3}
- Links to soil biodiversity measurements: relationship of microbial and invertebrate biomass and diversity to BD (Section 8 and 10)

3.17. Linkages to other surveys

- Links to other UK soil monitoring programmes and inventories such as the National Soil Inventories and Environmental Change Network soil monitoring, both of which contain some BD measurements
- Links to soil monitoring programmes in other countries such as New Zealand.

4. Soil organic matter & carbon content

Task leader: Paul Chamberlain

4.1. Key question

Can we confirm the loss of soil carbon (0-15cm) as reported by Bellamy et al 2005?

4.2. Key products

National and country-level assessments of topsoil organic matter content (%) in 2007, and national change from 1978 and CS2000

National and country-level assessments of topsoil organic carbon content (%) in 2007, and national change from 1978 and CS2000 (based on a conversion calculation)

National and country-level assessments of topsoil organic carbon amount (g cm^{-3}) in 2007, and national change from CS2000 (based on a conversion calculation)

Assessment of the relative importance of different pressures and drivers of change for topsoil carbon content, in particular the land use change, climate and atmospheric pollution

4.3. Policy background

Measurements of soil carbon (C) content for policy reasons are necessary in many circumstances including verification of soil sinks and greenhouse gas inventories under the Kyoto protocol, the increased emphasis on soil protection for environmental health under CAP reform, and the efforts to establish reliable measures of soil quality. Soil organic C (SOC) is one of the headline indictors of soil quality and there is a wide acceptance that carbon is fundamental to soil functioning as it is the primary energy source in soils. All soils therefore need to retain carbon. However, soil C determinations are measured against large background stocks and high spatial heterogeneity, and more information is needed to be able to manage this resource better.

Soil is a major component in the global carbon cycle and vulnerable to impacts of human activity with about 2773 x 10^6 t of SOC in UK soils. Globally, twice as much carbon is stored in soils as in the atmosphere with peatlands contributing a third of this. Therefore, even small changes in soil C stocks might contribute significantly to global climate change, for example, due to a positive feedback as a result of global warming. Whereas above ground carbon cycling is well understood, there are great uncertainties in climate impacts on soil carbon cycling.

Changes to soil C content represent a major component of UK greenhouse gas emissions and under the Kyoto Protocol the UK is required to make estimates of net carbon emissions to the atmosphere. Soil C content changes will need to be included in inventories and reporting if these are to be meaningful. Knowledge of soil C stocks and changes is limited; recent work by the NSRI (Bellamy et al 2005) indicates that large changes have occurred recently, but these changes can only be related to broad habitat types. If stocks could be related to more detailed vegetation and other environmental data this would allow better mitigation targeting.

Both the UK government and EU legislation have introduced a number of soil protection measures that will help to conserve soil carbon. The reformed Common Agriculture Policy requires all farmers in receipt of the single payment to take measures to protect their soil from erosion, organic matter decline and structural damage. Further incentives for good soil/land management are available under the Environmental Stewardship scheme. CS soil C data will contribute to knowledge of how soil C is changing, and the effectiveness of soil protection legislation.

Soil samples in Countryside Survey 2007 will be the third in a time series of samples from 1978 and 2000; this will be the first soil time-series in Europe and possibly globally. Analyses of topsoil C in CS2007 will allow further quantification of topsoil C contents and change across all major UK land uses and, crucially, through the link to other CS and spatially relevant information, will allow the assignment of pressures and drivers to the observed changes, be they climate change, land use and management, atmospheric deposition, etc.

4.4. Rationale for measurement

Table 4-1 Carbon: rationale for measurement

	Facts	Comments
History in CS		
SOM content (%, from loss-on-ignition)	Measured in 1978 and CS200 for 256 squares. Expanded to 629 squares in CS2007	Repeat sampling will maintain the time series. The increased spatial coverage will support country-level reporting by giving greater statistical power, especially for Wales (see below)
Topsoil organic carbon (SOC) content (g cm^{-3})	An SOC conversion will be obtained from CS2000 and CS2007	Combining bulk density with SOC allows change in SOC stocks to be determined (all squares CS2007) and change CS2000 to CS2007 (256 squares)
Topsoil total C content (%)	Gives total soil C composition; measured by elemental analyser in CS2000	Data obtained from same analytical run as that of total soil N content (see WP 4.4)
Links and compatibility to other monitoring programmes	*National Soil Inventory- England & Wales.* Information from 5500 locations in England & Wales including %C and soil horizon information *National Soil Inventory - Scotland* Data includes %C and soil horizon information. A repeat survey in Scotland is planned for 2007 *Environmental Change Network* Soil monitoring at 12 terrestrial sites every 5 & 20 years. Total organic C and bulk density in both 5 and 20-year determinands. Dissolved organic C measured in soils every 2 weeks *Representative Soil Sampling Scheme (RSSS)* Carried out since 1969, soil samples	Data comparability exercise for CS, NSI, RSSS and ECN carried out for Defra (Project SP0515). Soil C data directly comparable between NSI, CS & ECN ECN produces high-resolution long-term data on soil DOC concentrations and captures seasonality and inter-annual differences where CS cannot. ECN data may help explain results observed in CS.

	taken from stratified random sample of 180 farms. Runs on a five-year sampling cycle, with a subset of the selected farms sampled each year. However, SOM not measured since 1984.	
Uniqueness of CS		
SOM content (LOI)	CS soil samples are spatially linked to many other data collected at the same time. It is unique in that the results can be linked to pressures and drivers, e.g. vegetation, deposition, land management	Sampling of soil in close proximity to the detailed vegetations characterisations is vital in the investigation of relationships between land use/habitat and soil characteristics
Topsoil SOC content	CS measures the bulk density of each soil sample, leading to an accurate measure of topsoil C content	Bulk density has not been measured within any large-scale soil survey until now. The data can be used to determine how stable BD is over time and whether BD measured or estimated are required assess soil carbon changes
Value for money (Policy priority or interpretative value X cost)		
SOM content (LOI)	High	High policy and interpretative value (time series), low cost. Expansion to all CS squares future-proofs data for further Surveys
Topsoil SOC stock	High	High policy and interpretative value, low cost.
Topsoil total C content (%)	High	Data obtained 'free' as part of total soil N content determinations

4.5. Proof of concept

Loss-on-ignition (LOI) is a simple and inexpensive method for determining SOM, and SOC using an appropriate conversion equation. LOI values were determined for 256 squares in the Ecological Survey of 1978 and in CS2000; therefore CS2007 will be the first national survey to have three measurements over which to assess SOM

change. In addition to the 256 squares previously sampled for LOI, an extra 373 squares (giving a total of 629) will be sampled for soils in CS2007. This is necessary 1) to ensure adequate coverage for country-level reporting in Wales, for which there were only 20 squares in previous Surveys; 2) to ensure adequate statistical power to detect change within all three GB countries (see Section 4.15).

Alongside LOI measurements, total soil C content was determined by elemental analyser analysis in CS2000 and will be obtained again in CS2007. This measurement complements the LOI data since it is an actual measurement of C content in soil, however in soils with high amounts of carbonates the total C content may be considerably larger than the organic C content. The total C content is determined 'for free' in the same analytical run as that of total N and thus is extra information at no extra cost (see Section 7), however, total C determinations cannot replace LOI as a method of measuring soil C because 1978 samples were only analysed by LOI and not by total C.

To calculate topsoil SOC amount on a g cm^{-3} basis, a measurement of C per unit volume is needed. Combining bulk density measurements with LOI/SOC values will result in estimates of topsoil C on an area basis, leading to the first national Survey assessment of topsoil C content and change, CS2000 to CS2007.

4.6. Key models which require analyte data

Many SOM models have been produced (e.g. Century, RothC, DNDC, EPIC, Ecosse); the majority, including all those listed, require SOM content, SOC content or %C, but all are calibrated to a specific site rather than soils over a large area.

However, there are efforts to quantify ecosystem roles in the carbon cycle using models for which CS data will be important. The NERC QUEST programme is seeking to produce a better qualitative and quantitative understanding of the earth system, and within QUEST the QUERCC project is looking to use soil C:N:P ratio data as a benchmark with which to judge the ability of models to predict soil processes.

GBMOVE is an empirically-based static model which uses information on soil acidity, nitrogen and moisture status, together with information on light availability derived from the SUMO vegetation-type model, to predict plant species' probability of occurrence, and thereby predict vegetation composition. GBMOVE needs soil C to derive soil C:N, and to be able to respond to modelled changes in C in its own right. CS data will act as input variables for this model.

Lab experiments and some General Circulation Models (GCMs) have suggested a feedback between soil C and climate change; modelling the soil C changes observed in CS using UK climate and atmospheric deposition data will aid in assigning the drivers of change. Combining GCMs and the CS soils C data may lead to better predictions of climate change over the next 100 years.

4.7. QA

The Defra/NERC joint Codes of Practice will be followed throughout.

In 1978 and CS2000 LOI was performed on 10 g soil at 375℃ for 16 hours and on 1 g soil at 550℃ for 3 hours, respectively. In the preparation phase of CS2007 it was

identified that the CS2000 LOI method results in higher values of LOI across the entire SOM range relative to the 1978 method, and it was decided that all available CS2000 soils would be reanalysed using the 1978 method. Additionally, it was decided to use the 1978 LOI method in CS2007, as 1) this would yield a consistent dataset across 1978, CS2000 and CS2007, and 2) the use of 10 g soil for LOI is preferable since it is more representative than 1 g.

4.8. Field protocol

See Section 2 for full details. Samples for LOI & SOC were collected using a 15 cm long by 5 cm diameter black plastic core following the detailed field protocol described in Annex 1. Using the black core, a sample was collected from a point 15 cm to the south of the southern corner of the centre quadrat in each X plot in each 1 km square, giving 5 samples per 1 km square. The surface vegetation was removed to reveal the soil surface and the core was inserted to the full 15 cm depth. In stony or shallow soils, the sampling point was moved if a full core depth could not be obtained. Any such variations in the protocol were recorded. On removal from the ground, the outside of the core was cleaned and any excess soil was trimmed from the bottom of the core. The core was then placed in a labelled plastic bag which was sealed and stored in the surveyor's vehicle pending delivery to Lancaster.

4.9. Laboratory protocol

All 3145 black cores will be analysed for LOI at CEH Lancaster. For standard operating procedure see Annex G. LOI is measured on a 10 g air dried sub-sample taken after sieving to 2 mm. The sub-sample is dried at 105℃ for 16 hours to remove moisture, weighed, then combusted at 375℃ for 16 hours. The cooled sample is then weighed, and the loss-on-ignition (%) calculated.

Through the total N analysis, total C will be determined, using a total elemental analyser. This will be for the 1280 samples from the original 256 squares. The method used was CEH Lancaster UKAS accredited method SOP3102. Details of the method are given in Section 7.9.

4.10. Methods for sample storage and archiving

Table 4-2 Carbon: methods for sample storage and archiving

Requirements	**LOI**
Type of sample (e.g. wet/dry soil, extract, both)	Air dry soil
Mass / volume of sample	All sample remaining after processing
Storage container (e.g. glass, plastic)	Plastic
Storage requirements (e.g light dark, controlled humidity, temperature)	Dark, dry, cool
Storage location	CEH Lancaster
Length of time samples are stable	Indefinitely

4.11. Future use of material

All future use to be approved by CS topic group or steering group. Possible use of archived soils includes re-analysis to check methodology and QC for future surveys. Other possibilities include analysis for other chemical methods e.g. metabolomics, NMR etc.

4.12. QC

All LOI analytical batches contained one standard material that was cross-checked after analyses to validate the data, and one repeat sample. Results from LOI of the standard materials are included in the ORACLE SOM datasets for future reference. Results indicate good repeatibility and precision (Figure 4.1).

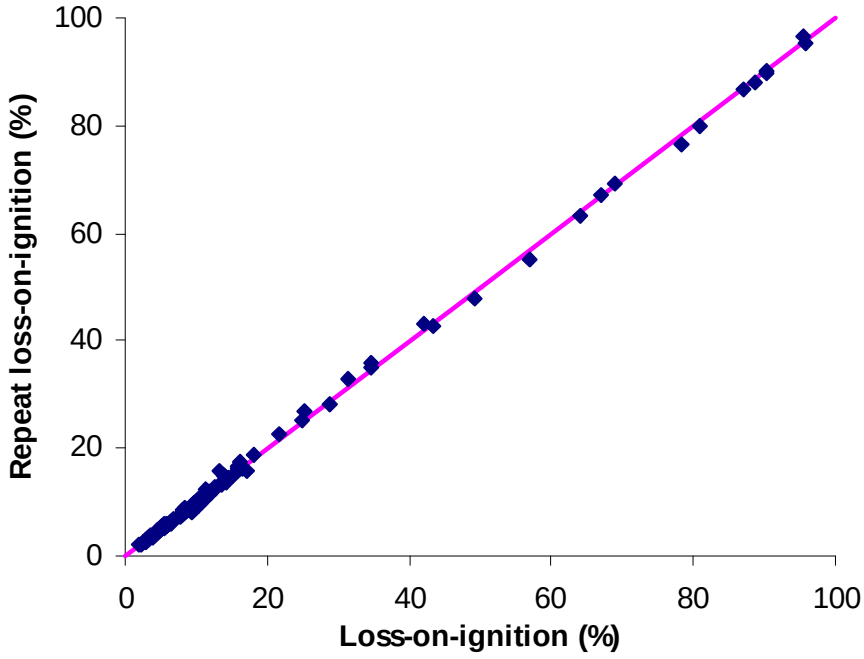


Figure 4.1 Comparison of results for LOI on repeat samples

4.13. Calculations/Units

LOI (%SOM) is obtained directly from the experimental protocols and will be directly input into the main CS database.

LOI-SOC conversion factors will be produced from linear regression analysis of data retrieved from this database, and an appropriate conversion applied to all LOI data, to produce %SOC estimates.

Topsoil SOC stock data (in g m^{-3}) will be produced as follows:

SOC stock (g cm^{-3}) = bulk density (in g cm^{-3}) x SOC content (%) / 100

4.14. Data storage

Data will be stored in the CS database in the following format:

Table 4-3 Carbon data storage

ABBREVIATION	EXPLANATION
SQUARE_NUM	CS2000 1km square number
PLOT_TYPE	CS2000 X-plot
REP_NUM	CS2000 subplot within the 1km square
ARRIVAL_DATE	Date of arrival of cores at CEH
TRAY_WEIGHT	empty tray weight
CORE_TRAY_WEIGHT	Tray weight plus sample.
PIPE_WEIGHT	weight of empty core
WET_PH	wet pH value
QC_WET_PH	Quality Control code for missing values
CORE_TRAY_REWEIGHT	Reweight after sample for pH removed
AIR_DRY_START	date for starting air-drying
AIR_DRY_WEIGHT_BEFORE_SEIVING	weight of air-dry sample before sieving
AIR_DRY_WEIGHT_AFTER_SEIVING	weight of air-dry sample after sieving
DRY_PH	dry pH value
QC_DRY_PH	Quality Control code for missing values
DRY_PERCENTAGE	Dry (percent) - of the sample
QC_DRY_PERCENTAGE	Quality Control code for missing values
LOI	Loss-on-Ignition
QC_LOI	Quality Control code for missing values
BATCH	Batch Number as samples completed within batches of 25 samples
LAB_NUM	Laboratory Number - No of sample within each batch
STORAGE_START_DATE	Start date for storage of samples.
NOTES_PRESENT	if notes are present (Y)
NOTES	Notes of disturbance to the sample.

4.15. Statistical analysis

Power analysis of the existing CS dataset (1978 & CS2000) was been performed to determine the number of squares needed in CS2007 to give adequate reporting power for soils in Wales, and greater power for soils in Scotland and England.

LOI varies substantially across the country, being much higher in Scotland that England or Wales (Table 4-4). As a result the changes in LOI, which in absolute terms are not markedly different, are proportionally much smaller in Scotland. Only in England was the change from 1978 to 2000 significant. Estimates of LOI in Wales

are poor largely because of the small sample size. With only 20 sample squares in Wales previously sampled for soils, several Land Classes that occur in Wales were not represented and several were only represented in one square.

The current sample sizes enable changes of about 10% to be detected with reasonable power in Scotland and England (Table 4-5). For example, the analysis shows that the number of squares sampled for soils in CS2000 in England gives a 64.6% chance of observing a 10% change in LOI with a significance of 5% (the usual level below which results are not considered significant). However, in Wales however the soils sample size is too small to detect any reasonable level of change with any certainty. In Wales, the number of squares previously analysed for soils only yields a 18% chance of observing a 5% change in LOI. Hence, the number of squares in CS2000 is not sufficient to allow country-level reporting for Wales.
Table 4-6 summarises the statistical power of various sample sizes in Wales. Whilst 20 squares have previously been sampled for soils in Wales, there were a total of 65 squares in Wales in CS2000. However, to enable reasonable reporting for Wales separately in CS2007, it has been recommended (Clarke, Howard, & Scott, Countryside Survey: Sampling for Wales-Only Reporting. Available on Confluence) that Wales has a total of 124 squares in CS2007. If all these 124 squares were sampled for soils, there would be a 72.5% chance of detecting a 10% change in LOI at 5% significance. To ensure that there is a significant chance of detecting changes in soil C of the magnitude reported in the NSI England & Wales (x% since the 1970s; Bellamy et al 2005), it is therefore recommended that all CS2007 squares in all 3 countries be sampled for topsoil C.

Table 4-4 Estimates of LOI and change in LOI 1978-CS2000 for England, Scotland and Wales.

	LOI 1978	LOI 2000	Change in LOI	% change	se of change	Num Squares
England	12.4	14.6	2.2	16.3	0.6	114
Scotland	45.9	47.6	1.3	2.8	1.4	98
Wales	14.4	26.8	1.4	6.9	2.0	20

Note: Estimated amounts and changes in LOI are national estimates calculated from weighted averages using the ITE Land Classification as strata. The large apparent change for Wales between 1978 & CS2000 is due to the addition of an extra square in CS2000 which contained highly organic soils. The change in LOI data is calculated only on squares sampled in both years.

Table 4-5 Power to detect various degrees of change in LOI for England, Scotland and Wales.

		Percentage change in LOI			
	Significance	**5%**	**10%**	**20%**	**30%**
England	**1%**	12.3	50.3	99.0	100.0
	5%	21.4	64.6	99.7	100.0
	10%	31.6	75.5	99.9	100.0
Scotland	**1%**	24.1	82.1	100.0	100.0
	5%	36.8	90.1	100.0	100.0
	10%	49.1	94.5	100.0	100.0
Wales	**1%**	3.6	10.0	40.6	79.0
	5%	7.5	18.0	55.1	88.0

	10%	13.1	27.4	67.2	93.2

Table 4-6 Power to detect various degrees of change with increased sample sizes in Wales.

Sample size	Significance	Percentage change in LOI			
		5%	**10%**	**20%**	**30%**
20	**1%**	3.6	10.0	40.6	79.0
(1978/2000)	**5%**	7.5	18.0	55.1	88.0
	10%	13.1	27.4	67.2	93.2
65	**1%**	8.3	32.9	92.5	100.0
(current)	**5%**	15.4	46.9	96.5	100.0
	10%	24.1	59.4	98.3	100.0
90	**1%**	11.2	45.6	98.2	100.0
	5%	19.7	60.1	99.3	100.0
	10%	29.6	71.6	99.7	100.0
120	**1%**	14.8	59.2	99.7	100.0
(proposed)	**5%**	24.8	72.5	99.9	100.0
	10%	35.7	82.0	100.0	100.0

The statistical approach used for analysing the data for changes in 1978 – 1998, 1998 – 2007 and 1978 – 2007 for the 2007 report is reported in a separate CS technical report. Essentially, bootstrapping is used which involves treating sample data as a population from which to resample. Each resample produces a separate estimate of some quantity of interest, for example stock or change. A large number of resamples (typically 1000 or 10,000) then gives an approximation to the distribution of the required estimate, from which any statistic can be extracted. The main advantage of this method of estimation for CS is that it allows for non-normality in the data, without the necessity of knowing details of the actual distribution, and as such provides more accurate measurements of significance. For a background to the approach see ANNEX F.

4.16. Linkages to other tasks and work packages

- Links to other chemical measurements such as soil pH and total N concentrations.
- Links to contaminants - SOM as an explanatory variable of metal and POP concentrations
- Links to biodiversity measurements for relationship of soil C content to invertebrate and microbial diversity
- Links to available N determinations – relationship of soil C content to available N
- Links to Integrated Ecosystem Assessment (WP5)

- Needs input form WP1

4.17. Linkages to other surveys

- Links to other UK soil monitoring programmes and inventories:
  - National Soil Inventory – England and Wales
  - National Soil Inventory – Scotland
  - Environmental Change Network Soil C monitoring
  - BIOSOIL
  - British Woodland survey
- Opportunity to test depth issues for total soil C stock with NSI Scotland
- Links to UK Soil Indicators Consortium (UK SIC) – for which SOM content is a proposed chemical indicator for soil quality as it defines soil fertility, stability and erosion extent
- Links to monitoring programmes in other countries (e.g. the Netherlands, New Zealand) and EU projects, particularly ENVASSO (ENVironmental ASsessment of Soil for mOnitoring)

5. pH

Task leader: Brian Reynolds

5.1. Key question

Has recovery from acidification continued?

5.2. Key products

- National and country level assessment of soil pH in 0 – 15 cm depth of soil
- Time series of soil pH change: 1978 to 2000 to 2007
- Assessment of plant species composition change in relation to N and acidity
- Assessment of soil acidity in relation to declining acid deposition
- Key parameter for calibration and validation of biogeochemical models

5.3. Policy background

Soil pH is probably the most commonly measured soil chemical parameter. It gives an indication of soil acidity and therefore has direct policy relevance in a number of areas ranging from agricultural productivity to recovery from acidification. Soil pH is a key variable for predicting the mobility and bioavailability of metals in soils and helps determine the response of plant species to changes in atmospheric nitrogen and acid deposition.

Soil pH data from CS2000 provided unique nationwide evidence of soil pH change since the first measurements in 1978. The preliminary data were incorporated within the report of the National Expert Group on Transboundary Air Pollution (NEGTAP 2001) as providing evidence of a possible response to the reduction in acid deposition over the twenty year period between the two surveys (Figure 5-1). Although the MASQ (Monitoring and Assessing Soil Quality) report itself was rather more cautious in its interpretation (Black et al., 2000).

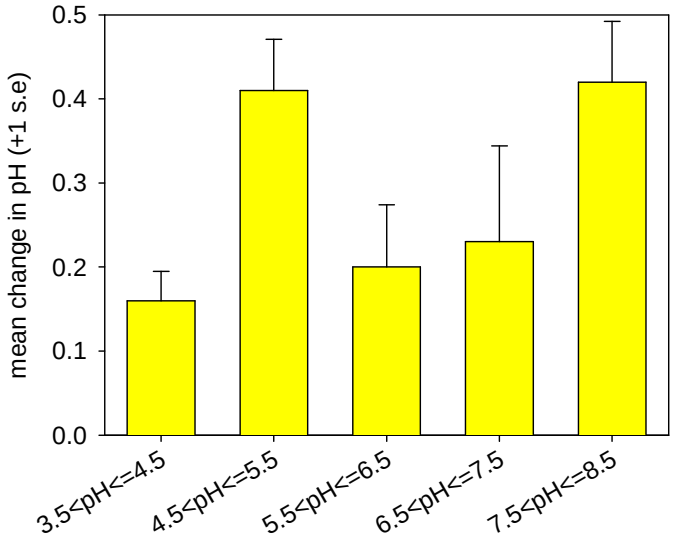


Figure 5-1 Soil pH change between 1978 and CS2000

However, the MASQ report confirmed an increase in soil pH between 1978 and 2000 in all but coniferous woodlands irrespective of whether the data were analysed by environmental zone, broad habitat, ITE Land Class, Aggregate Vegetation Class or major soil group. More importantly, the vegetation survey within CS2000 indicated a change in vegetation structure towards more nitrophilous, less acid tolerant species from 1990 to CS2000 (Haines-Young et al., 2000) providing corroboration for the soil data and an excellent example of the power of the CS approach in combining co-located, synchronous data collection of a wide range of variables.

5.4. Rationale for measurement

The rationale for including measurement of soil pH can be summarised in the following points:

- To maintain and extend time series of soil pH from 1978
- To increase reporting power for individual countries (especially Wales) by making measurements in all squares sampled during CS2007 (potentially up to 620).
- To determine whether the decrease in soil acidity across all major soil groups and environmental zones observed between 1978 & 2000 has continued
- To improve the prediction of plant species composition change using linked biogeochemical / plant species models

Table 5-1 pH rational for measurement

	Facts	**Comments**
History in CS	Measured on soils collected from X plots in the original 256 squares which formed the "ITE Ecological Survey of the UK". CS2000 re-measured pH from the same 256 squares.	256 squares essential to maintain time series data. 620 squares would provide better spatial information. Soil pH is a critical interpretative variable.
Country level reporting	Power analysis has indicated only 256 squares needed to quantify pH change at country level based on change between 1978 and 2000	It is unlikely that the pH change between CS2000 and CS2007 will be as great or consistent as between 1978 and CS2000. The time period is shorter and the deposition change has been less. Soil pH is a critical parameter for interpretation of plant species change (Ellenberg pH), bioavailability of P, N and metals. Recommendation is therefore for soil pH to be measured in all 620 squares.
Links and compatibility to other monitoring programmes	Comparable data to those collected in the NSI, RSSS for same horizon (0-15cm). ECN provides more information to depth and more frequent monitoring of soil solution but from few sites.	Neither NSI or RSSS have data between CS2000 and CS2007. Soil pH was measured at ECN sites in 2003. CS2007 will provide the only update for spatial data on soil pH in GB Comparison of these surveys reported in Defra project SP0515 (Defra 2003)
Uniqueness of CS	Time series is unique. The integrated approach enabling links to vegetation, management and water quality change is unique.	
Value for money (Policy priority or interpretative value X cost)	Very high	High policy and interpretative value, low cost

5.5. Proof of concept

The measurement of soil pH in a suspension of de-ionised water is a well established technique and was used in both 1978 and for CS2000. However, measurements of pH in water are subject to a number of uncertainties some of which are fundamentally related to the effects of changes in the ionic strength of the soil-water suspension on ions sorbed to the soil particles. One of the main consequences of this is the so called dilution effect, whereby different pH values are measured depending on the soil:water ratio in the suspension (Table 1). A solution of 0.01M $CaCl_2$ has approximately the same ionic strength as the soil solution in fertilised temperate soils and has been recommended as a more appropriate medium for soil pH measurement (Schofield & Taylor 1955). Measurement of pH in $CaCl_2$ yields more consistent results (Table 5-2). The pH value in $CaCl_2$ is lower than measured in water but closer to the value observed under equilibrium conditions using repeated extractions (White 1969).

Table 5-2 Effect of soil:solution ratio and solution ionic strength on soil pH measurements (from White 1969)

Soil:liquid ratio	pH in H_2O	pH in 0.01M $CaCl_2$	pH in equilibrium solution
1:2	5.08	4.45	4.45
1:5	5.29	4.45	4.45
1:10	5.43	4.46	4.45
1:50	5.72	4.52	4.45

Unfortunately, the relationship between soil pH measured in water and $CaCl_2$ is not consistent (Figure 5-2, Figure 5-3). Over a wide soil pH range (Figure 5-2), soil pH in $CaCl_2$ is approximately 0.8 pH units lower than the corresponding measurements in deionised water but there is considerable scatter, particularly for more acid soils. This can be seen more clearly in Figure 5-3, where the difference between the two measurements is about 0.4 pH units.

In the 1978 Ecological Survey, soil pH in water was measured using the method employed at ITE Bangor which itself was based on the method published by the Soil Survey of England and Wales (Avery and Bascomb 1974). This used a soil to water ratio of 1:2.5 by weight which was achieved by adding 25 ml of deionised water to 10 g of soil. In CS2000, soil pH was measured at CEH Merlewood using the protocol described in Allen et al., (1989). For quickness, this uses a volumetric scoop of soil (half a 50 ml beaker) which is topped up with deionised water. The resulting suspension has a soil to water ratio of approximately 1:2 by weight. It is unlikely that this small difference in ratio will result in a significant difference in pH between the two methods. However, as soil pH in $CaCl_2$ is to be measured in 2007, the original method used in 1978 has been chosen as pH in $CaCl_2$ can easily be measured by adding a few millilitres of concentrated $CaCl_2$ solution to the water pH suspension. This saves the time required to re-weigh and mix a second sample.

The comparability of the 1978 and CS2000 methods was checked in CS2000 using archive soils taken in 1971 from woodland habitats across GB (Black et al., 2000). The method used in 1971 was the same as that used in 1978 and the re-analysis on

dried archived soils used the CS2000 method. No statistically significant differences were found between pH values measured on dried archive soils from 1971 using the CS2000 method and the original data obtained from field moist soils in 1971.

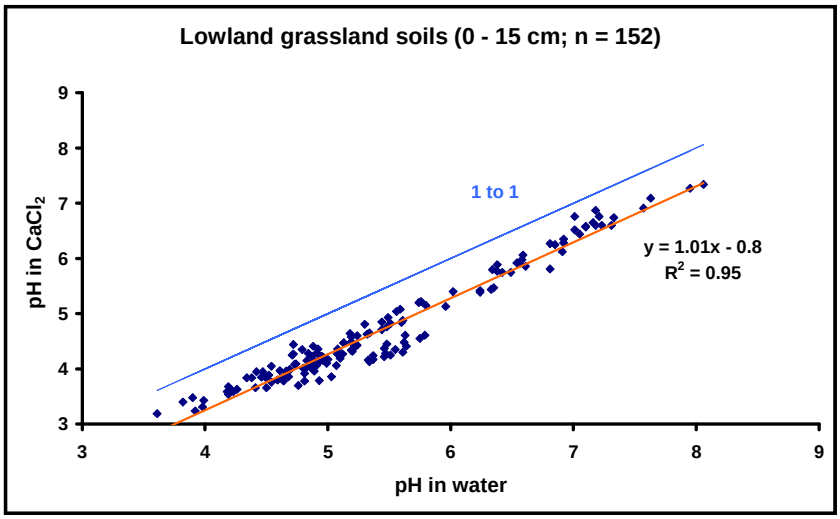


Figure 5-2 Soil pH measured in deionised water and 0.01M $CaCl_2$ for a range of lowland grassland soils (unpublished data CEH Bangor and CCW)

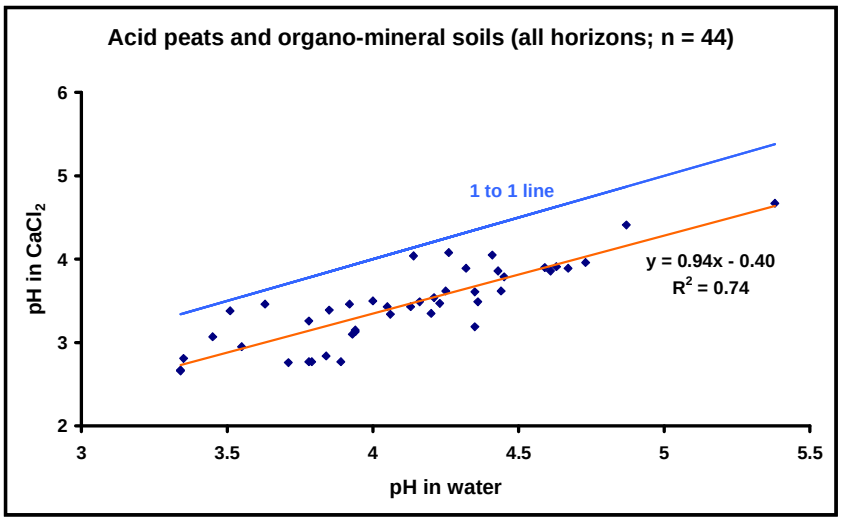


Figure 5-3 Soil pH measured in deionised water and 0.01M $CaCl_2$ for acid grassland, wet heath, forest and woodland soils.

5.6. Key models which require analyte data

The biogeochemical models commonly used to predict soil and surface water acidification trends in response to changes in acid deposition and land use can use soil pH as a calibration term in the absence of data on soil solution chemistry. As the latter are generally only available for a few research sites, soil pH is of major importance for regional model applications. These are used to predict target loads for

reductions in the emissions of acidic pollutants and nitrogen for both terrestrial and fresh water ecosystems.

5.7. QA

The Defra/NERC/BBSRC Joint Codes of Practice will be followed.

A set of soil pH measurements in deionised water and 0.01M $CaCl_2$ will also be made on a subset of 200 soils taken from the CS2000 soil sample archive. These measurements will be used to check the comparability of the method used in CS2007 with those used previously.

5.8. Field protocol

See Section 2 for full details. The field method described here is the same as that used in CS2000. Samples for pH were collected using a 15 cm long by 5 cm diameter black plastic core following the detailed field protocol described in Annex 1. Using the black core, a sample was collected from a point 15 cm to the south of the southern corner of the centre quadrat in each X plot in each 1 km square, giving 5 samples per 1 km square. The surface vegetation was removed to reveal the soil surface and the core was inserted to the full 15 cm depth. In stoney or shallow soils, the sampling point was moved if a full core depth could not be obtained. Any such variations in the protocol were recorded. On removal from the ground, the outside of the core was cleaned and any excess soil was trimmed from the bottom of the core. The core was then placed in a labelled plastic bag which was sealed and stored in the surveyor's vehicle pending delivery to Lancaster

5.9. Laboratory protocol

See ANNEX G for standard operating procedure. Two soil pH measurements (one in deionised water and one in 0.01M $CaCl_2$) are to be made on subsamples of fresh, field moist soil taken from each black core collected in CS2007 (3145 samples). Analysis will take place at CEH Lancaster.

A set of dry soil pH measurements in deionised water and 0.01M $CaCl_2$ will also be made on a subset of 200 soils taken from the CS2000 soil sample archive. These measurements will be used to check the comparability of the methods used in CS2007 with those used previously.

The methods to be used in CS2007 are based on those published by the Soil Survey of England and Wales (Avery and Bascomb 1974) and are described in full detail in Annex 2. The method for soil pH in water differs from that used in CS2000, but is the same as that used in 1978.

Fresh soil pH in water: Soil pH in water is measured using 10 g of field-moist soil in a 50 ml plastic beaker to which 25 ml of deionised water is added giving a ratio of soil to water of 1:2.5 by weight. The suspension is stirred thoroughly and left to stand for 30 minutes after which time the pH electrode is inserted into the suspension and a reading taken after a further 30 seconds.

Fresh soil pH in 0.01M $CaCl_2$: Following the measurement of the soil pH in water, 2 ml of 0.125M $CaCl_2$ is added to the suspension, which on dilution with the 25 ml of water results in a solution concentration of approximately 0.01M $CaCl_2$. The

suspension is stirred thoroughly and left for 10 minutes after which time the pH electrode is inserted into the suspension and a reading taken after a further 30 seconds.

Dry soil pH in water: Soil pH in water is measured using 10 g of air dried < 2mm sieved soil in a 50 ml plastic beaker to which 25 ml of deionised water is added giving a ratio of soil to water of 1:2.5 by weight. The suspension is stirred thoroughly and left to stand for 30 minutes after which time the pH electrode is inserted into the suspension and a reading taken after a further 30 seconds.

Dry soil pH in 0.01M $CaCl_2$: Following the measurement of the dry soil pH in water, 2 ml of 0.125M $CaCl_2$ is added to the suspension, which on dilution with the 25 ml of water results in a solution concentration of approximately 0.01M $CaCl_2$. The suspension is stirred thoroughly and left for 10 minutes after which time the pH electrode is inserted into the suspension and a reading taken after a further 30 seconds.

The following operational points are to be observed:

The fresh soil pH measurements should be made as soon as possible after the sample is opened.

Care should be taken to ensure that the temperature of the buffer solutions used to calibrate the pH meter differ by no more than 1℃ from the temperature of the soil suspensions.

The pH electrode should be carefully rinsed and dried between each measurement. Particular care is to be taken to clean the electrode following calibration with buffer solutions.

5.10. Methods for sample storage and archiving

Table 5-3 pH methods for sample storage and archiving

Requirements	
Type of sample (e.g. wet/dry soil, extract, both)	Dry, < 2mm sieved soil
Mass / volume of sample	Minimum of 10 g
Storage container (e.g. glass, plastic)	Plastic
Storage requirements (e.g light dark, controlled humidity, temperature)	Dark, cool and dry
Storage location	CEH archive
Length of time samples are stable	At least 25 years

5.11. Future use of material

All future use to be approved by the CS topic group, the CS steering group or a delegated responsible person within CEH. Possible uses of archived soils include re-analysis to check methodology and QC for future surveys. Other possibilities include analysis for other chemical methods e.g. metabolomics, NMR etc. Biological methods are unlikely to be appropriate.

5.12. QC

The calibration of the pH meter should be checked after a batch of 25 samples using pH 4 and pH 7 buffer solutions. If either of the buffer solution calibration values is more than 0.02 pH units from the expected value, the meter is to be re-calibrated.

A standard soil, a certified reference soil and a duplicate analysis should be performed on every batch of 25 samples.

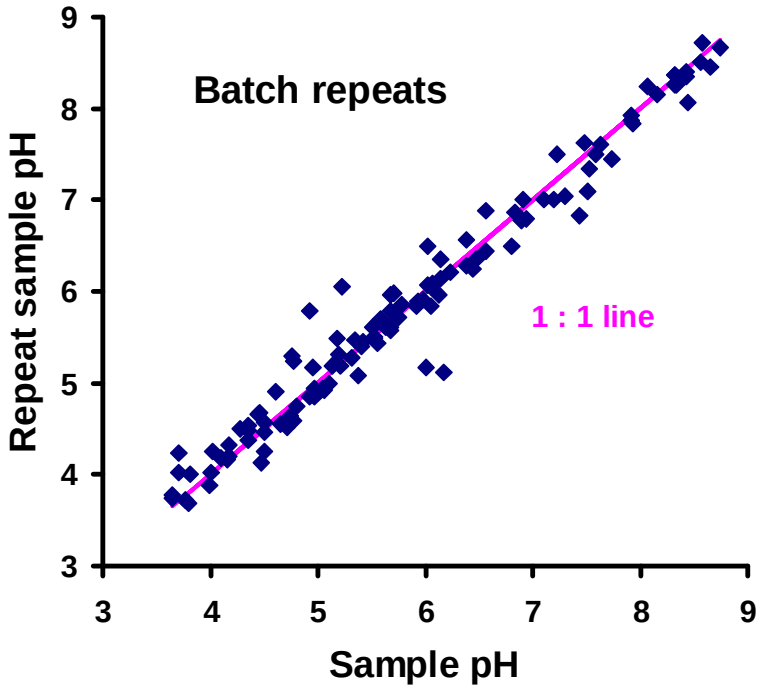


Figure 5-4 Results for pH for batch repeats

5.13. Calculations/Units

Soil pH is reported to 0.01 pH units.

5.14. Data storage

Data for soil pH from CS2000 are currently held in two data tables in the MASQ database. The MASQ_BLACKCORE_INFO table holds the following information directly relevant to soil pH as shown below (Table 5-4).

Table 5-4 pH relevant fields in the MASQ_BLACKCORE_INFO data table.

ABBREVIATION	EXPLANATION
SQUARE_NUM	CS2000 1 km square number
PLOT_TYPE	CS2000 X-plot number
REP_NUM	CS2000 subplot within the 1 km square
WET_PH	wet pH value

QC_WET_PH	quality control code for missing values
DRY_PH	dry pH value
QC_DRY_PH	quality control code for missing values
BATCH	batch number as samples completed in batches of 25 samples
LAB_NUM	laboratory number – number of sample within each batch
STORAGE_START_DATE	start date for storage of samples
NOTES_PRESENT	if notes present (Y)
NOTES	notes describing extent of sample disturbance

For CS2007, it is important that the MASQ_BLACKCORE_INFO data table currently held in the database is modified so that it can be distinguished from new data collected in CS2007. A single repository for all black core processing data should be retained.

Some of the soil pH information is also held in the MASQ_PHLOI_DATA. The table also includes the pH data from samples collected in 1978, difference in pH values between 1978 and 1998/9, plus similar information for LOI. There seems to be no reason why pH and LOI data should be held in one table. From the structure of the table it appears to have been generated from the data analysis for the 'Accounting for Nature' report. With the proposed extension of the time series and the intention to determine bulk density and other measures of carbon apart from LOI, the table should be split to provide a pH table and a separate soil carbon table.

The new pH table should include information from 1978, CS2000, CS2007 and any subsequent data. Recommended fields are shown in Table 5-5.

Table 5-5 Proposed data fields for the new MASQ_SOILPH_DATA table.

Field name	Description
SQUARE_NUM	Countryside Survey 1 km square number
PLOT_TYPE	Countryside Survey X-plot number
REP_NUM	Countryside Survey subplot within the 1 km square
PH1978	pH value in 1978
QC_PH1978	quality control code for missing values
PHW2000	wet pH value 2000
QC_PHF2000	quality control code for missing values
PHD2000	dry pH value 2000
QC_PHD2000	quality control code for missing values
PHD2000_REPEAT07	dry pH value for CS2000 samples repeated in 2007
QC_PHD2000_REPEAT07	quality control code for missing values
PHW2007	wet pH value 2007
QC_PHF2007	quality control code for missing values
PHD2007	dry pH value 2007
QC_PHD2007	quality control code for missing values

5.15. Statistical analysis

Soil pH data can be analysed to provide information on the state of soil acidity in 2007 and the change in soil acidity since 1978.

Reporting level

The data from CS2000 describing soil pH and change in pH since 1978 was reported at the GB level in the MASQ report (Black et al., 2000). Within CS2007 there is a requirement for reporting at the level of individual countries. A power analysis has been undertaken of the sampling requirements to reliably detect change in soil pH at the country level. The analysis is based on the data from 1978 and CS2000 (Table 5-6) which shows that the percentage change in pH was between 5 and 10% depending on the country

Table 5-6 Estimates of soil pH change between 1978 and CS2000

	Estimates of					
	pH 1978	**pH 2000**	**Change in pH**	**% change**	**se of change**	**num squares**
England	6.098	6.406	0.301	4.8	0.056	115
Scotland	4.738	4.954	0.221	4.6	0.048	101
Wales	4.976	7.054	0.546	9.1	0.068	21

The results of the power analysis are shown in Table 5-7. Thus within Wales, for example, there is 98.3% chance of detecting a 5% change in soil pH at the 1% significance level, based on the 21 squares measured in 1978 and in CS2000.

Table 5-7 Results of the power analysis to detect change in soil pH at the individual country level based on data from 1978 and CS2000

		Percentage change in pH			
	Significance	**5%**	**10%**	**20%**	**30%**
England	**1%**	99.9	100.0	100.0	100.0
	5%	100.0	100.0	100.0	100.0
	10%	100.0	100.0	100.0	100.0
Scotland	**1%**	99.7	100.0	100.0	100.0
	5%	99.9	100.0	100.0	100.0
	10%	100.0	100.0	100.0	100.0
Wales	**1%**	98.3	100.0	100.0	100.0
	5%	99.4	100.0	100.0	100.0
	10%	99.8	100.0	100.0	100.0

There are some very important caveats on the interpretation of this analysis. Firstly the increase in pH from 1978 to CS2000 was relatively consistent across the whole of GB. As a result, the measurements of change have small standard errors and hence the power to detect change is high, even with the small sample sizes. All three countries show significant change. The apparently greater change in Wales may be a reflection of the much smaller sample size including some influential squares. It is unlikely that the change in soil pH between CS2000 and CS2007 will be as consistent across country in either direction or magnitude. The interval between the measurements will be smaller (8-9 years compared to 20-21 years) and the change in acid deposition (one of the main drivers for change) during the last 8 years has been less than between 1978 and CS2000.

State of soil acidity

Sampling and analysis of soil from all CS2007 squares will be required to provide rigourous country-level reporting. On the basis of the analyses reported following CS2000 (Black et al., 2000), the following analyses for soil pH can be undertaken to provide information on the state of soil acidity at various levels of reporting and in relation to factors such as land use and soil type. Summary statistics reported in Black et al. (2000) included mean, median, minimum, maximum and standard deviation.

Change in soil acidity

Analysis of change in soil acidity can be assessed for pH in water only. Two approaches can be used:

- Compare summary statistics for all samples collected in each year
- Use the repeat data set to calculate differences in pH between surveys.

Comparisons and differences can be analysed with respect to GB level and factors such as soil group, broad habitat etc (Table 5-9).

Table 5-8 State of soil acidity

Summary stats by level	pH in water				pH in $CaCl_2$
	ES1978	CS2000	CS2007	Repeat data set	CS2007
GB	Y	Y	Y	Y	Y
England			Y		Y
Wales			Y		Y
Scotland			Y		Y

Summary stats at GB level by factor	pH in water				pH in $CaCl_2$
	ES1978	CS2000	CS2007	Repeat data set	CS2007
Environmental zone	Y	Y	Y	Y	Y
Broad habitat	Y	Y	Y	Y	Y
ITE Land class	Y	Y	Y	Y	Y
CVS Aggregate Veg Class	Y	Y	Y	Y	Y
Major soil groups	Y	Y	Y	Y	Y

Where:
EC1978 = Ecological Survey of GB in 1978

Repeat data set = those sites for which data are available from the same site for all three surveys

Table 5-9 Change in soil acidity

By	Comparison		Difference
	ES1978 to CS2000	CS2000 to CS2007	Repeat data set
GB	Y	Y	
England			
Wales			
Scotland			
Environmental zone	Y	Y	Y
Broad habitat	Y	Y	Y
ITE Land class	Y	Y	Y
CVS Aggregate Veg Class	Y	Y	Y
Major soil groups	Y	Y	Y

The statistical approach used for analysing the data for changes in 1978 – 1998, 1998 – 2007 and 1978 – 2007 for the 2007 report is reported in a separate CS technical report. Essentially, bootstrapping is used which involves treating sample data as a population from which to resample. Each resample produces a separate estimate of some quantity of interest, for example stock or change. A large number of resamples (typically 1000 or 10,000) then gives an approximation to the distribution of the required estimate, from which any statistic can be extracted. The main advantage of this method of estimation for CS is that it allows for non-normality in the data, without the necessity of knowing details of the actual distribution, and as such provides more accurate measurements of significance. See ANNEX F for a background document describing this approach.

5.16. Linkages to other tasks and work packages

Soil pH will be used as an explanatory variable to interpret the data on soil-P, available N and metals (Section 6,7, and 9).

Soil pH data will link directly to WP1 (habitats and landscapes) in relation to explaining plant species distribution in CS2007 and changes since the earlier surveys. The data will also provide contextual information for the interpretation of water chemistry results from WP3.

5.17. Linkages to other surveys

In the past, CS data have been compared with other spatial surveys of soil chemical properties within the National Soil Inventory and the Representative Soil Sampling Scheme. As far as can be ascertained, there will be no contemporaneous data from the NSI and RSSS with which to compare CS2007. However, there are ongoing soil monitoring programmes within ECN and the Level II forest plots which may have data spanning the period between CS2000 and CS2007, for both surface (0 – 15 cm) and from deeper in the soil profile.

6. Phosphorus

Task leader: Brian Reynolds

6.1. Key question

Can the trend of increasing P status in intensive grasslands be confirmed and is it matched in other habitats?

6.2. Key products

- Whole GB assessment of soil available-P status in 2007
- GB-level assessment of change in soil available-P status between CS2000 and CS2007
- Explanatory data to contribute to the analysis of observations arising from analysis of other CS data sets eg. changes in vegetation species, trophic status of freshwaters

6.3. Policy background

The Olsen-P data collected by CS2007 can contribute to the condition assessment of Broad and Priority Habitats in relation to soil fertility. The data wil provide a baseline assessment for a recommended UK-SIC indicator.

6.4. Rationale for measurement

Table 6-1 Phosphorus rational for measurement

	Facts	**Comments**
History in CS	Olsen-P measured on samples from 256 squares in CS2000	Olsen-P used as a measure of available-P
Links and compatibility to other monitoring programmes	NSI and RSSS used Olsen-P as a measure of available-P to the same depth (0-15 cm) as CS	Defra project SP0515 concluded measurements were comparable between NSI and RSSS.
Uniqueness of CS	No repeat surveys for RSSS or NSI since CS2000 Unique combination of soil, vegetation & land use measured together	High interpretative value for: i) terrestrial vegetation species change in response to fertility in combination with soil N ii) trophic status of linked freshwaters
Value for money (Policy priority or interpretative value X cost)	Recommended UK-SIC indicator for environmental interaction cheap, simple measurement; High value for money	Measurement will be made on 'black core' soil sample used for pH & LOI core measurements

6.5. Proof of concept

A wide range of extractants has been used to measure the more soluble, weakly bound or 'available' forms of phosphorus in soils. Some of the more commonly used extractants include 1% citric acid, 2.5% acetic acid, dilute buffered sulphuric acid (Truog's reagent), acetic acid-sodium acetate buffer (Morgan's reagent) and sodium bicarbonate buffered at pH 8.5 (Olsen-P). There have also been numerous studies comparing the performance of the different tests with each other, with plant response and with other factors (see eg Allen, 1989).

Olsen-P (Olsen et al., 1954) has been widely used in England and Wales to assess the fertility of agricultural soils (MAFF 2000). It has also been used in conjunction with phosphorus sorption index (PSI) to provide an index of the leaching risk of dissolved P from soils to freshwaters (Hughes et al., 2001). There has, however, been a long standing debate as to the most appropriate measure of soil available-P in relation to soil type. Olsen-P, which uses a bicarbonate extraction at pH 8.5, is considered applicable for limed, fertilised agricultural soils and semi-natural ecosystems on base rich or circum-neutral soils. Methods based on an acid extraction are thought to be more appropriate for more acidic soils found in many semi-natural and woodland ecosystems.

Olsen-P was measured in the 256 'soil squares' during CS2000. The data were not reported in the MASQ report (Black et al., 2000) although they are held on the MASQ data base (Wood 2006). The data were not included in the Defra funded study which examined the comparability of soil properties measured by different surveys and monitoring schemes where it was reported that total-P was measured in CS2000 (Bradley et al., 2003). Subsequently, the performances of Olsen-P and Truog's reagent have been compared across a range of soils collected in CS2000 (Rowland pers comm, 2006 and Figure 1). Allen (1989) recommends Truog's extraction for all but the most calcareous soils, whilst Olsen-P is considered more appropriate for calcareous soils. The scatter plot in Figure 1 shows there is a significant correlation between the two data sets with a slope approaching one, but there is considerable scatter in the relationship. Further analysis using other soil and contextual data from the sample sites is required to identify what factors are contributing to the scatter. This may lead to a more robust, multi-factorial relationship between the two methods.

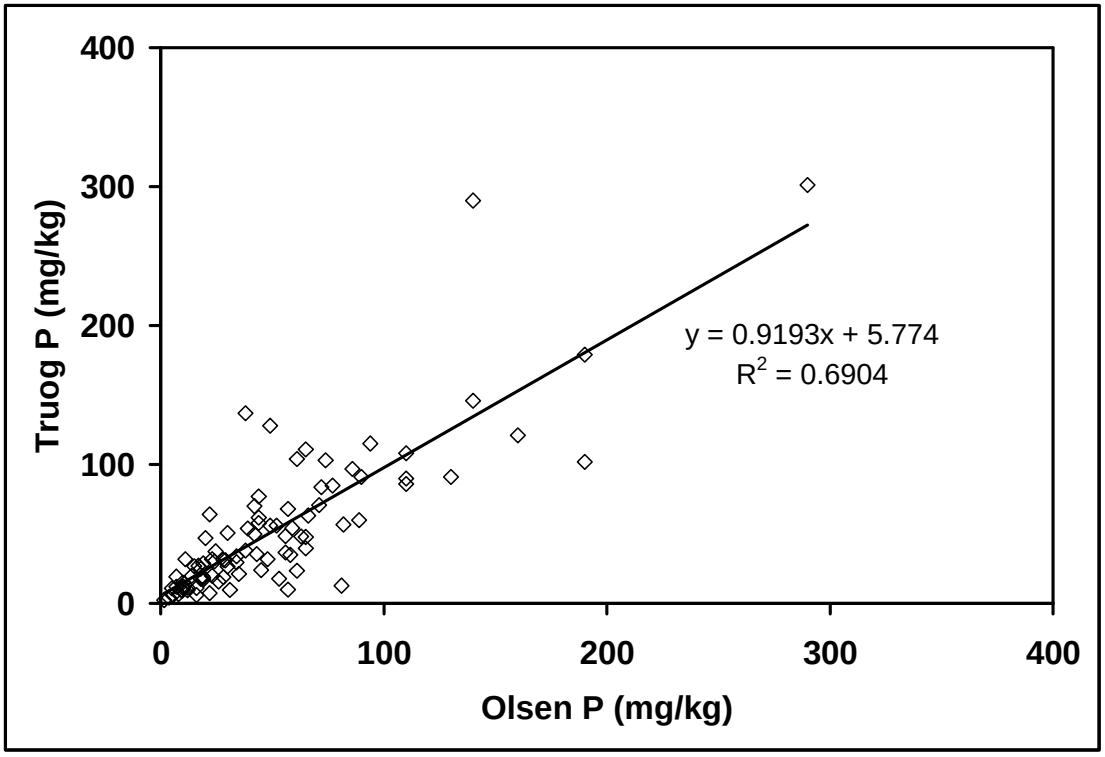


Figure 6-1 Scatter plot of Olsen-P and Truog-P for 90 soils collected during CS2000.

A similar comparative study study using 199 soils from the Irish Republic and Northern Ireland (Foy et al., 1997) showed that soil pH had a significant influence on the relationship between Olsen-P and Morgan-P (sodium acetate / acetic acid at pH 4.8) concentrations. Some studies have shown that increasing soil pH over a range from 5.0 to 6.5 can depress Olsen-P values (Sorn-srivichai et al., 1984) and Olsen-P values may reach a minimum between pH values of 5.6 and 6.0 increasing at both lower and higher pH values (Naidu et al., 1987). Other data sets, however, have failed to show a consistent effect of pH on soil extractable P (Pimplaskar et al., 1982 plus data from Poulton et al., 1997 re-analysed by Foy et al., 1997). In the Irish study, differences in the intercepts of the relationship between log Olsen-P and log Morgan-P were also observed when geology and county were included as factors in the regression, although regression slopes did not vary significantly. Overall, Foy et al. (1997) concluded that whilst soil pH, county and geology were factors contributing to the variability in the relationship between Olsen-P and Morgan-P, much of the scatter remained unaccounted for.

Choice of method for CS2007

Olsen-P was used to measure available phosphorus in the soil samples collected in CS2000 and that alone provides a strong argument for using the same method in CS2007 in order to establish a time-series.

The literature indicates that no one extractant is ideal for use across the range of soil fertility and pH gradients encountered in CS. Thus, one option might be to use more than one extractant with the choice being determined by the characteristics of each soil sample. For example, an acid extractant for semi-natural, acidic, low fertility soils and Olsen-P for more base-rich, fertile soils. However, this is likely to make the data very hard to interpret, particularly in relation to changes since CS2000 and for those soils with ‘intermediate’ characteristics where the choice of extractant is not clear.

The evidence so far from the methodological comparison conducted by Rowland (pers comm 2006) and from other studies reported in the literature suggests that it is unlikely that a simple, robust relationship can be established between Olsen-P and another extractant within the timescale required for CS2007. Therefore, in order to maximise the use of pre-existing CS2000 data, it would be hard to justify using another extractant, even though Olsen-P might not be ideal across the range of soil conditions encountered in CS.

Olsen-P has been widely used in England and Wales in soil monitoring schemes such as the Representative Soil Sampling Scheme, the National Soil Inventory and the Environmental Change Network sites. The Defra comparability study shows that where the same analytical methods have been used the data for phosphorus are comparable (Bradley et al., 2003). This provides further justification for retaining Olsen-P for CS2007. Olsen-P is also used in the Soil Geochemical Atlas of Northern Ireland, although the sampling depth for this survey was 25 cm.

Olsen-P has also been recommended by UK-SIC as an indicator for environmental interactions. CS will provide the opportunity to explore the performance of this indicator in relation to the chemical and ecological data collected in the Freshwater Work Package(3).

6.6. Key models which require analyte data

So far, measures of soil available-P have not been included in models exploring the relationships between vegetation species change recorded in CS2000 and soil N enrichment through atmospheric nitrogen deposition. However, soil-P data are likely to have strong interpretative power in the analysis of model simulations and predictions.

6.7. QA

Joint code of practice will be followed. Re-analysis of a subset of soils collected in CS2000 will be used to test method comparability (see Annex 2 - Laboratory protocol section).

6.8. Field protocol

The soil used for phosphorus analysis is taken from the ‘Black core’. There are no specific sampling protocol requirements beyond those normally employed for collecting the ‘black core’ sample (See Annex 1).

6.9. Laboratory protocol

A total of 1280 black cores will be analysed for Olsen-P at CEH Lancaster following their standard operating procedure. These will be from the original 256 squares, with 5 X-plots in each square.

The method for Olsen-P is well established and involves extraction of 5 g of air dried, sieved soil with 100 ml of 0.5M sodium bicarbonate at pH 8.5. The phosphorus in the

extract is then determined colorimetrically using a skalar continuous flow analyser. The skalar method uses molybdenum blue at 880nm with the addition of a dialysis step to overcome the effect of the Olsen's reagent.

There are a number of factors which can contribute to errors in the analysis:

- Effect of drying – drying soil affects the release of phosphorus with enhancements of up to 30% possible from drying at 40 degC (Jackson 1958). The effect varies with soil type (Allen, 1989). If field moist soil is used, larger quantities are recommended for the extraction (10 g to 100 ml for mineral soil and up to 25 g to 100 ml for peats; Allen, 1989). Methodological consistency is therefore very important. Since the Olsen-P measurements made on CS2000 soils used air-dried soils, the same procedure will be used for CS2007.

- Extraction temperature – the extraction is temperature sensitive and must therefore be performed under constant temperature conditions

- Soil:solution ratio and extraction time – these may affect the amount of phosphorus extracted and thus a consistent method should be employed.

- Effect of organic matter – the high pH of the Olsen-P extraction means that some organic matter is also extracted. Organic phosphorus will not, however be measured by the molybenum blue method.

6.10. Methods for sample storage and archiving

Table 6-2 Phosphorus: methods for sample storage and archiving

Requirements	
Type of sample (e.g. wet/dry soil, extract, both)	Air-dried and sieved soil to < 2 mm
Mass / volume of sample	Minimum 10 g
Storage container (e.g. glass, plastic)	Plastic
Storage requirements (e.g light dark, controlled humidity, temperature)	Dark & dry
Storage location	CEH sample archive
Length of time samples are stable	Indefinitely

6.11. Future use of material

Stored samples can be used for re-analysis to check method comparability in subsequent surveys. It should be remembered that the use of fresh or air-dried soil can affect the amount of phosphorus extracted. Soil would be available for other analytes measurable on sieved, air dried soils. Access to archived soil should be under the control of a designated responsible person in CEH.

6.12. QC

A replicate analysis, plus one standard soil and one certified reference soil to be analysed per batch of 25 samples. Extraction procedure and conditions to be matched with those used for the analysis of CS2000 soils.

6.13. Calculations/Units

Olsen-P is expressed in mg kg^{-1} dry soil.

6.14. Data storage

Data for phosphorus is currently stored in the MASQ database in the following Oracle data tables:

MASQ_PHOSVALID_DATA
MASQ_PHOSVALID_METADATA

Table 6-3: Information for the PHOSVALID_DATA table

Field name	Description
SQ	CS2000 1km square number
X	CS2000 X-plot
N	CS2000 subplot within the 1 km square
batch	Environmental Chemistry batch number
SampDesc	SQ, X and N combined comma separated
SampNo	Environmental Chemistry replicate number
Residue (g)	Leftover weight of soil in container
PO4-P mg kg^{-1}	phosphorus (mg per kg)

For C20007 it will be necessary to add a date identifier and a year. An identifier should be added to the current table to uniquely identify data collected by CS2000. The field labelled PO4-P mg kg^{-1} should be re-labelled Olsen-P mgP kg^{-1}

6.15. Statistical analysis

State

Sampling from all CS survey squares will be required to provide adequate data for country-level analysis of the Olsen-P status of soils in 2007. Summary statistics will include mean, median, minimum, maximum and standard deviation.

The data will be analysed by the main reporting categories of:

- ITE Land Class
- Environmental Zone
- Broad Habitat
- CVS Aggregate Vegetation Class
- Major Soil Group

Change

Change in Olsen-P status between 1998/9 and 2007 can be assessed at GB level using data collected from the 256 soil squares sampled in CS2000.

Two approaches can be used:

- Compare summary statistics for all samples collected in each year
- Use the data from the set of sample sites (X-plots) where repeated measurements have been made to calculate differences in Olsen-P between surveys.

Comparisons and differences can be analysed with respect to GB level and factors such as soil group, broad habitat etc.

Overall approach

The statistical approach used for analysing the data for changes in 1978 – 1998, 1998 – 2007 and 1978 – 2007 for the 2007 report is reported in a separate CS technical report. Essentially, bootstrapping is used which involves treating sample data as a population from which to resample. Each resample produces a separate estimate of some quantity of interest, for example stock or change. A large number of resamples (typically 1000 or 10,000) then gives an approximation to the distribution of the required estimate, from which any statistic can be extracted. The main advantage of this method of estimation for CS is that it allows for non-normality in the data, without the necessity of knowing details of the actual distribution, and as such provides more accurate measurements of significance. Background information on approach used can be found in ANNEX F.

6.16. Linkages to other tasks and work packages

WP1 - Explanatory variable contributing to the interpretation of any plant species change attributable to the influence of soil fertility.

WP3 – Important explanatory variable contributing to the interpretation of data describing the trophic status of fresh waters surveyed in CS.

6.17. Linkages to other surveys

Fertiliser use data could be used as an interpretative variable for CS Olsen-P data.

Linkage to agri-environment scheme data could provide information on the effectiveness of such schemes in achieving a reduction in fertiliser use and decrease in fertility in sensitive habitats.

Could be used for comparison with NSI

6.18. References

Allen, S.E. (ed.) 1989. Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford, 368 pp.

Black, H.I.J. et al. 2000. MASQ: Monitoring and assessing soil quality in Great Britain. Countryside Survey Module 6: Soils and Pollution. R&D technical Report E1-063/TR, Environment Agency, Bristol.

Bradley, R.I., Bellamy, P.H., Loveland, P.J., Black, H.I.J., Hornung, M., Scott, A., Lane, A., Webb, J. and Jackson, D.R. 2003. Comparability of soil properties derived from different data sources. Defra, project code SP0515.

Foy, R.H., Tunney, H., Carroll, M.J., Byrne, E., Gately, T., Bailey, J.S. and Lennox, S.D. 1997. A comparison of Olsen and Morgan soil phosphorus test results from the cross-border region of Ireland. Irsh Journal of Agricultural and Food Research, 36, 185-193.

Hughes, S., Reynolds, B., Bell, S.A. & Gardner, C. 2000. Simple phosphorus saturation index to estimate risk of dissolved P in runoff from arable soils. Soil Use and Management, 16, 206-210.

Jackson, M.L., 1958. Soil Chemical Analysis. Prentice Hall, London.

MAFF 2000. Fertilser recommendations for agricultural and horticultural crops (RB209) Seventh Edition, The Stationery Office, 177pp.

Naidu, R., Tilman, R.W., Syers, J.K. and Kirkman, J.H. 1987. Effect of liming on phosphate extracted by two soil testing procedures. Fertiliser Research 14, 143-152.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington DC, Circular No. 939, 19pp.

Poulton, P.R., Tunney, H. and Johnston, A.E. 1997. Comparison of TEAGASC (Ireland) and MAFF (England and Wales) fertiliser P recommendations. In: Phosphorus Loss from Soil to Water, eds H. Tunney, P.C. Carton, P.C. Brookes and A.E. Johnston)

Pimplaskar, M.S., Floate, M.J.S. and Newbold, P. 1982. The suitabiltiy of different methods for assessing the phosphorus status of hill soils. Journal of the Science of Food and Agriculture 33, 957-963.

Sorn-srivichai, P., Tilman, R.W., Syers, J.K. and Cornforth, I.A. 1984. The effect of soil pH on Olsen bicarbonate phosphate values. Journal of the Science of Food and Agriculture 35, 257-264.

Wood, C. 2006. Database documentation. MASQ (Monitoring and Assessing Soil Quality) A data base of UK Soil Chemistry and Biology data. 1978-2000. February 2006, version 3 (Final), CEH Lancaster.

7. Mineralisable and total N

Task leader: Ed Rowe, Bridget Emmett

7.1. Key question

Mineralisable N: Can the trend of eutrophication of the countryside be detected in the soil as well as the vegetation using this sensitive soil process method?

Total N: Can the trend of eutrophication of the countryside be detected in the soil as well as the vegetation using this basic soil property?

7.2. Key products

Eutrophication (nitrogen):

- National and country-level assessments of soil nitrogen and change in total N since 1998
- Attribution of change in, and form of, soil N under pressures and drivers
- Attribution of change in plant species composition in relation to internal N status versus N deposition
- Assessment of soil N in relation to changes in soil carbon status

7.3. Policy background

Changes in plant species composition were observed following CS2000. This has been ascribed to ecosystem eutrophication following an enhanced deposition of atmospheric nitrogen compounds. The purpose of the measurement of mineralisable N within CS2007 is to develop a simple surrogate measure which links different measures of soil N status and N mineralisation potential with plant species composition across a wide range of broad habitats, geographical locations and soil types. This will help determine causal drivers of change in species composition and to improve the link between models of soil biogeochemistry and plant species diversity.

Policy decisions concerned with the abatement of atmospheric emissions of sulphur dioxide, oxides of nitrogen and reduced forms of nitrogen are currently informed through the critical load approach. The general definition of the critical load is: “a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge” (UNECE 2004). Critical loads for nitrogen are calculated using either empirical or steady-state mass balance approaches (UNECE 2004). The former relies on data from experiments with critical loads for each habitat agreed by consensus at an EU/UNECE level.

However, neither of these approaches allow a timescale of changes to be identified and thus dynamic models are currently being developed to enable forecasting of both soil and plant species change. Countryside Survey will provide valuable input into

this development due to the uniqueness of the data in linking soil, water and vegetation sampling at each location.

Figure 7-1 shows the links between the biogoechemical model MAGIC which predicts the soil chemistry response to changes in nitrogen deposition and acidity. GB-MOVE then uses the soil chemical conditions output by MAGIC to predict plant species occurrence based on the calibration data set derived from CS2000. The interactive effect of grazing can also be incorporated using the SUMO model.

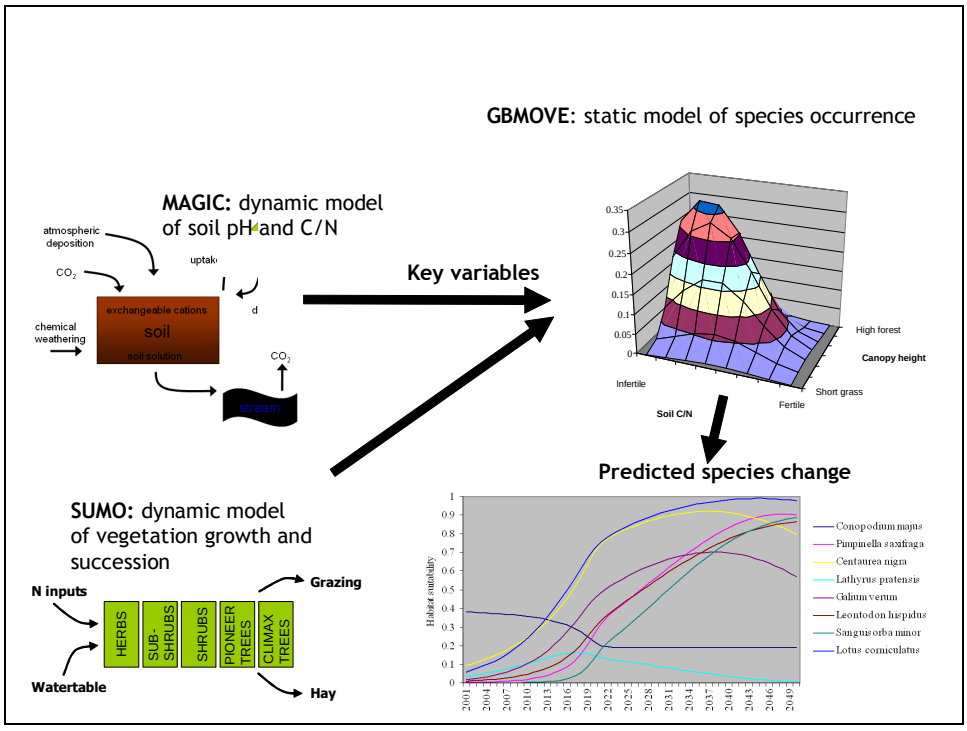


Figure 7-1 The MAGIC-SUMO-GBMOVE model chain for predicting plant species change in response to changing atmospheric nitrogen deposition**.**

7.4. Rationale for measurement

Table 7-1 Total and mineralisable N: rational for measurement

	Facts	Comments
History in CS		
Total N	Total N in 2000	Maintain time series. Enables stock and change
Mineralisable N	No measurements previously made	Better measure of plant-available N than total or soluble N
Links and compatibility to other monitoring programmes		
Total N	Not NSI	
Mineralisable N	No known datasets at national scale	
Uniqueness of CS		
Total N	No known national datasets	Only national dataset Only integrated sample which can be linked to vegetation and land management
Mineralisable N	No known national datasets	Only national dataset Only integrated sample which can be linked to vegetation and land management
Value for money (Policy priority or interpretative value X cost)		
Total N	High	High policy and interpretative value, low cost
Mineralisable N	Medium	High policy and interpretative value, medium cost

Enhanced soil nitrogen status can influence plant species assemblages in two ways. Reactive N limits plant production in many terrestrial ecosystems, so increased exposure to anthropogenic N is likely to result in increased plant growth. Consequent changes to competitive interactions have been implicated as a cause of plant diversity loss. Secondly, some plants are known to respond to changes in the ratio of mineralisable ammonium to mineralisable nitrate in the soil.

Plant species' preferences for relatively fertile and infertile sites have been scored on a scale of 1-9 (Ellenberg, 1974; Hill et al., 1999). The mean Fertility score of the plant species assemblage is presumably an accurate indicator of exposure to plant-available N, at least on N-limited sites. However, relating mean Fertility score to biophysical measurements of soil nutrient status is problematic. Total soil N content

and C/N ratio are weak predictors of mean Fertility score, since N may be strongly bound within organic matter. Extractable soluble N is highly subject to fluctuation due to leaching and changes in microbial activity, and measurements taken from cores which have undergone different conditions during transit to the lab are therefore of limited value. The mineralisation or immobilisation of N during an incubation has been used as a relatively robust measure of available N. The relative production of ammonium and nitrate provides additional information which may help explain species distributions.

Currently, soil C/N ratio is used as an index of N availability to relate Fertility score to nitrogen availability in CS datasets, although this measure only explains around 60% of the variation in Fertility score. CEH have also used the ratio to develop linked biogoechemical / plant species models to predict changes in plant species composition in response to changes in atmospheric nitrogen deposition within the Defra Terrestrial Umbrella project.

It is intended to enhance this simple surrogate measure by combining C/N ratio with soil / broad habitat and/or environmental zone classification; pH; measurements of extractable nitrate and ammonium; and net mineralisation/immobilisation of nitrate and ammonium, and thus improve predictions of Fertility score. Mineralisable N measurement has been tested in the CS Pilot Project, using a combination of novel and well-established protocols to provide a proof of concept.

A measurement of conductivity was included in the protocol, with the aim of distinguishing more and less saline soils and thus providing more information on plant species' environmental niches.

7.5. Proof of concept

Net N mineralisation potential of a soil has been measured by comparing the amounts of extractable nitrate and ammonium before and after a period of incubation under standard conditions of temperature and moisture. Usually two adjacent soil samples or a split soil sample are used for this measurement. Diekmann & Falkengren-Grerup (1998) measured ammonium and nitrate release after an incubation of 15 weeks at 18 ^{o}C and approximately 40-60 % of water-holding capacity, and defined a functional index of nitrogen availability as the linear combination of ammonium and nitrate release which best correlated with plant species occurrence. This measure was found to be a robust indicator of species' response to nitrogen availability across a range of soils. However, parts of their procedure are difficult to justify for a large number of samples, and so improvements have been developed and tested in the CS Pilot Project.

Water tension during incubation affects mineralisation flux, and also nitrification and denitrification fluxes and hence the ratio of ammonium to nitrate. However, different soils can have widely differing gravimetric water content at the same water tension (Figure 7-2). Measuring water tension is time-consuming, and calculating the amount of water that needs to be added or evaporated to reach approximately half of water-holding capacity is not straightforward. Instead we propose standardising the water content to approximately field capacity, by thoroughly wetting the soil, allowing it to drain, and then applying suction to drain the larger pores.

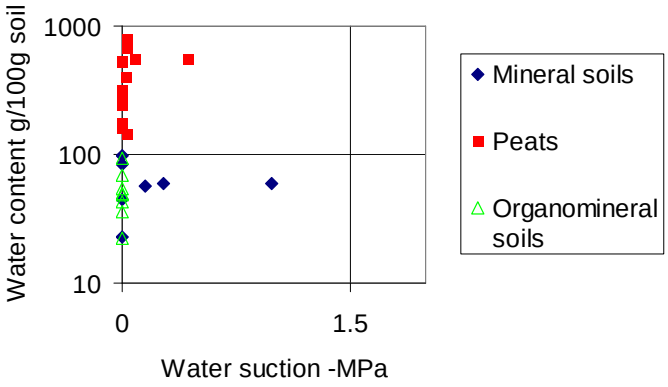


Figure 7-2 Water content and suction of field-moist cores from the CS Pilot Project. Plant-available water holding capacity is approximately that between -0.05 and -1.5 MPa.

Initial measurements of soluble N on receipt by the lab are of limited value, since samples may have undergone different conditions during transit. The proposed method thus dispenses with this initial measurement, instead standardising the soil solution N concentration by flushing with a weak salt solution until approximately 4 pore-volumes have passed through, sufficient to leach out most ammonium as well as nitrate. In the CS pilot study, 1 mg N L^{-1} NH_4NO_3 was used, to allow strongly immobilising soils to be distinguished from those with little activity. This method was successful in standardising soil solution mineral N concentration (Figure 4.6-3). However, in the main survey it was decided to use a solution without N to ensure that all N in the final extract is soil-derived. A NaCl solution intended to mimic the ionic strength of UK rainfall will be used, to allow potentially useful analyses of the leachate for other base cations. A sample of the leachate will therefore be collected and frozen.

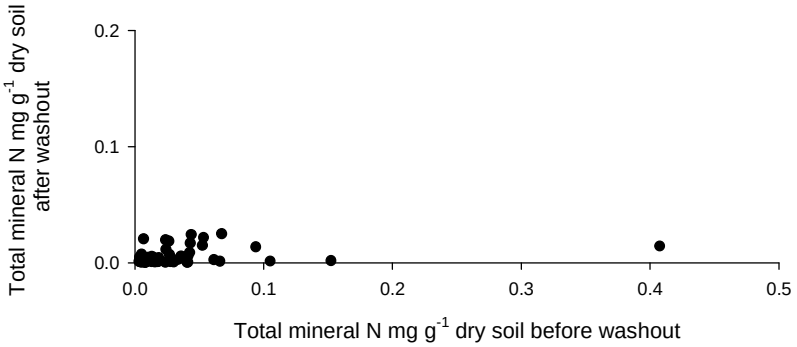


Figure 6-3 Relationship between total mineral N content before and after washing of cores from the CS Pilot Project. Washthrough solution was 1 mg N L^{-1} NH_4NO_3 solution

7.6. Key models which require analyte data

Measurements of soil “fertility” are required for a variety of applications, including species occurrence models, and models of plant production such as those used for forecasting vegetation dynamics or carbon fluxes. Mineralisable N will be used in regression approaches to predicting species occurrence or habitat suitability (e.g. the GBMOVE model). Mineralisable N may also be valuable for parameterising and testing dynamic models of plant succession (e.g. SUMO, PIPS) and soil carbon

dynamics (e.g. DECOMP), since a measure of the labile organic matter pool is useful for estimating both soil N availability and the rate of organic matter turnover.

7.7. QA

Defra/NERC/BBSRC Joint Codes of Practice will be followed

7.8. Field protocol

See Section 2 for full details. Samples for mineralisable nitrogen will be collected using a 15 cm long by 4 cm diameter white plastic core following the detailed field protocol described in Annex 1. Using the white core, a sample was collected from a point 30 cm to the south of the southern corner of the centre quadrat in each X plot in each 1 km square, giving 5 samples per 1 km square. The surface vegetation was removed to reveal the soil surface and the core was inserted to the full 15 cm depth. In stoney or shallow soils, the sampling point was moved if a full core depth could not be obtained. Any such variations in the protocol were recorded. On removal from the ground, the outside of the core was cleaned and any excess soil was trimmed from the bottom of the core. Caps are gently pushed over the end of each pipe and the pipe placed in a sample bag. The bag will be placed in an envelope and posted to CEH Bangor.

Total N and C analyses will be done on the long black core (C).

7.9. Laboratory protocol

A total of 768 cores will be analysed for mineralisable N; 3 cores will be randomly selected from the 5 X-plots in the original 256 squares. This analysis will take place at CEH Bangor. See also Section 17.6 of ANNEX G for standard operating procedure.

Cores were be sawn down both sides and placed on their sides in racks which allow leachate to be collected. Solution was applied by repeated misting of the surface of the core until 150 ml of leachate has been collected. The cores were kept at 4 ^{o}C during this period. The solution used is equivalent to the mean concentrations, weighted by land-use classes, of the major ions in UK rainfall as reported by Ron Smith, CEH Edinburgh, in March 2007. These were (all in $\mu eq\ L^{-1}$) 17.6 Ca^{2+}, 30.1 Mg^{2+}, 125 Na^{+}, 140 Cl^{-} and 57.2 SO_4^{2-}, resulting in a solution with a pH of approximately 4.6. After washing out the cores, a small amount of suction was applied to drain larger pores. Cores were then incubated under anaerobic conditions for 4 weeks, at 10 ^{o}C, approximately UK mean summer soil temperature. Cores were then extracted with 1 M KCl, and ammonium and nitrate concentrations determined as a measurement of mineralisable N.

For total N, 1280 samples were analysed (5 X-plots from each of the 256 squares) at CEH Lancaster. The method used was CEH Lancaster UKAS accredited method SOP3102. Samples were analysed on an Elementar Vario-EL elemental analyser (Elementaranalysensysteme GmbH, Hanau, Germany).

The Vario EL is a fully automated analytical instrument. It works on the principle of oxidative combustion followed by thermal conductivity detection. Following combustion in the presence of excess oxygen the oxides of nitrogen and carbon flow through a reduction column which removes excess oxygen.

Carbon is trapped on a column whilst nitrogen is carried to a detector. Carbon is then released from the trap and detected separately. Calibration is performed infrequently, with daily runs being factorised to this calibration through the use of a certified standard (acetanilide). Quality control is achieved by use of two in-house reference materials analysed with each batch of samples.

Sample weights are usually 15mg for peat and 15-60mg for mineral soil samples.

7.10. Methods for sample storage and archiving

Table 7-2 Total and Mineralisable N: methods for sample storage and archiving

Requirements	**Total N**	**Mineralisable N**
Type of sample (e.g. wet/dry soil, extract, both)	Air dry Soil	Frozen KCl extract
Mass / volume of sample	20g	20mls
Storage container (e.g. glass, plastic)	Plastic	Plastic
Storage requirements (e.g light dark, controlled humidity, temperature)	Dark, dry, cool	Freezer -4º C
Storage location	Lancaster	Bangor
Length of time samples are stable	indefinitely	1 month

7.11. Future use of material

All future use to be approved by CS topic group or steering group. Possible use of archived soils includes re-analysis to check methodology and QC for future surveys. Other possibilities include analysis for other chemical methods e.g. metabolomics, NMR etc. Biological methods unlikely to be appropriate.

7.12. QC

a) Include one each of the CEH Bangor standard soil samples with each batch of 40 samples and record the value(s) on the laboratory QA sheet.
b) Include three duplicate samples per batch, selected at random, as specified in the CEH Bangor laboratory QA procedures sheet
c) Include three blank extracts per batch of 40 samples to provide a test for contamination from glassware, filter papers, etc.
d) GPR grade KCl must be used because of the unacceptably high concentration of N in ‘Analar’ grade KCl
e) Whatman No. 44 filter papers must be used because No. 542 papers contain unacceptably high concentrations of nitrate-N and ammonium-N.
f) To avoid cross-contamination with ammonium based extractants, dedicated glassware, bottles, phials, funnels etc should be used.
g) Dispenser volumes should be calibrated by weight rather than volume.
h) Calibrate the Jenway 4320 Conductivity Meter against 0.01 M KCl (148 $\mu S\ cm^{-1}$ at 25 ^{o}C) at the start of each batch.

7.13. Calculations/Units

The calculation of mineralisable nitrate and ammonium is performed on an Excel spreadsheet (A1_template for data reporting for labs_v9.xls held at CEH Bangor), which includes corrections for blanks and dilutions and includes results from standard soils.

The results will be expressed in terms of mg/g dry weight of soil.

7.14. Data storage

Raw data will be stored as follows:

- Raw data as in QA files stored at Bangor
- Final data only in CS database

Metadata

Field name	Units and Description
N_not_NF	True/False. True if core is N (i.e. analysed for mineralisable N), false if core is NF (i.e. frozen without analysis) or was not received.
N_arrival	Date. Date of arrival at CEH Bangor. Null if core never arrived.
N_notes_arrival	Text. Notes taken on arrival of the core in Bangor, including notes written by field surveyors on the bag.
NF_Freezer_No	Code. Storage freezer location.
NF_Freezer_Date	Date. Date put into freezer.
NE_gap_top	cm. Length of gap at top of core.
NE_gap_bottom	cm. Length of gap at bottom of core.
NE_Total_Length	cm. Total length of the core.
NE_Org_Length	cm. Length of organic part of core
NE_Min_Length	cm. Length of mineral part of core
N_setup_date	Date. Date leaching rack was set up.
NE_conductivity	uS / cm at 25 ^{o}C. Conductivity (microSieverts per cm) in leachate.
NE_Leachate_Vol	mL. Volume of leachate collected after passing through core.
NE_Incub_Start_Date	Date. Date incubation started.
NE_Incub_End_Date	Date. Date incubation ended.
NE_Soil_Roots_Stones	g. Total fresh weight of core.
NE_Tot_Soil	g. Total fresh weight of core after removing roots and stones.
NE_notes_analysis	Text. Notes taken during analysis of the core in Bangor.
NE_BatchCode	Code. Code of this core's N mineralisation batch
NE_Wt_Crucible	g. Weight of crucible
NE_Wt_CrucPlusFreshSoil	g. Weight of crucible plus moist soil
NE_Wt_CrucPlusSoilAfter105	g. Weight of crucible plus soil after drying at 105o
NE_Wt_CrucPlusSoilAfter375	g. Weight of crucible plus soil after combustion at 375o
NE_MCFC_PCDW	percent. Moisture content as % of soil dry weight
NE_MCFC_PCFW	percent. Moisture content as % of soil fresh weight
NE_LOI_pcdw	percent. Organic matter by loss on ignition as % of soil dry weight
NE_FreshSoilExtracted	g. Exact fresh weight of approx 10 g soil extracted
NE_VolExtract	mL. Volume of extract analysed.
NE_NO3N_ConcRecorded	mg N / L. Conc. of nitrate-N in extractant

NE_NO3N_DilutionFactor	factor. Number of times by which soil extract was diluted before analysis.
NE_NO3N_ConcBlank	mg N / L.
NE_NH4N_ConcRecorded	mg N / L. Conc. of ammonium-N in extractant
NE_NH4N_DilutionFactor	factor. Number of times by which soil extract was diluted before analysis.
NE_NH4N_ConcBlank	mg N / L.
NE_NO3N_Soil	mg N / g dry soil. Conc. of nitrate-N in soil.
NE_NH4N_Soil	mg N / g dry soil. Conc. of ammonium-N in soil.
NE_NO3N_SOM	mg N / g loss-on-ignition. Conc. of nitrate-N in soil organic matter.
NE_NH4N_SOM	mg N / g loss-on-ignition. Conc. of ammonium-N in soil organic matter.
NE_NMINTOT_Soil	mg N / g dry soil. Conc. of nitrate-N plus ammonium-N in soil.
NE_NMINTOT_SOM	mg N / g loss-on-ignition. Conc. of nitrate-N plus ammonium-N in soil organic matter.
NE_NO3N_PROP_NMIN	proportion. Nitrate-N as prop of (nitrate-N plus ammonium-N).
QA_DUP_SAMPLE	Text. "QA" if a standard reference soil (in which case Rep_Num is the code for the reference soil). "Duplicate" if a duplicate of the soil with the same SQUARE_NUM, PLOT_TYPE and REP_NUM in the main dataset.

7.15. Statistical analysis

Country-level statistics confirm the need to analyse all samples in Wales to deliver country level products on soil-N.

The statistical approach used for analysing the data for changes in 1978 – 1998, 1998 – 2007 and 1978 – 2007 for the 2007 report is reported in a separate CS technical report. Essentially, bootstrapping is used which involves treating sample data as a population from which to resample. Each resample produces a separate estimate of some quantity of interest, for example stock or change. A large number of resamples (typically 1000 or 10,000) then gives an approximation to the distribution of the required estimate, from which any statistic can be extracted. The main advantage of this method of estimation for CS is that it allows for non-normality in the data, without the necessity of knowing details of the actual distribution, and as such provides more accurate measurements of significance. See ANNEX F for a background document on approach used.

7.16. Linkages to other tasks and work packages

- LOI, SOM – organic matter content will contribute to variability in N
- Soil pH – N deposition can lead to acidification

- Total and available N are used to interpret change in WP1 and WP3

7.17. Linkages to other surveys

- Total N: Comparison to NSIS (Scotland), BIOSOIL and British Woodland survey.

- No other surveys currently measure mineralisable N.

7.18. References

Diekmann, M. & Falkengren-Grerup, U. (1998) A new species index for forest vascular plants: development of functional indices based on mineralization rates of various forms of soil nitrogen. Journal of Ecology, 86(2), 269-83.

Ellenberg, H. (1974) Zeigerwerte der Gefasspflanzen Mitteleuropas. Scripta Geobotanica, 9, 1-97.

Hill, M.O., Mountford, J.O., Roy, D.B. & Bunce, R.G.H. (1999) Ellenberg's Indicator Values for British Plants. Institute of Terrestrial Ecology, Huntingdon, UK.

8. Invertebrates

Task leader: David Spurgeon, Paul Chamberlain

8.1. Key question

Is there robust evidence of a decline in soil biodiversity as stated by the EU?

8.2. Key products

Assessment of soil invertebrate diversity across all major UK land uses using standard diversity indices i.e. Shannon-Weiner, Simpson Evenness, community structure and rank abundance analysis of Collembola and mites (*microarthropods*)

- Determine whether long-term change can be detected in soil invertebrate diversity against a background of spatial and temporal heterogeneity (by comparison with CS2000 results)

- Assessment of the relative importance of different pressures and drivers on soil invertebrate diversity, in particular land use change, climate and pollution

Provision of data to the Biological Record Centre to support the production of atlases of soil invertebrates, in particular Collembola and Oribatid mites that will build on existing data resources.

8.3. Policy background

The properties, activities of, and interactions between soil biota are critical requirements for the provision of most soil functions through their role in the provision of "ecological services", in particular food and fibre production, environmental interactions, and support of habitats and biodiversity. The biological components of soils have considerable potential as indicators of soil quality since they are a fundamental requirement for maintaining soil health. National-level requirements for biological indicators were outlined in the Soil Action Plan for England (Defra, 2004).

Little is known about soil biodiversity compared to other environments, although the biological component of soils drives many soil functions such as C storage, transformation of pollutants and the supply of nutrients to plants. A number of soil dwelling invertebrates and some fungi are covered by biodiversity action plans (BAPs), but since our knowledge of soil biodiversity is sparse it is not known how climate change, land management and aboveground vegetation, amongst other factors, affect their diversity. Beyond those species covered by BAPs, there is a need to quantify soil biodiversity and determine whether it is possible to observe consistent trends in this biodiversity against a dynamic background of spatial and seasonal variability. If so, the key questions are: how is soil biodiversity changing over time, and what are drivers of that change?

CS2007 represents the first time that a UK Survey has sampled soil invertebrates for a second time, yielding the opportunity to produce a time-series of invertebrate diversity across the UK. CS2007 will therefore provide data on the long-term stability of soil communities and also test whether it is possible to detect long-term changes in soil invertebrate diversity against the background of the temporal and spatial dynamics of soil communities.

CS2007 invertebrate diversity measurements are also closely allied with the UK Soil Indicators Consortium (UK SIC), a body consisting of 14 public stakeholders which is developing a national set of soil indicators and a soil monitoring scheme. UK SIC in turn has been developed in the light of the EU thematic strategy for soil protection, national soil strategies and action plans and other natural resource protection programmes, which highlight the need to identify robust physical, chemical and biological indicators of soil quality. CS2007 is primarily linked to UK SIC through the SQID-II project (Scoping Biological Indicators of Soil Quality), which is funded by Defra on behalf of UK SIC. SQID-II is currently assessing 13 potential biological soil quality indicators, of which soil invertebrate diversity is the only determinand occurring in both SQID-II and CS2007. Amongst their other measurements, SQID-II will analyse the invertebrate diversity in 100 soil samples (i.e. 100 x-plots) from CS2007 as a discrimination trial of the proposed indicators. Within CS2007, 256 squares, a repeat of CS2000, will be sampled for invertebrates. However, only inverts from 768 samples, 3 out of 5 x-plots for each square, will initially be analysed for invertebrate diversity.

8.4. Rationale for measurement

Table 8-1 Invertebrates: rational for measurement

	Facts	Comments
History in CS		
Invertebrate diversity	First measured in CS2000 on 256 squares	Repeat in CS2007 to initiate the first ever survey time-series for soil biodiversity
Links and compatibility to other monitoring programmes		
Invertebrate diversity	No other national Surveys or monitoring programmes	CS unique in UK, but other countries such as the Netherlands, Germany and Canada have programmes involving biodiversity assessments; measurements of microarthropods are directly compatible with these schemes
Uniqueness of CS		
Invertebrate diversity	No other national datasets	Only national UK dataset Only dataset with time series
Value for money (Policy priority or interpretative value X cost)		
Invertebrate diversity	High	High policy and interpretative value (time series), low cost. Important to establish whether it is possible to detect meaningful changes against spatial and temporal variability. Important in interpreting other data e.g. metals effects on biodiversity

8.5. Proof of concept

Invertebrate diversity was first measured as part of Countryside Survey in CS2000, and CS is the first and only national scale monitoring of soil biodiversity in the UK. The inclusion of invertebrate diversity measurements in CS2000 was partially driven by a 1996 report of the Royal Commission on Environmental Pollution, the Sustainable Use of Soils, which identified the development of indices of soil biological activity and diversity as a key research priority. However, a major difficulty in developing such indices was the requirement for baseline data from which a set of standards could be developed. It was within this context that an assessment of soil biodiversity was deemed timely within CS2000, which provided a cost-effective

framework for integrating a soil biological survey with existing and subsequent soil and land use data.

The aim of the soil invertebrate diversity measurements in CS2000 was not to sample and identify all invertebrate diversity within a soil, since such an all-taxa biodiversity inventory would require a wide range of techniques and would be a massive undertaking and highly expensive. Instead, the aim of invertebrate diversity in CS2000 was to produce a large baseline dataset across all major soil groups and habitats of Great Britain that could be used to examine the potential for using soil invertebrates in soil quality assessment. Therefore, the strategy was to capture soil invertebrates that would be abundant and relatively cost-effective to sample and identify. The category of soil invertebrates that best suited these criteria was the soil meso-fauna.

In CS2000, a combined approach to soil biodiversity assessment by looking at the discriminating power of functional and taxonomic groups of soil biodiversity was achieved. Efforts focused on groups that could be sampled, extracted and characterised with relative ease and within a limited budget. Hence whilst all individual invertebrates were identified to Taxa level, the Collembola and mites were further identified: the mites (Acari) to species level, and the Collembola to family or genus. In CS2007, Collembola will be identified to family and mites to the groups: phoretic, mesostigmatic, oribatid and prostigmatid.

The detailed protocols developed within CS2000 for sampling, analyses and data-management are available for CS2007. The CS2000 dataset now forms valuable baseline data for CS2007 and a means to place specific site, region and country-scale issues within context e.g. regional, national, European and the wider international environment.

CS2000 detected significant differences in invertebrate diversity between differing Environmental Zones, Broad Habitats, ITE Land Classes and other measures. CS2007 is an opportunity to resample these sites and determine whether consistent changes in invertebrate diversity can be detected against a background of spatial and temporal heterogeneity.

8.6. Key models which require analyte data

Not applicable

8.7. QA

The Defra/NERC joint Codes of Practice will be followed throughout.

8.8. Field protocol

Samples will be collected using a 8 cm long by 4 cm diameter white plastic core following the detailed field protocol described in Annex 1. Using the white core, a sample will be taken from a point 15 cm to the south and 15 cm to the east of the southern corner of the centre quadrat in each X plot in each 1 km square, giving 5 samples per 1 km square. The surface vegetation will be removed to reveal the soil surface and the core inserted to the full depth. In stoney or shallow soils, the sampling point will be moved if a full core depth cannot be obtained. Any such variations in the protocol will be recorded. On removal from the ground, the outside of the core is cleaned and any excess soil trimmed from the bottom of the core. Caps

are gently pushed over the end of the pipe. The core is then placed in a labelled plastic bag, placed in an envelope and posted to CEH Lancaster.

8.9. Laboratory protocol

A total of 768 cores will be analysed for invertebrates; 3 cores will be randomly selected from the 5 X-plots in the original 256 squares.

Soil invertebrates are extracted from a soil core as soon as possible after removal from the field using a dry Tullgren extraction method. Each Tullgren funnel consists of an aluminium funnel base supported in the funnel bank. There is a rubber seal on the end of each funnel to keep the collection bottles in place during the extraction period. An aluminium sieve unit balances above the funnel, and the soil cores are placed onto the sieve to extract soil fauna over a period of 5 days. A light which houses a 40 W bulb is suspended above the sieve and is used to provide heat to drive the soil fauna from the soil cores and into the collection bottles filled with a 70% ethanol preservative. Once collected, the soil invertebrates are identified to major taxa (Taxonomic level 1) and counted. Further identification of Collembola and mites to morphotype level, is then carried out.

8.10. Methods for sample storage and archiving

Table 8-2 Invertebrates: Methods for sample storage and archiving

Requirements	
Type of sample (e.g. wet/dry soil, extract, both)	Invertebrate preservation in 70% ethanol
Mass / volume of sample	n/a
Storage container (e.g. glass, plastic)	Plastic tubes in plastic bags
Storage requirements (e.g light dark, controlled humidity, temperature)	Cool and dark storage location
Storage location	CEH Lancaster long term storage facilities
Length of time samples are stable	Indefinitely

8.11. Future use of material

All future use to be approved by CS topic group or steering group. Possible use of archived invertebrates includes re-analysis to check methodology and QC for future surveys.

8.12. QC

After identification to Taxonomic level 1, every one in ten of the first 500 samples will be re-counted and identified by another member of staff. This second identification and count will be compared with the original. Differences in identifications will be resolved at this stage. Any mislabelling will be corrected at this stage. Any changes will be noted on the record sheets. The process will be repeated at a reduced rate as the identifications proceeded (5 percent for the next 300 down to 2 percent for the final 252).

In CS200, t-test analysis indicated that there were no significant differences between the original fauna numbers and the validated data. This process, however, highlighted that a small percentage of the smallest invertebrates could be lost in transfer from the original extraction sample tube to the colour-coded tubes. Future re-assessment of identifications or counts will take this factor into account.

8.13. Calculations/Units

All invertebrates numbers are reported on a per soil sample (i.e. per white core) basis. These will be converted to a N m^{-2} where relevant. Diversity indices will be calculated using Primer6 software.

8.14. Data storage

For a complete account of all invertebrate data held, see the MASQ Data Catalogue. As an example, the Fauna_Data table contains faunal counts from dry extractions of soil samples from white soil cores:

Table 8-3 Fauna data table

Field name	Description	ENGLISH_NAME
SQUARE_NUM	CS2000 1km square number	
PLOT_TYPE	CS2000 X-plot	
REP_NUM	CS2000 subplot within the 1km square	
SOIL_TYPE	Soil Major Group Abbreviation	
QC_CODE	Codes for missing samples, etc	
ACTO	acari	Mites
ACPH	acari - phoretic	Mites
ACME	acari - mesostigmata	Mites
ACOR	acari - oribatid	Mites
ACPR	acari - prostigmata	Mites
ARAN	araneae	Spiders

NOTE: This is not the complete table – only a selection as an example

8.15. Statistical analysis

CS2007 will resample the 256 squares sampled in CS2000. This preserves the time series from CS2000. However, no country-level reporting will be possible since there are insufficient squares. Country-level reporting would require much more sampling and identification of invertebrates from additional CS squares, which will not be carried out in CS2007.

8.16. Linkages to other tasks and work packages

Within WP4

- Links to soil type, pH, soil C and N content for interpretation of invertebrate diversity distribution
- Link to main SQID-II invertebrates analyses, which are a sub-set of this
- Link to microbial diversity for interrelationship of microbial and invertebrate diversity
- Link to available N content as potential driver of invertebrate diversity
- Link to contaminants to assess effects of metals and organic contaminants on invertebrate diversity

Links to other WPs

- Links to aboveground diversity, habitats, Ellenberg values of plant community structure & areas of conservation status (WP1)
- Links to Integrated Ecosystem Assessment (WP5) - relationships between above- and belowground diversity

8.17. Linkages to other surveys

- Links to various invertebrate records held by the Biological Records Centre –atlases of the invertebrates such as nematodes, beetles etc
- Links to various UK invertebrate recording schemes
- Links to Dr Steve Hopkins' Collembola distribution map of UK
- Links to Dr Malcolm Luxton's mite biogeography map of GB
- Links to the National Soil Inventory (NSI) Scotland, which will sample invertebrates in its next survey, 2007
- Links to NSI Scotland 1980 data on soil nematode community
- Links to various national soil monitoring initiatives in other countries, including the Netherlands, Canada and Germany - see documents produced by the OECD Expert Meeting on Soil Erosion and Soil Biodiversity Indicators, available on the web at:
- http://webdomino1.oecd.org/comnet/agr/soil_ero_bio.nsf/
- Links to UK SIC and its efforts to establish reliable indicators of soil quality
- Links to the Framework 6 EU project ENVASSO: Environmental Assessment of Soil for Monitoring

9. Metals

Task leader: David Spurgeon

9.1. Key question

Is the decline in atmospheric deposition reported by the Metal Deposition Network reflected in soil metal concentrations?

9.2. Key products

Heavy metals in soils:

- Nationwide assessment of levels of heavy metals in soil and change since 1998
- Attribution of stock and change in metal concentrations to pressures and drivers

A comprehensive approach has been agreed for analysis of metals in soils collected in CS 2007 that will both ensure that any new data will be both consistent with CS2000 and also deliver concentrations for an extended analyte suite. This strategy involve a 5 stage approach based on aqua regia digest; analysis by ICP- OES for Cd, Cr, Cu, Pb, Ni, V, Zn and P and S; further analysis of the same samples for further metals by ICP-MS; careful data evaluation and targeted remeasurement for any problem analytes. This approach provides the best way of delivering quality data for the main metals (Pb Cd Cu Zn Ni Cr V), a wide suite of measurements for other metals (including Hg) and also P and S concentrations.

9.3. Policy background

In the European Union and internationally, a set of risk assessments has focused on the ecological effects of metals in both aquatic and terrestrial ecosystems. This work is driven by a number of policy initiatives, including new procedures for the mandatory risk assessment of existing priority chemicals according to European Commission regulation 1488/94. Methods have also been proposed and developed for the application of critical loads for the control of heavy-metal emissions. In support of the 1998 Convention on Long-Range Transboundary Air Pollution Aarhus Protocol on Heavy Metals (that came into force on 29 December 2003), the UK government has funded a series of projects to support the development of a critical loads approach for metals. Also to provide the required background data that is necessary to apply this method for national scale mapping of critical limit and critical load exceedances (e.g. the heavy metal deposition network).

The focus on the risk assessment of metals recognises the harm that they can do to soil ecosystems. A number of keystone soil taxa, such as earthworm and springtails are particularly sensitive to metals and high concentration of these contaminants can cause reduction in both the abundance and diversity of communities of these taxa. The result of this can be a breakdown in soil function including the breakdown of

organic matter and turnover of essential elements. Because these are not broken-down over time and so can be removed only by the relative slow process of leaching (or cropping), the accumulation of metals in soils due to pollution offers one of the most serious threats to long-term soil sustainability. Put simply, if soil becomes contaminated with metals there is no simple solution to ameliorating the effects of this contamination. Concepts that exist for organic pollutants, such as “managed natural attenuation” simply do not apply to metals within reasonable time scales. Accurate knowledge on the rate of accumulation of metals in soils is therefore an important aspect of soil management.

The current project work concerning the derivation of critical loads for metals in the UK is funded by Defra and aims both to contribute to the development of improved critical loads methods for application within UN/ECE and to develop improved tools to assess the effects of changing rates of atmospheric deposition on pools of metals in soils and freshwaters. Full details of this project can be review at the project website available at URL http://www.york.ac.uk/depts/eeem/research/projects/criticalloads(stage3)/overview.htm. The project is a collaboration between The University of York, CEH and the Department of Agriculture and Rural Development , N Ireland. The aims of the project are:

- To calculate and produce national maps of effects-based critical limits (at which biodiversity and ecosystem function is protected) for Cu, Zn, Cd and Pb
- To calculate and produce national maps of steady-state critical loads (i.e. atmospheric metal deposition levels that can be sustained without soils and freshwaters reaching critical limits)
- To develop catchment-scale models of metal dynamics that can predict the effects of future atmospheric deposition scenarios on metal concentrations in soils and surface waters

The ‘Heavy Metal Deposition Monitoring Network’ is one project, that the critical loads work draws on to supply data for assessment. This project, also funded by Defra, is run by CEH and has collected information on the concentrations of a suite of metals present in rainwater and particulate materials. Samples are collected from 15 sites and the data has been used to provide essential information on patterns and trends in metal deposition rates across the UK.

To provide essential information concerning the spatial distribution of heavy metals in soils, the collection and analyses of heavy metals in soils were conducted in the previous Countryside Survey. The integrated analyses of both soil chemical composition and biodiversity (plant, invertebrate, microbial) have provided a unique opportunity to ascribe environmental pressure to biodiversity change. During the next survey, the temporal stability of these relationships can be established over a time series. This temporal understanding in the distribution and possible effects of metals (including entry into food chains) will be unique for the UK and to our knowledge in the world.

9.4. Rationale for measurement

Table 9-1 Metals: rationale for measurement

	Facts	Comments
History in CS	CS 2000	Give capability to establish a time series for the first time
Country level reporting	No	CS2000 did not give country level statistics, although the data showed regional trends
Links and compatibility to other monitoring programmes	UK heavy metal monitoring network BGS Gbase for stream sediment Comparable data to those collected in the NSI, for same horizon (0-15cm).	Link soils metal levels with predicted deposition Links soil metal levels with stream sediment database NSI metal concentrations are only for one sampling period
Uniqueness of CS	Would be the only national and world wide dataset that will encompass temporal stability and links soil metal concentrations to soil type, habitat and land use over time	
Value for money (Policy priority or interpretative value X cost)	Priority - medium Value for money - high	Sound fit with current prioritise, but not listed among the top priorities by customers. Favoured by CEH due to fit with existing projects ICP-MS method will give data on multiple metals (27) and also potentially data on total P and total S (although this is being validated in a pilot test).

Metal pollution can have direct adverse impacts on soil and freshwater biota, and the long-term sustainability of soils, and indirect effects on human health through the food chain. Past and present deposition of metals to land (e.g. from atmospheric deposition and solid waste disposal) has resulted in the presence of metal concentrations in soils that exceed environmental quality standards in some parts of the country. Although UK emissions of metals such as lead, cadmium, copper and zinc have decreased over the past two decades, recent measurements from the heavy metal deposition network have established that significant deposition is still occurring. During CS2000, over 1200 soil samples were successfully analysed for seven heavy metals (Pb, Zn, Cu, Cd, Zn, V, Ni). The metal concentrations showed regional trends in the concentrations of metals (higher values in England and Wales than in Scotland) and in different broad habitats (highest in arable/horticultural and

improved grassland soils, lowest in dwarf shrub heaths and bogs). The measurements also provided a measurement of concentrations of metals in soils that are primarily influenced by diffuse, rather than point source, inputs. To provide knowledge of the prevailing dynamic of metal concentrations in GB soils, a repeat (or partial repeat) of the previous metal analysis would allow a validation of modelled prediction of the measurements made through the heavy metal deposition network. It would also provide a change statistic with the previous measurements that can be used to populate dynamic approaches to predicting future metal loads and associated risks for metals in soil. Modelling in support of these analyses can also be applied to predict concentrations of metals of the highest concern for human health in crop species and home grown vegetables.

9.5. Proof of concept

Within the pilot study two methods of analysis were compared ICP-MS and ICP-OES. The results indicate that ICP-MS has the same/better sensitivity and reliability than ICP-OES and in addition would be able to deliver for the first time National scale data on soil concentrations for a suite of many metals including not only cadmium, copper, lead, zinc, nickel, chromium and vanadium that were measured in CS2000, but also selenium, mercury arsenic, antimony, manganese and molybdenum among many others. Additionally it is anticipated that the ICP-MS method will also provide reliable data on total phosphorous and sulphur, although this is currently being validated.

In CS2000, concentrations of the 7 analysed metals (Cd, Cu, Cr, Ni, Pb, Zn) were measured in soils collected from all X plots from the squares that were sampled in the original 1978 survey. This gave a total of 1256 soils for metals analysis. A repeat analysis of this number of squares would be the option that would provide the maximum amount of data concerning current metals concentration and would establish the most robust time series. However, we recognise that there are competing priorities for analysis and so the protocol used for the selection of soils for analysis should be cost effective as well as robust. For this reason, as part of the pilot phase CEH instigated a power analysis of the CS2000 data to evaluate within and between square variability and also to assess the implications of changing sample number for the power of the analysis to detect change. This was done to help the CSSTG identify the analytes and products that would be included in the coming survey. For metals the modelling showed a higher proportion of variance was found between, rather than within squares.

The power analysis indicated a high probability to detect at change as low as 10% between surveys, especially if Land Class is considered. Importantly, a repeat analysis of more than 2 samples per square gives only a small amount of extra power for detecting change and would be wasteful in terms of resources. On this basis we were able to recommend to the SCSTG that the best approach for a repeat survey of soil metal concentrations would be to analyse soils from 2 X-plot from all the CS1978 squares analysed in CS2000. This design giving both a high power to detect change and attribute concentrations to particular drivers and also a saving on 3/5 effort compared to a full analysis of metal concentrations in all X plots from 1978 squares as was conducted in CS2000.

9.6. Key models which require analyte data

The metals data collected through the new CS campaign could also be used to estimate metal bioavailability, based on principals taken for soil solution chemistry and metal speciation modelling. As part of the Defra critical load for metals project,

one workpackage aimed to derive effect based EQSs ("critical limits") for each metal applicable for physicochemically diverse soils and waters. This work conducted jointly by CEH Lancaster and Monks Wood (Lofts, Spurgeon, Tipping, Svendsen) has developed a method to calculate critical limits of cationic heavy metals accounting for variations in soil chemistry. The approach used for predicting bioavailability is based on the principals developed in the so called "biotic ligand model". This is based on principal that the exposure of an organism to metals in different soil or waters is governed by the amount of metal that is present in the free ion form in solution coupled to the competition of this free ion with other ions to bind to ion import channels and cell surface receptors.

In the ongoing Defra critical load for metals project, simple regression based predictive models have been developed that allow estimation of both free ion metal concentrations in soil solution and also the magnitude of competition effects of counter ions (principally the H^+ ion). The result of this work is the capability to predict with much greater accuracy the exposure of species to metals (so far Cd, Cu, Pb, Zn and Ni, although development of model for additional metal is in progress). This prediction is for any given soil and can be determined either from measurement of total metal concentration alone, or the use of weak salt extract (e.g. weak $CaCl_2$, NH_4-salt, EDTA solutions). The regression model relies on three simple parameters for estimation of availability, total soil metal concentration, soil pH, soil organic matter content, all of which are proposed to be collected in the new CS campaign. Therefore, it will be a relatively simple matter to estimate available concentrations of each of these metals for UK soil, based on the available data. Currently CEH is about to start a 3 year PhD project that will aim to link these speciation and bioavailability models with existing principals for mixture toxicity. This work could provide the basis for a more complete estimation of combined metal exposure at the samples sites.

9.7. QA

Measurements of metals in soils by Aqua Regia digestion will follow the method of The Standing Committee of Analysts 1986. Methods for the examination of waters and associated materials: Methods for the determination of metals in soils, sediments and sewage sludge and plants by the hydrochloric-nitric acid digestion, Method 'A'.

Analyses will be conducted in accordance with the existing system at CEH Lancaster. Procedures have been audited and the laboratory is ISO 17025 accredited by UK Accreditation Service (UKAS)

9.8. Field protocol

The field method described here is the same as that used in CS2000. Samples for metals were collected using a 15 cm long by 5 cm diameter black plastic core following the detailed field protocol described in Annex 1. Using the black core, a sample was collected from a point 15 cm to the south of the southern corner of the centre quadrat in each X plot in each 1 km square, giving 5 samples per 1 km square. The surface vegetation was removed to reveal the soil surface and the core was inserted to the full 15 cm depth. In stony or shallow soils, the sampling point was moved if a full core depth could not be obtained. Any such variations in the protocol were recorded. On removal from the ground, the outside of the core was cleaned and any excess soil was trimmed from the bottom of the core. The core was then placed in a labelled plastic bag which was sealed and stored in the surveyor's vehicle pending delivery to Lancaster.

9.9. Laboratory protocol

The soil from 512 cores will be analysed for metals. These will be 2 cores, randomly selected from the 5 X-plots in the original 256 squares.

Analyses of the soil digests for the 2000 survey were conducted by ICP-OES. For future analyses, however, this method will be augmented by use of a further rinductively coupled plasma mass spectrometry (ICP-MS) analysis. This is planned becasue ICP-MS enables detection of a greater number of analytes from each run. The current standard suite at CEH Lancaster is up to 27 analytes (including As, Hg, as well as the seven metals analysed in the 1998 samples - Pb, Zn. Cu, Cd, Zn, V, Ni). Details of the analytical methods are given in Appendix 2. For the ICP-OES method, QA in the past Countryside Survey showed consistent and low blanks during the analysis of the 60 analysed batches. The method was sufficiently sensitive that only a very small proportion of the analytical measurements (0.2%) were below the limit of detection. Coefficients of variation for CRM were between 3 to 7%.

9.10. Methods for sample storage and archiving

Table 9-2 Metals: methods for sample storage and archiving

Requirements		
Type of sample (e.g. wet/dry soil, extract, both)	air dry soil	digests
Mass / volume of sample	10g	25 ml
Storage container (e.g. glass, plastic)	Plastic	Glass
Storage requirements (e.g light dark, controlled humidity, temperature)	Dark, dry, cool	Acid store
Storage location	Lancaster	Lancaster
Length of time samples are stable	Indefinitely	Indefinitely

9.11. Future use of material

- All future use to be approved by CS topic group or steering group or a delegated responsible person within CEH.
- Archived soils can be used to validate the predictions of metal deposition for monitoring and modelling and also for critical loads assessment work. Funding is being sought from FSA, European Union, UN-ECE in support of critical load, Environment and Human Health initiatives, particularly if As and Hg, Pb and Cd can be measured.

9.12. QC

QC procedures are based on a total analytical error target of 20%. Made up of 10% error in precision, and 10% error in bias (but see below.). If a QC standard falls outside the action limit, or two successive QC standards fall outside the warning limits the analytical data associated with that batch or batches should be rejected. Similarly, for CRM where the results exceed the bias target the associated analytical data should be rejected. After 100 samples are analysed, it may become necessary to review the analytical error targets for some determinands. Persistent consecutive warning limits and action limits exceedance will demonstrate what is achievable in

routine analysis. Some previously rejected batches of results may then become acceptable and be re-instated.

9.13. Calculations/Units

All values for metal concentration will be expressed as ppm (equivalent to mg/kg and µg/g). Analysis of blanks, spiked samples and reference materials will be undertaken. If these show either the presence of ubiquitous contamination in the case of blanks or a consistent inaccuracy in measurement for spikes and standard reference material, the raw concentration data can be corrected to account for this. Based on past experience, this is not, however, expected to be a major issue.

9.14. Data storage

Raw data will be stored as follows:

- Raw data as in QA files stored at Bangor
- Final data only in CS database as follows

The example below is given for cadmium and chromium for respective analysis by ICP-MS and ICP-OES

Field name	**Description**
SQUARE_NUM	CS2007 square number
PLOT_TYPE	CS Plot type
REP_NUM	Replicate number
CHEM_BATCH_NUM	Environmental Chemistry Lancaster Batch number
CHEM_ID	Number of sample with Environmental Chemistry Batch (1-25)
Cd_ICP_OES_MG/KG	Cadmium by ICP-OES
Cd_ICP_OES_LOD	Cd by ICP-OES Limit of Detection
Cr_ICP_OES_MG/KG	Chromium by ICP-OES
Cr_ICP_OES_LOD	Cr by ICP-OES Limit of Detection
Cd_ICP_MS_MG/KG	Cadmium by ICP-MS
Cd_ICP_MS_LOD	Cd by ICP-MS Limit of Detection
Cr_ICP_MS_MG/KG	Chromium by ICP-MS
Cr_ICP_MS_LOD	Cr by ICP-MS Limit of Detection
COMMENTS	

9.15. Statistical analysis

Data concerning metal concentrations in soils will be analysed using a range of standard analysis of variance, single variable, multiple and non-linear regression techniques will be used for attribution of soil metal concentrations to particular drivers, such as land-use, broad habitat, soil type and so on. In addition spatial statistical algorithms will be applied to assess spatial aggregation and relation to major and diffuse sources. This will be based on existing approaches for data analysis within CS. Modelling work using regression based approaches will be used for estimation of free metal ion and also the amount of metal ion that is bioavailable to biota. This work will be based on the principals of the “biotic ligand model”, as used in the current Defra critical loads for metals project.

9.16. Linkages to other tasks and work packages

- WP4 explanatory variables - Soil pH and organic matter content data for calculation of metal bioavailability in CS soils

- WP4 explained variables - Invertebrate and microbial biodiversity data for assessment of metal effects

- Links to other CS work packages - Fields Survey for samples, Vegetation survey for information on plant diversity, Freshwaters for metal analysis to assess linkage between soil metal content and percolation of metals into surface waters

9.17. Linkages to other surveys

- NSI England and Wales, NSI Scotland, GBase, Critical Loads, Metal Deposition Network, EA Soil and Herbage Survey, EU project ALARM, PhD projects.

10. Microbial Diversity

Task leader: Roger Pickup and Rob Griffiths

10.1. Key question

What is diversity of soil bacteria at a national scale and what determines the distribution?

10.2. Key products

- National and country-level assessments of soil bacterial biodiversity
- Attribution of changes to either soil chemistry, land use, or location
- Assessment of distribution of bacterial pathogens and relationships with land-use practices

10.3. Policy background

The Royal Commission on Environmental Pollution (RCEP) raised the importance of soils in the U.K. with the publication of its nineteenth report, *Sustainable Use of Soil* (RCEP, 1996). The RCEP report (RCEP, 1996) identified the development of indices of soil biological activity and diversity as a key research priority. The major difficulty in developing such indices is the development of meaningful biological indicators and methodologies, coupled with the need for baseline data from which a suitable set of standards can be formulated.).

A nationwide survey was proposed to establish a framework for comprehensive baseline datasets of soil biological properties. The Countryside Survey 2000 (CS2000) provided a cost-effective framework for integrating an assessment of soil biological properties with detailed landscape, land-use, soils and vegetation data.

Soil biodiversity

Following the 1992 Earth Summit in Rio de Janeiro three reasons were highlighted on why soil biodiversity should be protected.

- The first, and most researched, is the fundamental role that soil organisms have in maintaining soil processes that are essential to the functioning of all terrestrial ecosystems; their “ecological” significance.
- The second is their usefulness; the “utilitarian” reason. Soil organisms have been used widely in biotechnology, e.g. to improve nitrogen fixation, for the bioremediation of contaminated soils and in screening for potential pharmacological compounds. The immense genetic pool within the soil suggests that there is, still, significant potential for novel products and applications.
- The third reason is ethical. The soil contains some of the oldest organisms on earth that should, as identified by the Convention on Biological Diversity, have value in their own right. While it is acknowledged that the scale of effort and taxonomic expertise imposes serious limitations on our ability to assess soil biodiversity), some of these limitations are being tackled by the development and application of novel genetic and functional analytical techniques for the characterization of soil communities.

Currently within CEH we have extensive experience in monitoring of bacterial communities using molecular profiling techniques, and they have provided a deeper understanding of the dominant bacterial taxa present in a variety of soils. Furthermore, we are now beginning to develop an understanding of the main environmental factors dictating the structure and diversity of soil bacterial communities. We now seek to apply these techniques to a wider range of soil types to provide the first nationwide map of soil bacterial biodiversity, and to seek a greater understanding of how anthropogenic activities such as land use change may impact on bacterial communities. Furthermore, the application of such methodologies to a subset of samples from the Countryside survey will provide valuable information on their utility and usefulness as biological indicators of soil health/quality.

10.4. Rationale for measurement

Table 10-1 Microbial diversity: rational for measurement

	Facts	**Comments**
History in CS	Only microbial functional attributes previously examined (CS 2000)	
Links and compatibility to other monitoring programmes		
Uniqueness of CS	Would be the only world wide dataset examining patterns of soil bacterial biodiversity, and environmental determinants of community structure.	
Value for money (Policy priority or interpretative value X cost)	Provide baseline data on utility of microbial measures as indicators of soil quality. Fits within current CEH research priorities examining the links between above and belowground organisms.	

10.5. Proof of concept

Soil Biodiversity

Perhaps the most abundant and diverse group of microbes in soil are the bacteria. These organisms sit base of the soil food web and play an important role in the turnover of both soil detritus and plant exudates, and are therefore critical to the

cycling of nutrients necessary for sustaining plant growth. Little is known of the ecology of microorganisms for two reasons; firstly their small size makes them difficult to study, and secondly there are technical difficulties associated with studying their ecology in what is essentially a hidden environment. It is however essential that we determine their taxonomic diversity, their functional roles and interactions in the soil ecosystem, and lastly establish the anthropogenic and natural drivers which influence or alter beneficial microbial traits. Molecular methods based on the extraction of nucleic acids from soil and amplification of ribosomal RNA genes allow assessment of the phylogenetic diversity of organisms present in a sample (O'Donnell and Gorres, 1999). Whilst the detection of these organisms may be biased by inefficient recovery of nucleic acids from all organisms, and also preferential PCR amplification, they are generally considered to generate a clearer picture of the identities of dominant organisms compared to traditional culture-based methods. The application of such methods to environmental samples has provided a clearer picture of the true extent of microbial taxonomic diversity, as many new sequence types have been discovered compared with those found through traditional culture-based methods (Hugenholtz *et al*., 1998). More recently the adoption of rapid fingerprinting approaches to study microbial communities has permitted routine typing of microbes in their natural environment (Muyzer *et al*., 1993).

From the numerous cloning and sequencing projects that have been carried out on soil samples across the globe we are beginning to develop a clearer picture of the identities of the dominant soil bacteria. Essentially, no matter where you sample geographically, you will find that most of the clones sequenced will either belong to the alphaproteobacterial or acidobacterial lineages (Figure 10-1). Whilst it is appreciated that these two lineages contain a vast diversity of bacterial "species", there are often several intra lineages that are apparently geographically dominant eg the alphaproteobacterial Bradyrhizobia clades. Whilst it may be possible to infer certain functional roles for such taxa, eg such as in nitrogen fixation, the other main groups of bacteria, the acidobacteria, have only recently been discovered solely through molecular surveys and so their functions in the environment are almost entirely unknown.

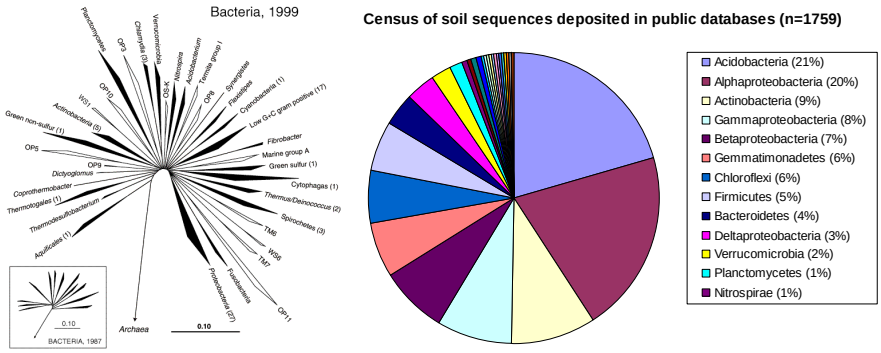


Figure 10-1 Showing main lineages of soil bacterial sequences deposited in Genbank. Griffiths, Unpublished.

Ecologically, it has been postulated from a meta-analysis of soil molecular studies that the ratio of alpha proteobacteria to acidobacteria may be related to the nutrient status of the soil (Smit *et al*., 2001) (Table 10-2). Furthermore Acidobacteria are

difficult to grow in culture, requiring low nutrients and long incubation times, whereas alphaproteobacteria taxa are relatively easy to grow in the lab. It may therefore be hypothesised that these two groups of organisms may be carrying out similar functions in the soil but have different growth requirements. There is an obvious relevance of this to the ongoing debate about microbial functional redundancy in soil systems.

Table 10-2 Proposed relationship between soil nutrient status and the ratio and alphaproteobactiera to acidobacteria. (Taken from Smit *et al*. 2001)

Soil Type	**Ratio Proteobacteria:Acidobacteria**
Oligotrophic arid	0.16
Low input agricultural	0.34
Low input agricultural	0.46
High input agricultural	0.87

To advance our understanding of soil bacterial ecology there is therefore a need to identify the factors that influence the abundance of these recognised dominant organisms as a prelude to elucidating the their functional roles in the soil environment, and understanding how soil functions may be altered by shifts in bacterial composition. In order for this to be achieved, a survey approach is required incorporating molecular methodologies to assess both diversity and identity; appropriate collection of environmental and physical-chemical data; and rigorous data collection and analyses methodologies.

CEH is in a unique position to initiate such a survey due to its access to multiple field sites and involvement in the upcoming Countryside survey. Indeed CEH's commitment to having a soils component the CS, will require substantial groundwork prior to the main survey to establish methodologies for sampling and analyses.

In order to make microbial diversity assessments it is anticipated that the main methodology used will be tRFLP. Since this is a capillary sequencer based method, it is particularly suited to data basing, and has been shown to be of use in determining drivers of change in soil bacterial communities. In particular, CEH have recently established that specific diagnostic peaks exist for the alphaproteobacteria and acidobacteria using our standard tRFLP methodologies (Figure 10-2, courtesy Thomson *et al*.) and an in-house developed computer program.

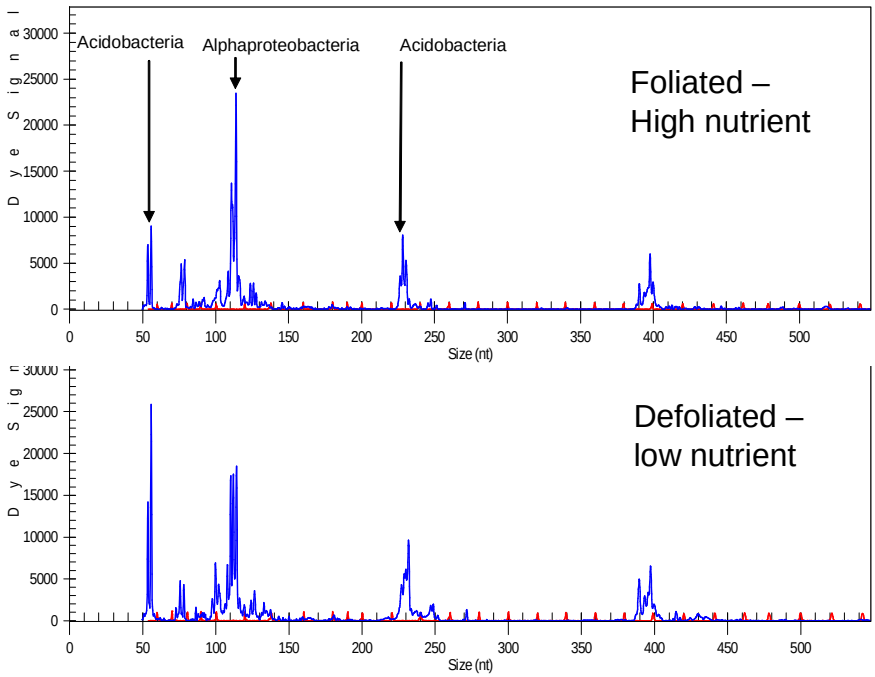


Figure 10-2 tRFLP profiles from either foliated or defoliated soil showing diagnostic peaks for dominant soil bacterial lineages. Each profile is representative of 4 replicates, and clearly shows increased abundance of acidobacteria in the low nutrient soil caused by defoliation.

10.6. Key models which require analyte data

N/A

10.7. QA

Sample labelling and tracking will be facilitated using a "barcode" system and associated reader. All molecular analyses will be carried out according to strict set laboratory protocols.

10.8. Field protocol

Samples will be collected using a 15 cm long by 4 cm diameter grey plastic core following the detailed field protocol described in Annex 1. Using the core, a sample will be taken from a point 15 cm to the south and 15 cm to the west of the southern corner of the centre quadrat in one X-plot. The surface vegetation will be removed to reveal the soil surface and the core inserted to the full depth. These cores are marked with top and bottom and it is important that top is at the soil surface. In stoney or shallow soils, the sampling point will be moved if a full core depth cannot be obtained. Any such variations in the protocol will be recorded. On removal from the ground, the outside of the core is cleaned and any excess soil trimmed from the bottom of the core. Caps are gently pushed over each end of the pipe. The core is then placed in a labelled plastic bag, placed in an envelope and posted to CEH Lancaster.

10.9. Laboratory protocol

A total of 1280 grey cores, from the 5 X-plots in the original 256 squares will be analysed. These cores will be removed from the freezer and split lengthways, the remainder being kept frozen for further analysis.

Sieves should be cleaned and autoclaved prior to each use. 10g of bulk soil from 2 mm sieved soil will be retained in sterile sealed plastic bottles and transferred from handling facility to the Lancaster molecular biology laboratory prior to DNA extraction. DNA extraction will be carried out using either the MoBio Ultraclean Soil DNA Mega Prep Kit, the Tepnel Nucleoplex 96 well extraction procedure, or an in-house proprietry method (currently under evaluation see S:\SAFETY\Risk assessment\MMECOL_ MICROBIAL DIVERSITY\BBCTAB.doc). DNA will be divided into smaller volumes so that deterioration by freeze/thaw is minimised and stored at -20 ^{o}C. We estimate a maximum yield of 40 µg per sample (based upon the assumption that 1 g of soil contains 10^9 bacterial cells) of which CEH Lancaster would be aliquotted use 50 % of the final volume, CEH Oxford would use 20 %, and 30 % would be stored as an archive at CEH Lancaster. This would allow limited repletion of the pathogen analysis and full repetition of the tRFLP analysis. The reproducibility of results is not expected to diminish after storage of DNA without thawing for 5 years. All stored samples would be logged with the central data archive.

Community Analysis by tRFLP: The terminal restriction fragment length polymorphism (tRFLP) method of profiling bacterial communities is emerging as being one of the most useful methods for examining across many samples5. Whilst it may lack the phylogenetic resolution of other gel-based techniques, the use of a capillary sequencer and size standards for data collection allows comparison of multiple samples without the constraints of gel to gel based comparisons. Furthermore the presence of taxon specific cut sites (albeit at low phylogenetic resolution) allows identification of peaks in the community profile. We will therefore use it to examine broad scale changes in the communities arising from the environmental variables examined. Standardised quantities of soil DNA will be amplified using universal 16S rRNA bacterial primers, with a phosphoamidite labeled forward primer. The PCR products will then be digested using the restriction enzyme Msp I and digested products run on a Beckman CEQ2000 capillary sequencer. Once profiles have been obtained from all samples we will use binning methodologies within the BECKMAN CEQ2000 sequence analysis software to generate a data matrix containing the relative heights of and identities detected peaks in all the samples.

Full protocol: S:\SAFETY\Risk assessment\MMECOL_ MICROBIAL DIVERSITY\TRFLP Protocol.doc

Pathogen Assesments

Samples will be interrogated for the presence of a range of acterial/protozoan pathogens hazardous to human health. The presence of 8 pathogens will be determined quantitatively by real time PCR (Lancaster). Distribution will be assessed in relation to land-use, biogeography, soil type, chemical/physical characteristics and above ground diversity.

Table 10-3 Pathogen assessment

Pathogen	Classification	Type of pathogen
E. coli O157:H7	bacterium	Human
Mycobacterium avium subspecies *paratuberculosis*	bacterium	Human/animal
Salmonella typhimurium	bacterium	Human
Listeria monocytogenes	bacterium	Human
Xylella fastidiosa	bacterium	Plant
Campylobacter jejuni	bacterium	Human
Shigella spp.	bacterium	Human
Crytosporidium parvum	protozoan	Human

10.10. Methods for sample storage and archiving

Table 10-4 Micobial Diversity: methods for sample storage and archiving

Requirements	
Type of sample (e.g. wet/dry soil, extract, both)	Wet soil
Mass / volume of sample	2-5g
Storage container (e.g. glass, plastic)	No preference. Must be clean
Storage requirements (e.g light dark, controlled humidity, temperature)	Frozen asap
Storage location	CEH Lancaster
Length of time samples are stable	

10.11. Future use of material

All data generated will be made publicly available. Soil nucleic extracts will be archived at -80^{o}C for future use.

10.12. QC

Sample labelling and tracking will be facilitated using a “barcode” system and associated reader. All molecular analyses will be carried out according to strict set laboratory protocols.

10.13. Calculations/Units

Following extraction of nucleic acids, quantities recovered will be assessed using a spectrophotometer and given as ug/gram wet weight soil. After molecular analyses (trflp), individual peak lengths (base pairs).

10.14. Data storage

Data generated from the project will be analysed ‘in house’ and archived with co-operation from the NERC Environmental Bioinformatics Centre. Specifically, past collaborations between ASW’s section and NEBC have involved the development of a tRFLP classifier (TRFLPMAP), Barcode system development and sequencing of microbial genomes. NEBC staff will aid in archiving and databasing of tRFLP data, advise on MIAME compliance for trflp data and provide strategic advice on novel

analyses methods for environmental gene analyses. After analyses of primary data, two main data matrices will be generated as part of this project. The environmental variable matrix will contain all the environmental variables measured as part of the Countryside Survey. This will include details on location, chemistry, soil type and dominant vegetation. A second "community matrix" will contain the relative abundances of matched t-RFLP peaks for all the samples.

10.15. Statistical analysis

Initially we propose to perform exploratory analysis using standard multivariate methods such as principal components analyses to identify major groupings in both the community and environmental datasets, and also to examine the associations of particular variates with major groupings. Secondly we will seek to use constrained ordination (e.g. canonical correspondence analyses) to interrogate associations between the environmental variables and the community data. The environmental variables we will test will all be measured as part of the Countryside Survey. Specifically these will include measures of soil chemistry such as C, N, P, pH and moisture content, but may also include measures of organic and inorganic pollutants (under negotiation). To assess any changes in bacterial communities due to vegetation composition the first axis scores of each of unconstrained ordinations performed on the vegetation data will be included as environmental variables in the constrained ordinations for the bacterial community data (ter Braak and Schaffers, 2004). Similarly, if soil fauna is to be studied as part of the Countryside Survey (currently under negotiation), a similar approach could be used to assess to correlate changes in soil bacteria with soil fauna. Other nominal variables will be included in the analyses such as soil classification (Avery, 1990), broad habitat classification and aggregate vegetation classification (Firbank *et al*., 2003; Haines-Young *et al*., 2003). Once major correlates are determined between particular environmental variables and bacterial community variables, we will then seek to use standard statistical tests to draw explicit conclusions on the quantitative relationship between the environmental variables and the relative abundances of microbial taxa.

10.16. Linkages to other tasks and work packages

- Soil type, pH and organic matter content data for explaining differences in bacterial community structure

- Metals component – examination of any microbiological consequences of metal pollution – indicators.

- Vegetation survey for information on plant diversity, and linkages to below-ground microbial diversity

10.17. References

Avery, B. W. 1990. Soils of the British Isles. CABI International.

Firbank, L. G., C. J. Barr, R. G. H. Bunce, M. T. Furse, R. Haines-Young, M. Hornung, D. C. Howard, J. Sheail, A. Sier, and S. M. Smart. 2003. Assessing stock and change in land cover and biodiversity in GB: an introduction to Countryside Survey 2000. Journal of Environmental Management. 67:207-218.

Haines-Young, R., C. J. Barr, L. G. Firbank, M. Furse, D. C. Howard, G. McGowan, S. Petit, S. M. Smart, and J. W. Watkins. 2003. Changing landscapes, habitats and vegetation diversity across Great Britain. Journal of Environmental Management. 67:267-281.

Hugenholtz, P., B. M. Goebel, and N. R. Pace. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Journal of Bacteriology 180:4765-4774.

Muyzer, G., E. C. Dewaal, and A. G. Uitterlinden. 1993. Profiling of Complex Microbial-Populations by Denaturing Gradient Gel-Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes-Coding for 16s Ribosomal-Rna. Applied and Environmental Microbiology 59:695-700.

O'Donnell, A. G., and H. E. Gorres. 1999. 16S rDNA methods in soil microbiology. Current Opinion in Biotechnology 10:225-229.

Smit, E., P. Leeflang, S. Gommans, J. van den Broek, S. van Mil, and K. Wernars. 2001. Diversity and Seasonal Fluctuations of the Dominant Members of the Bacterial Soil Community in a Wheat Field as Determined by Cultivation and Molecular Methods. Appl. Environ. Microbiol. 67:2284-2291.

ter Braak, C. J. F., and A. P. Schaffers. 2004. Co-correspondence analysis: A new ordination method to relate two community compositions. Ecology 85:834-846.

11. ANNEX A: Power analyses- Metals and POPs

Detailed Analysis of the Statistical Power for Detecting Changes in Concentrations of Metals and Organic Compounds

Peter Rothery and David Spurgeon

11.1. Introduction

This report discusses the effectiveness of the resampling scheme for Countryside Survey that would estimate changes in concentrations of metals and organic compounds (polycyclic aromatic hydrocarbons – PAHs and polychlorinated biphenyls - PCBs) in polluted soils. A model is developed for estimating sampling error of estimated change and statistical power for detecting changes between two temporally repeated surveys. The approach is applied using data from the 1998 survey (AKA CS2000)to evaluate the efficacy of repeating the scheme used in 2007 and from that to make recommendations for sampling in 2007. Our particular aim was to attempt to identify the optimum possible sampling design for the metals and organic. This was done to assist the CS Soils Topic Steering Group to identify the analytes and products that would be included in the coming survey.

11.2. Methods

Data used

Concentrations of Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), Vanadium (V) and Zinc (Zn) made from 1098 samples in 243 1 km squares in the CS survey were used to estimate within and between square variability. For the organics, the number of analytes (over 60) that were measured meant that we rationalised the analysis to concentrations of selected compounds or structurally related groups. These were the sum total concentrations of all, tri, penta and octa chlorinated PCBs (which as groups represent the range of physical properties for these compounds) and the sum of all PAHs, as well as concentrations of a representative 5 ringed (benzo[a]pyrene) and 3 ringed (fluorine) PAHs.

For both metals and the organics any observations recorded as below the limit of detection (LOD) are allocated a value equal to half the LOD for the particular chemical analysis run. This is a major issue for some organics, but not really for the metals, for which less than 10 measurement from the complete 1998 survey gave values below the detection limit. For statistical analysis all concentrations are $\log_e$ transformed to stabilise variability and to reduce the high degree of positive skewness in the concentrations. Differences on the logarithmic scale correspond to multiplicative (percentage) changes in concentrations.

11.3. Detailed approach to statistical analysis of change

Estimating precision and power for estimating change is hampered by the fact that data are only available from a single survey. There is no available to data to estimate directly the variation in change in concentrations from one survey to the next at the sampling locations. This problem is addressed by using a statistical model to estimate indirectly variation in change and hence the sampling errors of the estimated change and statistical power for specified schemes.

The statistical analysis is based on a model which partitions variation in concentrations into effects for (a) differences between successive surveys; (b) variation between 1 km squares within years; (b) variation between samples taken at locations within 1 km squares within years. We consider a design with repeat visits to the same locations, i.e. samples taken at the *same* locations within the *same* 1 km squares If y_{ijk} denotes the $\log_e$ transformed concentration for the *i*th survey, the *j*th km square and *k*th location within the *j*th square, the model is written as follows

$$y_{ijk} = m_i + S_{ij} + \varepsilon_{ijk}$$

where m_i denotes the mean $\log_e$ concentration for the *i*th survey (i = 1, 2), S_{ij} is a random effect for the *j*th square in the *i*th survey (j = 1,..., S) and ε_{ijk} is a random effect for the *k*th sample in the *j*th square and the *i*th survey (k = 1,…, n_j). The random effects S_{ij} and ε_{ijk} are assumed to vary with mean zero, and variance V_S and V_e, respectively.

The difference in mean $\log_e$ concentrations between surveys (i.e. $m_2 - m_1$) corresponds to an R-fold change given by $\log_e R = m_2 - m_1$, or $R = \exp(m_2)/\exp(m_1)$, i.e. a percentage increase/decrease of $100\times(R - 1)$. For example, a 10% increase corresponds to R = 1.10, a 10% decrease R = 0.90.

Let d_{jk} denote the difference in $\log_e$ concentrations for the two surveys at a particular location, i.e.

$$d_{jk} = y_{2jk} - y_{1jk}$$

For the above model the variance of the difference is given by

$$\text{var}[d_{jk}] = 2V_S(1 - r_S) + 2V_e(1 - r_e)$$

where r_S denotes the correlation between the average concentration in squares in successive surveys, and r_e denotes the corresponding correlation between concentrations at the same location within a square. Note that these correlations arise because of repeated samples at the same locations in successive surveys. When samples are located independently in each survey the correlations are zero, i.e. $r_S = r_e = 0$.

The change in mean concentrations between successive surveys can be estimated by the mean difference, i.e. $d = \Sigma\, d_{jk}/n$, with summation of sample locations, and where n is the total number of samples in each year, i.e. $n = \Sigma\, n_j$. The variance of d is given by

$$\text{var}[d] = 2V_S(1 - r_S)\,\Sigma\, n_j^2/n^2 + 2V_e(1 - r_e)/n$$

with corresponding standard error

s.e.[d] = $\sqrt{\{2V_S\ (1 - r_S)\ \Sigma\ n_j^2\ /n^2 + 2V_e\ (1 - r_e)/n\}}$

The null hypothesis of no change in mean concentrations, i.e. H_0: $m_1 = m_2$ can be tested using the statistic

$t = d$/s.e.[d]

On the null hypothesis, t follows (approximately) Student's t-distribution with (S -1) degrees of freedom. In practice, the standard error is calculated using estimates of the variance components derived from the mean squares in a one-way analysis of variance between and within 1 km squares.

Statistical power

The statistical power of the test for detecting a difference in $\log_e R$ is determined primarily by the so-called non-centrality parameter $\theta = \log_e R$/s.e.[d], and also by the degrees of freedom. For a difference of 3 standard errors ($\theta = 3$) the power exceeds 80% (two-tailed test at the 5% level) for $S > 15$. Power for detecting changes of ±5% (R = 1.05 & 0.95), ±10% (R = 1.10 & 0.90) and ± 20% (R = 1.20 & 0.80) were calculated for different sample designs. Note that power for a given percentage *increase* is not equal to the power for the corresponding percentage *decrease* because power depends on $\log_e R$, e.g. for a 20% increase (R = 1.20, $\log_e R$ = 0.182) whereas for a 20% decreases (R = 0.80, $\log_e R$ = -0.223). Power for a decrease is always greater than that for the corresponding increase. Power is calculated for a range of scenarios: (a) a scheme with repeated visits to the same locations used in 1998; (b) schemes with reduced number of samples per 1 km square; (c) for the organic compounds, schemes with number of 1 km squares increased to the same number used in 1998 for metals).

Stratification and analysis of data from successive surveys

The analysis was first applied ignoring the stratification of 1 km squares in to Land Classes, i.e. effectively regarding the data as a random sample from the population. In a further analysis the model was applied by allowing for differences in Land Classes by estimating the variation between squares within Land Classes (assuming equal variances for each Land Class). The power analysis tests the null hypothesis of no change in any of the Land Classes. When data are available for two consecutive surveys it will be possible to (a) test for differences in change across strata; (b) allow for the stratification to reduce the error of estimated change and to increase power for detecting change.

11.4. Results

Metals

The component of variation between squares accounts for an average of 63% of the total (Table A-1), and is relatively consistent for the different metals - lowest for Cadmium (55%) and highest for Nickel (77%). The average total variance of the $\log_e$ concentration is 0.92 which corresponds to an approximate coefficient of variation CV = 92%. Table A-2 shows standard errors of estimated change in mean $\log_e$ concentrations for a scheme using repeated samples at each location. For a plausible value of r = 0.8, the average standard error is 0.034 (CV = 3.4%). For a reduced value r = 0.5, average is 0.054 (CV = 5.4%) , whereas for zero correlation

(effectively no matching of locations in successive surveys) the average is 0.076 (CV = 7.6%).

Table A-3 shows corresponding power for detecting changes of 5%, 10%, and 20% not considering stratification. For the plausible correlation (r = 0.8), the power of the scheme for detecting a 10% change is high (> 80%) for Cadmium, Copper, Lead and Zinc, and lower for Chromium (60%-68%), Nickel (64%-73%) and Vanadium (77%-85%). The power for detecting a change of 5% is low in all cases (r = 0.8: 20%-41%). A change of 20% will almost certainly be detected r = 0.8, and in most cases with high power (> 90%) for r = 0.5. Importantly for the design of any future survey, reducing the number of samples used per square (to simulate a reduction in the intensity of within square measurement in the coming survey) reduces precision of the estimated change (Table A-4) and the statistical power (Table A-5). However, in general, the reductions are relatively small, with little loss of power when taking only 2 samples per square for a large reduction (60%) in the number of samples to be analysed. For example, the power for detecting an increase of 10%, or more, exceeds 80% using 2 samples for Cadmium, Copper, Lead and Zinc (for r = 0.8).

Allowing for differences between Land Classes significantly reduced the component of variation between squares, and produced a greater consistency for different metals. The average component between squares was 0.343 (range = 0.223-0.502) compared with 0.631 (range =0.454-0.994) ignoring Land Classes, accounting for 54% of the total variation. The lower variability between squares increases precision and power for estimating and detecting change. As noted above the gains by taking more than two samples per square are relatively small for a large reduction in analysis indicating that this may be an efficient approach.

Organic compounds

The component of variation between squares accounts for an average of 41% of the total (Table A-5). However, there is relatively large variation between compounds - lowest for PCB-octa (19%) and highest for PCB-tri (75%). The average total variance of the $\log_e$ concentration is 2.08, with large variation between compounds being lowest for PCB-sum (1.19) and highest for Flourene (3.97). Table A-6 shows standard errors of estimated change in mean $\log_e$ concentrations for a scheme using repeated samples at each of location in the 107 1 km squares for different values of correlation (r) between observations at the same location in successive surveys. For a plausible value of r = 0.5, the average standard error is 0.125 (CV = 12.5%). For zero correlation (effectively no matching of locations in successive surveys) the average is 0.177 (CV = 17.7%). Table A-7 shows that the corresponding power for detecting a change of 10% is relatively low for both r = 0.5 (< 22%) and r = 0.8 (< 47%). Increasing the number of 1 km squares from 107 to 243, and the number of samples per square reduces the standard error of the estimated change (Table A-8) and increases power (Table A-9). However, the power for detecting a 10% increase (r = 0.5) remains relatively low in all cases even using 5 samples per square being highest for PCB-octa (64%) and lowest for Flourene (21%).

If stratification is considered, for the PCBs, this gives no significant reduction in the component of variation between 1 km squares. In fact, estimates were slightly *higher* which may be a chance effect or due to the fact that some Land Class are represented by only a small number of squares due to the restricted analysis that was conducted. For PAH compounds there was a significant reduction in variation between squares. However, the total variance remains relatively large because of the variation between samples within squares. Overall, considering stratification lead to

increased precision and power for estimating and detecting change. However, gains from stratification appear less marked than those for metals because of the relative large component of variation between samples within squares.

11.5. Discussion & Recommendations

Metals

The variation in $\log_e$ concentrations between 1 km squares was on average 68% of the total variance. Values were similar for each metal. The average variance component between squares was 0.63 (CV = 79%) and within squares was 0.29 (CV = 54%). Based on this, a repeat survey with observations at the same locations within the 243 1 km squares used in the 1998 survey should give high power for detecting changes of 10% or more. There are substantial gains in sampling at the same locations in successive in successive surveys.

Importantly for the design of any future survey, using more than 2 samples per square gives a relatively small increase in precision and statistical power for estimating and detecting change, and is potentially wasteful in terms of the number of samples analysed. For example, in a scheme with 2 samples per square the total number of samples for analysis is would of course use only 2/5 of the resources that were needed in the 1998 survey. The saving made in such analyses could be either used to analyse for square for metals, analyse for more metals, or be used for another type of analysis entirely. Consideration of the Land Class stratification leads to a further significant reductions in the variation between 1 km squares, and worthwhile increases in precision and power.

Organic compounds

The variation in $\log_e$ concentrations between 1 km squares was on average 41% of the total variance. However, there was considerable variation between the different compounds (range 19%-75%) of the total variance. The average variance component between squares was 0.86 (CV = 93%) and within squares was 1.23 (CV = 111%). Variation between and within 1 km squares was generally higher for organic compounds than for metals, with a higher percentage variation within squares. We propose that this is associated with the spatial scale at which the origins /source of the metals and organics may operate. Thus, while organic pollutant in soil are derived to a large extent by human activities (especially for PCBs) which can be heterogeneous on a small scale, metals in soil come from both human activity and from weathering of the base rock. This later source is likely to be more uniform over a larger area – especially in rural soils, leading to a lower within square variability.

The analysis shows that a repeat survey with observations at the same locations within the 107 1 km squares used in the 1998 survey has fairly low power for detecting even a 20% change, although using plausible value for the correlation between observations at the same locations in successive locations should give high power for detecting changes of 10% or more. Increasing the number of samples per square reduces the required number of 1 km squares but increases the total number of samples for analysis. For PCBs the Land Class stratification there was no

significant reduction in the variation between 1 km squares, and therefore no gains in precision or power. For total PAHs, flourene and benzo[a]pyrene there was a significant reduction in the between square component of variation. However, the total variation and variation between samples (within squares) remained relatively large and the above comments on efficacy of different schemes apply.

The analysis conducted raises the question of how to reduce variability within squares and the possibility of bulking samples and taking a sub-sample was considered. This would, however, raise the issue of comparability of estimates derived from bulked sample with previous ones from averages over separate samples and would also break the direct link between the organic analyses conducted for the CS squares and the other measurements made at the X-plots. For these reasons, we rejected this approach. Given that organic analysis is a comparatively expensive measurement, we would suggest, therefore, that the most effective use of resources would not be to undertaken multiple analyses per square for these compounds. Instead, the option we would recommend would be to analyse a single X-plot sample per square, making sure that when a square has had at least one previous analysis, it is this X-plot that is included in the repeat analysis. This would increase the spatial and land class coverage of the survey at the minimum resource cost.

Table A-1 Estimated variance components between and within 1km squares of $\log_e$ transformed metal concentrations. The component of variation between squares is statistically significant ($p < 0.001$) in each case.

Metal	Estimated variance components (% of total variance)		Total variance
	Between squares	Between samples within squares	
Cadmium (Cd)	0.444 (55)	0.357 (45)	0.801
Chromium (Cr)	0.994 (73)	0.366 (27)	1.361
Copper (Cu)	0.500 (69)	0.225 (31)	0.725
Nickel (Ni)	0.903 (77)	0.277 (23)	1.180
Lead (Pb)	0.478 (65)	0.262 (35)	0.740
Vanadium (V)	0.647 (68)	0.307 (32)	0.954
Zinc (Z)	0.454 (67)	0.225 (33)	0.678
Mean	0.631 (68)	0.288 (32)	0.919

Table A-2 Standard error of estimated change in mean $\log_e$ metal concentration between two successive surveys using repeated samples at each location in 243 1 km square with number of samples per square as in Table 1. Note that the standard error can be interpreted as the coefficient of variation of the estimated R-fold change in concentration between successive levels.

Metal	Standard error		
	r = 0	*r* = 0.5	r = 0.8
Cadmium (Cd)	0.067	0.047	0.030
Chromium (Cr)	0.096	0.068	0.043
Copper (Cu)	0.069	0.049	0.031
Nickel (Ni)	0.091	0.064	0.041
Lead (Pb)	0.068	0.048	0.030
Vanadium (V)	0.078	0.055	0.035
Zinc (Z)	0.066	0.047	0.029
Mean	0.076	0.054	0.034

Table A-3 Statistical power (two-tailed, 5% level) for detecting 20%, 10% & 5% increases (decreases) in metal concentrations between two successive surveys using repeated samples at locations in 243 1 km squares with number of samples per square as in Table 1. Values in bold are power of 80% or more.

	Statistical power								
	r = 0			*r* = 0.5			*r* = 0.8		
Metal	5%	10%	20%	5%	10%	20%	5%	10%	20%
Cadmium	11 (12)	29 (35)	77 (**91**)	18 (19)	52 (60)	**97** (**99+**)	37 (40)	**89** (**94**)	**99+** (**99+**)
Chromium	8 (8)	17 (19)	47 (64)	11 (12)	29 (34)	76 (**90**)	20 (22)	60 (68)	**99** (**99+**)
Copper	11 (12)	28 (33)	75 (**90**)	17 (18)	50 (58)	**96** (**99+**)	35 (38)	**87** (**93**)	**99+** (**99+**)
Nickel	8 (9)	18 (21)	51 (68)	12 (12)	31 (37)	**81** (**93**)	22 (24)	64 (73)	**99** (**99+**)
Lead	11 (12)	29 (34)	76 (**91**)	17 (19)	51 (59)	**97** (**99+**)	36 (39)	**88** (**93**)	**99+** (**99+**)
Vanadium	10 (10)	23 (27)	64 (**81**)	14 (15)	40 (47)	**91** (**98**)	28 (31)	77 (**85**)	**99+** (**99+**)
Zinc	11 (12)	30 (36)	79 (**92**)	18 (20)	53 (62)	**97** (**99+**)	38 (41)	**90** (**95**)	(**99+**) (**99+**)

Table A-4 Statistical power (two-tailed, 5% level) for detecting 20%, 10% & 5% increases in metal concentrations between two successive surveys using repeated samples at locations in 243 1 km squares with *m* = 1,2…,5 samples per square as in Table 7, based on values for components of variation between 1 km squares and within squares as in Table 3. Values in bold are power of 80% or more.

		Power for correlation between successive surveys:								
		r = 0			r = 0.5			r = 0.8		
Metal	*m*	5%	10%	20%	5%	10%	20%	5%	10%	20%
Cadmium	1	9	22	61	14	38	**89**	27	74	**99+**
	2	10	26	72	16	47	**95**	33	**84**	**99+**
	3	11	29	76	17	50	**96**	36	**88**	**99+**
	4	11	30	78	18	53	**97**	37	**89**	**99+**
	5	12	31	**80**	18	54	**98**	39	**90**	**99+**
Chromium	1	7	15	40	10	25	68	18	52	**97**
	2	8	16	45	11	28	74	20	58	**98**
	3	8	17	47	11	29	76	21	60	**99**
	4	8	17	48	11	30	78	21	61	**99**
	5	8	17	49	11	30	78	21	62	**99**
Copper	1	10	23	65	14	41	**91**	29	78	**99+**
	2	11	27	73	16	47	**95**	33	**85**	**99+**
	3	11	28	75	17	50	**96**	35	**87**	**99+**
	4	11	29	77	17	51	**97**	36	**88**	**99+**
	5	11	29	77	18	52	**97**	37	**89**	**99+**
Nickel	1	8	16	45	11	28	74	20	58	**98**
	2	8	18	50	12	31	79	22	63	**99**
	3	8	18	52	12	32	**81**	22	65	**99**
	4	8	19	53	12	32	**82**	23	66	**99+**
	5	8	19	53	12	33	**82**	23	67	**99+**
Lead	1	10	23	64	14	41	**91**	29	78	**99+**
	2	11	27	73	16	47	**95**	34	**85**	**99+**
	3	11	29	76	17	50	**96**	36	**88**	**99+**
	4	11	29	77	18	52	**97**	37	**89**	**99+**
	5	11	30	78	18	53	**97**	38	**89**	**99+**
Vanadium	1	9	19	54	12	33	**83**	23	67	**99+**
	2	9	22	61	14	38	**89**	27	74	**99+**
	3	9	23	64	14	40	**91**	28	77	**99+**
	4	10	23	65	14	41	**91**	29	79	**99+**
	5	10	24	66	15	42	**92**	30	79	**99+**
Zinc	1	10	25	68	15	44	**93**	31	**81**	**99+**
	2	11	28	76	17	50	**96**	36	**87**	**99+**
	3	11	30	79	18	53	**97**	38	**90**	**99+**
	4	12	31	**80**	19	54	**98**	39	**91**	**99+**
	5	12	32	**81**	19	55	**98**	40	**91**	**99+**

Table A-5 Estimated variance components between and within 1km squares of $\log_e$ transformed organic compound concentrations. The component of variation between squares is statistically significant ($p < 0.01$) except for PCB-octa ($p = 0.062$)

Organic compound	Estimated variance components (% of total variance)		Total variance
	Between squares	Between samples within squares	
PCB:			
Sum	0.380 (32)	0.814 (68)	1.194
Tri	2.379 (75)	0.782 (25)	3.161
Penta	0.421 (31)	0.946 (69)	1.367
Octa	0.220 (19)	0.948 (81)	1.169
PAH:			
Sum	0.626 (33)	1.292 (67)	1.917
Benzo[a]pyrene	0.955 (52)	0.893 (48)	1.848
Flourine	1.050 (26)	2.915 (74)	3.965
Mean	0.862 (41)	1.23 (59)	2.08

Table A-6 Standard error of estimated change in mean $\log_e$ organic compound concentration between two successive surveys using repeated samples at each location in 107 1 km squares with number of samples per square as in Table 1. Note that the standard error can be interpreted as the coefficient of variation of the estimated R-fold change in concentration between successive levels.

Organic compound	Standard error		
	r = 0	*r* = 0.5	r = 0.8
PCB:			
Sum	0.133	0.094	0.060
Tri	0.254	0.179	0.113
Penta	0.142	0.101	0.064
Octa	0.125	0.088	0.056
PAH:			
Sum	0.170	0.120	0.076
Benzo[a]pyrene	0.179	0.127	0.080
Flourine	0.238	0.168	0.106
Mean	0.177	0.125	0.079

Table A-7 Statistical power (two-tailed, 5% level) for detecting 20%, 10% & 5% increases (decreases) in metal concentrations between two successive surveys using repeated samples at locations in 107 1 km squares with number of samples per square as in Table 1. Values in bold are power of 80% or more.

	Statistical power for detecting change								
	r = 0			r = 0.5			r = 0.8		
Organic Compound	5%	10%	20%	5%	10%	20%	5%	10%	20%
PCB:									
Sum	7 (7)	11 (12)	27 (38)	8 (8)	17 (20)	48 (65)	13 (14)	35 (42)	**86** (**96**)
Tri	5 (5)	7 (7)	11 (14)	6 (6)	8 (9)	17 (23)	7 (7)	13 (15)	36 (50)
Penta	6 (6)	10 (11)	25 (34)	8 (8)	16 (16)	44 (59)	12 (13)	32 (38)	**81** (**94**)
Octa	7 (7)	12 (13)	31 (43)	9 (9)	19 (22)	54 (71)	14 (15)	40 (47)	**90** (**98**)
PAH:									
Sum	6 (6)	9 (9)	19 (26)	7 (7)	12 (14)	32 (45)	10 (10)	24 (28)	66 (**83**)
Benzo[a]pyrene	6 (6)	8 (9)	17 (23)	7 (7)	12 (13)	30 (41)	9 (10)	22 (26)	61 (79)
Flourene	5 (6)	7 (7)	12 (15)	6 (6)	9 (10)	19 (26)	7 (8)	14 (17)	40 (55)

Table A-8 Standard error of estimated change in mean $\log_e$ organic concentration between two successive surveys using repeated samples at each location in 243 1 km square for 1, 2,…,5 samples per square in each survey based on components of variation between 1 km squares and within squares as in Table 7. Note that the standard error can be interpreted as the coefficient of variation of the estimated R-fold change in concentration between successive levels.

Organic Compound	Number of samples per 1 km square	$r = 0$	$r = 0.5$	$r = 0.8$
PCB:				
Sum	1	0.099	0.070	0.044
	2	0.080	0.057	0.036
	3	0.073	0.052	0.033
	4	0.069	0.049	0.031
	5	0.067	0.047	0.030
Tri	1	0.161	0.114	0.072
	2	0.151	0.107	0.068
	3	0.147	0.104	0.066
	4	0.146	0.103	0.065
	5	0.144	0.102	0.065
Penta	1	0.106	0.075	0.047
	2	0.086	0.061	0.038
	3	0.078	0.055	0.035
	4	0.074	0.052	0.033
	5	0.071	0.050	0.032
Octa	1	0.098	0.069	0.044
	2	0.076	0.053	0.034
	3	0.066	0.047	0.030
	4	0.061	0.043	0.027
	5	0.058	0.041	0.026
PAH Sum	1	0.126	0.089	0.056
	2	0.102	0.072	0.046
	3	0.093	0.066	0.042
	4	0.088	0.062	0.040
	5	0.085	0.060	0.038
Benzo[a]pyrene	1	0.123	0.087	0.055
	2	0.107	0.076	0.048
	3	0.102	0.072	0.045
	4	0.098	0.070	0.044
	5	0.097	0.068	0.043
Flourene	1	0.181	0.128	0.081
	2	0.144	0.102	0.064
	3	0.129	0.091	0.058
	4	0.121	0.086	0.054
	5	0.116	0.082	0.052

Table A-9 Statistical power (two-tailed, 5% level) for detecting increases of 5%, 10% and 20% in organic compound concentrations between two successive surveys using repeated samples at locations in 243 1 km squares with 1,2,…,5 samples per square. Values in bold are power of 80% or more.

		r = 0			*r* = 0.5			*r* = 0.8		
Organic Compound	*M*	5%	10%	20%	5%	10%	20%	5%	10%	20%
PCB: Sum	1	8	16	45	11	27	74	19	57	**98**
	2	10	22	62	14	39	**89**	27	75	**99+**
	3	11	25	70	16	45	**94**	32	**83**	**99+**
	4	11	28	75	17	49	**96**	35	**86**	**99+**
	5	12	29	78	18	52	**97**	37	**89**	**99+**
Tri	1	6	9	20	7	13	36	10	26	71
	2	6	10	23	7	14	40	11	29	77
	3	6	10	23	8	15	41	11	30	79
	4	6	10	24	8	15	42	12	31	**80**
	5	6	10	24	8	15	43	12	31	**80**
Penta	1	8	15	40	10	24	68	18	52	**97**
	2	9	20	56	13	35	**85**	24	70	**99+**
	3	10	23	65	14	41	**91**	29	78	**99+**
	4	11	25	69	15	45	**94**	32	**82**	**99+**
	5	11	27	73	16	47	**95**	34	**85**	**99+**
Octa	1	8	16	46	11	28	74	20	58	**99**
	2	10	24	67	15	43	**92**	30	**80**	**99+**
	3	12	30	78	18	52	**97**	37	**89**	**99+**
	4	13	34	**84**	20	59	**99**	43	**93**	**99+**
	5	14	37	**88**	22	64	**99**	46	**95**	**99+**
PAH: Sum	1	7	12	30	8	19	53	14	39	**90**
	2	8	15	43	10	26	71	19	55	**98**
	3	9	17	50	11	30	79	21	62	**99**
	4	9	19	54	12	33	**83**	23	67	**99+**
	5	9	20	57	13	35	**85**	25	70	**99+**
Benzo[a]-pyrene	1	7	12	31	9	19	55	14	41	**91**
	2	8	14	39	10	24	67	17	51	**97**
	3	8	15	43	10	26	72	19	55	**98**
	4	8	16	45	11	28	74	20	58	**98**
	5	8	17	47	11	28	76	20	59	**99**
Flourene	1	6	8	17	7	12	30	9	22	61
	2	6	10	24	8	15	43	12	32	81
	3	7	11	29	8	18	51	13	38	**88**
	4	7	12	32	9	20	56	15	42	**92**
	5	7	13	35	9	21	60	16	45	**94**

12. ANNEX B: Power analysis for soil pH

B Reynolds and A Scott

12.1. Introduction

The original Ecological Survey of Great Britain 1978 contained 256 squares, each of which contained 5 x-plots. In CS2000, the number of squares was 569, but soil analyses in CS2000 were limited to the original 256 squares. The data from CS2000 describing soil pH and change in pH since 1978 was reported at the GB level in the MASQ report (Black et al., 2000). Within CS2007 there is a requirement for reporting at the level of individual countries. A power analysis has been undertaken of the sampling requirements to reliably detect change in soil pH at the country level.

12.2. Method

The analysis is based on the data from 1978 and CS2000 (Table B-1) which shows that the percentage change in pH was between 5 and 10% depending on the country. Estimates were obtained as weighted averages using the ITE Land Classification as strata. The estimates of change and their standard errors were then used to provide a power analysis for the ability to detect change from CS2000 to CS2007. This uses assumptions of normality to estimate the probability of detecting changes of specified size at a variety of significance levels. Altering the sampling sizes used in the power calculations allowed the calculation of the increase in power possible through an increase in the number of sample squares.

Table B-1. Estimates of soil pH change between 1978 and CS2000

	Estimates of					
	pH 1978	**pH 2000**	**Change in pH**	**% change**	**se of change**	**num squares**
England	6.098	6.406	0.301	4.8	0.056	115
Scotland	4.738	4.954	0.221	4.6	0.048	101
Wales	4.976	7.054	0.546	9.1	0.068	21

12.3. Results

The results of the power analysis are shown in Table B-2. Thus within Table B-2 for Wales, for example, there is 98.3% chance of detecting a 5% change in soil pH at the 1% significance level, based on the 256 squares measured in 1978 and in CS2000.

Table B-2. Results of the power analysis to detect change in soil pH at the individual country level based on data from 1978 and CS2000

		Percentage change in pH			
	Significance	**5%**	**10%**	**20%**	**30%**
England	**1%**	99.9	100.0	100.0	100.0
	5%	100.0	100.0	100.0	100.0
	10%	100.0	100.0	100.0	100.0
Scotland	**1%**	99.7	100.0	100.0	100.0
	5%	99.9	100.0	100.0	100.0
	10%	100.0	100.0	100.0	100.0
Wales	**1%**	98.3	100.0	100.0	100.0
	5%	99.4	100.0	100.0	100.0
	10%	99.8	100.0	100.0	100.0

12.4. Discussion

The results indicate that no more squares beyond the orginal 256 sampled in 1978 are required for reliable reporting of country level changes in soil pH. However, there are some very important caveats on the interpretation of this analysis. Firstly the increase in pH from 1978 to CS2000 was relatively consistent across the whole of GB. As a result, the measurements of change have small standard errors and hence the power to detect change is high, even with the small sample sizes. All three countries show significant change. The apparently greater change in Wales may be a reflection of the much smaller sample size including some influential squares. It is unlikely that the change in soil pH between CS2000, CS2007 and future surveys will be as consistent across the country in either direction or magnitude. The interval between the measurements will probably be smaller (8-9 years compared to 20-21 years) and the change in acid deposition (one of the main drivers for change) during the last 8 years has been less than between 1978 and CS2000 and is likely to become more regionally variable into the future.

12.5. Conclusions

Whilst the results of the power analysis might suggest that change in soil pH can be detected reliably at the country level with data from 256 sample squares, this result is influenced by the nature of the pH change detected between 1978 and CS2000. Given the uncertainties in the expected pH change between CS2000, CS2007 and into the future, soil pH should be determined on the X plots from all 1 km squares visited in CS2007 to safeguard future country-level reporting of soil pH change.

Black, H.E. et al., 2000. MASQ: Monitoring and Assessing Soil Quality in Great Britain. Countryside Survey Module 6: Soils and Pollution. Environment Agency R&D Technical Report E1-063/TR

13. ANNEX C: Power analysis of soil C determinations in Countryside Survey

Paul M Chamberlain and Andy Scott

13.1. Introduction

The original Ecological Survey of Great Britain 1978 contained 256 squares, each of which contained 5 x-plots. In CS2000, the number of squares was 569, but soil analyses in CS2000 were limited to the original 256 squares. The increased need for accurate measures of soil organic matter (SOM) and soil C (C) stocks, and the need for country-level reporting in CS2007, mean that greater numbers of CS squares will have soil measurements made in CS2007. Power analysis was therefore carried out using the loss-on-ignition (LOI, a method of determining SOM content) data from 1978 and CS2000, in order to determine the number of squares needed in CS2007 to give adequate reporting power for soils in Wales, and greater power for soils in Scotland and England.

13.2. Method

The existing CS data was used to calculate national estimates of average LOI for 1978 and 1998 (the year of sampling for CS2000) and the average changes over this period. Estimates were obtained as weighted averages using the ITE Land Classification as strata. The estimates of change and their standard errors were then used to provide a power analysis for the ability to detect change from CS2000 to CS2007. This uses assumptions of normality to estimate the probability of detecting changes of specified size at a variety of significance levels. Altering the sampling sizes used in the power calculations allowed the calculation of the increase in power possible through an increase in the number of sample squares.

13.3. Results

LOI varies substantially across the country, being much higher in Scotland that England or Wales (Table C-1). As a result the changes in LOI, which in absolute terms are not markedly different, are proportionally much smaller in Scotland. Only in England was the change from 1978 to 2000 significant. Estimates of LOI in Wales are poor largely because of the small sample size. With only 20 sample squares in Wales previously sampled for soils, several Land Classes that occur in Wales were not represented and several were only represented in one square.

The current sample sizes enable changes of about 10% to be detected with reasonable power in Scotland and England (Table C-2). For example, the analysis shows that the number of squares sampled for soils in CS2000 in England gives a 64.6% chance of observing a 10% change in LOI with a significance of 5% (the usual level below which results are not considered significant). However, in Wales however the soils sample size is too small to detect any reasonable level of change with any certainty. In Wales, the number of squares previously analysed for soils only yields a 18% chance of observing a 5% change in LOI. Hence, the number of squares in CS2000 is not sufficient to allow country-level reporting for Wales.

Table C-1 Estimates of LOI and change in LOI 1978-CS2000 for England, Scotland and Wales.

	LOI 1978	LOI 2000	Change in LOI	% change	se of change	Num Squares
England	12.4	14.6	2.2	16.3	0.6	114
Scotland	45.9	47.6	1.3	2.8	1.4	98
Wales	14.4	26.8	1.4	6.9	2.0	20

Note: The large apparent change for Wales between 1978 & CS2000 is due to the addition of an extra square in CS2000 which contained highly organic soils. The change in LOI data is calculated only on squares sampled in both years.

Table C-2 Power to detect various degrees of change in LOI for England, Scotland and Wales.

		Percentage change in LOI			
	Significance	**5%**	**10%**	**20%**	**30%**
England	**1%**	12.3	50.3	99.0	100.0
	5%	21.4	64.6	99.7	100.0
	10%	31.6	75.5	99.9	100.0
Scotland	**1%**	24.1	82.1	100.0	100.0
	5%	36.8	90.1	100.0	100.0
	10%	49.1	94.5	100.0	100.0
Wales	**1%**	3.6	10.0	40.6	79.0
	5%	7.5	18.0	55.1	88.0
	10%	13.1	27.4	67.2	93.2

Table C-3 summarises the statistical power of various sample sizes in Wales. Whilst 20 squares have previously been sampled for soils in Wales, there were a total of 65 squares in Wales in CS2000. However, to enable reasonable reporting for Wales separately in CS2007, it has been recommended (Clarke, Howard, & Scott, Countryside Survey: Sampling for Wales-Only Reporting. Available on Confluence) that Wales has a total of 124 squares in CS2007. If all these 124 squares were sampled for soils, there would be a 72.5% chance of detecting a 10% change in LOI at 5% significance. To ensure that there is a significant chance of detecting changes in soil C of the magnitude reported in the NSI England & Wales (0.6% yr^{-1} in the period 1978 – 2003; Bellamy et al 2005), it is therefore recommended that all CS2007 squares in all 3 countries be sampled for topsoil C.

Table C-3 Power to detect various degrees of change with increased sample sizes in Wales.

		Percentage change in LOI			
Sample size	**Significance**	**5%**	**10%**	**20%**	**30%**
20	**1%**	3.6	10.0	40.6	79.0
(1978/2000)	**5%**	7.5	18.0	55.1	88.0
	10%	13.1	27.4	67.2	93.2
65	**1%**	8.3	32.9	92.5	100.0
(current)	**5%**	15.4	46.9	96.5	100.0
	10%	24.1	59.4	98.3	100.0
90	**1%**	11.2	45.6	98.2	100.0
	5%	19.7	60.1	99.3	100.0
	10%	29.6	71.6	99.7	100.0
120	**1%**	14.8	59.2	99.7	100.0
(proposed)	**5%**	24.8	72.5	99.9	100.0
	10%	35.7	82.0	100.0	100.0

13.4. Conclusion

Power analysis demonstrates that the number of squares in Wales must be significantly increased in order to increase the chance of detecting even a large change in SOM content. Since England and Scotland already contain more soil squares, analysis indicates that the chance of detecting significant changes in SOM content is much greater. However, it is recommended that in CS2007 all squares are sampled for SOM determinations. This will further increase the statistical power in for reporting in Scotland and England and give future surveys far greater power than currently available.

14. ANNEX D: Method development for bulk density

Assessment of soil bulk density determinations in Countryside Survey

Paul M Chamberlain and Jan Poskitt

14.1. Introduction

Soil bulk density (BD), the amount of soil per unit volume, is the most useful parameter of soil physical structure, and influences soil porosity, macro- and micropore volume, and soil biodiversity. Bulk density determinations are also necessary in converting soil carbon (C) content (in %) into a stock (in g m^{-3}). It is possible to estimate BD from other measurements of the soil, chiefly %C and the soil type, but it was deemed necessary to actually measure BD in CS2007, rather than rely on a relationship to another variable. Bulk density is the only measure of soil physical structure to be measured in CS2007, but as BD has not been measured previously in CS, testing of the proposed method was necessary to ensure robust results.

There is an ISO standard method of measuring soil BD (ISO 11272:1998); this method requires the drying all the soil in a core at 105℃, which would change the soil to such a significant extent that no other analyses (e.g. pH, metal content) would be possible. Hence to follow the ISO method would require a soil core dedicated to BD alone, which is impractical in the context of CS. An alternative method has therefore been devised, based on the black 5 x 15 cm core protocol in CS2000. Indeed, all the measurements necessary for estimating BD were made in CS2000, with the exception of stone volume. The aims of this part of the CS2007 Pilot phase were therefore:

1. To test the proposed method of estimating BD on soil cores taken in the CS2007 Pilot Survey, and to compare with values estimated by other means to check the utility of the method.

2. To ascertain whether it is possible to use stone density data derived in CS2007, combined with measurements made in CS2000, to estimate the BD of CS2000 soils.

14.2. Method

BD was calculated in the following way for both CS2000 and CS2007 Pilot soils unless otherwise noted**:**

A black 5 x 15 cm core was taken from each X-plot location as specified in the Surveyors Handbook. This core was bagged and returned to CEH for analysis. The black core has multiple measurements made on it: loss on ignition, wet pH, BD, Olsen-P content, metal contents and %N. However, most of these measurements are made on sieved air-dried soil and are not discussed below. Determinations of BD, LOI and wet pH are carried out in a continuous analytical process, detailed below.

Sub sampling for wet pH and Air-Drying

1. Remove a black core from labelled bag
2. Measure with a ruler and record the exact depth of sample taken (**depth**) on recording sheet (*not done in CS2000*)
3. Label a clean foil tray (tray 1)with the SQXN
4. Weigh foil tray 1 and record (**tray 1**) on sheet
5. Weigh black core + soil + tray 1 and record weight (**wt 1**)
6. Remove soil from core into tray, using core extruder
7. Weigh soil + tray 1 and record weight (**wt 2**)
8. Break up the soil, mix and take a sub-sample for wet pH. Avoiding stones and roots, fill a numbered beaker up to the 20 ml mark. Set sub-samples aside on a tray until you have a batch of 25
9. Weigh soil + tray 1 again, record weight (**wt 3**) against SQNX on recording sheet
10. Put to dry on rack in drying room at 25^{o}C
11. Leave until dry enough to sieve, typically two weeks

Air-dry weight and sieving

1. Identify 25 dried soil samples in drying room
2. Weigh dried soil + tray 1 and record weigh (**wt 4**)
3. Sieve soil using 2mm stainless steel mesh sieve and wooden paddle taking care to retain all material which cannot be sieved.
4. Return all material which cannot be sieved to foil tray 1, set aside to weigh later
5. Place sieved soil in plastic container and seal

Stone weight and volume

1. Take the set aside trays and weigh stones and all unsieved material + tray 1 and record (**stone wt**)
2. Measure volume of stones by placing in a measuring cylinder in a known volume of water.
3. Measure the change in water volume in the measuring cylinder and record (**stone vol**) on BD recording sheet (*not done in CS2000*)

Moisture content after drying at 105℃ and LOI

1. Place trays of crucibles in oven to dry at 105^{o}C for 24 hours
2. Cool crucibles in desiccator
3. Once cool, weigh all crucibles (**wt5**)
4. Mix sieved soil and remove sub-sample of approx. 1 g of soil into prepared crucible, check crucible no against SQXN
5. Set aside batch of 25 to weigh on 4 place balance.
6. Weigh sub-sample + crucible (4 place balance) and record (**wt 6**) on sheet
7. Heat all crucibles + soils in oven at 105^{o}C for 24 hours
8. Cool crucibles in desiccator
9. Weigh (4 place balance) and record (**wt 7**) on BD sheet
10. Heat crucibles + soil in furnace at 550℃ for 2 .5 hr
11. Cool crucibles in desiccator
12. Weigh (4 place balance) and record (**wt 8**) on BD sheet

This method produces the following measurements, which allows the determination of soil BD:

BD calculation

Air dry soil and stone weights

Wt soil + stones	=	**Wt 4**	-	**Tray 1**
Wt soil + stones (air dried)	=	tray 1 + soil + stones air dried at 25^{o}C (g)	-	first foil tray weight (g)

Stone weight	=	**Stone wt**	-	**Tray 1**
Weight of stones in core	=	tray 1 + stones and unsieved debris weight (g)	-	first foil tray weight (g)

Soil weight	=	**Wt soil + stones**	-	**Stone weight**
Soil weight after air drying	=	Wt soil + stones (air dried)	-	Weight of stones in core

Wt sub sample	=	**Wt 2**	-	**Wt 3**
Weight wet pH sub-sample	=	tray 1 + wet soil + stones weight (g)	-	tray 1+ wet soil + stones weight after wet pH sub-sample removed, before drying (g)

	=	**Wt 3**	-	**Tray 1**	-	**Stone weight**
Wt soil to be air dried	=	tray 1+ wet soil + stones weight after wet pH sub-sample removed, before drying (g)	-	first foil tray weight (g)	-	Weight of stones in core

Wt moisture lost on air drying	=	**Wt soil to be air dried**	-	**Soil weight**
	=	Wt soil to be air dried	-	Soil weight after air drying

% moisture lost on air drying	=	**Wt moisture lost on air drying**	/	**Wt soil to be air dried**	**x 100**
	=		/	Wt soil to be air dried	x 100

Wt sub-sample if dried at 25℃	=	**Wt sub sample**	x	**(100 –**	**% moisture lost on air drying)**	**/ 100**
	=	Weight wet pH sub-sample	x			/ 100

Total weight of soil if all dried at 25℃	=	**Soil weight**	+	**Wt sub-sample if dried at 25℃**
	=	Soil weight after air drying	+	

Drying sub-sample of soil at 105℃

Air dried soil in crucible	=	**Wt 6**	-	**Wt 5**
	=	crucible + sub-sample, air dried at 25°C	-	weight of dried, cooled, empty crucible

Soil wt after 24 hr at 105℃	=	**Wt 7**	-	**Wt 5**
	=	crucible + sub-sample, oven dried at 105°C, 24 hr	-	weight of dried, cooled, empty crucible
Weight moisture lost after 24 hr at 105℃	=	**Air dried soil in crucible**	-	**Soil wt after 24 hrs at 105℃**

% moisture lost on drying air dried soil at 105℃ (%)	=	**100 – (**	**Soil wt after 24 hrs at 105℃**	/	**Air dried soil in crucible**	x 100)

Total weight of soil if all dried at 105℃	=	**Total weight of soil if all dried at 25℃**	**x (**	**100 – % moisture lost on drying air dried soil at 105℃ (%)**	/ 100)

Bulk density estimation

Bulk density	=	**Total weight of soil if all dried at 105℃**	**/**	**(core volume**	-	**stone vol)**
Bulk density of soil in g cm^{-3}				Calculated core volume (ml)		stone volume (ml)

SQXN	unique ID number
depth	soil depth sampled in cm
tray 1	foil tray weight (g)
wt 1	tray 1 + black core + soil & stones weight (g)
wt 2	tray 1 + soil & stones weight (g)
wt 3	tray 1+ soil & stones weight after wet pH sub-sample removed, before drying (g)
wt 4	tray 1 + soil & stones weight air dried at 25°C (g)
stone wt	tray 1 + stones and unsieved debris weight (g)
stone vol	stone volume (cm^3)
wt 5	weight of dried, cooled, empty crucible (g)
wt 6	crucible + sub-sample, air dried at 25°C (g)
wt 7	crucible + sub-sample, oven dried at 105°C , 24 hr (g)
wt 8	crucible + sub-sample, oven dried at 550°C, 2.5 hr (g)

14.3. Results

CS2007 Pilot

The BD values of CS2007 Pilot cores are shown in Figure D-1. Estimated BD values ranged from 0.08 to 1.0 g cm^{-3}, and varied considerably even within a single square.

As a check to ascertain whether the estimated BD values were reasonable, BD values were also calculated from LOI measurements, assuming that 50% of SOM is SOC (C_{org}), using the equation of Howard et al. (1995):

$$BD = 1.3 - (0.275 \ln (C_{org})) \quad (1)$$

This equation has recently been used in the estimation of total topsoil C stocks in England and Wales (Bellamy et al, 2005). It is not anticipated that this equation will be used in Countryside Survey to estimate soil BD, since the BD of individual samples often deviates significantly from the value predicted by the equation (see below). The equation is simply used here as a method of determining reasonable BD estimates for CS soils, and for comparison with the estimated BD obtained from the measurements detailed in the method section above.

Comparison of the estimated values from the two methods produced a significant relationship with a line approx. y = x (Figure D-2); the two methods therefore predicted similar BD values for the same soils. The method proposed for determining BD in CS2007 therefore yields reasonable BD values based on a literature-derived equation.

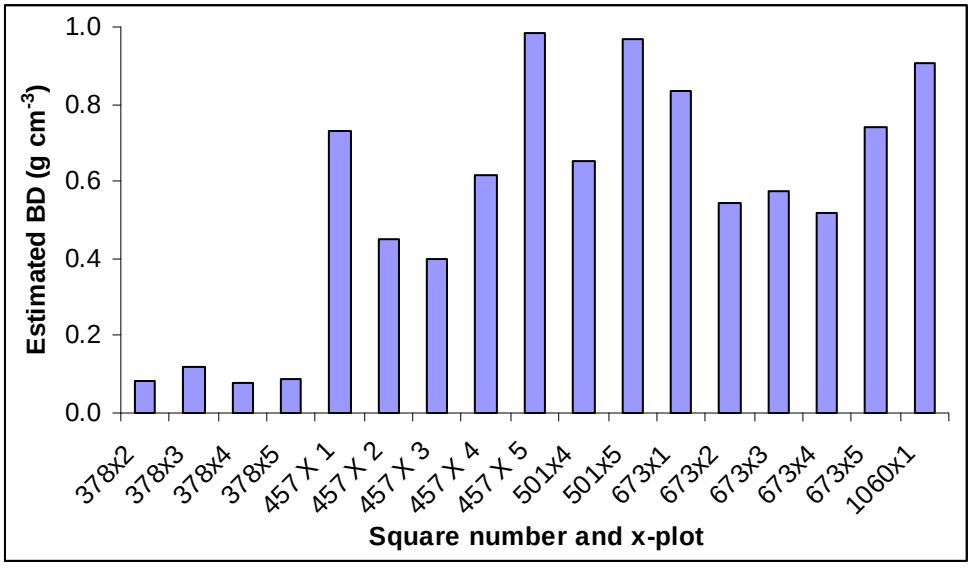


Figure D-1 Estimated BD values of soil cores taken in CS2007 Pilot

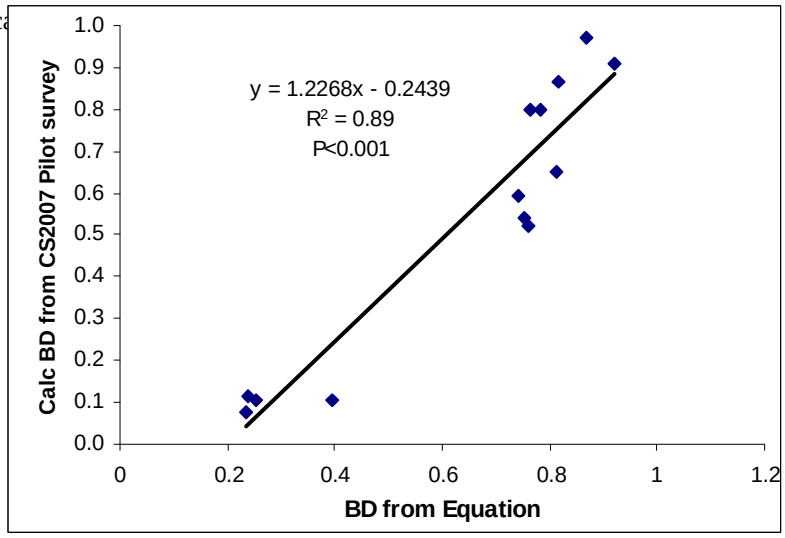


Figure D-2 Comparison of BD from equation 1 (from LOI) and estimated BD (from weight measurements) in CS2007 Pilot.

Back-calculating CS2000 BD

In the light of the success of the BD method in determining CS2007 Pilot BD values, the second aim of exploring the possibility of back-calculating CS2000 BD values was addressed.

In CS2000, all measurements necessary for the calculation of BD were made, with the exception of the soil volume and the stone volume. However, stone weight values were recorded in CS2000, and assuming that the properties of the stones across an x-plot do not vary, it is possible to calculate stone volume using stone weight (from CS2000) and stone density (from CS2007) using the equation:

$$\text{Stone volume} = \text{stone weight} / \text{stone density} \quad (2)$$

Stone volume estimates can then be combined with the other CS2000 data to estimate BD, with the second assumption that the soil occupied the full 15 cm depth of the core. This is reasonable, since the surveyors in CS2000 were trained to insert the core such that the top of the core was level to the soil surface, yielding a depth of 15 cm.

The resulting BD estimate was then compared with the BD estimated from CS2000 LOI values (Figure D-3), and also with CS2000 total %C values (determined using an elemental analyser; Figure D-4). Both comparisons gave good agreement with the estimated BD values from the weight measurements, although the equation using 50% LOI produced a slightly stronger relationship.

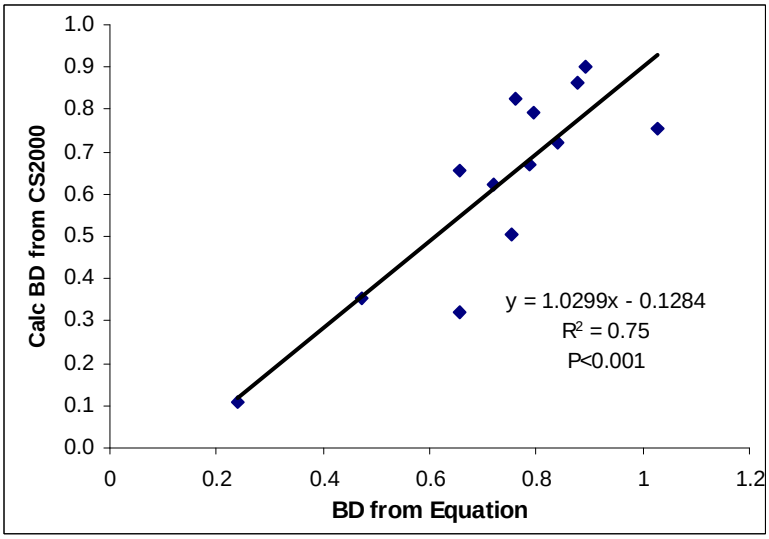


Figure D-3 Comparison of BD from equation 1 (from LOI) and estimated BD (from weight measurements) in CS2000, using stone density data derived from the same x-plots in CS2007.

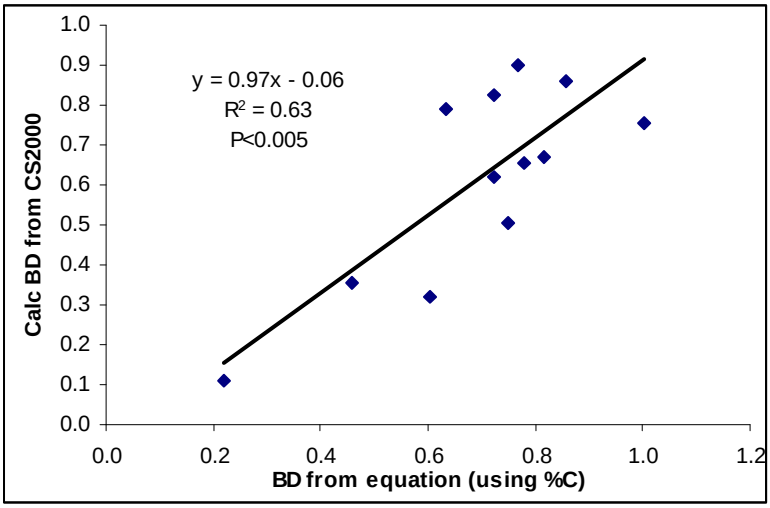


Figure D-4 Comparison of BD from equation 1 (from total %C) and estimated BD (from weight measurements) in CS2000, using stone density data derived from the same x-plots in CS2007.

These comparisons indicate that is possible to back-calculate CS2000 BD values by combining with CS2007 Pilot stone density data. However, measured BD values in the CS2007 Pilot, which is the only dataset in which all necessary variables were measured (Figure D-2), are consistently overestimated by the equation. Differences between the two values range from -0.29 to +0.1, with a mean difference (±SE) of -0.11 ± 0.04. Hence whilst there is a good average relationship between the two values for BD, the equation does not adequately predict the BD in individual samples. Since one of the main uses of CS2007 BD data is in the estimation of C stocks, using average values rather than the actual BD of a soil core would lead to varying estimations of soil C stocks and is not acceptable. BD must be measured on every sample in order to determine the actual BD of that soil core, rather than an average value derived from the equation.

14.4. Conclusion

Soil BD was determined on soil samples collected in the CS2007 Pilot Survey using the proposed CS2007 BD method. Results gave good agreement with BD values derived from a literature equation for BD and indicate that the proposed BD method is acceptable for use in CS2007.

Values of stone density were taken from the Pilot samples and combined with CS2000 data to back-calculate CS2000 BD. These results also compared favourably with BD values estimated from the literature equation, indicating that it is possible to back-calculate CS2000 BD values.

However, the equation-derived BD estimates consistently underestimated the actual BD values for the CS2007 Pilot samples, suggesting that BD values must be measured for each CS2007 soil sample and cannot simply be estimated from LOI measurements. It is therefore essential to measure soil BD on every soil sample in CS2007, since it cannot be estimated by another method.

15. ANNEX E: Method development of nitrogen mineralization method

Ed Rowe & Bridget Emmett

15.1. Introduction

Plants are generally considered good indicators of soil "fertility", since they integrate fluctuating environmental exposure to nutrients. Fertility indicator scores such as Ellenberg N (EbN) are available for most UK plant species, and can be used to derive mean indicator scores for a plant species assemblage. Current research on pollutant effects is focused on identifying measurable abiotic parameters which correlate with mean floristic fertility indicator score. Such parameters can be used as explanatory variates in predictive models of plant assemblage change.

The selection of abiotic explanatory variates for predicting mean EbN should not rely entirely on statistics. Ideal variates would: a) explain a large proportion of the variation; b) be orthogonal to other selected explanatory variates; and c) be directly causal, i.e. affect mean EbN directly rather than via secondary mechanisms.

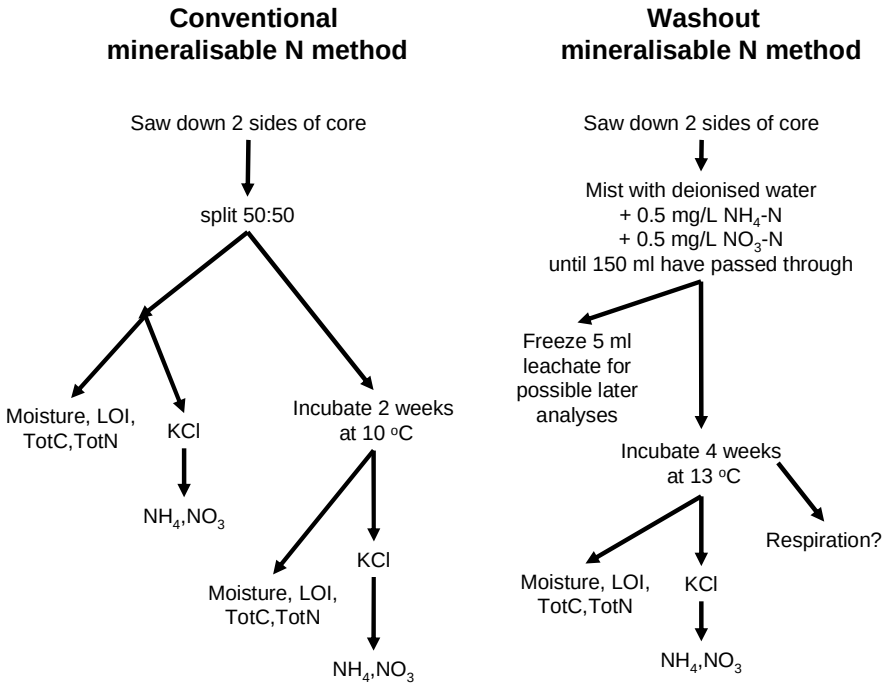
A study was carried out to assess abiotic measurements on cores taken in the CS Pilot Study in 2006 for their ability to explain variation in mean EbN. A conventional method for measuring mineralisable N (subtracting initial mineral N from mineral N measured after a 2-week incubation) was compared with a new method involving washing out the initial soil solution with a weak N solution (0.5 mg NH_4-N and 0.5 mg NO_3-N L^{-1}) and measuring after a 4-week incubation. The new method was intended to:

a) by washing through, standardise mineral N contents in cores which had variable treatment before arriving in the lab
b) by adding a little N, differentiate low-activity from strongly N-immobilising soils
c) by saturating the soil and then applying suction, standardise water tension before incubation at approximately field capacity.
d) by incubating warmer and for longer, allow differences between soils to develop

Aims of the study were:

a) to identify abiotic variates strongly correlated with mean EbN
b) to assess the explanatory power of mineralisable N for EbN
c) to compare mineralisable N measured by conventional and washout methods
d) to compare costs of the two methods
e) to assess whether the new method was successful in standardising initial mineral N contents, differentiating immobilising soils, and increasing the range of measured mineralisable N.

15.2. Methods


Conventional mineralisable N method
Saw down 2 sides of core
split 50:50
Moisture, LOI, TotC,TotN
KCl
NH4,NO3
Incubate 2 weeks at 10 oC
KCl
Moisture, LOI, TotC,TotN
NH4,NO3
Washout mineralisable N method
Saw down 2 sides of core
Mist with deionised water
+ 0.5 mg/L NH4-N
+ 0.5 mg/L NO3-N
until 150 ml have passed through
Freeze 5 ml leachate for possible later analyses
Incubate 4 weeks at 13 oC
Respiration?
Moisture, LOI, TotC,TotN
KCl
NH4,NO3

15.3. Results

a) Costs

Staff costs were similar for the two methods, with 100 samples taking approximately 10 and 9 person.days for conventional and washout methods respectively (Table 15-1). There may be economies of scale for batches of more than 60 samples with the washout misting process. Recurrent costs were £500 and £250 per 100 samples for conventional and washout methods respectively.

Table 15-1 Time taken for activities relating to mineralisation measurement (not including pH, Total C Total N, etc.)

Activity	**Time for 100 samples (person.hours)**	
	Conventional	**Washout**
a) essential activities for both methods		
Sawing cores open down two sides	3.3	3.3
LOI + moisture (postincubation)	7.4	7.4
KCl extraction and min N analysis (postincubation)	29.6	29.6
b) for conventional mineralisable N method only		
LOI + moisture (preincubation)	7.4	
KCl extraction and min N analysis (preincubation)	29.6	
c) for washout mineralisable N method only		
Mixing 1 mg N L^{-1} solution		1.7
Misting, suction, bagging for incubation		22.7
Total	77.3	64.7

b) Comparison of conventional mineralisable N and new method

The process of flushing the soil with 1 mg L^{-1} solution was successful in standardising mineral N concentrations (Figure 15-1). Mean initial mineral N concentration was 0.038 mg N g^{-1}, whereas mean mineral N concentration after flushing was 0.005 mg N g^{-1}.

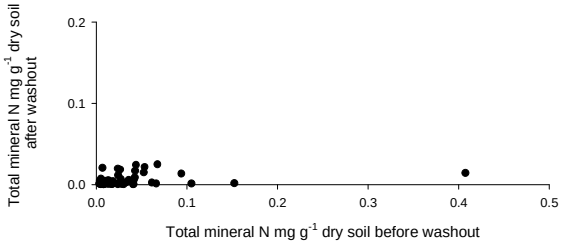


Figure 15-1 Relationship between initial mineral N (NH_4 + NO_3), measured in cores used for conventional incubation, and mineral N measured in washout cores before incubation.

Soil total C/N ratio was in general weakly negatively correlated with measurements of mineralisable N (Table 15-2). The nitrate proportion of mineral N was low on initial measurement (conventional method), but high after incubation in both conventional and washout methods. Mineral N measurements using the conventional method were generally weakly correlated with mineral N measurements using the washout method; the greatest correlation coefficient of 0.51 was between initial nitrate (conventional method) and total mineral N (washout method).

Table 15-2 Correlation coefficients among total soil measurements and mineralisable N measurements using conventional and washout methods.

Variate	CNgpg	NCgpg	Conv_InitNH4	Conv_InitNO3	Conv_InitTotMinN	Conv_FinNH4	Conv_FinNO3	Conv_FinTotMinN	Conv_NetTotNMinbld	Conv_NetTotNMinbld_pgOM	Wash_NH4	Wash_NO3	Wash_TotminN
NCgpg	-0.869												
Conv_InitNH4	0.348	-0.374											
Conv_InitNO3	-0.171	0.224	-0.124										
Conv_InitTotMinN	0.231	-0.228	0.856	0.407									
Conv_FinNH4	0.213	-0.322	0.724	0.036	0.686								
Conv_FinNO3	-0.365	0.535	-0.17	0.384	0.043	-0.026							
Conv_FinTotMinN	-0.194	0.28	0.25	0.347	0.411	0.524	0.838						
Conv_NetTotNMinbld	-0.387	0.468	-0.478	-0.003	-0.442	-0.064	0.788	0.636					
Conv_NetTotNMinbld_pgOM	-0.232	0.372	-0.151	-0.11	-0.197	-0.066	0.755	0.607	0.764				
Wash_NH4	-0.117	0.058	0.131	0.027	0.135	0.262	0.007	0.149	0.033	-0.007			
Wash_NO3	-0.071	0.085	-0.119	0.505	0.153	0.122	0.321	0.34	0.205	-0.004	0.192		
Wash_TotminN	-0.072	0.085	-0.119	0.505	0.154	0.123	0.321	0.34	0.205	-0.004	0.195	1	
Wash_TotminN_pgOM	-0.412	0.52	-0.262	0.122	-0.177	-0.175	0.445	0.284	0.429	0.409	0.129	0.509	0.509

c) Correlations between floristic fertility indicator and soil variables

The individual soil variables most strongly correlated with mean Ellenberg fertility score (Table 15-3) were pH (more correlated when measured in $CaCl_2$ than in water), moisture content (slightly more correlated when measured after saturation and suction than at field moisture content), C/N ratio, Total C, and mineralisable N per g organic matter (washout method).

Table 15-3 Correlation coefficients between mean floristic Ellenberg N score (in 1998) and measured soil parameters (in 2006): CC = correlation coefficient.

Variate	CC	Variate	CC	Variate	CC
pHCaCl2	0.804	Conv_FinNO3	0.504	Conv_InitNH4	-0.293
Wash_MC	-0.744	Conv_PropNO3Init	0.468	Conv_InitNO3	0.289
CNgpg	-0.719	Wash_NH4_pgOM	0.442	Conv_FinTotMinN	0.263
InitMC	-0.706	TotNmgpgdw	-0.421	Conv_RNI	0.2
TotCmgpgdw	-0.692	Conv_NitrifRate	0.377	Wash_NO3	0.15
pH_water	0.681	Wash_LOI	-0.366	Wash_TotminN	0.15
Wash_TotminN_pgOM	0.641	Conv_NetTotNMinbld	0.36	Conv_InitTotMinN	-0.121
Wash_NO3_pgOM	0.64	Conv_NetTotNMinbld_pgOM	0.331	SPPRICH98	0.115
InitLOI	-0.637	Conv_FinNH4	-0.307	Wash_NH4	0.044
Conv_PropNO3Fin	0.631	Wash_Prop_NO3	0.294		

d) Explanatory variates for floristic fertility indicators

The best single explanatory variate for mean Ellenberg fertility score was N/C ratio, explaining 72% of the variation (

Table 15-4). Mineralisable N (washout method, per g organic matter) explained 40%, but was somewhat orthogonal to N/C ratio; together these variates explained 78 % of the variation.

Stepwise regression among all single effects models (i.e. without interactions) selected a model including pH, moisture content as measured after saturation and suction (MC), mineralisable N, and N/C ratio which explained 83 % of the variation. Moisture Content was strongly correlated with Total C (Correlation coefficient = 0.90), Total N (0.72) and C/N ratio (0.77), and is considered less causally connected than total C and N contents to plant nutrient exposure. Models based on total C and N contents were therefore favoured. Models with single effects of pH, mineralisable N and either C/N or N/C ratio explained 80% or 82% of the variation, but separating the effects of total C and total N improved the fit to 84%. When interactions were also included, this “best causal model” explained 89 % of the variation, approaching the best possible fit of 90% obtained by including all variates and interactions in the model.

Table 15-4 Proportion of variance in mean Ellenberg fertility score explained by selected regression models. + = only main effects included; * = main effects and interactions included; pH = pH in $CaCl_2$ solution; Mineralisable_N = total mineral N g^{-1} organic matter, after washout with 1 mg N L^{-1} and 4 week incubation; Tot_C = total C g^{-1} soil; Tot_N = total N g^{-1} soil; MC = water content (g g^{-1} fresh soil) after saturation and mild suction; CN = g C g^{-1} N; NC = g N g^{-1} C;

Model	**Notes**	**R^2**
NC		72.1
pH		64.5
MC		56.3
CN		53.0
Tot_C		49.6
Mineralisable_N		39.8
Mineralisable_N * NC		77.5
pH + MC + Mineralisable_N + NC	best model selected by stepwise regression	82.7
pH * MC * Mineralisable_ N * NC	as above, including interactions	87.6
pH + Mineralisable_N + CN		80.1
pH + Mineralisable_N + NC		82.4
pH + Mineralisable_N + TotN + TotC		84.2
pH * Mineralisable_N * TotN * TotC	“best causal model”	88.9

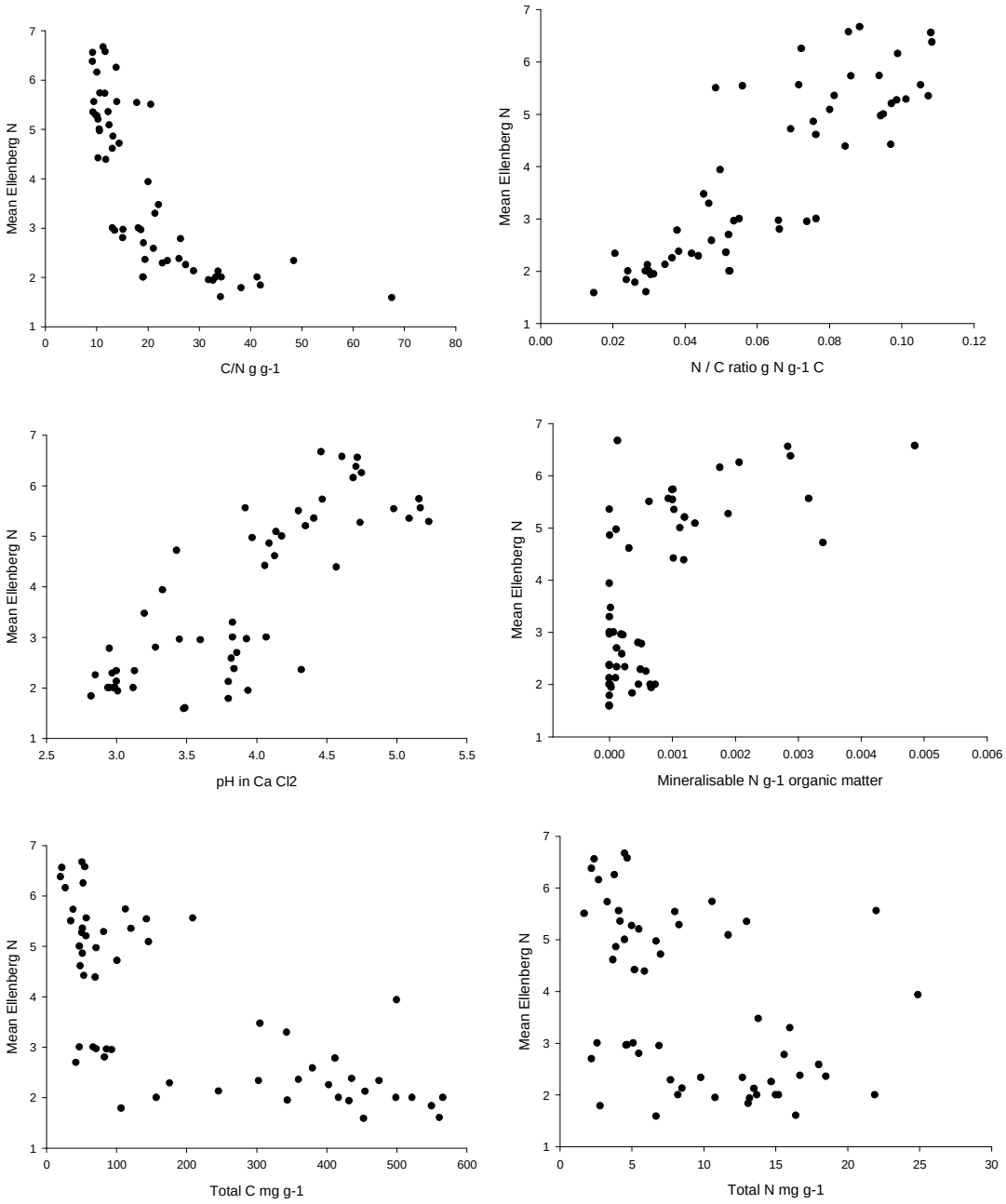


Figure 15-2 Scatter plots of mean Ellenberg fertility score against selected abiotic measurements.

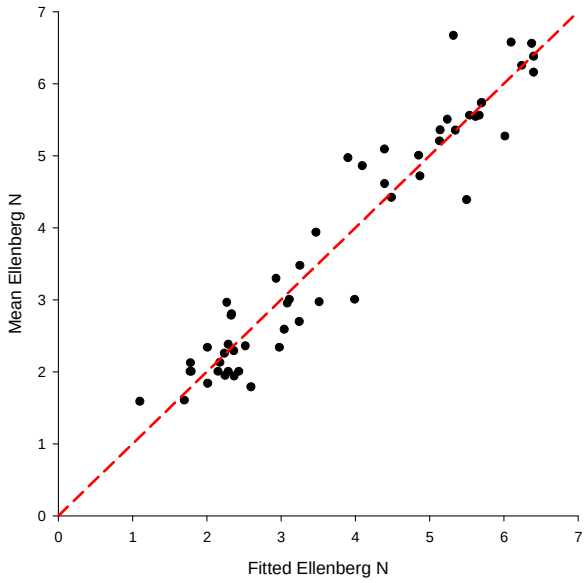


Figure 15-3 Measured mean Ellenberg score against mean Ellenberg score predicted using the best causal model (R^2 = 88.9). EbN = -16.9 + 5.178.pHCaCl2 + 0.0387.TotCmgpgdw + 1.533.TotNmgpgdw + 6291.Wash_TotminN_pgOM - 0.01175.pHCaCl2.TotCmgpgdw - 0.357.pHCaCl2.TotNmgpgdw - 0.00262.TotCmgpgdw.TotNmgpgdw - 1372.pHCaCl2.Wash_TotminN_pgOM + 15.5.TotCmgpgdw.Wash_TotminN_pgOM - 693.TotNmgpgdw.Wash_TotminN_pgOM + 0.000661.pHCaCl2.TotCmgpgdw.TotNmgpgdw - 2.1.pHCaCl2.TotCmgpgdw.Wash_TotminN_pgOM + 136.pHCaCl2.TotNmgpgdw.Wash_TotminN_pgOM - 2.14.TotCmgpgdw.TotNmgpgdw.Wash_TotminN_pgOM + 0.554.pHCaCl2.TotCmgpgdw.TotNmgpgdw.Wash_TotminN_pgOM

15.4. Discussion

Indicators not often directly associated with fertility are strongly associated with EbN. EbN has been shown to be correlated with other Ellenberg indicators, and is best used as a composite indicator of fertility rather than an indicator of nitrogen or nutrient exposure. Nevertheless, a model based simply on soil total N/C ratio and mineralisable N was able to explain 78% of the variation in EbN. The “best causal model” chosen, including pH, total N, total N, mineralisable N and interactions, explained an astonishing 89% of the variation in EbN.

Moisture content is a surprisingly good indicator of EbN. The correlation was better for MC measured after saturation and suction, which presumably gives a more consistent measure of water content (approximately field capacity) than that measured at sampling when previous wetting was not controlled.

The proportion of nitrate after conventional incubation was well-correlated with EbN, which may reflect increased activity of nitrifiers in soils with larger typical N fluxes. However, after the longer incubation used in the washout method, nearly all mineral N was nitrate and so the nitrate proportion was poorly correlated with Eb N. The nitrate proportion may reflect aeration during incubation, which is a difficult factor to control.

16. ANNEX F: Consistent Estimation of Stock and Change

Andy Scott

16.1. Executive Summary

1. This report, produced at the request of the CS Reporting Topic Group, examines the feasibility of providing consistent estimates of stock and change from Countryside Survey data. It provides a description of the statistical methodology required for consistent estimation, discusses the implications for CS estimation procedures, and makes recommendations with regard to implementation for CS2007. A case study based on CS broad habitat data is used to illustrate the methodology and its implications.

2. Current methods of estimation of stock and change in CS make minimal assumptions about the data and are therefore robust. Estimates of stock are calculated using all the data from a particular survey while change is calculated from only the repeated measurements across pairs of surveys. Estimating stock and change from different subsets of the data has the consequence that only in exceptional circumstances will these estimates be consistent.

3. Modification to the existing methods to report change as the difference between stock estimates can compromise the precision of change estimates, the reason this approach has not been used in previous surveys. With the existing methodology a choice has to be made between consistency and precision.

4. A modelling approach to the CS data can be used to produce consistent estimates, and is in theory straightforward to implement, but requires additional assumptions about the distribution of data. Furthermore consistency across the complete timescale of CS requires estimates to be produced from the complete dataset (i.e. all surveys taken together). One consequence is that estimates can not be made consistent across reporting occasions since the introduction of data from each new survey will influence the estimates of previous surveys.

5. A case study using CS Broad Habitat data suggests that consistent estimation via modelling is feasible and can be made reasonably robust. In general estimates derived using these methods differ from estimates obtained using the old methods by less than the inconsistencies already arising from the old methods.

6. However the modelling approach can require much more computer time than previous methods since it requires the iterative fitting of a model rather than the formulaic calculation of a mean. In addition there are a number of practical issues affecting implementation that arise from the CS sampling methodology.

7. Specific recommendations for modifications to the current estimation procedures are made. In particular it is recommended that modelling methods for consistent estimation are adopted for CS2007 and any future surveys, but that care should be taken to check the validity of results, especially those for small subsets of the data.

16.2. Background

The results of statistical analysis are usually reported in two forms. Point estimates, the expected or most likely value of a variable of interest, and interval estimates, the range of plausible values. Point estimates of stock and change reported from previous Countryside Surveys have been considered inconsistent since reported changes in the extent of specific habitats between any two surveys have not been the same as the differences in the reported extent of those habitats in the two surveys.

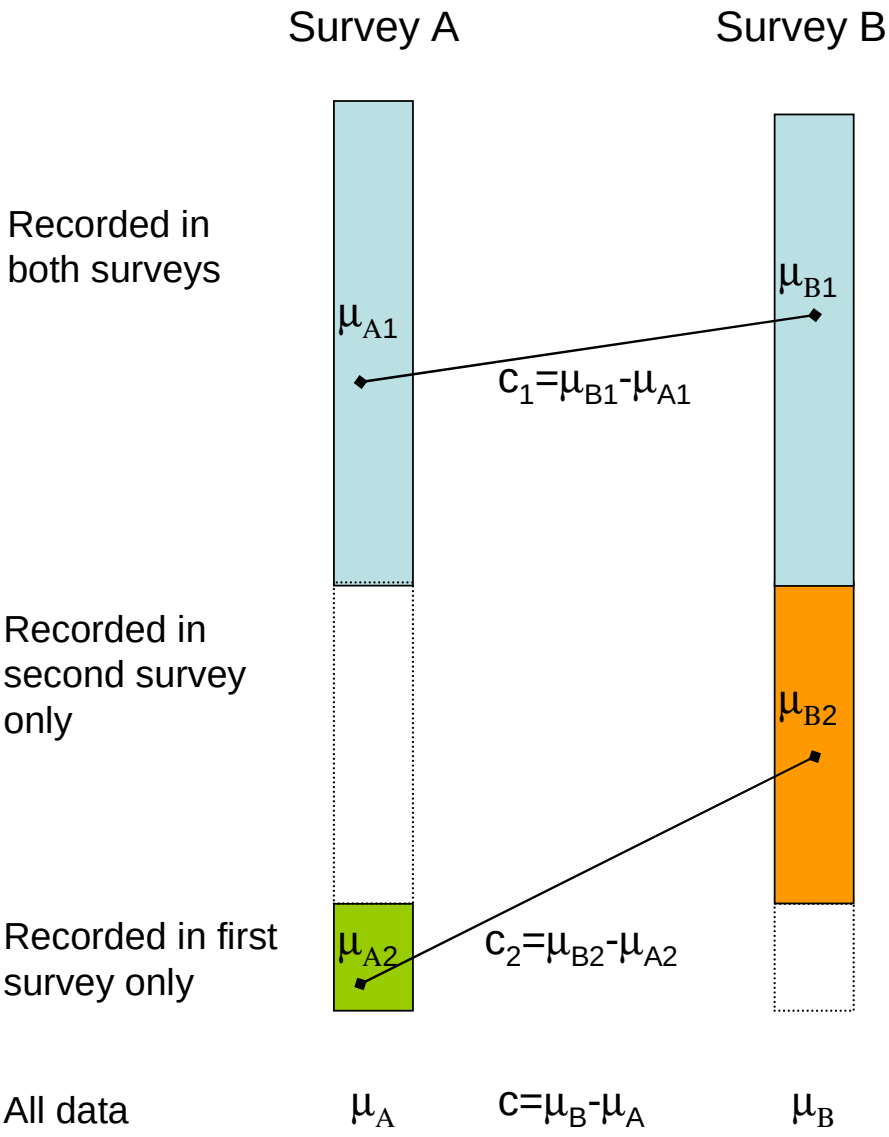
The reason for this discrepancy is illustrated in Figure 1. For each pair of surveys some sample squares (or plots) are not recorded in one or other of the surveys. The cause of the majority of this missing information has been the introduction of new squares as CS has developed, so that most of the unrepeated data is from the later survey in each pair, but loss of squares/plots recorded in an earlier survey, through landowner refusal for example, can also occur. Figure 1 illustrates three methods of estimating stock and change (others are possible):- from all squares, from repeated squares only, and from un-repeated squares only. Each method produces a consistent set of estimates. The inconsistencies in point estimates reported by CS arise because the reported estimates of stock are calculated using all the data from a particular survey while change is calculated from repeated measurements only. This automatically means that only in exceptional circumstances will these estimates be consistent.

In should be emphasised that the inconsistencies do not represent a problem with the data or its analysis. The inconsistencies arise from random sampling variation in the data (in particular missing information) coupled with the methods of estimation used in CS. Estimating stock using all the data from a survey maximises information use from that survey, while estimating change using only repeated measurements minimises the distributional assumptions needed and hence ensures robust estimation. The difficulty lies with the interpretation of point estimates outside the context of their standard errors and confidence intervals. If results were presented solely in the form of confidence intervals, (e.g. stock in 1990 was between *a* and *b*, stock in 1998 was between *c* and *d*, change was between *x* and *y*), it would be clear that any inconsistency was more apparent than real.

In CS2007 the emphasis on reporting has changed, from describing the current survey and changes since the immediately preceding survey, to timelines spanning the interval from the first survey to the present. This change will highlight the apparent inconsistencies and could also introduce additional ones since, using the same methods of analysis, estimated changes between adjacent surveys would not sum to estimates of change between non-adjacent surveys.

As a result the CS Reporting Topic Group has requested that the question of producing consistent estimates be examined and the feasibility of their introduction in the reporting of CS2007 be considered. This report briefly reviews the current methods (Section 2), describes statistical methodology and estimation procedures that can be used to ensure consistency (Section 3), provides a case study of their use with the CS Broad Habitat data (Section 4), discusses their limitations and the implications of their introduction (Section 5), and makes recommendations with regard to any required modifications for CS2007 (Section 6).

Figure 1 Current reporting of stock and change



Estimates:- (stock μ, change c)

μ_A, μ_B, c	all squares,
μ_{A1} , μ_{B1}, c_1	repeat squares
μ_{A2}, μ_{B2}, c_2	unrepeated squares

Reported stock μ_A and μ_B, reported change c_1

16.3. Current CS Methodology

This section gives a brief, and simplified, overview of the sampling and analysis procedures used for Countryside Survey data, providing sufficient background information for an understanding of the implications of the proposed changes to the basic CS estimation procedure. It can be ignored by those familiar with the CS sampling and analysis schemes. Fuller expositions of the details of CS sampling and estimation can be found in Scott (2007) and Scott *et al*. (1996).

Sampling

CS Field Survey data comprises information collected from a stratified sample of the 1 km squares at the intersection of a 15km grid covering GB. Each selected square is mapped and detailed measurements made of selected features, for example a number of quadrats are laid out and used to collect additional information on vegetation, soils etc. Thus there are two levels of sampling. Measurements are made at both levels so that some relate to the whole square while others describe features within the square. Measurements are of varied types ranging from binary (yes/no) variables to continuous variables such as areas or lengths.

The strata used for square selection are defined by the ITE Land Classification. The details of the classification have changed somewhat from its original form, largely as a result of the need for separate country reporting. Originally the classification comprised 32 Land Classes. For CS2000, due to the requirement for separate reporting in Scotland, the classification was modified and currently contains 42 classes. For CS2007, as a result of modifications to the classification brought about by the requirement of Wales only reporting, the classification is likely to comprise 49 Land Classes (Clarke *et al*., 2007). Effectively each country will have a separate classification, 21 classes in England, 12 in Wales and 16 in Scotland, although the classes in each of these national classifications are strongly related through their derivation from the original GB classification.

Estimation

The basic procedure originally used in calculating regional or national estimates was to produce estimated means and standard errors for the quantity of interest for each Land Class and then to combine these to produce an estimated mean or total (with standard error) for the specified region. The method of combination differs depending on whether a total or mean figure is required but in both cases involves weighting the individual land class estimates by values proportional to the area of land within the Land Class.

This procedure makes minimal assumptions about the form of the data. Estimates of means and standard errors are unbiased regardless of the distribution involved, as are the formulae for combining them. It is assumed that mean estimates for any Land Class are independent of estimates within any other Land Class and of estimates of total available land but this assumption is assured by the sampling scheme used.

Bootstrapping

Testing for significance requires more information about the distribution of an estimate than just its standard error. Prior to CS2000 significance was assessed by assuming normality of estimates. In CS2000 because of concerns about the validity of this assumption, largely because of the skewness of some of the features being

estimated, standard errors and confidence intervals for square level data were estimated using the bootstrap (Efron and Tibshirani, 1993). Minitab macros to produce bootstrap estimates were written for CS2000 by Ralph Clarke (CEH Dorset). These were converted to SAS macros by John Watkins(CEH Lancaster).

Essentially bootstrapping involves treating sample data as a population from which to resample. Each resample produces a separate estimate of some quantity of interest, for example stock or change. A large number of resamples (typically 1000 or 10,000) then gives an approximation to the distribution of the required estimate, from which any statistic can be extracted. The main advantage of this method of estimation for CS is that it allows for non-normality in the data, without the necessity of knowing details of the actual distribution, and as such provides more accurate measurements of significance.

16.4. Consistent Estimation

Possible approaches

A number of approaches could be used to ensure consistency. Figure 1 illustrates three sets of consistent estimates that can be derived from a pair of surveys. Each of these three sets estimate the same values and could, in principle at least, form the basis for reporting. If almost all squares were measured on every sampling occasion then the unrepeated squares could be discarded with little loss of precision. This is not a practical approach for CS as a substantial number of extra squares has been added with each survey. Equally using just the unrepeated squares is clearly not sensible.

The first potentially usable approach is to use the stock estimates for all measurements, as is done at present, but to estimate change as the difference between stock estimates. The statistics relevant to this approach were described in the CS1990 main report (Barr et al. 1993, p171) but the method has not so far been used as a general approach in CS. It has a number of advantages. Estimates are consistent and robust and estimates from one survey do not change following the implementation of later surveys. The methodology is easy to implement and quick to run, and standard errors and confidence limits can be estimated with the bootstrap. There are however a number of disadvantages as well. Most importantly, the method is only efficient for measuring change when the covariance (or correlation) between measurements in successive surveys is not large. For many CS measurements estimating change as the difference between stock estimates would produce change values with larger standard errors than by estimating change just from repeat squares. Furthermore the approach is not directly applicable to plot level data. Thus there would be inconsistencies in methodology between estimates of square level and plot level data. On the technical side using this method requires modification to the bootstrapping macros to allow bootstrapping from two surveys simultaneously.

An alternative approach is to use modelling techniques to estimate stock and change. This approach could be applied to both square and plot level data and would produce consistent as well as efficient, i.e. more precise, estimates of both stock and change. As with the previous approach, however, there are a number of drawbacks. To fully explain these the modelling approach is described in detail in the remainder of this section.

Modelling basics

The discrepancies between estimates of stock and change arise, as explained above (Section 1, Figure 1), because missing information means that stock and change are estimated from different sets of data. Effective statistical methods for dealing with incomplete data were only devised in the late 1970's (Dempster *et al*., 1977) and it was some time before their regular use spread from the statistical to the user community. At first a computational slow and demanding set of techniques, their practical utility and computational efficiency has been gradually increased by the introduction and theoretical justification of more effective estimators and algorithms over the last two decades (see e.g. Scott, 2002). Many statistical models fitted by proprietary software can now cope with incomplete data. Such techniques do, however, require assumptions about data distributions for implementation. Thus ensuring consistency of CS estimates of stock and change involves making additional assumptions about the data which may in some instances not be met. The advantage of consistency has to be balanced against a possible loss of accuracy arising from distributional mis-specification.

The way in which incomplete data techniques work can be illustrated by considering ways in which missing information might be replaced. Change and stock estimated from the completed dataset would then be automatically consistent. Two extremes are possible, depending upon whether stock or change values are used to replace the missing data. If missing values are replaced by the appropriate survey mean then stock estimates are unchanged but a new value for change is found. Alternatively if the average change found from repeated measurements is used to predict the missing values then the change estimate from the completed dataset is the same as the change from the repeated measurements but the stock estimates will change. In reality, of course, these are extremes and a procedure somewhere between will be most appropriate. Missing information techniques in effect use the correlation structure from the repeated measurements to judge where between these two extremes the most appropriate estimates lie. In practise the techniques work directly with the observed data and not by filling in missing values.

For CS, because of its hierarchical sampling scheme, implementation of consistent estimation via modelling requires fitting a mixed effects and/or repeated measures statistical model to data from all surveys. Such models contain two types of parameter: fixed effects parameters are functions of stock and change values while random effects parameters have a specification that reflects the random variation in the data as determined by the sampling structure. After model fitting the estimates of the fixed effect parameters are then transformed to estimates of stock and change.

Such models require more assumptions than the current methods, which, following the introduction of the bootstrap, essentially only require calculation of means. In essence they require calculation of variances and covariances as well as means and specification of the distributional form of the random and repeated effects. Models appropriate to measurements made, or summarised, at the square and plot level are described below.

Square level data

For measurements applicable to complete 1 km squares the CS dataset can be considered as made up of a random sample of squares within each Land Class, each square providing a value on each survey (apart from missing observations). Statistically the appropriate model for this form of data is a repeated measures

model. Such a model comprises two separate model components, one for fixed effects and one for random elements (hence the generic name mixed model).

The fixed effect component is just a standard regression model. For CS square level data the simplest fixed effect model treats the mean value within a Land Class on each sampling occasion as a fixed effect to be estimated, i.e. a simple regression of the variable of interest on year (or, equivalently, survey) treated as a categorical variable. The fitted effects are then, when scaled by the land class area, just the required estimates of stock in each survey. Estimates of change are just the differences between fitted stock estimates and hence are automatically consistent. More complex models, with additional explanatory variables can be used to break down the stock, or change, estimates into additional categories.

The random effects component of the overall model describes the variation of individual recorded measurements about the fitted fixed effects. Standard regression models specify one random element per observation, usually referred to as a residual, and all residuals are assumed to be independent. A mixed model differs from a standard regression model in including parameters describing the structure of the residuals. For CS square level data each square within a land class is assumed to have a constant random difference from the land class average. Measurements from the same square in successive surveys vary about this square level residual, and these survey deviations from the square level residual are allowed to be correlated.

Plot level data

CS measurements are made not just at the whole square level but also within squares. Vegetation and soil data, for example, are recorded for a number of plots within each sample square. In previous surveys the full hierarchical nature of the plot level data was not explicitly dealt with. A variety of approaches were adopted for different analyses. In some, measurements were summarised at the square level prior to analysis. This approach is robust but clearly does not make full use of the data and hence will generate standard errors that are larger than necessary. In other analyses plots were treated as independent observations within a land class and the square level variation not allowed for. This approach is efficient if the variation among plots within squares is the same as their variation across squares but can produce biased results, or incorrect standard errors, if this is not true. In CS2000 mixed models that allowed for square level variation but ignored the sampling structure in terms of land classes were used for some plot level data. In addition, because the bootstrapping macros written for CS2000 were produced for square level data only, results for plot level data had standard errors calculated from, possibly incorrect, distributional assumptions rather than from bootstrapping.

The model described above for square level data can be extended by the inclusion of a plot level residual, or random effect, in addition to the square level random effect. The correlated survey residuals now vary about the average level for the plot, not the square. Both forms of model can be embedded within bootstrapping procedures.

Model specification

Exposition of the proposed models and the assumptions on which they are based requires at least some mathematical specification for clarity. Let y_{ijk} represent an observation in survey *k* from square *j* in land class *i*. Then a general model for square level data can be written as

$$y_{ijk} = a_{ik} + s_{ij} + e_{ijk}$$

where the *a* parameters (the fixed effects) represent land class means in successive surveys, the *s* values are the square random effects and the *e* values are the repeated measures effects. To complete the model requires specification of the distribution of the random and repeated effects. The *s* values are assumed to be normally distributed with mean zero and standard deviations σ_i which differ across land classes. The *e* values are also normally distributed with zero mean, standard deviations σ_{ik} which vary across land classes and surveys, and covariances, for individual squares, which vary across land classes and pairs of surveys.

For K successive surveys this general model includes, for each land class, K fixed effect parameters but 1+K(K+1)/2 random parameters (the variances and covariances of the random and repeated values). Thus the number of random effect parameters is greater than the number of fixed effect parameters and this imbalance increases with the number of surveys. Unfortunately estimates of variances and covariances are much less precise than estimates of means, which the fixed effect parameters effectively are. Because of the large number of land classes used for CS sampling there are relatively few sample squares in each class. The result is that the full model tends to be unstable and difficult to fit, increasingly so as the number of surveys increases. An additional technical complication is that the computer time for model fitting also increases with the number of parameters.

To make consistent estimation via modelling practicable, therefore, it is desirable to reduce the number of random effect parameters. A helpful property of mixed effect models is that estimation of the fixed effects parameters is relatively robust to mis-specification of the distribution of the random values. Thus the number of random effect parameters can often be reduced considerably without substantially affecting the accuracy or precision of the fixed effect parameters. Reducing the number of parameters can be done in a variety of ways, giving a choice of models to fit. It is not usually sensible to set random parameters to zero, the usual method of reduction for regression or fixed effect parameters. The alternative is to assume certain sets of parameters are equal or can be specified as functions of a smaller number of parameters.

One possibility is to assume that variance and/or covariance parameters do not vary with land class. However for many CS variables this is demonstrably not true, variability is very different across land classes. A more realistic assumption is that random effect parameters do not vary across surveys. Thus it can be assumed that the standard deviations, σ_{ik}, take a common value, σ_i, for all surveys. This assumption reduces the number of repeated measures variance parameters per land class to one. Many theoretical structural models have been proposed for covariances. A particularly effective model is the autoregressive model of order one which assumes that the covariance between repeated measure values in successive surveys is constant and that non-adjacent survey values are conditionally independent given the values of intervening surveys. This assumption reduces the number of repeated measures covariance parameters per land class to one. Using both of these assumptions (giving a model that will be referred to as the AR1 model) reduces the total number of random effect parameters to three per survey, regardless of the number of surveys.

Although estimation of fixed effects is relatively robust to mis-specification of distributional assumptions this is not the case for variance and covariance parameters. Thus parametric calculation of standard errors may produce erroneous values and this applies to the standard errors automatically output by the modelling software. However bootstrap estimation, which requires only the fixed effect values, will also be robust. The AR1 model with bootstrap estimation of standard errors has

therefore been investigated in detail as a means of providing consistent estimation with CS data.

16.5. Case study – Broad habitats

Broad Habitats in CS

One of the main outputs of previous CS surveys (Barr *et al*., 1993: Haines-Young *et al*., 2000) has been an assessment of the stock of, and change in, acreage of a variety of habitats. In CS2000 standard Broad Habitats were used. Broad Habitat information is recorded at the square level as the proportion of rural land within the square that falls into each category. Information on Broad Habitats is available from the 1984, 1990 and 1998 (CS2000) surveys. Habitat information from the 1978 survey, long before the definition of Broad Habitats, was coded differently so is not directly comparable.

As an example of the application of the methods discussed in this report, data from the 1984, 1990 and 1998 surveys for seven Broad Habitats have been analysed. Throughout the remainder of this section values for stock and change are presented as percentages of the rural land in specified regions. Separate estimates are given for three regions:- Great Britain, England & Wales, and Scotland. The figures presented here are derived for illustrative purposes and do not make adjustment for the amount of urban land or sea within individual squares as is done in the main survey reports. Such adjustments are an added complication in the analysis which is not relevant to the choice of method. This does mean, however, that the results presented here differ slightly from those given in published survey reports.

Results

Table 1 shows estimates of stock and change, with their standard errors calculated using the methods employed in CS2000 to date. Estimates of stock (Table 1a) are obtained from the data for individual surveys. Two forms of change estimate are presented for each pairs of surveys, change estimates from repeated squares (Table 1b) and the differences between stock estimates (Table 1c). The inconsistencies between stock and change from repeated squares, evident from previous reports, are clear. Differences between the stock estimates for any pair of surveys do not equal the corresponding change estimates. Additional discrepancies, not obvious from previous surveys because of the reporting structure used, can be seen in the change figures. Using only repeated squares, estimates of changes from 1984 to 1990 and from 1990 to 1998 do not sum to the estimates of change from 1984 to 1998. Table 1d gives the ratio of the standard errors for the two methods of calculating change. Almost all of these ratios are greater than 1 and many substantially higher. This emphasises the fact that estimating change from stock values gives less precise estimates in general than estimating change from repeat squares. This is the reason that CS has used the methods that it has to date.

Table 2 shows estimates of stock and change, with their standard errors, obtained from fitting mixed effect/repeated measures models to the data from the three surveys. Separate models were fitted for each land class. The form of model used (denoted AR1) assumed constant within land class variance of each variable across surveys with correlation between surveys represented as a first order autocorrelation process. The estimates in Table 2 do not exhibit the discrepancies shown in Table 1. Each change estimate is equal to the difference between the corresponding stock

estimates and change estimates from consecutive inter-survey periods sum to the change estimate for the change over the whole period.

Table 3 summaries the effect of changing from the current methodology to a modelling approach. Each value in this table gives the difference in estimates from the two methods as a percentage of the standard error of the estimate using the old CS method of analysis. For stock only one estimate has changed by as much as a standard error (neutral grassland in England & Wales in 1990) and most changes are much less than this. For change estimates also only one estimate has altered by more than a standard error (the change in Coniferous woodland in England & Wales from 1984 to 1990). Overall none of the estimates obtained using the new modelling approach are outside the error bounds of the estimates from the old methodology and most are well within them.

The particular form of comparison of old and proposed methods used in Table 3 was chosen because it emphasises the lack of significance of the reported differences. However it can appear to exaggerate the actual alterations that occur. The estimated stock of Improved Grassland in GB in 1984, for example, changes by a fifth of a standard error when modelling is used in place of the older methods but the actual change in the estimate is less than one percent. The change is only substantial in terms of the standard error because the extent of this broad habitat is large and it is well estimated with a relatively small standard error

Table 4 puts these results in context. This table summarises the inconsistencies arising from the old methodology. The values listed are the differences between changes derived from two stock estimates and those estimated directly from repeated squares, as a percentage of the change standard error. The majority of these values are substantially greater than the corresponding values in Table 3b. Some discrepancies are more than two or even three standard errors. Thus in general the estimates derived using consistent estimation methods differ from estimates obtained using the old methods by less than the inconsistencies already arising from the old methods.

Table F-1 Estimates using current CS methods of analysis.

a) Stock

Great Britain

		1984	1990	1998
Broadleaved, mixed & yew woodland	mean	5.660	5.945	6.406
	se	0.484	0.379	0.387
Coniferous woodland	mean	5.450	5.926	5.968
	se	0.776	0.638	0.622
Boundary and linear features	mean	2.108	2.150	2.164
	se	0.094	0.077	0.078
Arable and horticultural	mean	22.990	22.897	22.919
	se	1.137	0.946	0.903
Improved grassland	mean	25.656	23.962	23.784
	se	1.205	0.996	0.898
Neutral grassland	mean	2.000	2.449	2.645
	se	0.206	0.198	0.216
Calcareous grassland	mean	0.305	0.330	0.262
	se	0.145	0.123	0.101

England & Wales

		1984	1990	1998
Broadleaved, mixed & yew woodland	mean	7.155	7.304	7.862
	se	0.693	0.515	0.508
Coniferous woodland	mean	2.627	2.724	2.548
	se	0.613	0.475	0.421
Boundary and linear features	mean	2.691	2.742	2.751
	se	0.138	0.113	0.114
Arable and horticultural	mean	30.945	31.459	30.949
	se	1.635	1.359	1.268
Improved grassland	mean	32.476	29.785	29.636
	se	1.700	1.305	1.184
Neutral grassland	mean	2.099	2.469	2.958
	se	0.264	0.238	0.292
Calcareous grassland	mean	0.283	0.321	0.249
	se	0.179	0.136	0.109

Scotland

		1984	1990	1998
Broadleaved, mixed & yew woodland	mean	2.883	3.420	3.699
	se	0.590	0.525	0.595
Coniferous woodland	mean	10.696	11.878	12.323
	se	1.877	1.579	1.583
Boundary and linear features	mean	1.024	1.049	1.072
	se	0.083	0.077	0.080
Arable and horticultural	mean	8.208	6.987	7.997
	se	1.119	0.966	1.021
Improved grassland	mean	12.984	13.142	12.912
	se	1.354	1.325	1.178
Neutral grassland	mean	1.816	2.412	2.064
	se	0.330	0.361	0.302
Calcareous grassland	mean	0.345	0.346	0.286
	se	0.229	0.230	0.195

b) Change from repeated squares only

Great Britain

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.272	0.303	0.607
	se	0.076	0.099	0.136
Coniferous woodland	mean	0.293	-0.016	0.417
	se	0.150	0.155	0.309
Boundary and linear features	mean	0.075	-0.009	0.050
	se	0.022	0.025	0.039
Arable and horticultural	mean	-0.274	0.413	0.005
	se	0.412	0.283	0.465
Improved grassland	mean	-1.863	-0.444	-2.165
	se	0.489	0.336	0.523
Neutral grassland	mean	0.675	0.033	0.665
	se	0.159	0.167	0.246
Calcareous grassland	mean	0.026	-0.060	-0.043
	se	0.023	0.031	0.026

England & Wales

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.295	0.298	0.578
	se	0.106	0.126	0.166
Coniferous woodland	mean	0.003	-0.114	-0.040
	se	0.035	0.064	0.065
Boundary and linear features	mean	0.099	-0.024	0.053
	se	0.031	0.037	0.058
Arable and horticultural	mean	-0.142	0.359	0.076
	se	0.566	0.389	0.640
Improved grassland	mean	-2.696	-0.650	-3.072
	se	0.687	0.460	0.752
Neutral grassland	mean	0.711	0.244	0.934
	se	0.219	0.222	0.319
Calcareous grassland	mean	0.040	-0.060	-0.035
	se	0.035	0.037	0.029

Scotland

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.228	0.312	0.662
	se	0.088	0.155	0.230
Coniferous woodland	mean	0.831	0.168	1.267
	se	0.425	0.426	0.869
Boundary and linear features	mean	0.032	0.021	0.045
	se	0.023	0.019	0.032
Arable and horticultural	mean	-0.521	0.514	-0.127
	se	0.547	0.337	0.604
Improved grassland	mean	-0.315	-0.061	-0.480
	se	0.549	0.391	0.597
Neutral grassland	mean	0.608	-0.358	0.167
	se	0.200	0.238	0.361
Calcareous grassland	mean	0.001	-0.059	-0.059
	se	0.001	0.052	0.051

c) Change as difference between stock estimates

Great Britain

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.285	0.461	0.746
	se	0.286	0.174	0.330
Coniferous woodland	mean	0.477	0.041	0.518
	se	0.372	0.265	0.481
Boundary and linear features	mean	0.042	0.014	0.056
	se	0.058	0.037	0.068
Arable and horticultural	mean	-0.093	0.022	-0.071
	se	0.728	0.344	0.771
Improved grassland	mean	-1.694	-0.178	-1.872
	se	0.829	0.494	0.904
Neutral grassland	mean	0.449	0.195	0.645
	se	0.154	0.205	0.247
Calcareous grassland	mean	0.025	-0.067	-0.042
	se	0.071	0.032	0.086

England & Wales

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.149	0.559	0.708
	se	0.427	0.248	0.496
Coniferous woodland	mean	0.097	-0.176	-0.079
	se	0.267	0.280	0.414
Boundary and linear features	mean	0.051	0.009	0.060
	se	0.087	0.054	0.100
Arable and horticultural	mean	0.514	-0.510	0.005
	se	1.070	0.479	1.124
Improved grassland	mean	-2.691	-0.150	-2.841
	se	1.188	0.676	1.312
Neutral grassland	mean	0.370	0.488	0.858
	se	0.176	0.292	0.330
Calcareous grassland	mean	0.038	-0.072	-0.034
	se	0.109	0.039	0.129

Scotland

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.537	0.279	0.816
	se	0.254	0.171	0.250
Coniferous woodland	mean	1.182	0.445	1.627
	se	0.934	0.534	1.099
Boundary and linear features	mean	0.025	0.023	0.048
	se	0.044	0.036	0.059
Arable and horticultural	mean	-1.221	1.010	-0.211
	se	0.639	0.380	0.739
Improved grassland	mean	0.158	-0.230	-0.072
	se	0.845	0.568	0.903
Neutral grassland	mean	0.596	-0.349	0.248
	se	0.283	0.232	0.351
Calcareous grassland	mean	0.000	-0.059	-0.059
	se	0.001	0.052	0.051

d) Ratio of se's of change estimates

(se for stock difference over se for change from repeated squares)

Great Britain

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	3.77	1.75	2.42
Coniferous woodland	2.48	1.71	1.56
Boundary and linear features	2.69	1.50	1.76
Arable and horticultural	1.77	1.21	1.66
Improved grassland	1.69	1.47	1.73
Neutral grassland	0.97	1.22	1.00
Calcareous grassland	3.12	1.04	3.23

England & Wales

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	4.03	1.96	2.99
Coniferous woodland	7.63	4.39	6.38
Boundary and linear features	2.77	1.46	1.74
Arable and horticultural	1.89	1.23	1.76
Improved grassland	1.73	1.47	1.74
Neutral grassland	0.80	1.31	1.03
Calcareous grassland	3.12	1.06	4.39

Scotland

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	2.88	1.10	1.09
Coniferous woodland	2.20	1.25	1.27
Boundary and linear features	1.90	1.93	1.86
Arable and horticultural	1.17	1.13	1.22
Improved grassland	1.54	1.45	1.51
Neutral grassland	1.42	0.98	0.97
Calcareous grassland	1.02	1.00	1.00

Table F-2 Consistent Estimates (using AR1 model).

a) Stock

Great Britain

		1984	1990	1998
Broadleaved, mixed & yew woodland	mean	5.849	6.111	6.416
	se	0.373	0.374	0.381
Coniferous woodland	mean	5.448	5.786	5.770
	se	0.628	0.647	0.655
Boundary and linear features	mean	2.090	2.153	2.141
	se	0.074	0.077	0.079
Arable and horticultural	mean	22.906	22.501	22.893
	se	0.904	0.898	0.906
Improved grassland	mean	25.871	24.034	23.621
	se	0.972	0.905	0.892
Neutral grassland	mean	2.016	2.616	2.634
	se	0.217	0.230	0.217
Calcareous grassland	mean	0.294	0.312	0.252
	se	0.122	0.127	0.105

England & Wales

		1984	1990	1998
Broadleaved, mixed & yew woodland	mean	7.311	7.592	7.892
	se	0.513	0.512	0.502
Coniferous woodland	mean	2.601	2.658	2.556
	se	0.438	0.444	0.428
Boundary and linear features	mean	2.700	2.783	2.752
	se	0.105	0.110	0.115
Arable and horticultural	mean	30.920	30.644	30.966
	se	1.285	1.285	1.277
Improved grassland	mean	32.708	30.083	29.492
	se	1.334	1.180	1.172
Neutral grassland	mean	2.111	2.731	2.948
	se	0.271	0.291	0.292
Calcareous grassland	mean	0.266	0.295	0.234
	se	0.134	0.145	0.117

Scotland

		1984	1990	1998
Broadleaved, mixed & yew woodland	mean	3.132	3.361	3.674
	se	0.506	0.508	0.596
Coniferous woodland	mean	10.739	11.597	11.744
	se	1.585	1.650	1.688
Boundary and linear features	mean	0.957	0.982	1.004
	se	0.087	0.090	0.091
Arable and horticultural	mean	8.013	7.369	7.893
	se	0.998	0.954	1.000
Improved grassland	mean	13.166	12.793	12.713
	se	1.208	1.217	1.196
Neutral grassland	mean	1.840	2.403	2.052
	se	0.350	0.362	0.301
Calcareous grassland	mean	0.345	0.346	0.287
	se	0.229	0.229	0.195

b) Change

Great Britain

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.263	0.305	0.567
	se	0.078	0.099	0.120
Coniferous woodland	mean	0.338	-0.015	0.322
	se	0.150	0.147	0.242
Boundary and linear features	mean	0.063	-0.012	0.051
	se	0.024	0.025	0.032
Arable and horticultural	mean	-0.405	0.392	-0.012
	se	0.422	0.286	0.434
Improved grassland	mean	-1.837	-0.413	-2.250
	se	0.491	0.338	0.508
Neutral grassland	mean	0.600	0.018	0.618
	se	0.158	0.167	0.212
Calcareous grassland	mean	0.019	-0.060	-0.041
	se	0.011	0.031	0.026

England & Wales

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.281	0.300	0.581
	se	0.112	0.127	0.152
Coniferous woodland	mean	0.057	-0.103	-0.045
	se	0.044	0.075	0.068
Boundary and linear features	mean	0.084	-0.031	0.053
	se	0.035	0.037	0.046
Arable and horticultural	mean	-0.276	0.322	0.046
	se	0.584	0.393	0.593
Improved grassland	mean	-2.625	-0.591	-3.217
	se	0.703	0.461	0.729
Neutral grassland	mean	0.620	0.216	0.836
	se	0.214	0.223	0.276
Calcareous grassland	mean	0.028	-0.061	-0.032
	se	0.018	0.038	0.027

Scotland

		1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	mean	0.229	0.313	0.542
	se	0.088	0.155	0.192
Coniferous woodland	mean	0.858	0.147	1.005
	se	0.418	0.398	0.675
Boundary and linear features	mean	0.025	0.022	0.047
	se	0.024	0.019	0.033
Arable and horticultural	mean	-0.643	0.524	-0.120
	se	0.530	0.339	0.591
Improved grassland	mean	-0.373	-0.080	-0.453
	se	0.518	0.393	0.567
Neutral grassland	mean	0.563	-0.351	0.212
	se	0.199	0.233	0.310
Calcareous grassland	mean	0.001	-0.059	-0.059
	se	0.001	0.051	0.051

Table F-3 Differences between Current and Consistent estimates (as a percentage of the standard error of the current estimates).

a) Stock

Great Britain

	1984	1990	1998
Broadleaved, mixed & yew woodland	39.0	44.0	2.7
Coniferous woodland	-0.2	-22.1	-31.8
Boundary and linear features	-19.2	4.2	-29.7
Arable and horticultural	-7.4	-41.9	-2.8
Improved grassland	17.8	7.2	-18.2
Neutral grassland	7.9	84.3	-4.9
Calcareous grassland	-7.6	-14.0	-9.8

England & Wales

	1984	1990	1998
Broadleaved, mixed & yew woodland	22.6	55.9	5.8
Coniferous woodland	-4.2	-13.7	1.9
Boundary and linear features	6.1	36.3	0.9
Arable and horticultural	-1.5	-60.0	1.3
Improved grassland	13.6	22.8	-12.2
Neutral grassland	4.6	110.2	-3.4
Calcareous grassland	-9.3	-19.4	-14.1

Scotland

	1984	1990	1998
Broadleaved, mixed & yew woodland	42.1	-11.3	-4.2
Coniferous woodland	2.3	-17.8	-36.6
Boundary and linear features	-80.7	-87.1	-85.1
Arable and horticultural	-17.4	39.5	-10.2
Improved grassland	13.4	-26.3	-16.9
Neutral grassland	7.3	-2.6	-4.0
Calcareous grassland	0.1	0.2	0.1

b) Change

Great Britain

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	-12.3	1.5	-29.4
Coniferous woodland	29.8	0.2	-30.8
Boundary and linear features	-57.4	-15.6	0.7
Arable and horticultural	-31.6	-7.4	-3.7
Improved grassland	5.2	9.4	-16.3
Neutral grassland	-47.2	-9.4	-19.4
Calcareous grassland	-33.1	-0.1	6.1

England & Wales

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	-14.0	1.6	1.9
Coniferous woodland	155.3	18.1	-7.8
Boundary and linear features	-49.3	-17.6	-0.6
Arable and horticultural	-23.8	-9.6	-4.8
Improved grassland	10.2	12.8	-19.3
Neutral grassland	-41.6	-12.6	-30.5
Calcareous grassland	-33.0	-0.1	8.5

Scotland

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	1.1	0.3	-52.3
Coniferous woodland	6.2	-4.9	-30.2
Boundary and linear features	-28.9	5.8	4.3
Arable and horticultural	-22.4	2.9	1.2
Improved grassland	-10.6	-5.0	4.4
Neutral grassland	-22.7	3.0	12.4
Calcareous grassland	-14.8	-0.1	-0.2

Table F-4 Inconsistencies in Current estimates (figures are the difference between stock estimates minus change estimate as a percentage of the change standard error).

Great Britain

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	17.0	158.8	101.5
Coniferous woodland	122.5	36.7	32.6
Boundary and linear features	-155.6	90.5	14.1
Arable and horticultural	44.1	-138.3	-16.3
Improved grassland	34.4	79.3	56.0
Neutral grassland	-141.9	96.9	-8.4
Calcareous grassland	-5.7	-23.8	2.2

England & Wales

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	-138.2	206.2	78.4
Coniferous woodland	268.0	-96.5	-59.6
Boundary and linear features	-153.8	90.3	12.4
Arable and horticultural	116.0	-223.4	-11.1
Improved grassland	0.7	108.8	30.7
Neutral grassland	-155.7	109.8	-23.6
Calcareous grassland	-5.3	-30.2	3.5

Scotland

	1990-1984	1998-1990	1998-1984
Broadleaved, mixed & yew woodland	349.689	-21.726	66.862
Coniferous woodland	82.369	65.100	41.410
Boundary and linear features	-28.406	10.211	7.152
Arable and horticultural	-127.988	147.168	-13.831
Improved grassland	86.126	-43.220	68.217
Neutral grassland	-5.871	4.105	22.419
Calcareous grassland	-47.675	0.000	-0.447

16.6. Discussions and conclusions

Analysis of the CS Broad Habitat data confirms the feasibility of producing consistent estimates for the CS2007 report. Consistent estimates obtained through modification of existing methods to estimate change as the difference between stock estimates lacked precision when compared to estimates of change from just repeated squares. The current methodology therefore requires a choice between consistency and precision. Adopting a model based approach, in contrast, provides both consistency and precision.

Implementation of model based analysis within a bootstrapping envelope for square level data, although computationally challenging at times, proved to be reasonably straightforward. Using the AR1 model, programs took substantially longer to run than when using the old methodology but not sufficiently so as to suggest that extension of the technique to the large number of analyses required for the complete survey is impractical. Experimentation with a variety of models confirmed that the estimation of fixed effect parameters, e.g. stock and change, was robust to model variation. Fully parameterised models were, however, extremely slow to fit, to the extent that use of this model is probably impractical for the analysis of large number of variables.

In addition to the Broad Habitat data consistent analyses have been undertaken for the CS soil data as part of a separate project. This is a plot level dataset available so far only for 1978 and 1998 but repeated in 2007. The results confirm the feasibility of producing consistent estimates at this level as well as at square level. This would not only make such estimates numerically consistent but would also produce a consistency of approach across plot and square level data, something not achieved in previous surveys. The results of the analyses are currently being compiled into a separate report to Defra.

The basic square and plot level analyses described so far comprise the core of results presented in previous main survey reports. However a number of more involved analyses are also required. In the past these extended results have been analysed in a variety of ways, as was done for the plot level data. They were therefore not consistent with the core analyses although, because of the structure and graphical presentation of the results, this was not as obvious as the inconsistencies between stock and change estimates. Plot level results, for example, are often broken down by vegetation type. The introduction of such additional explanatory variables complicates the model quite considerably. This problem has been examined to some extent during the analysis of the CS soil data and appears to be amenable to incorporation within the consistent estimation framework but further investigation is required to confirm this.

Other, more complex, analyses, such as the estimation of net flows between habitat types, have not yet been examined as part of the preparation of this report. Ideally all analyses would be performed within the same consistent framework but the practicality of this also needs further investigation. The standardisation of just the core analyses would, by itself, be a major advance over previous surveys with the inclusion of other forms of analysis a bonus.

There are other reasons than just consistency for adopting a consistent methodological approach to estimation and analysis. Although robust, previous methods of analysis were not always fully efficient in that they did not utilise all the available information in producing individual estimates and did not always incorporate the hierarchical structure of the data. The modelling approach investigated here does

utilise all available information as well as correctly representing the data hierarchy and hence, assuming of course that the distributional approximations are sufficiently reasonable not to bias the analyses, should produce more precise estimates.

Adoption of this approach has other implications for results. Because analyses involve data from all surveys then estimates for any one survey are influenced by information from all others. A consequence of this is that estimates can not be made consistent across reporting occasions since the introduction of additional data with each new survey will produce updated estimates for previous surveys. Such updating is conceptually different to the inconsistencies currently present in the reporting from previous surveys. The latter arise from not fully utilising available information. In contrast it does not seem unreasonable for the acquisition of new information to be expected to produce small revisions to previous findings.

Choice of a suitable model is clearly an important part of ensuring estimates are accurate. While the examples tried so far appear to perform well in practise this may not be the case for all variables or for more complex models. The AR1 model whose results for Broad Habitat data are presented in Section 4 above has many desirable properties; it is stable, relatively quick to fit, has a small number of random parameters that does not increase with the number of surveys, and appears to give estimates of fixed effects that are robust to distributional mis-specification. When producing large numbers of analyses, as for Countryside Survey, it is clearly not possible to spend substantial amounts of time on model selection and checking. The need is for a standard model that can be applied in an automated manner to a large number of variables to produce robust results. The AR1 model appears to meet these criteria. If adopted for CS2007, however, it would be prudent to implement some form of check on performance and accuracy. One simple check would be to compare the revised stock and change estimates for surveys prior to CS2007 with previously published results. Discrepancies, as shown by Tables 3 and 4, should be small and comparable to the discrepancies between stock and change arising from the old methodology. An additional check would be to produce stock and change estimates for the new survey from both the old and new methods to check that differences are small.

In addition to model structure, defined by the chosen parameter set, the distributional assumptions of the model will affect estimation. For the models considered here, the effect of treating the distributions of random effects as normal when they are not does not appear to markedly affect fixed effect estimation. However for more complex models or for very non-normal distributions this may not be true. Examining plots of variable distributions prior to analysis would focus attention on those analyses requiring more detailed examination for validity.

For very non-normal data a standard approach to non-normality is to transform data prior to analysis. For CS, however, it is important to present results on the original scale of measurement. Analysis could be performed on some transformed scale but it would then be necessary to convert fitted parameter values to measures on the original scale of measurement. Such conversions almost always involve random as well as fixed effects and so are susceptible to the less precise estimation of these parameters.

Implementation of consistent estimation via modelling presents a number of technical challenges including the modification of existing analysis programs and macros. Computing time for models with many parameters can be long but appropriate model simplification seems to overcome this problem. A possible difficulty, that did not actually occur with the analyses presented here, is the inability to fit a specified

model to some bootstrap dataset because the random data selection process has produced a dataset with insufficient information on some parameters. The chance of this happening has increased recently with the trend for CS estimates to be required for smaller regions, for example Wales only reporting (Clarke *et al*., 2006) or the use of spatial masks to estimate priority habitats (Scott *et al*., 2007). This problem is not specific to model based analysis but affects all CS estimation methods using the bootstrap. With the modelling approach the software used for analysis can fit a partial model in such situations, resulting in biased results. It is important that such anomalies be detected and discarded.

16.7. Recommendations

The modelling methods described in Section 3 appear to provide a successful basis for consistently estimating stock and change from CS data. The following recommendations are therefore made:-

1) Methods for consistent estimation should be adopted for estimation of stock and change in CS2007 and any future surveys.

2) These should be based on repeated measures mixed models for simultaneous estimation of stock and change.

3) For quality assurance purposes, comparison of revised with old estimates should be undertaken and any gross differences investigated.

4) Preliminary plotting of variable distributions should be used to pinpoint variables “at risk” of biased results. The sensitivity of such variables to model assumptions can then be checked.

5) Programs and macros for consistent estimation should include error trapping to detect possible inadequate model fitting due to bootstrap dataset variation..

6) Further investigation of the possible extension of consistent estimation to more complex analyses should be made.

16.8. References

Barr, C.J., Bunce, R.G.H., Clarke, R.T. , Fuller, R.M., Furse, M.T., Gillespie, M.K., Groom, G.B., Hallam, C.J., Hornung, M., Howard, D.C., and Ness, M.J. (1993). *Countryside Survey 1990 Main Report*, DETR, London.

Clarke, R..T., Howard, D.C. and Scott, W.A. (2006). Sampling for Wales-Only Reporting. CS2007 Preparation Phase II product.

Dempster, A.P., Laird, N.M., and Rubin, D.B. (1977).
Maximum likelihood from incomplete data via the E.M. Algorithm.
J.R. Statist. Soc. B. 39: 1-38.

Efron, B. and Tibshirani, R.J. (1993). *An introduction to the bootstrap*. Chapman and Hall; London.

Haines-Young, R.H., C.J. Barr, H.I.J. Black, D.J. Briggs, R.G.H. Bunce, R.T. Clarke, A. Cooper, F.H. Dawson, L.G. Firbank, R.M. Fuller, M.T. Furse, M.K. Gillespie, R. Hill, M. Hornung, D.C. Howard, T. McCann, M.D. Morecroft, S. Petit, A.R.J. Sier, S.M. Smart, G.M. Smith, A.P. Stott, R.C. Stuart, R.C. & J.W. Watkins. (2000). *Accounting for nature: assessing habitats in the UK countryside*, DETR, London.

Scott, W.A. (2002).
Maximum likelihood estimation using the empirical Fisher information matrix.
J. Statistical Computation and Simulation. 72(8): 599-611.

Scott, W.A. (2007). Review of CS Sampling and Estimation procedures. CS2007 Preparation Phase II product.

Scott, W.A., Hallam, C.J. and Watkins, J. (1996). *Standard Errors for Land Classification Data.* Internal report for Module1a of the ECOFACT project.

Scott, W.A., Smart, S.M., Wright, S.J., Stuart, R.C., Scott, R.J. and Maskell, L. (2007). Scope for producing national estimates of stock and change for Priority Habitats. CS2007 Preparation Phase II product.

17. ANNEX G: Laboratory protocol

17.1. Sample information and reception

Black core (C) 15cm by 5cm
One "black" core soil sample will be collected from all X plots using the CS2007 Soil Sampling Protocol. These samples will be returned at the earliest opportunity to Jan Poskitt at CEH Lancaster, logged in and stored buy arrival date at 4^{o}C until processed.

Table G- 1 Information recorded for black soil core (C)

SQXNC	Square, plot type (X), rep no. (N) and core code (C) ready listed
C arrival	Date of black core sample arrival
C notes	Information about unusual black core samples

Table G-2 Example of recording sheets for soil cores

SQ	X	Code	Arrival date	Notes
Square number	X-plot number	Core code	Date of sample arrival and login	
732	1	C		
732	2	C		

Short white core (F) or (FF) 8cm by 4cm
Cores will be taken from all X-plots, using the CS2007 Soil Sampling Protocol. These samples are posted to CEH Lancaster as soon as possible. All samples will be logged in; samples coded FF will be immediately frozen in trays according to core type and square number, those coded F will be processed on day of arrival.

Table G- 3 Information recorded for short white soil cores (F) or (FF)

SQXNF	Square, plot type (X), rep no. (N) and core code (F or FF) ready listed
F/FF arrival	Date of arrival at Lancaster (short white core)
F/FF notes	Information about unusual short white core
F depth	Record if incomplete core
Start	Start date & time (for extraction)
Stop	Stop date & time (for extraction)
Rack no.	Storage rack number

Table G- 4 Example of recording sheets for short white soil core

SQ	X	Code	F depth	Start	Stop	Rack no.	Notes
Square number	X-plot number	Core code	Record if incomplete core	Extraction start date & time	Extraction stop date & time	Storage rack number	
732	1	F					
732	2	F					

Long grey core (PF) of (PFM) 15cm by 4cm

This core will be collected from all X plots using the CS2007 Soil Sampling Protocol. These samples will be posted to CEH Lancaster, logged in and immediately frozen in trays according to core type and square number. Cores labelled PFM will be used for microbial diversity and POPs. Cores labelled PF will be archived (by freezing). The core will be split in half lengthways. One half will be used for microbial diversity and the top 8cm of the other half will be used for POPs. A sub-sample of the 0-15cm mixed soil will be stored air-dried for repeat analyses for other analytes as required.

Table G-5 Information recorded for long grey soil core (P)

SQXNP	Square, plot type (X), rep no. (N) and core code (P) ready listed
PF/PFM arrival	Date of arrival at Lancaster (long grey core)
PF/PFM notes	Information about unusual long grey core

Table G-6 Example of recording sheets for long grey soil core

SQ	X	Code	Arrival date	Notes
Square number	X-plot number	Core code	Date of sample arrival and login	
732	1	PFM		
732	2	PFM		

Long white soil core (N) or (NF) 15cm by 4cm

Cores will be taken from all X-plots, using the CS2007 Soil Sampling Protocol. These samples are posted to CEH Bangor as soon as possible and stored at 4^{o}C until processed. Samples coded NF were stored at 4^{o}C at CEH Bangor before being transferring to CEH Lancaster and then frozen. After analysis, subsamples will be stored air-dried for repeat analyses for other analytes if required.

Table G-7 Information recorded for long white soil core (N) or (NF)

SQXNN	Square, plot type (X), rep no. (N) and core code (N or NF) ready listed
N/NF arrival	Date of arrival at Bangor (long white core)
N/NF notes	Information about unusual/missing long white cores
NF Freezer no.	Storage box number in freezer
NF Freezer date	Date sample frozen

Table G-8 Example of recording sheets for long white soil core (N or NF).

SQ	X	N	N or NF	Arrival date	Notes
732	X	1	NF		
732	X	2	N		

Sample reception

The following flow diagrams (Figure G-1 and G-8) illustrate the procedures for sample reception at Lancaster and Bangor.

Summary of sample numbers and codes

Table G-9 Summary of sample numbers (assuming all CS squares and X plots sampled).

Analyte	Core	Sample number
Bulk density	Black core (C)	3145
Loss on ignition	Black core (C)	3145
pH	Black core (C)	3145
Phosphorus	Black core (C)	1280
Mineralisable N	Long white core (N)	768
Total N & C	Black core (C)	1280
Faunal extraction	Short white core (F)	1280
Metals	Black core (C)	512
Microbial diversity[1]	Long grey core (P)	1280
PAH and PCB[2]	Long grey core (P)	256
Glomalin[2]	Black core (C)	200
Soil aggregation[2]	Black core (C)	1280

1. Desirable analyte (funded)
2. Desirable analyte (currently unfunded)

Table G- 10 Summary of codes used for cores during CS2007

Code	Description
C	Black core
CA	Archived air dried and sieved soil from core C
CG	Archived soil from core CA after grinding by NRM using metal equipment
CMG	Archived soil from core CA after grinding using non-metallic equipment suitable for metals analysis
F	Short white faunal core (from original 256 squares)
FF	Short white faunal core to freeze (from remaining squares)
FE	Faunal extract (from original 256 squares)
FA	Remaining soil from faunal core to be archived (from original 256 squares)
PFM	Long grey microbes and POP's core frozen (from original 256 squares)
PF	Long grey microbes and POP's core frozen (from remaining squares)
P15	P core cut to produce 15cm soil sample
P8	P core cut to produce an 8cm soil sample
N	Long white Nitrogen core (from 3 of 5 x-plots in original squares)
NF	Nitrogen core frozen (from remaining squares and x-plots)
NL	Leachate from the N core
NE	Extract from the N core for NH_4 and NO_3
NA	Archived air dried and sieved soil from N core after incubation

Core Analytes

Note: All samples at CEH Lancaster were processed in batches of 23 or 25 (23 + repeat + QC soil). The order of these samples was determined by the arrival of samples at the site, and the order was strictly maintained throughout the processing as all record sheets were listed in this order.

Software was developed at CEH Lancaster for the logging in of samples, and the generation of batches for processing. The documentation below reflects the usage of this software, but the lab methods themselves do not rely on access to the processing software itself.

17.2. Bulk Density

Health & Safety
The following should be read and understood

- The risk assessment associated with this project
- Code of Practice for CEH Science Support Facilities: Grinding Areas

Pay special attention to the following:

- Work at a fume hood with dust extraction system when handling dried soil samples
- Wear appropriate PPE at all times.

Equipment
Trolley, access to drying room and oven, balance, large aluminium foil trays, knives, core extruders, 25 numbered glass beakers, printed labels, recording sheets with column headings as in table above.

Table G- 11 Measurements needed for bulk density

SQ	Square number
X	X-plot number
Code	Core code
Depth top	depth of gap from top if core not full (cm)
Depth bottom	depth of gap from bottom if core not full (cm)
Tray 1	weight of foil tray (g)
wt 1	weight of tray + black pipe + soil (g)
wt 2	weight of tray + soil (g)
Depth	Depth of soil core when removed from pipe (cm)
Photo	Tick when photo taken
Depth O	Depth of top organic layer (cm)
wt 3	weight of tray + soil after taking pH sub-sample (cm)
wt 4	weight of tray + soil after drying at 25^{o}C (g)
stone wt	weight of tray + stones and un-sieved debris
wt 5	weight of sieved soil sample (g)
stone vol	stone volume (ml)

Core processing and removing sample for pH

1. Label a clean foil tray with the appropriate SQXN printed label
2. Weigh foil tray and record (**Foil tray weight**) on sheet. Two decimal places only.
3. Remove a black core from labelled bag
4. Measure distance between the soil surface and the top of the black pipe to nearest 5 mm and record (**Depth top**) on recording sheet. (Use a white plastic ruler cut off at 0 cm for accurate measurement)
5. Measure distance between the soil and the bottom of the black pipe to nearest 5 mm and record (**Depth bottom**) on recording sheet.
6. Clean soil from outside of black pipe and discard, do not include in sample.
7. Weigh pipe + soil + tray and record weight (**Foil tray weight + pipe + soil**)
8. Remove soil from core into tray, using core extruder. If absolutely

necessary, use a knife or spoon to loosen soil and dig out if it is compacted (but note we would like to be able to photograph the intact core – see 11)

9. Weigh soil + tray and record weight (**Foil tray weight + soil**)
10. Measure soil depth and record (**Depth of soil when removed from pipe**)
11. Take a photograph of the soil core, with a ruler and the SQXN code clearly visible (use the code from the bag) (**Photo**). Cut cores lengthways down the middle if necessary to make photograph clearer.
12. Measure and record the depth of the upper organic horizon (**Depth of top organic layer**)
13. Break up the soil, mix and take a sub-sample (10g) for pH avoiding stones and roots (See 17.4. pH).
14. Weigh soil + tray again, record weight (**weight of tray + soil after taking pH sub-sample**) against SQNX on recording sheet
15. Put to dry on rack in drying room at 25^{o}C.
16. Check soils daily and use a rubber mallet to break lumps of soil apart if required. Crumble damp soils apart whilst drying. These steps make soils easier to sieve later.
17. Leave until dry enough to sieve, (typically 2-4 days for most soils).
18. Wash, dry and store black plastic cores.

Sieving and moisture determination at 25^{o}C

Equipment
Dried soil samples, balance, 2mm stainless steel sieves, receiving bowls/sieve bases, wooden paddles, plastic storage tubs, balance, recording sheets.

Method
When soil samples in the drying room have dried sufficiently to sieve (

Figure G- 5):

1. Weigh air-dried soil + tray and record weigh (**weight of tray + soil after drying at 25℃**)
2. Sieve soil using 2mm stainless steel mesh sieve and wooden paddle taking care to retain all material which cannot be sieved.
3. Return all material which cannot be sieved to the foil tray and weigh (**weight of tray + stones and unsieved debris**).
4. Take a photograph of the stones and unsieved debris in the foil tray, making sure the sample code is visible.
5. Put tray and unsieved material to one side for use later (see A2.2.5 below)
6. Label a plastic storage tub with sample code and add soil
7. Seal tub immediately and store in labelled box, with LOI batch sheet (for later) and cover sheet.

NB: Moisture determination and LOI should be carried out on the air-dried samples as soon as possible (See Soil Organic matter and carbon content). Until then they should be sealed and stored closed.

Stone weight and volume

Equipment:
Balance, plastic cylinders, bucket for wet waste, access to sink.

1. Full a measuring cylinder with water to a known volume. Record (**volume of water before stones added (ml)**).
2. Add stones and unsieved debris to cylinder. If unsieved debris includes roots etc, put them in the cylinder and add the stones on top to weigh them down.
3. Record final volume of water (**volume of water after stones added (ml)**).
4. Record volume again, this time as initial volume before next sample added (**volume of water before stones added (ml).**
5. Add next sample to cylinder and repeat steps 2 and 3.
6. Calculation:
   BD = (soil dry weight-stone weight)/(soil volume-stone volume)

Barcode	SQ	X	Code	pH of sample in water	pH in CaCl2	Weight of tray + soil after drying at 25C	Weight of tray + stones and unsieved debris	Volume of water before stones added	Volume of water after stones added	Notes

Table G-12 Example of recording sheets for black core measurements

17.3. Soil Organic matter and carbon content

Health and Safety
The following should be read and understood

- Safety Guidance Note - SGN Safe Working in Analytical Laboratories
- The risk assessment associated with the SOP
- The risk assessment from the Project Leader or sampler relating to the sample (if present)

Sample preparation for determination by loss on ignition (LOI)
Soil should be pre-dried at 25ºC and sieved as preparation for analysis (see A2.2.4). Do not bring more than one or two batches of samples into the lab at once. Move samples into storage after analysis.

Data collection
Loss-on-ignition measurements were made using the CEH Lancaster Chemistry balance software, which records values electronically and automatically generates the %moisture and %loss-on-ignition values. See CEH Lancaster SOP-3503 for details of the software.

Measurements necessary for %moisture and %LOI are shown in Table G-13.

Table G-13 Measurements needed for moisture determination and Loss on Ignition

Sample ID
Crucible ID
Weight of dried & cooled crucible
Weight of crucible + 10g air-dried soil sample
Weight of crucible + soil dried at 105^{o}C
Weight of crucible + soil dried at 550^{o}C

Equipment
Electronic 4-decimal place balance, balance software, oven, muffle furnace, tongs for picking up crucibles. In-house reference material (QC samples).

Method for Loss on Ignition
(see Figure G- 6)

Dry %

1. Check the balance using a test weight and record in lab record book if you are the first person to use it on the day of use.
2. Dry 25 crucibles (23 samples, 1 repeat & 1 QC standard soil sample) in oven at 105ºC (± 5℃) for about 30-40 minutes, or in the mu ffle furnace at 375℃ for 10 min.
3. Cool the crucibles to room temperature in desiccator and weigh
4. Add 10±0.2 g air dry soil and weigh
5. Place the crucibles on an aluminium tray and place in the oven set at 105ºC (± 5℃) to dry the samples overnight.
6. Cool the crucible to room temperature in desiccator and weigh

Loss on ignition

1. Place the crucibles containing the oven-dried sample in muffle furnace.
2. Do not place the tray in the furnace. Space the crucibles apart and away from the wall and leave space for the inset on the inside of the door.
3. Ash the material for 16 hours at 375℃
4. Turn the muffle heater off and allow the muffle to cool to below 150℃.

NB. Samples that have cooled to room temperature in the muffle must be re-dried in the oven at 105ºC (±5ºC) for approx. 30 mins before cooling in a dessicator and weighing.

1. Transfer to a desiccator and when cool weigh
2. Retain sub-samples until calculation complete, then dispose.
3. Wash & dry crucibles for re-use
4. Store tubs containing air-dry soil samples at SSU.

Method for Total C

For method see Mineralisable and Total Nitrogen, and conductivity

17.4. pH

Health and Safety
The following should be read and understood

- The risk assessment associated with this project
- Code of Practice for CEH Science Support Facilities: Grinding Areas

Pay special attention to the following:

- Work at a fume hood with dust extraction system when handling dried soil samples
- Wear appropriate PPE at all times.

Equipment Required
1 l volumetric flask
50ml beakers
Glass rod
Waste container
25 ml dispenser
wash bottle
Hanna HI-111 pH/ORP Meter
pH reference materials
In-house reference material (QC samples)

Reagents Required

1. *Preparation of 0.125M $CaCl_2$ solution*
   Weigh out 27.4g of calcium chloride 6-hydrate 'Analar' grade, transfer to a 1 litre volumetric flask and make up to the mark with deionised water.
2. *pH 4, 7 & 10 buffers*
   Calibrate the pH meter using the 3 buffers according to the manufacturers' instructions.

Method
See also Figure G- 4 and
See Table G-12.

1. Samples are processed in batches of 25 – 23 samples plus repeat and reference soil. Beakers are labelled 1-25.
2. Weigh 10g of field moist soil into the correctly numbered beaker.
3. pH is determined on half a batch at a time (due to time constraints because of pH measurement times – see below).
4. To half the batch, add 25ml of deionised water using a dispenser. Stir the suspension thoroughly with a glass rod.
5. Allow to stand for 30 minutes.
6. Stir the suspension thoroughly before measurement of pH and measure pH in the settling suspension.
7. Leave the electrode in the suspension for at least 30 seconds.
8. Record a stable reading. It can be considered stable when over a period of 5 seconds it varies by not more than 0.02 pH unit.
9. Note the recorded values to two decimal places (**pH of sample in water**).
10. Add 2ml of 0.125M $CaCl_2$, which on dilution with the 25ml of water results in solution concentration of approximately 0.01M $CaCl_2$.

11. Stir thoroughly and allow to stand for 10 minutes. Stir thoroughly and remeasure as in 7 -9 above (**pH in $CaCl_2$**).

Check pH buffers regularly within a sample batch e.g. every 25 samples. If either buffer is more than 0.02 of a pH unit from the correct value, repeat calibration according to manufacturers' instructions.

Notes

1. Thoroughly rinse the pH and temperature probes between samples/ buffers with a stream of water from a deionised water wash bottle. Ensure the glass bulb of the pH probe is cleared of soil and be particularly thorough after probes have been immersed in pH buffers.
2. At high pH values, it may be more difficult to reach stabilization.
3. If there are large differences in pH between samples it may take longer to stabilise.
4. The quality of the electrode will effect the ability to reach stabilization.

See Table G-12 for an example of recording sheets for pH measurements.

Reference

Avery, B.W. and Bascomb, C.L. 1974. Soil Survey Laboratory Methods. Soil Survey Technical Monograph No. 6, Harpenden.

British Standard 1995. BS775: Section 3.2: ISO 10390:1994.

17.5. Phosphorus

Health and Safety
The following should be read and understood

- The risk assessment associated with this project
- Code of Practice for CEH Science Support Facilities: Grinding Areas

Pay special attention to the following:

- Work at a fume hood with dust extraction system when handling dried soil samples
- Wear appropriate PPE at all times.

Equipment required
Electronic 4dp balance
5 l volumetric flask
250 ml polythene bottles
end-over-end shaker

Reagents required
Preparation of Olsen's reagent (0.5 M $NaHCO_3$ buffered at pH 8.5)

1. Dissolve 210g $NaHCO_3$ in about 4 litres distilled water (do not dry $NaHCO_3$)
2. Dilute to 5 litres and mix well
3. Adjust to pH 8.5 with 1M NaOH

Method

1. Weight 5g air-dry sieved soil into a polythene bottle
2. Add ~ 0.5g Activated charcoal
3. Add 100ml extractant
4. Shake ½ hour on end-over-end shaker
5. Filter using no. 44 paper. Reject first few ml filtrate
6. Run two blanks with extractant only
7. Determine phosphorus concentration in extractant using a continuous flow analyser and the molybdenum-blue method (Allen et al., 1989)

Notes

1. Moistures should be determined at the time of weighing for subsequent correction to an oven-dry basis.
2. Do not extract oven-dry material.
3. If the molybdenum blue method is udes to determine phosphorus, the extract must be neutralised with 10% v/v sulphuric acid (01% nitrophenol in alchohol as indicator) before colour development in the molybdenum blue procedure

Reference
Allen, S.E. (ed), 1989. Chemical Analysis of Ecological Materials, 2nd Edition, Blackwell Scientific Publications, London.

17.6. Mineralisable and Total Nitrogen, and conductivity

1. Leaching, conductivity and incubation
2. KCl extraction, moisture and LOI measurements
3. Total N and C

The long white core (N) is first leached and incubated, then homogenised. Samples are taken for mineral N analysis and moisture / LOI analysis. Extra samples are taken for analysis by Prof Davey Jones, University of Wales, Bangor (UWB) Total N and C analysis is done on the long black core (C).

Leaching and incubation for mineralisable N

Equipment required
42 horizontal leaching racks
42 conical flasks or beakers marked at 150 mL
misting pump sprayer
waterproof bench in 4 ^{o}C room
10 ^{o}C Incubator

For preparing rain solution
Large funnel
2 L Beaker
20 L plastic bottle

Reagents Required

Preparation of stock solution

1. Weigh the following salts into separate clean 1000 ml beakers:
    a. 73.05 +/- 0.02 g (i.e. 73.03 to 73.07 g) GPR-grade NaCl
    b. 17.78 +/- 0.02 g (i.e. 17.76 to 17.8 g) GPR-grade CaCl2.6H20
    c. 2.47 +/- 0.02 g (i.e. 2.45 to 2.49 g) GPR-grade CaSO4.2H2O
    d. 42.05 +/- 0.02 g (i.e. 42.03 to 42.07 g) GPR-grade MgSO4.7H2O
2. Dissolve salts in several aliquots of deionised water, transfer to clean 10 L bottle using a large funnel.
3. Weigh 13.75 +/- 0.02 g (i.e. 13.73 to 13.77 g) of 'GPR' grade 98% sulphuric acid into a clean 1000 ml beaker. Add this to the 10 L bottle. **NB do not add water to concentrated sulphuric acid**. Dissolve traces of acid remaining in the beaker by quickly adding a large aliquot of deionised water, and add to the 10 L botte.
4. Record date on 10 L bottle.
5. Make up to 10 L with deionised water.
6. Shake thoroughly.

Preparation of "UK rain minus N" solution

1. Place exactly 10 ml of stock solution in a clean 10 L bottle.
2. Add 2 L deionised water and shake thoroughly
3. Make up to 10 L, mixing thoroughly.
4. Check conductivity is 34-36 □s cm^{-1} at 25 ^{o}C.
5. Decant into pump sprayer.
6. Approximately 20 L of UK rain -N solution is required to leach 42 samples. Fresh solution should be made up for each batch and a sample of the solution kept for QA purposes

Method

1. Cut core down two opposite sides, nearly through the plastic, using a bench saw and finishing with a sharp knife if necessary.
2. Lay each core on a tray on the original labelled plastic bag.
3. Remove core intact, using a blunt knife if necessary, and place horizontally into a leaching rack.
4. Photograph core together with labelled plastic bag
5. Record total, mineral and organic lengths.
6. Place a ~ 170 mL plastic beaker under the spout of the rack. Tuck the labelled plastic bag in next to the rack.
7. Once all 42 racks have been set up, begin misting with the artificial rain solution, applying only as much solution as will soak into the soil at any one time.
8. As leachate from each rack reaches approx. 150 mL, move this rack and beaker along the bench. Only apply a little more solution to these racks in subsequent mistings, to keep the surface moist but not cause further leaching.
9. Continue misting until all the soils have passed 150 mL. If after 4 days the core has still not passed 150 mL, proceed anyway.
10. Measure conductivity in the leachate beakers, about half way down, using the Jenway 4320 Conductivity Meter. Give a slight swirl with the probe to mix the leachate but avoid disturbing settled mud.
11. Record the exact volume leached by decanting into a measuring cylinder, then return to the original flask (this will also mix the leachate and suspend solids).
12. Remove a sample, unfiltered, into the 50 mL plastic bottle provided by UWB, leaving a small air gap to prevent splitting on freezing. Freeze at -18 ^{o}C. Discard the remaining leachate.
13. Connect the rack to a vacuum line, using a trap to stop water entering the line, and suck air through the soil in the rack for 20 seconds to remove excess water.
14. Place the rack, together with the original labelled bag, into a lightweight partially breathable plastic bag (PB121015 Clear Light Duty Polythene Bags 250 x 375 mm, Transpak.co.uk).
15. Place the batch in an incubator at 10 ^{o}C for 32 days.
16. Respiration measurements will be done on these racks after 21 days.

Extraction procedure for mineral N

Equipment required

250 ml Conical flasks
Plastic bottles (green marked)
Plastic funnels (green marked)
Cling-film
Whatman No. 44 filter papers
Flatbed shaker

For preparing KCl solution

2 L Beaker
10 L Plastic bottle
Large filter funnel
Wash-bottle
Dispenser (labelled KCl)

Reagents Required

Preparation of 1M KCl solution

1. Weigh-out 745.6g of 'GPR' grade potassium chloride in a clean 2000 mL beaker.
2. Dissolve salt in several aliquots of deionised water, transfer to clean 10 L bottle using a large funnel. Record date on 10 L bottle.
3. Make up to 10 L with deionised water.
4. Shake thoroughly and measure conductivity.
5. Transfer to dispenser bottle (eg "Zippette") labelled 1M KCl.
6. Ensure enough 1M KCL is made for both extraction and analysis (approximately 2 L for 60 sample analysis). Use a new KCL solution for each batch.

Method

1. After incubation, remove core from rack onto clean laboratory paper, and thoroughly homogenise together by hand.
2. Weigh 10 (±0.1) g of the moist soil into a 250ml conical flask
3. Add 100 mL of 1M KCl to the conical flask.
4. Shake for 1 hr on the flatbed shaker after covering with cling-film.
5. Pre-wash Whatman No. 44 filter papers by pouring through and discarding 50 mL of 1M KCl solution.
6. Filter the sample into a labelled plastic bottle and analyse for ammonium and nitrate. If analysis cannot be carried out immediately the extract can be frozen at -18 ^{o}C and analysed up to 3 months later.
7. For each batch, also freeze a bottle of the stock KCl solution for mixing standards and laboratory QC.
8. Ammonium-N and nitrate-N are analysed by autoanalyser (Indol-phenol blue and sulphanilamide/NEDA/Cd/Cu reduction methods respectively).
9. Place 10 g +/- 0.2 g moist soil into a crucible, and determine moisture content (105 ^{o}C for 72 hours) and loss-on-ignition (375 ^{o}C for 16 hours) for each core.
10. Place a further 50 mL (approx) of moist soil into a plastic container provided by UWB, for immediate analysis of microbial biomass by chloroform fumigation.
11. Store remaining soil moist until analyses completed, in case moisture / LOI need to be recalculated. Then air dry for archiving.

Total C & N (DUMAS)

This analysis is done on the long black core (C). The method used was CEH Lancaster UKAS accredited method SOP3102.

1. A representative portion of air dried soil is finely ground and dried to 105^{o}C for 3 hours prior to analysis.
2. Samples are accurately weighed into a tin combustion capsule, with weights of 15mg for peat and 15-60mg for mineral soil samples
3. Samples are analysed on an Elementar Vario-EL elemental analyser (Elementaranalysensysteme GmbH, Hanau, Germany). Where carbon is trapped on a column whilst nitrogen is carried to a detector. Carbon is then released from the trap and detected separately.
4. Calibration is performed infrequently, with daily runs being factorised to this calibration through the use of a certified standard (acetanilide).

5. Quality control is achieved by use of two in-house reference materials analysed with each batch of samples.

	SQ	X	N	N Rack No.	Photo (tick)	Depth of Gap (cm) Top	Depth of Gap (cm) Bottom	N Org Length (cm)	N Min Length (cm)	N Setup date	N Leachate Vol (mL)	N Incub Start Date	N Incub End Date	N Soil Roots Stones (g)	N Total Soil (g)	N notes
1	720	X	1	5	√	0.5	0	3	11.5	12/6/07	152	14/6/07	12/07/07	214.1	178.5	
2	720	X	3	6	√	0	1	14	0	12/6/07	147	14/6/07	12/07/07	196.3	190.2	

Table G-14 Lab sheet for mineralisable N

Table G-15 Lab sheet for mineralisable N (extraction)

	SQ	X	N	Wt of moist soil (g)	Flask No.	Bottle No.	Time on shaker	Time off shaker
1	720	x	1	10.095	5	55	10.15	11.15
2	720	x	3	9.903	6	56	10.15	11.15

Table G-16 Lab sheet for mineralisable N (moisture and LOI weights). Weights recorded on this sheet should include crucible (cruc) weights. Crucible weight to be extracted before data transferred to CS database.

	SQ	X	N	Cruc No.	Cruc weight (g)	Cruc + N fresh soil (g)	Cruc + soil after 105ºC (g)	Cruc + soil after 375ºC (g)
1	720	x	1	43	23.048	48.468	44.925	44.023
2	720	x	3	54	19.464	39.640	22.981	20.178

17.7. Faunal extraction

Health and Safety

- The risk assessment associated with the SOP
- Store Ethanol and all samples containing Ethanol in a flammables cupboard

Sample preparation

White faunal cores should be extracted on day of arrival. Soil cores should be as fresh as possible but if immediate extraction is not possible samples may be stored at 4^{o}C for two days. i.e. over a weekend period.

Data Collection

Record the following onto recording sheets and transfer data regularly to Excel spreadsheets.

Table G-17 Example of recording sheets for Faunal extraction

SQ	X	Code	F depth	Start	Stop	Rack no.	Notes
Square number	X-plot number	Core code	Record if incomplete core	Extraction start date & time	Extraction stop date & time	Storage rack number	
123	1	F					
123	2	F					

Dry Extraction

Setting up the Tullgren funnels

There are at least 6 banks of 12 Tullgren funnel units. Each unit consists of an aluminium funnel with a rubber seal to attach a collection bottle during the extraction period and an aluminium sieve on top. Each unit is positioned (through a hole) beneath a light bulb holder. Soil cores are placed in the sieve with the bulb turned on to extract soil fauna over an extended period; typically 5 days. A 40W bulb provides heat to drive the soil fauna from the soil core into collection bottles filled with preservative; usually 70% ethanol.

Equipment

- Core extractor, 70% Ethanol (30% deionised water, 70% Ethanol or use IMS)
- Tullgren units complete with clean mesh holders, clean funnels and rubber tubes, 40W bulbs, beakers, spray bottles, buckets
- Plastic Sterilin bottles with lids
- Paper labels with square and X-plot numbers for inside the bottles; printed on paper and cut as required
- Sticky labels with square and X-plot numbers to put on the bottle lids;
- A4 binder labelled "**Faunal Extraction"** and recording sheets with square and plot numbers (SQXN)

Pre-extraction

- Locate free positions and set up white boards by each bank of funnel units.
- Check that all light bulbs are working and set correctly and replace duds
- Check funnels units are complete and clean
- Fill collection bottles 3/4 full with 70% Ethanol

Method
On same day as arrival of cores (see also Figure G-3; Task 3 flow diagram)

1. Identify white cores to be extracted and print labels.
2. Record **SQXN** on recording sheet.
3. Put paper labels with SQXN into extraction tubes and sticky labels with SQXN on lids.
4. Gently screw tubes onto the rubber seals on the bottom of the funnels.
5. Take one of the white cores from the envelope; Please do this one by one, otherwise the cores may get mixed up!
6. Remove core from the bag onto a clean sheet of paper and remove caps from either end; take care not to lose soil from the core.
7. Measure distance between the soil surface and the top of the white pipe to nearest .5 cm and record (**depth**) on recording sheet. (Use a white plastic ruler cut off at 0 cm for accurate measurement)
8. As gently as possible, push the soil out of the core onto the paper.
9. Holding the paper to prevent soil escaping, carry it to the correct funnel (with labelled bottle) and place soil pieces on the mesh.
10. Gently pour any loose soil from the paper into the mesh unit.
11. Turn light on and leave extractor on for 5 full days (or until Monday am if 5 days fall on a weekend).
12. Record start date and time and stop date and time against funnel number on the whiteboard.
13. Record start date and time (**start**) and stop-date and time (**stop**) on recording sheets.
14. After 5 days remove tube from funnel, screw labelled lid tightly onto tube and store in numbered storage rack.
15. Put soil back into labelled plastic bag, seal and store in labelled boxes until all samples have been completed.
16. Place a beaker under the funnel and clean the funnel using a spray bottle of deionised water.
17. Wash and store cores and lids; soak in buckets, wash, allow to dry and store in bags at SSU.

Faunal Identification Protocol

Task
To identify and enumerate soil fauna to the taxonomic level one from soil invertebrate samples; first level based on Field Studies AIDGAP key, with extra sorting of Collembola and mites to **morphotype**; all samples separated into appropriate colour-coded vials.

Data Collection
Data of taxonomic name and number of individuals to be entered onto Excel spreadsheets. Record sheets to be copied and sent to Claire Wood, CEH Lancaster.

Procedure
1. Note down the details of the sample to be processed on record sheet along with the present day's date (not that on which the sample was extracted).
2. Remove approximately 1-2 ml of alcohol and soil/organic matter from the sample tube using a plastic disposable pipette.

3. Place the liquid and soil into a suitable container, either a watch glass or small Petri-dish.
4. Initially scan the sample on low magnification and remove the identified fauna to the appropriately coloured, size and type of tube (See list below).
5. Label the specimen with sample ID and date of identification.
6. Note group/species of organisms discovered in faunal ID book.
7. Each time an animal of the same species or group is added to the tube place a tally mark in the correct place in the faunal ID book.
8. Once the sample has been completed, tally marks should be added up and a total for that group/species noted in the *Total* number column of the faunal ID book.
9. After scanning the sample on a low magnification, it should be re-examined on a higher power to enable such organisms as small mites, pauropoda etc to be located.
10. Soil particles and organic debris should be moved around so that fauna hidden underneath can be located.
11. When the initial sub-sample has been thoroughly searched and the soil fauna removed, identified and enumerated both the alcohol and soil/organic matter should be returned to a new empty container containing a label giving full details of the sample and a note that the contents of the tube have been examined. If the label does not indicate that the tube contains checked material it may become confused with the tube bearing the same label from which it was withdrawn!
12. Next another sub-set of the sample should be removed and examined following steps 2-11.
13. In addition, for the purpose of quality control, another member of staff should check every tenth sample for the first 500 samples. Fauna will then be identified and enumerated by both members of staff to ensure that the identification and counting procedures employed be both individuals produces comparable results. This process will be repeated at a reduced rate as the identifications proceeded (5 percent for the next 300 down to 2 percent for the final 252).
14. Data from the record book should be entered into Excel as soon as is convenient.

Species	Tube description	Lid colour
Oligochaeta	Small screw top bottle	yellow
Diptera adults and larvae	“”	green
Coleoptera adults and larvae	“”	red
Acari	“”	purple
Araneae	“”	purple
Pulmonata	“”	orange
Isopoda	“”	orange
Lepidoptera adults and larvae	“”	blue
Psocoptera	“”	white
Copepods	“”	black
Opiliones	“”	brown
Pseudoscorpions	“”	brown
Collembola – Entomobryoidea	0.5 ml	Green
Collembola – Poduroidea	0.5 ml	Purple
Collembola – Sminthuridae & Neelidae	0.5 ml	Orange
Hemiptera	0.5 ml	Blue
Chilopoda or Diplopoda	0.5 ml	Pink
Hymenoptera	0.5 ml	Yellow
Symphyla	0.2 ml	Clear
Nematoda	0.2 ml	Blue
Pauropoda or Protura	0.2 ml	Green
Thysanoptera	0.2 ml	Pink
Diplura or Thysanura	0.2 ml	Yellow

17.8. Metals

Analysis method for ICP-OES used in CS 2000
Analysis will be carried out on air-dried ball-milled material. 3g of dried milled material is needed for analysis. The total sample required is between 10g – 20g of wet weight sample.

Pre-treatment

- Soils should be ball-milled or ground fine with a mortar and pestle.
- Soak/reflux all glassware/plasticware in HNO_3/HCl or 12.5% HNO_3 before use. N.B condensers could have previously been used for Tinsley-C ($K_2Cr_2O_4$).

Digestion
The below method is adapted from The Standing Committee of Analysts 1986. Methods for the examination of waters and associated materials: Methods for the determination of metals in soils, sediments and sewage sludge and plants by the hydrochloric-nitric acid digestion, Method 'A'. 35pp. Her Majesty's Stationery Office: London.

Reagents

a) aqua regia: 38.5% ultra-pure water
39% 'Primar' HCl (1.18)
22.5% 'Primar' HNO_3 (1.42)
b) 12.5% HNO_3: 875 ml ultra-pure water + 125 ml 'Primar' HNO_3 (1.42).

- Prepare aqua regia mixture immediately prior to use – do not store except as a washing/refluxing solution.
- Prepare only the amount required i.e. 1 batch (25 samples, 2 refs, 2 blanks, 1 duplicate) requires 900 ml $\rightarrow$ make 1000 ml.
- For all reagents, use only glassware designated for 'trace' work.

Equipment
12 x100ml vol flasks
12 x plastic funnels
30 x 250 ml flat bottomed flasks (fbf)
12 x reflux condensers
30 ml bottle top dispenser

Procedure for aqua regia digestion

1. Weigh 3 g finely ground sample into 250 ml fbf, using M (MAFF) heading for the balance record.
2. Add 30 ml aqua regia mixture and carefully swirl flasks to ensure the entire sample is wetted.
3. Leave covered in fume-cupboard overnight (at least 16 hours).

4. Start refluxing using water-cooled condensers on electromantles on setting 4, after any initial reaction increase to 7. Reflux for 2 hours and then cool fbf until cool to touch (quicker if fbf is lifted out of heating mantle socket)
5. Rinse inside surfaces of condensers into fbf with 12.5% HNO_3 from wash bottle.
6. Filter through 541 filter papers into 100ml volumetric flasks within fume-cupboard. Rinse out fbf x3 with 12.5% HNO_3 from wash bottle, make up to volume, transfer to scintillation vials.
7. Rinse down from top edge of filter paper with 12.5% HNO_3 (x3 if possible).
8. Wash fbfs in hot soapy water using brush or sanding to dislodge 'caked on' soil.
9. Rinse in deionised water, dry quickly in hot oven so they are ready to weigh on next batch to stand in aqua regia overnight.

Procedure for aqua regia inductively coupled plasma-optical emission spectrometer analysis

1. Samples to be analysed for zinc, vanadium, lead, nickel, copper, chromium and cadmium by Centre for Ecology and Hydrology (CEH) Lancaster using Inductively Coupled Plasma-Optical Emission Spectrometers (ICP-OES) using the technical method and operational limits set out below.
2. A minimum of one blank, and one certified reference material (CRM), and a local reference material (LRM) to be analysed with each batch of 20 samples.
3. Results of LRM (SR3) to be plotted on a Shewhart control charts as an AQC sample. The mean should be set as the current mean of analyses, and warning and action limits to be set at 2sd and 3sd of current analyses. Current analyses should include data obtained in the performance testing exercise.
4. The 2sd warning limit should not exceed the analytical target level for precision of 10% of the mean value.
5. Results obtained for the CRM (141R) should be plotted against the certified reference value with limits set at the analytical target for bias of + or – 10%. (Results for the CRM obtained during the performance exercise should be plotted on this chart.)

-

Table G-18. Brief ICP-OES technical details.

Element	Wavelength	Detection limit (ppb in solution)	Detection limit (mg/kg)
Cd	228.802	1	0.02
Cr	357.869	5	0.1
Cu	324.754	2	0.04
Ni	341.476	10	0.2
Pb	220.282	10	0.2
V	292.402	6	0.1
Zn	213.856	12	0.2

n.b. Values below the detection limits are stored as zero in the database.

Task 1: Lancaster

CS2007 LOGGING IN ALL CORES
Each day check for core arrival at 11:30am
Take envelopes to A14, follow steps 1-3 on the same day

Step 1: Log in all cores	Record
Open logging in software	
Remove cores from envelopes	
Scan barcodes of all samples to register them in database	Barcode
Print out log in sheet and put in folder	

↓

Step 2: Fauna sample (F)
Take all short white cores coded F to the extraction lab and put them on to extract (Task 2)

↓

Step 3: FF, PF and PFM cores
Take all cores coded FF, PF or PFM to the walk-in freezer and put them in the correct trays corresponding to their code and square number

↓

Step 4: C cores
Put all C cores in a tray labelled with today's date and put them in the cold room on a shelf next to the previous day's C core arrivals

Figure G- 1. Flow diagram for logging in cores at Lancaster (Task 1).

Task 2: Lancaster

CS2007
FAUNAL EXTRACTION

Step 1: Preparation in advance
Locate free positions and set up Tullgren unit with clean funnels
Check and replace bulbs if necessary
Prepare printed sheets of SQXNF labels (one paper label for collection tube & sticky label for lid)

Step 2: Preparation immediately before handling soil samples
Have Faunal Extraction folder with spreadsheets for required square & plot numbers ready
Print & cut SQXNF paper label and place in collecting bottle
Fill collection bottles to ¾ full with 70% ethanol
Gently insert collecting bottle into rubber seal on each funnel unit
Stick SQXNF label on lid
Place lid beside correct extraction bottle
On white board record start & stop date against SQXNF

Step 3: Faunal Extraction	Record
Remove core from sealed bag, remove end caps	
Record depth of core, especially note if core is not complete (8 cm) *by measuring space at both ends*	F depth
Place mesh holder on a clean sheet of paper	
Carefully extract soil from plastic core into mesh holder using core extractor if necessary	
Carry to extractor unit, place holder onto funnel and pour lose soil from paper onto mesh.	
Place each bag next to funnel	
When all cores are in place, turn on extractor and leave for 5 days	
Turn on lights and leave on for 5 days	
Record start & stop date against SQXNF in folder	Start time Stop time

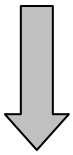
Step 4: Storing Faunal Samples	
After 5 days, remove tube screw on lid and store	
Record number of rack against SQXN in Faunal Extraction folder. Label SQXN + FE and bar code	Rack no.
Empty soil back into labelled bag and store in cardboard box.	
Clean funnels and replace; wash core & lids & store	

Figure G- 2. Flow diagram for faunal extraction at Lancaster (Task 2).

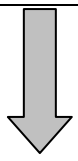
Task 3: Lancaster

CS2007 SOIL PROCESSING AND DRYING
Process samples in batches of 23. Only begin as many samples as can be processed in one day

Step 1: Fresh soil processing	Record:
Find unique code on recording sheet	SQXNC
Remove sample from bag, measure space from top and bottom of core	Depth top Depth bottom
Weigh foil tray	Foil tray weight
Weigh black core + soil + tray	Foil tray weight + pipe + soil
Remove soil from core, weigh tray+soil	Foil tray weight + soil
Take photograph with labelled bag (using digital camera)	Photo (tick)
Record depth of top organic layer	Depth of top organic layer
Sub-sample 10g into beaker & set aside for immediate pH (Task 5)	



Step 2: Dry soil sample	Record:
Weigh soil + tray again and record	wt 3
Dry soil in tray at 25℃, approx. 2-4 days, making sure to break up the lumps every day	



Step 3
Once sample is dry proceed to Task 6

Figure G- 3. Flow diagram for processing black core (Task 4)

Task 4: Lancaster

CS2007 pH MEASUREMENT
Only begin as many samples as can be processed in one day

Step 1 : Preparation in advance
Prepare 0.125M $CaCl_2$ 'working' solution (27.4 g in 1 L deionised water)

Step 2: pH measurement (25 samples)	Record:
Set up meter and calibrate with three buffer solutions, pH 4, 7 & 10	
Take 25 beakers with samples (see Task 4). Each batch of 25 should include 1 repeat and 1 reference soil. Process samples in half-batches. Add 25ml deionised water.	
Leave to stand for 30 minutes	
Stir and then measure pH in settling suspension	
Immerse electrode into supernatant leave for 30 seconds and record stable reading (varies by not more then 0.02 pH unit over 5 seconds	pH of sample in water
Store electrode in buffer solution when not in use	
Pipette 2 ml $CaCl_2$ solution into beaker	
Leave for 10 minutes and record pH	pH in CaCl2
When batch is complete, wash glassware for next batch	

Figure G- 4. Flow diagram for pH measurement at Lancaster (Task 5).

Task 5: Lancaster

CS2007 SIEVING SOIL, STONE WEIGHT & VOLUME
Take samples from drying room

Step 1: Air dry soil sieving and stones	Record:
When dry, weigh soil + tray and record	Weight of tray + soil after drying at 25℃
Sieve soil & retain stones and un-sieved material in labelled tray.	
Take photograph of unsieved material	

Step 2: Stone weight & volume	Record:
Weigh stones and un-sieved material + tray, record	Weight of tray + stones and unsieved debris
Measure volume of water before addition of unsieved material	Volume of water before stones added
Add unsieved material and measure volume of water again	Volume of water after stones added

Step 3: Store in labelled boxes	Record:
Add bar code to storage tub	
Put sieved soil in tub	
Seal tub and store	

Step 4
Proceed to loss on ignition analysis Task 7

Figure G- 5. Flow diagram for sieving soil, stone weight and volume (Task 6)

Task 6: Lancaster

CS2007 LOSS ON IGNITION
To be carried out on stored, air dried soil samples. Transport from Support Unit to Environmental Chemistry Lab, Main Building in batches for processing

Loss on ignition	Record:
Dry 25 labelled crucibles at 105℃, 30 mins	
Cool (30 mins) in desiccator, weigh and record	Weight crucible
Sub-sample from sealed tub into crucible (approx. 1g)	
Weigh crucible + sub-sample and record	Weight crucible + air-dry soil
Dry at 105°C for 16 hrs	
Cool (30 mins) in desiccator, weigh soil + crucible	Weight crucible + oven-dry soil
Dry at in muffle oven at 375°C for 16 hrs	
Cool (30 mins) in desiccator, weigh soil + crucible	Weight crucible + ash

Figure G- 6. Flow diagram for Loss on ignition analysis (Task 7)

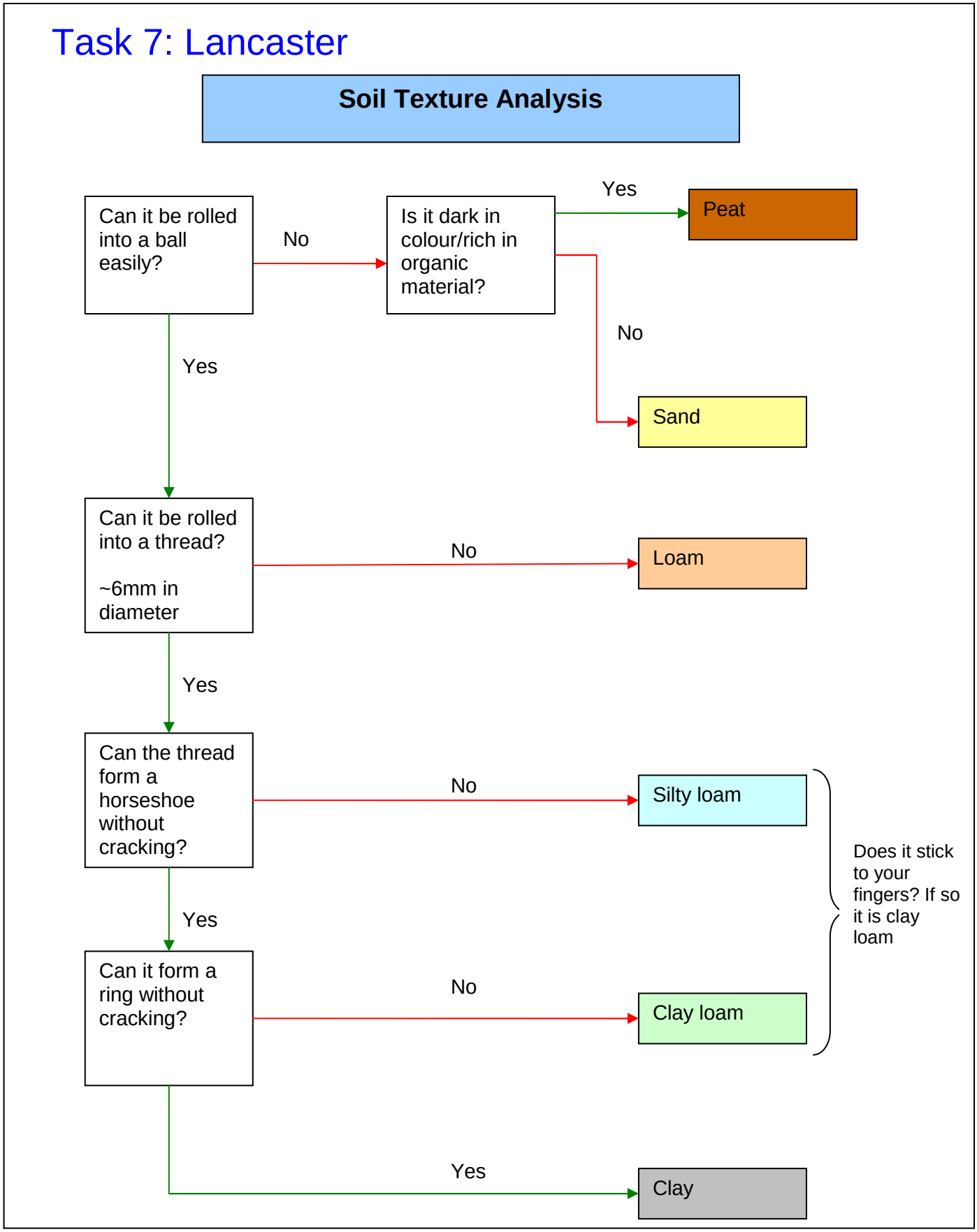


Figure G- 7. Flow diagram for soil texture

Task 1: Bangor

CS2007 LOGGING IN LONG WHITE CORES
Each day check for core arrival at 10:30am
Follow step 1 on the same day

Step 1: Log in long white cores N / NF (15cm)	Record
1.1 Record on LOG IN sheets against plot number	SQXN
1.2 Record date of arrival at Bangor	N_arrival
1.3 Remove cores from envelope	
1.4 Record information about unusual or missing samples	N_notes
1.5 Check logsheet to see if core is “N” (to be run) or “NF” (to be frozen) – this has already been randomly assigned. If there are only 2 “N” cores present, choose one of the “NF” cores at random. If only 1 or 0 “N” cores is present, use both of the “NF” cores	If any cores have changed from “NF” to “N”, change in N_or_NF, and add a note to N_notes
1.6 Store the 3 N cores in 4 ^{o}C cold room, in the appropriate labelled box (Monday_1 to Friday_2)	
1.7 Place the two NF cores in the courier box ready for weekly transfer to Lancaster (Annie to arrange). Store at 4 ^{o}C.	

Figure G-8 Flow diagram for long white core reception at Bangor.

Task 2: Bangor

CS2007
MINERALISABLE NITROGEN

Step 1: Selecting and opening long white cores N (15cm)	Record
1.1 At the start of each week select a batch of 42 N cores (NOT NF) on the basis of first-in first-out. If more come in during the week repeat as required in batches of 42.	
1.2 Saw N cores lengthwise down opposite sides, nearly through the plastic	
1.3 Lay each core on a tray on the original labelled plastic bag	

Step 2: Set up and leach mineralisable N racks	Record
2.1 Place exactly 10 ml of stock solution in a clean 10 L bottle. Add 2 L deionised water and shake thoroughly. Make up to 10 L, mixing thoroughly. Decant into pump sprayer. Approximately 50 L of solution is required to leach 42 samples. Use fresh solution for each batch. Take a 25 ml sample of the solution labelled with batch date for laboratory QC. Freeze sample at -18 ^{o}C.	
2.2 Set up 42 racks in the cold room (4 ^{o}C), each draining into a beaker marked at 150 mL	
2.3 Flip out core into rack, record SQXNN (e.g. 732X2N) and record rack and beaker number (should be the same number) on Lab Sheet 1a + 1b	**SQXNN** **Rack number**
2.4 Photograph core including sample bag with label showing	
2.5 Record the gap at the top and bottom of the core, and the length of organic and mineral horizon. If plant material or litter have been included (e.g. *Sphagnum*), describe and record the length of this material in N_notes	**N_Gap** **N_Org_Length** **N_Min_Length** **N_Notes**
2.6 Place the beaker under the rack and tuck in the labeled plastic bag alongside.	
2.7 Record set up date. Use pump sprayer to mist all the racks with leaching solution. Avoid lateral runoff, by applying only as much solution as will soak into the soil at any one time. When approx 150 mL leachate has been collected, move this rack and beaker to one side and apply only sufficient solution to keep the surface moist.	**N_Setup_date**
2.8 After 4 days, process all racks. Measure and record conductivity in □S cm^{-1}. Then record the exact leachate volume using a measuring cylinder, and mix by returning to the collection beaker.	**N_Conductivity** **NL_Leachate_Vol** (to 1 mL accuracy)
2.9 Remove a 40 ml sample, unfiltered, into a bottle provided by UWB. Freeze this sample at -18 ^{o}C and discard remaining leachate	
2.10 Apply suction to the rack spout for 20 seconds to drain off excess liquid (this need not be collected). Ensure labelled plastic bag is kept with rack.	

Step 3: Incubate	Record
3.1 Do not begin incubations until all 42 racks have finished leaching	
3.2 Place both the rack and the original labelled bag in a large thin plastic bag and seal the end with tape	
3.3 Place racks in incubator at 10 ^{o}C for 32 days. Randomise cores within the incubator. Record date	**N_Incub_Start_Date**
3.4 Respiration measurements will be done on these racks after 21 days (Rob Mills)	

Step 4: Measure mineralised N	Record
4.1 Remove racks from incubator, record date	**N_Incub_End_Date**
4.2 Remove plastic bag, remove soil from rack. Weigh all soil and record	**N_Soil_Roots_Stones** (to 0.1 g accuracy)
4.3 Spend 4 minutes breaking peds and removing roots and stones and discard stones and roots	
4.4 Spend 1 minute thoroughly mixing the soil and then weigh soil	**N_Tot_Soil** (to 0.1 g accuracy)
4.5 Weigh 10 g +/- 0.1 g fresh soil into conical flask. Record flask number (on Lab Sheet 2). Add 100 ml 1M GPR KCl solution. Use fresh solution for each batch.	**N_Soil_Extr** (to 0.001 g accuracy) **NE_Flask_Number**
4.6 Shake for 1 hour, recording time on and time off the shaker, filter using #44 Watmans. Record bottle number	**NE_Bottle_Number**
4.6 Decant 25 ml into a plastic container and freeze at -18 ^{o}C for later analysis by Davey Jones / UWB	
4.7 For each batch, also freeze a bottle of the stock KCl solution for mixing standards and laboratory QC.	
4.8 Weigh 10g of fresh soil into a pre weighed crucible (cruc) (Lab Sheet 3)	**Cruc_wt** **N_Fresh+Cruc** (to 0.001 g accuracy)
4.9 Dry soil at 105 ^{o}C for 72 hours and record weight	**N_Soil_105+Cruc** (to 0.001 g accuracy)
4.10 Oxidise soil at 375 ^{o}C for 16 hours and record weight	**N_Soil_375+Cruc** (to 0.001 g accuracy)
4.11 Place a further 50 mL (approx) of moist soil into a plastic container provided by UWB, for immediate analysis of microbial biomass by chloroform fumigation	
4.12 Air-dry all remaining soil for two weeks. When dry place in labelled plastic bag (732X2NA) for archiving. Send to Lancaster.	
4.13 Analyse filtrate for NH_4-N & NO_3-N and record as mg N L^{-1} together with blanks and standard soils	**NE_NH4N** **NE_NO3N**
4.14 Freeze extract once analysed	

Figure G-9. Flow diagram for Mineralisable N analysis

Desirable Analytes (Funded)

17.9. Microbial Diversity

Microbial diversity analysis will be carried out on each of the collected grey cores. Frozen soil cores (PF) will be removed from the plastic tube and sliced in half vertically. One half will be taken to Oxford for further processing whilst the remainder will be returned to the freezer at Lancaster.

At Oxford each core will be mechanically homogenised and aliquots removed for nucleic acid extraction, moisture determination, and LOI analyses. Moisture contents and LOI will be conducted as above. Procedures for nucleic acid extraction follow Griffiths *et al*. (2000), and are detailed below:

Reagents

- 120 mM K2PO4 buffer pH 8.0 with 5% CTAB (Hexadecyltrimethylammonium Bromide).
- phenol:chloroform:isoamly alcohol, 25:24:1
- chloroform:isoamly alcohol, 24:1
- 1.6M NaCl, 30 % PEG 6000
- 70% Ethanol solution (ice cold)

Procedure

1. Add 0.25 g soil sample to beat beating tube (Bio 101, Inc multimix 2, 6560-215)
2. Add 0.5 ml 120 mM K2PO4 buffer pH 8.0 with 5% CTAB.
3. Add 0.5 ml of phe:chl:iaa.
4. Agitate samples at 5500 rpm for 30 seconds in Fast Prep machine
5. Spin 14K, 4 oC, 5min.
6. Remove supernatant in to clean tube.
7. Wash with 0.5 ml chl:iaa.
8. Precipitate with PEG (1.6 M NaCl, 30 % PEG 6000) (if DNA looks dirty, brown from Humics) X2 volumes for at least 2 hours at room temp or over night at 4 oC.
9. Spin 14K, 4 oC, 10 min.
10. Wash 70 % EtOH
11. Air dry pellet
12. Resuspend in 100ul water.

Extracted nucleic acids will be labelled and stored at -70oC before subsequent processing for diversity analyses.

For further information on Countryside Survey see *www.countrysidesurvey.org.uk*

Or contact:
Countryside Survey Project Office,
Centre for Ecology and Hydrology,
Lancaster Environment Centre,
Library Avenue, Bailrigg, Lancaster LA1 4AP

Telephone: 01524 595811; Email: *countrysidesurvey@ceh.ac.uk*

Countryside Survey in 2007 was funded by a partnership of government-funded bodies led by the Natural Environment Research Council (NERC) and the Department for Environment, Food and Rural Affairs (Defra).