Soil Sampling For Long-term Monitoring Network Sites in England

1 Preparation

1.1.1 Sites

Between 6 and 9 sites are sampled each year. Soils sampling will be carried out either contractors or by Natural England staff. All sites are National Nature Reserves. Within each site, soil sampling and field assessments will be undertaken in areas representing one of the dominant semi-natural vegetation types.

The site managers must be contacted directly to make final arrangements to access the sites.

Most sites will have had vegetation surveys comprising assessments at up to 50 2 m by 2 m permanent plots (completed by Natural England staff or under different contracts). At each such vegetation plot a permanent "feno" marker will be placed at the SW corner of the vegetation plot. In some sites, the vegetation survey may not yet have been completed, and if this is the case surveyors will be provided with yellow "feno" markers to mark the location of the vegetation plot, to be surveyed later (see Figure 3).

At each site, aerial photographs and vegetation information from the sites will be used by Natural England to identify 5 vegetation survey plots which will be co-located with soil sampling plots, and additional plots to use if these prove unsuitable. These will be supplied to contractors once appointed.

1.1.2 Desk study

Before setting out to carry out fieldwork, surveyors are asked to develop a risk assessment for site-specific and general hazards and problems which might delay or prevent the soil sampling, or subsequent analysis, and present mitigation strategies for these risks. Surveyors are also asked to liaise with local contacts (contact details provided above) to inform this risk assessment.

Surveyors carrying out the fieldwork will be provided with the following field protocol. This will be discussed with the Natural England project manager and if any changes from the protocol are agreed, a revised version of the protocol will be produced by the contractor describing these changes. This will enable identification of any misunderstandings etc. in the sampling procedures.

Both the risk assessment and protocol documents should be agreed with Natural England before fieldwork commences. In the event of site risks or problems preventing sampling on a site, alternative sites will be identified by Natural England if possible.

The sampling period is constrained to support compatibility between surveys. **The field work should be carried out between 16th September and 16th October,** although this window can be extended with agreement by Natural England in the event of extreme weather conditions or any other unexpected events. Soil sampling should not be carried out following periods of drought, on flooded sites or in extreme cold or snowfall. In this event sampling should be rescheduled to a time when weather conditions are suitable.

1.1.3 Equipment

Surveyors are required to assemble the following field equipment

Equipment List for all sites
GPS with charger, preloaded with all site sample points
Compass – ideally sighted compass
Plant ID guides if required
Plastic sheet for soil examination
Self retracting tape measure
Knife - with long, flexible, sharp serrated blade and safety sleeve.
Munsell soil colour charts and soil texturing key
Narrow blade trowel – graduated or marked to 15 cm (such as http://www.rutlands.co.uk/sp++gd1027)
Spade
Plastic tube corers 15cm by 5cm diameter (40 per site)
Plastic tube corers 15cm by 4cm diameter (5 per site)
Plastic tube corer 8cm by 4 cm diameter with fine gauze cover (20 per site)
Scissors
Pruning/plasterboard saw and safety sleeve – for cutting into root mats and fibrous peat.
Secateurs
Pliers
Regular Trowel
Clingfilm for wrapping cores
Elastic Bands
Large sealable plastic bags for peat samples
Large plastic bags to bag multiple plot samples
Ziplock soil sample bags (medium to hold single cores)
Thick and thin marker pens
Waterproof paper
Digital camera with flash, batteries and memory card
2 x 50 m measuring tapes
2 x 20m measuring tapes
16 Bamboo canes with safety tape on the ends (eye protection) for marking out corners of 2 m
4 x ranging poles or similar for marking corners of sampling plots
Metre rule with large markings
Mallet
Driving blocks
Cool boxes for sample storage (a 42 L box should be sufficient for all samples from a single mineral soil site, but peaty sites may require additional cool storage) with freezer blocks
Field first aid kit
Nitrile Gloves
Heavy duty gloves
Wet Wipes and paper rolls
Propelling Pencils
Batteries AA and AAA as spares
Rucksacks
Dutch (Edelmann) auger
Feno marker (red?) for SW corner of soils plot and yellow for any unmarked vegetation plots. (use metal-capped marker, flush to ground for mown sites)
Metal detector (only where metal capped flush markers have been used on mown sites)
Weather writer / clip board - for writing
Bungee ropes for holding field equipment together
Field survey sheets on waterproof paper (see below).
Field survey protocol manual.
Map / aerial photo showing soil sampling plot locations and locations of reserve plots

For the peaty soil sites, the following equipment will also be needed:

Peat depth rods with threaded ends
Russian (Wardenaar) corer or box corer (to be agreed with project manager). Natural England has 2 russian corers that may be borrowed.
Additional sample bags

1.1.3.1 Health and Safety

Field teams should inform the project manager of planned travelling arrangements and keep the project manager informed of any changes to these plans. All field workers should make arrangements for pre- and post-field work check-in. All field staff involved should have sufficient personal protective equipment, and this should be informed by the site risk assessment, habitat and location of each site. The following should be included.

- Mobile phone with emergency and project manager numbers entered as speed dial
- Compass
- Torches (head or other)
- Whistle
- Waterproofs suitable for extended outdoor inclement weather conditions
- Robust walking boots or steel-top cap wellingtons
- Field gloves and hat
- Outdoor clothing appropriate for sampling locations in winter conditions (i.e. plan for the worst weather).
- Sufficient dry clothing to replace wet clothing as necessary
- Disinfectant for any areas of biosecurity concern.

It is recommended that the teams undertaking field assessment and sampling consist of at least 2 people, which should enable the work at a single site to be completed within 1.5 days working in a pairs. Field staff should determine time required to access / leave sites as well as field sampling duration within the risk assessment. Please take note that fieldwork may require driving fairly long distances to get to and back from the sites. Each field team should discuss and agree reasonable travelling arrangements which ensure that field staff share the driving and sufficient rest periods are taken. All field staff should follow their in-house risk assessment recommendations regarding fieldwork and driving.

2 Fieldwork

2.1.1 Layout of soil sampling plots and subplots

2.1.1.1 Soil sampling plots

Five sampling plots will be surveyed, these being SW of 5 vegetation survey plots, which are marked by a feno marker or post in their SW coner, and will be located using a GPS. Each soil sampling plot will be 20m by 20m square and is further subdivided into 100 2m by 2m sub plots, four of which are sampled at each sampling plot for each sampling visit. A schematic showing the suggested sampling scheme for one site/vegetation type is shown in Figure 2.

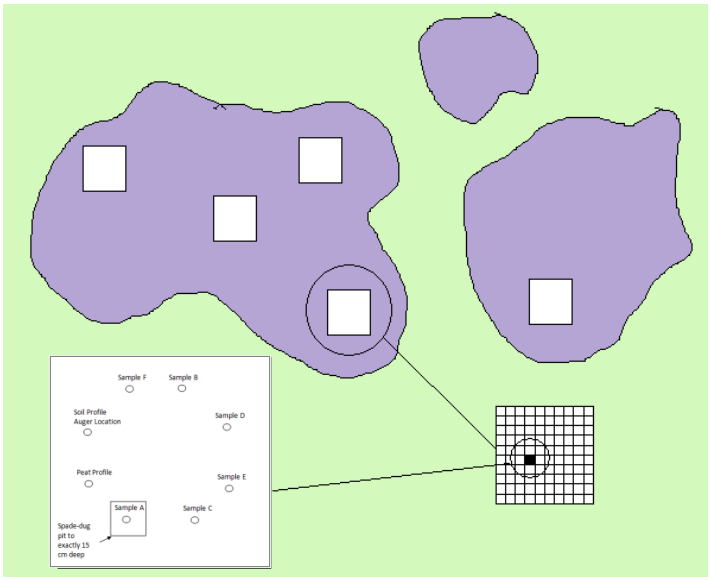


Figure 2. Schema showing arrangement of field sampling plots and subplots, where purple areas represent the homogeneous vegetation to be sampled, white squares represent 20 m by 20 m soil sampling plots, divided into 100 sub plots (bottom right), and showing how the soil sampling relates to a single sub-plot (bottom left).

Contractors will be provided with a list of vegetation plot numbers with appropriate vegetation cover, along with reserve plot numbers in case it is impossible or unsuitable to sample the pre-selected soil sampling plots. There are site maps for each field site which show the locations of the vegetation plots

Figure 3 shows how soil sampling plots relate to the vegetation plots. The Feno marker (Figure 4) is located at the south west corner of the 2 m by 2 m vegetation survey quadrat which is shown in the centre of this figure. This quadrat is surrounded by a larger 10m by 10m plot which is used when surveying in woodland. The five soil sampling plots will be located adjacent to existing 10m by 10m vegetation sampling plots but must be separate so that soil sampling does not affect the vegetation in the those plots.

In some sites, the vegetation survey may not been completed and plots may not yet have permanent markers in place. These plots should be located with a reasonably high precision GPS and marked with the Feno marker which will be provided. This will mark the SW corner of the 2m vegetation quadrat and the soil sampling plot should then be placed as described.

2.1.1.2 Plot and subplot layout protocol

Four subplots have been selected randomly for sampling at for all sampling plots on all sites for this baseline sampling period.

The subplots chosen for baseline sampling in 2013 are plots 2, 38, 64 and 100.

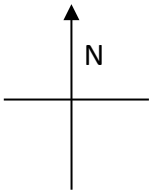
These subplots will be excluded from future sampling of subplots and a new set of sub-plots will be randomly selected for future monitoring re-visits. See Figure 5 below for details.

Figure 5 : Numbering and location of sub-plots within each soil sampling plot for baseline sampling

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

Random number allocation

2
38
64
100



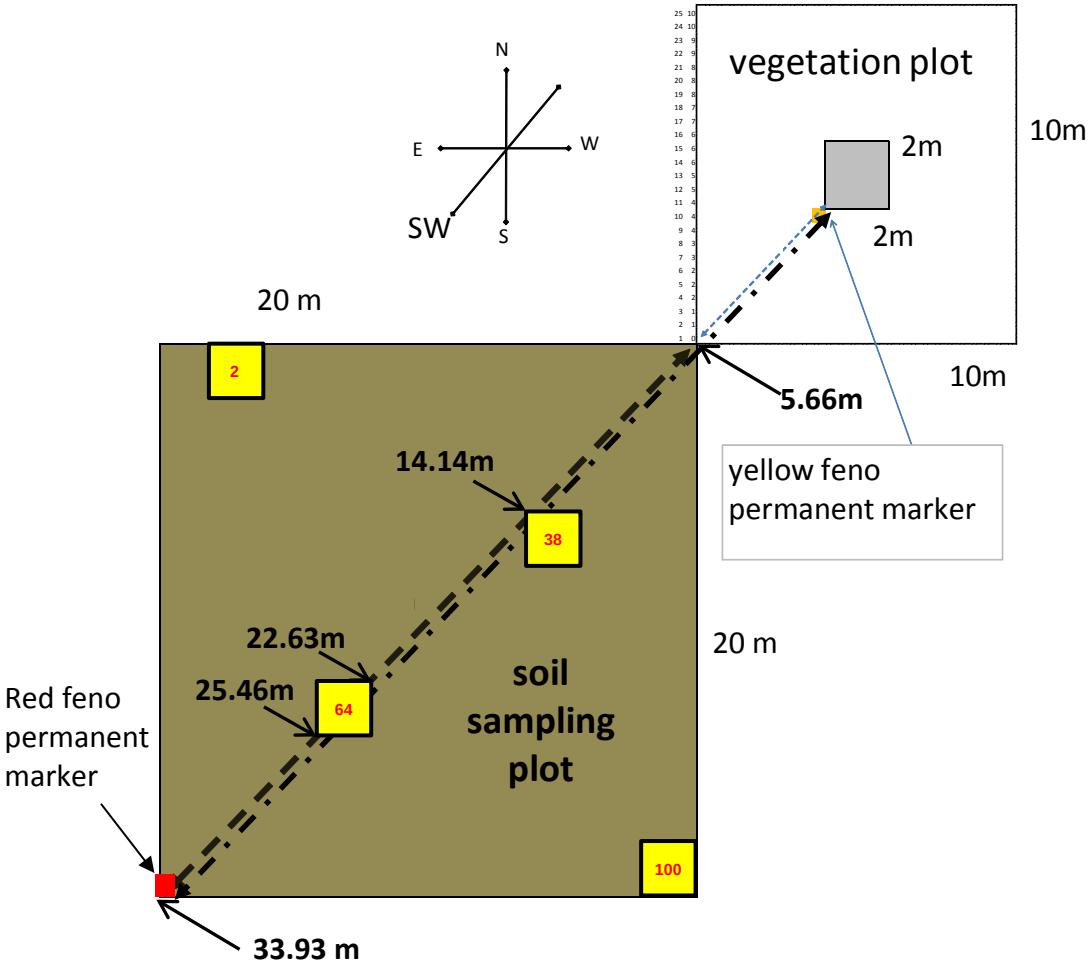


Figure 3 Locating soil sampling plots - layout of the vegetation plots at the sites. The yellow Feno marker off- centre of the 2mx2m vegetation quadrat is used to locate the edge of the 10x10m vegetation quadrat.

After locating the permanent marker (Figure 3), or its expected location, the soil sampling plots should be located by using tape measures. As much as possible, **avoid trampling over the plot prior to sampling**.

- Locate yellow veg plot feno marker or post.
- Stretch a 50m tape precisely SW from the feno marker using a sighted compass
- Place ranging poles or similar markers at:
  - **33.93 m** – SW corner of sampling plot
  - **5.66 m** - NE corner of the sampling plot.
- For baseline sampling (subplots 2, 38, 64, 100) – locate bamboo canes at
  - **14.14 m** - NW corner of subplot 38
  - **22.63 m** – NE corner of subplot 64
  - **25.46 m** – SW corner of subplot 64
- Pay out a 50m tape to 40m and hold firm at the NE and SW corners of the sampling plot, stretch to the NW and place a ranging pole at 20m to mark the NW corner of the sampling plot. (you will need to pace out holding the tape and following a compass where there are trees to avoid.
- Remove the 50m diagonal tape, and repeat the process above, stretching the tape to the SE, and mark with a ranging pole at 20m at the SE corner of the sampling plot.
- Leave the side tapes in place.
- Use a tape measure, compass and bamboo canes to mark out the remaining plots (if tapes are paid out from NE corner:
  - Subplot 2 is at N edge of plot between **16 and 18m**
  - Subplot 100 is at SE corner of plot with bamboo poles at **18 and 22 m**.
  - NOTE: you can check subplots are square checking diagonals are **2.83** m
- place a red feno marker at the SW corner of the sampling plot

Surveyors are required to make a judgement in the field as to whether the selected sampling plots adequately represent the habitats chosen, and change plots if necessary. If the plot identified is not suitably homogeneous in terms of soil, vegetation, rocks, etc., different plots should be selected and their exact location recorded and marked out. The first option is to establish the soil sampling plot offset from a different corner of the 10m vegetation survey plot (ie at the NW, NE or NE corners). The nature of the offset should be recorded and the soil plot feno marker placed at the corner most distant from the vegetation plot. If there are no suitable areas offset from the vegetation quadrat, then a replacement sampling point should be selected. Use the number of any replacement plots for the labelling of soil samples. If the pattern of vegetation does not allow for a square block then a different shape, covering the same area, with sides multiples of 2m should be chosen – eg 10m by 40m, or 8 m by 50 m, and this configuration carefully recorded.

If insufficient suitable plots then others must be identified in discussion with the site manager and/or the Natural England project manager.

Figure 4 Permanent markers of vegetation quadrats vegetation quadrats

2.1.1.3 Soil sampling sub-plots

If any of the pre-selected sub-plots are not suitable for soil sampling, please randomly select another sub-plot and change the numbers on the field sheet to reflect the new sub-plot chosen. Make a note on the field sheet explaining why the decision was made.

2.1.2 Labelling of photos and soil samples

Label all photographs and bags using the following system

Site number X Plot number X Subplot Number+Sample letter reference)

Thus a photo of sampling plot 24 at Malham tarn (site 16) will carry the label

16X24

This can be written on a piece of paper and held within the range of the photo.

A bulked soil sample (such as Sample D) from this plot will be

16X24D

And a soil sample (sample F) from plot 2 in this plot will be:

16X24X2F

All sample bags should also be labelled with the **date** and the **initials/unique identifier** of the surveyor.

2.1.3 Field sampling and data collection

At least **one photograph will be taken of the soil sampling plot from** the yellow feno marker or nearby towards the soil sampling plot to illustrate location in the landscape. Include in the photo a clear written sheet labelled with site number X plot (see section 10.2.4).

The exact position of the corners of the soil sampling plots should be recorded using GPS.

2.1.3.1 Photographs, vegetation height/structure and ground cover

At each subplot, **two photographs** should be taken, both including a written label.

- One photo from above to show the vegetation cover
- One photo from the northern side, to include a clearly marked metre rule to indicate sward / vegetation height.

Ground cover should be estimated and recorded to the nearest 5% (viewed from above) for all vascular plants (to species), and for the following broad groups: *Sphagnum*, other bryophytes, lichens, litter, bare soil, bare rock, and "other". Only covers exceeding 5% need be recorded. The presence and number of any other obvious biogenic structures (molehills, burrows, ant hills etc., fungal fruiting bodies) or other features of interest should be recorded in the field notes. Such biogenic structures should be avoided when taking soil samples.

2.1.3.2 Soil sampling and description

For most soil samples, there will be 1 bulked sample per plot = a total of 5 soil samples for that site for that year.

The locations of soil sampling /augering in each plot is shown in Figure 6.

All samples taken should be labelled with codes to represent site, sampling plot, subplot (if relevant) and core reference, along with the date and field surveyor's unique identifier.

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 made within the field record sheets.

If there is unusual vegetation, cow pat, boulder etc move minimum distance to get more homogenous sensible location and record problem. These should not be around the edge of the sub-plot to avoid interfering with other adjacent sub-plots.

All samples and bags must be labelled with corresponding site, plot, sub-plot and core codes.

N.B. where sampling is from one sub-plot only, ensure that this is the same sub-plot for all plots. In this instance this should be sub-plot "2".

It is recommended that the fieldwork at each plot should be taken, wherever possible in the order described below, but it is acceptable that some soil samples may be taken simultaneously, if disturbance to the soil to be sampled can be avoided.

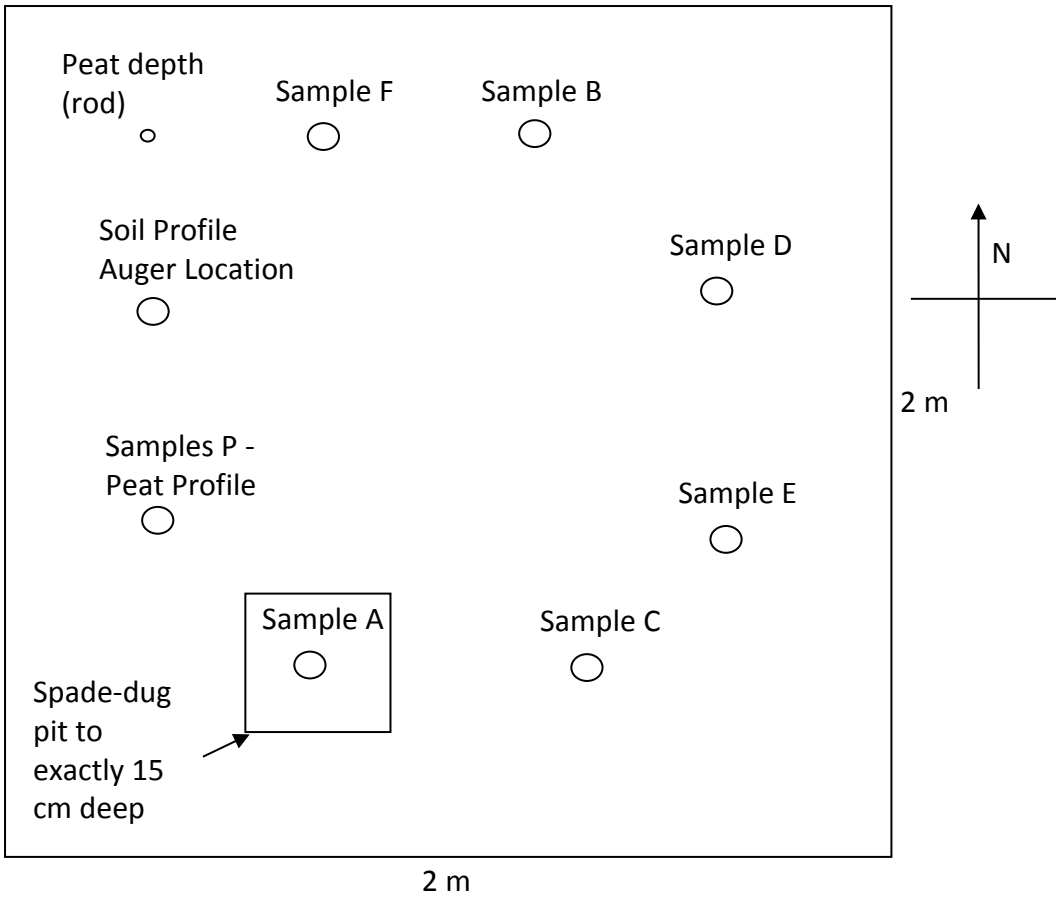


Figure 6. Arrangement of soil samples to be taken from sub-plots. Note that Sample P should be taken only in sites with peat deposits >30cm deep, and only in one sub-plot per sampling plot. Samples B and F should only be taken in one sub-plot (sub plot 2) for each sampling plot.

Sample B – 4 samples, one from each subplot – soil mesofauna

Take this sample in each sublot first to avoid disturbing any surface mesofauna. This sample should be taken at at all four subplots and placed together in a single labelled bag.

- One core 8cm long and 4 cm diameter with fine gauze over one end (secured with elastic band and insulating tape).
- Place open end of core carefully in an undisturbed area.
- Do not move from the litter surface as this is where the soil animals will be living.
- Carefully cut / drive the core into the soil using the block, mallet, knife or scissors. It may be easier to cut into the soil around bottom edge of the core before driving.
- Stop when the core top is flush with the soil surface (litter layer)
- Cut out a wedge of soil at the side of the soil core, remove this with a trowel, then use the tip of the trowel to lever out the intact core, taking care not to lose soil from the base of the core.
- On withdrawing this core, trim the bottom edge flush with core using scissors or a knife.
- Wrap core in cling film
- Place one core form each of the 4 sub-plots in the sample bag and label the bag with the siteXplotX+**B**
- Place zip-lock bag in cool storage as soon as possible following sampling and **keep cool 4^{o}C until analysis.**

Sample F - sub-plot 2 only –N mineralisation

- One core 15cm long and 4cm diameter from sub-plot 2 only.
- Locate position F in the sub-plot, an undisturbed area
- Gently remove any fresh litter from soil surface, taking care not to move decomposing litter beneath

- Place core on surface and cut/drive the core into the soil using the block, mallet and knife as necessary.
- It may be easier to cut into the soil around bottom edge of the core before driving.
- Stop when the core top is flush with the soil surface
- Cut out a wedge of soil at the side of the soil core, remove this with a trowel, then use the tip of the trowel to lever out the intact core, taking care not to lose soil from the base of the core.
- On withdrawing this core, trim the bottom edge flush with core using scissors or a knife.
- Wrap core in cling film
- Place core in the sample bag and label the bag with the siteXplotXsubplot+**F**
- Place zip-lock bag in cool storage as soon as possible following sampling and **keep cool 4^{o}C until analysis.**

Soil profile description – sub-plot 2 only

Record a description of the soil profile a dutch (Edelmann) auger from the position indicated on Figure 6.

- Take a series of cores sequentially down the soil profile to the depth of the C horizon or 1m (whichever is shallower), ejecting each core in sequence onto a plastic sheet and retaining their relative orientation and original total length.
- Identify the type of horizon, and record its depth rannge, texture and colour with reference to a Munsell colour chart.
- Record all information on field sheets.
- Take a photographs of the soil auger samples laid out in sequence, with a lable showing siteXplotXsub-plot clearly showing in the photograph.

Samples P – sub-plot 2 only, and only where peat depth exceeds 30 cm – peat depth and C storage

The following protocol for **peat depth and quality assessment** applies only to one sub-plot 2 aims to sample a known volume of peat from the entire peat profile. A box corer or Russian peat corer may be used depending on the depth of the peat.

If the top 30cm of the auger core is entirely organic (peaty) soil

- Starting at the litter surface, take sequential peat samples down the profile. Most Russian corers have a sampling chamber 50cm long, so samples would be 0-50 cm, 50-100 cm, 100-150 cm. Make a note of the dimension of the corer being used.cm) etc.
- Place each peat sample into a seperate sample bag labelled with siteXplotXsubplot+**P** followed by the depth increment sampled (eg. 16X24X2**P50-100**), and seal.
- Repeat this process until the entire profile has been sampled.
- When the final core is withdrawn, measure the length of peaty material present in the corer (above mineral-dominated material) and record this. Then use a knife to separate the mineral material from the peaty material in a perpendicular to the core, at this depth and place only the peaty material into the sample bag, labelling as before.
- Record the depth at which the peat is clearly water saturated as an indication of water table depth
- Note the total number of peat samples collected i.e. P1 to P5 inc

The following activities relate to all 4 sub-plots

Peat depth measurements

If the peat depth on the site exceeds 30cm when assessed at the auger core, **peat depth** should be assessed using threaded steel rods (or similar) driven into the peat until they meet a resistant mineral layer. This shoud be done at the location indicated at all 4 subplots and the depths recorded on the field sheet, but an alternative location within the subplot chosen if the rod hits eg. woody material.

The following cores are bulked from all 4 sub-plots sampled.

Sample C - taken from 0-15 cm at all four sub-plots – topsoil physico-chemical

- One black core 15cm long and 8cm diameter per sub-plot
- Locate position C, an undisturbed area

- Gently remove fresh litter from soil surface, taking care not to move decomposing litter beneath
- Place core on surface and cut/drive the core into the soil using the block, mallet and knife as necessary.
- It may be easier to cut into the soil around bottom edge of the core before driving.
- Stop when the core top is flush with the soil surface
- Cut out a wedge of soil at the side of the soil core, remove this and surrounding soil with a trowel, then use the tip of the trowel to lever out the intact core, taking care not to lose soil from the base of the core.
- On withdrawing this core, trim the bottom edge flush with core using scissors or a knife. PLEASE TAKE CARE WHEN REMOVING CORE TO RETAIN WHOLE CORE
- Wrap core in cling film securely.
- Place core in a large sample bag and label the bag with the siteXplot+**C**
- Repeat for all 4 sub-plots placing all C samples in the same bag – these will be bulked on return to the laboratory.
- Place zip-lock bag in cool storage as soon as possible following sampling.

Sample A - taken from 15–30 cm at all four sub-plots - lower soil physic-chemical

- One black core 15cm long and 8cm diameter per sub-plot
- Locate position A in the sub-plot
- Carefully remove the top 15 cm of the soil using a spade.
- Place core on top of exposed soil surface
- Cut/drive the core into the soil using the block, mallet and knife as necessary.
- It may be easier to cut into the soil around bottom edge of the core before driving.
- Stop when the core top is flush with the exposed soil surface
- Cut out a wedge of soil at the side of the soil core, remove this and surrounding soil with a trowel, then use the tip of the trowel to lever out the intact core, taking care not to lose soil from the base of the core.
- On withdrawing this core, trim the bottom edge flush with core using scissors or a knife. PLEASE TAKE CARE WHEN REMOVING CORE TO RETAIN WHOLE CORE On withdrawing this core, trim the bottom edge flush with core
- Wrap core in cling film securely.
- Place core in a large sample bag and label the bag with the siteXplot+**A**
- Repeat for all 4 sub-plots placing all A samples in the same bag – these will be bulked on return to the laboratory.
- Place zip-lock bag in cool storage as soon as possible following sampling.

Sample D – taken at all 4 sub-plots - **microbial analyses**

- This is a bagged sample of ca. 500 g of fresh soil made up of 8 to 12 sub-samples per plot.
- Locate position D in the sub-plot
- Use a graduated or marked long thin trowel is used to take at least 2-3 soil samples near position D to 15cm depth in each subplot.
- It is important to sample to the full 15cm and to obtain a core by turning the trowel in the soil. Use the knife to cut through root mats etc to the front and sides of the core before turning the corer.
- Lever out the samples and place soil directly into a ziplock bag labelled with siteXplot+**D**
- Repeat, once or twice depending on approximate weight / volume of sample (each sub-plot sample should be min. 1/ 4 of the total sample per plot.

Sample E – taken at all 4 sub-plots – **nematode extraction**

The sampling is the same as for CORE D

- This is a bagged sample of ca. 500 g of fresh soil made up of 8 to 12 sub-samples per plot.
- Locate position D in the sub-plot
- Use a graduated or marked long thin trowel is used to take at least 2-3 soil samples near position D to 15cm depth in each subplot.

- It is important to sample to the full 15cm and to obtain a core by turning the trowel in the soil. Use the knife to cut through root mats etc to the front and sides of the core before turning the corLever out the samples and place soil directly into a ziplock bag labelled with siteXplot+**E**
- Repeat, once or twice depending on approximate weight / volume of sample (each sub-plot sample should be min. 1/ 4 of the total sample per plot.

Table 3 summarises the soil samples per sub-plot.

Table 3. Check list for samples and sample numbers for each plot and for the entire site (5 plots).

Core Ref	Description	Bulking per plot?	Analysed for	Notes and total number of samples per site and for entire 2013 sampling nalysis
A	15-30cm depth	Taken at 4 subplots and bulked	Soil physico-chemical characteristics	5 samples per site 35 samples for analysis (7 sites x 5).
B	0-8cm deep, 8cm diameter, gauze top.	Taken at 4 subplots and bulked.	Soil mesofauna	5 samples per site 35 samples for analysis (7 sites x 5)
C	0-15 cm depth	Taken at 4 subplots and bulked	Soil physico-chemical characteristics	5 samples per site 35 samples for analysis (7 sites x 5)
D	8 to 12 trowel samples in total	Bulked from 2-3 sub-samples taken at all 4 subplots.	tRFLP, PLFA	5 samples per site 35 samples for analysis (7 sites x 5)
E	8 to 10 trowel samples in total	Bulked from 2-3 sub-samples taken at all 4 subplots.	Nematodes.	5 samples per site 35 samples for analysis (7 sites x 5)
F	0-15 cm depth	Taken at 1 subplot only – no bulking.	Potentially mineralisable N	5 samples per site 35 samples for analysis (7 sites x 5)
P(n)	Four* 50cm depth sections	Taken at subplot 1 only – no bulking	Bulk density, LOI	1 to N* samples per plot (ca. 4 x 5 x 2 peat sites = 40 samples.

*Actual number of samples will depend on total peat depth – a peat depth of 2m has been assumed here.

2.1.4 Sample storage and transport

- All samples should be kept in cool location out of the sun – ideally in cool boxes or on covered boxes
- Biological samples (CORES B, F, D and E) **must** be kept in cool boxes which include frozen freeze blocks.
- Return soil samples to for analysis as soon as possible – ensuring that biological samples are kept cool during transport and that laboratory staff know that they are arriving and can act to store and preserve them appropriately on arrival.

2.1.5 Field notes and records

On return from fieldwork:

- collate all field record sheets and transfer all data to relevant excel files and save to project folder. A standard data entry format will be agreed and data from those sites completed by Natural England will be sent to the contractor for collation with their data.
- Collate all notes relevant to the sites, and supply these with the data, bringing any issues, relocations, sampling problems etc. to the attention of the Natural England project manager.
- Edit all photographs to indicate site and transfer all photographs to the relevant project folder

- Send all field paperwork to the project manager and all digital data to Natural England, along with analysis data.

3 Soil Sample Analysis

3.1 Analysis of Physico-chemical attributes

The following analyses should be applied to the following soil samples collected above. In all cases analyses should as closely as possible conform to those used by the James Hutton Institute in for previous monitoring analysis. The JHI method codes for each analysis are provided in brackets below

Core A and Core C – 15cm by 5cm diameter – bulked samples from 0-15 and 15-30cm

For each set of bulked samples, entire sample collected should each be assessed using, where possible, UKAS approved method for:

- % gravimetric water content following drying at 30oC and after further drying at 105^{o}C
- % stone volume (volume of mineral particles >2mm diameter)
- dry bulk density g cm3
- fine earth bulk density (dry bulk density of non-stone material)

to give five measurements per site for A cores and five measurements for C cores.

For each sample, and for each core, aliquots of sieved soil should be measured out and analysed for the following parameters using UKAS approved methods:

- pH in water and in $CaCL_2$ (Method DM006)
- % gravimetric loss on ignition at 375^{o}C[1] (Method DM007)
- % gravimetric total C and total N (Method DM001)
- Cation Exchange Capacity and exchangeable cations, comprising Mg, Ca, Na, Mn, K, Fe, Al (Method DM004) and exchangeable acidity (Method DM002).
- Particle size distribution: gravimetric % sand, silt and clay, using both BSTC and international definitions for silt and sand (Method DM011)

CORES Pn – 50cm length cores taken down peat profile to full depth of peat.

The 50 cm peat samples of known volume must be weighed wet, dried at 105^{o}C, and reweighed to estimate bulk density following, where possible, a UKAS approved procedure. Dried cores will be subsampled by cutting lengthways and ground in a hammer mill or other suitable machine before being analysed for soil organic matter content by loss on ignition at 375^{o}C[1].

All analyses should be subject to adequate QC procedures, and documentation on the methods used should be available on request.

3.2 Analysis of soil biological parameters and functions

The following analyses and procedures should be undertaken for cores B, D and E.

Core B – four 8cm by 8cm diameter cores for soil arthropods by Tullgren extraction

This sample should be stored at 4^{o}C pending processing, for as little time as possible. Cores B should have its fine gauze carefully removed (to avoid loss of specimens), and each bulked set of cores carefully ejected to avoid core damage, and each set of cores mounted in a single Tullgren funnel with their original surface facing downward. Each funnel should be gently heated using a low-wattage incandescent bulb, for an appropriate extraction period (ie until the soil is no longer losing water), while collecting soil fauna in

[1] or optimum temperature for complete combustion of organic matter, while avoiding liberation of carbonate C.

ethanol following the protocols followed by Emmett et al 2008. Samples should be Collected in at least 90% ethanol and stored in 99% ethanol or suitable preservative to enable later genetic/metagenetic analysis of the specimens. Where 99% ethanol is used extra care will be needed when to avoid evaporative loss from sample containers, or absorption of moisture from the surrounding air. This contract does not extend to the counting or identification of animals extracted. Extracts should be carefully packed in protective material and sent to Natural England for identification or further processing, using a suitable courier or transport process.

Sample E – 8-12 bulked trowel samples

This sample must be stored at 4^{o}C pending analysis, and will homogenized, and sub-sampled before analysis using UKAS-approved methods where possible, for

- Soil microbial PFLAs (S.O.P. CP001; see Zogg et al, 1997; Frostegård et al, 1996, as adapted by Black et al, 2008; 2011). These would be extracted from an appropriate amount of homogenised soil (50-500g depending on organic matter content, Method SEP0000) and analysed by GC-FID or other suitable method (Method CM001) to indicate both total biomass PLFAs, and identification of a range of taxon-specific and unidentified PLFAs (see Appendix 3) following the methods used in the Black et al (2011). Data for individual PLFA species should be reported as ug g^{-1}, nmol g^{-1} and % total mol.

- A further subsample will be subject to microbial DNA analysis by terminal restriction fragment length polymorphism (tRFLP) on both ITS and 16S_AR genes, following the protocol described in Black (2008; 2011). The data will be reported on by relative abundance of all resulting fragment lengths. An internal size standard should be used which will allow for the reasonable calibration of size fragments between 30 and 550 base pairs.

Sample D – 8-12 bulked trowel samples
The bulked sample should be subject to a Baermann extraction (following the protocols established by Black *et al* (2008, 2011), for the SQID programme, which is based on a modified version of Brown and Boag (1988) for collection of soil nematodes. This contract is for extraction and archiving of samples only. A sub-sample of the bulked soils should be transferred to Baermann funnels for the extraction of nematodes and other soil water-dwelling organisms. The method followed should be that employed by the Hutton Institute for the 2011-2012 monitoring programme samples. The resulting extracts should be stored in 1% formalin/glycerol.

As an optional additional activity, contractors are asked to provide a quote for the analysis of 96 nematode samples by tRFLP following a protocol similar to that in Griffiths et al (2006), with the aim of characterising and quantifying soil nematode biomass to broad taxonomic groups, linked to functional feeding groups, in 19-24 of the sites sampled so far (analysing 4-5 samples from each). Samples analysed may be those from the current contract's extractions above, as well as from extractions carried out in previous years, which will be made available to the contractor in 1% formalin/glycerol solution.

Sample F – Rain extractable N and N mineralisation

Core F should be analysed for "rain extractable" mineral N and N mineralisation following the method described by Emmett (2008).

The intact core should be removed from its corer, and cut in half lengthways. One half of the core (Core F1) should be lain on its side on a rack and brought to field capacity and flushed through with "artificial rain" ("UK rain minus N", Emmett, 2008) to remove remaining mineral N (as described in Emmett 2008), and elute collected and analysed for "rain extractable" ammonium- and nitrate-N.

The core should then be subjected to standard suction to standardise soil water tension before N mineralisation. Soil cores should then be incubated for 28 days at 10 degrees C and extracted with 1M KCl as described in Emmett et al (2008) and soil ammonium-N and nitrate-N analysed and reported in terms of nitrogen mineralised per gramme of soil per day, by comparison with that extracted by water earlier. This does not produce true N mineralisation rates, but should provide a comparative index of N mineralisation.

3.3 Sample archiving

Sieved, dried (105^{o}C) samples from cores A and C which should be supplied in labelled bags/containers to Natural England for storage.

3.4 Data analysis and reporting

Raw data should be provided to Natural England, and be processed where this is required to provide biologically meaningful results (eg. profiling of PLFAs against soil organism groups, tRFLP). Data format should be provided to be as compatible as possible with other soil monitoring data sets.

- Content of clay, silt, sand, water, organic matter, carbon, nitrogen should be expressed in % values.
- Cations and CEC should be expressed as millequivalents 100g^{-1} dry soil
- Bulk density should be expressed in g cm^{-3}
- pH should be expressed in standard units
- Extractable ammonium and nitrate should be expressed as mg kg^{-1}
- Ammonium and Nitrate mineralisation should be expressed in mg kg^{-1} dry soil day^{-1}.
- PLFAs should be reported in the units described above
- tRFLP should be reported in units described above.

Site Name				**Site No.**	
Date		**Plot photo?**		**Plot No.**	
Surveyor Name(s)					
GPS NW			**GPS NE**		
GPS SW			**GPS SE**		

Sub-Plot	**2**	**38**	**64**	**100**
Photos taken from above and side? (tick)				
Cover - ID / cover of all exceeding 5%	**2**	**38**	**64**	**100**

*all vascular plants identified to species, and the following groups: Sphagnum, other bryophytes, lichens, litter, bare soil, rock/crustose lichens, other.

Auger profile - sub plot 2 only

Horizons	**Max depth (cm)**	**Texture**	**Colour**	**Notes**

Soil samples taken (tick)	**2**	**38**	**64**	**100**
Sample B (8cm, 4 cm diam., 0-8cm depth)				
Sample F (15 cm, 4 cm diam., 0-15 cm depth)		No sample required		
Sample C (15cm x 8cm diam, 0-15cm depth)				
Sample A (15cm x 8cm diam., 15-30cm depth)				
Sample D (long trowel, 0-15cm depth)				
Sample E (as sample E)				
Peat depth (probe)				
Number of peat cores taken				
Length of last peat core (cm)				
Water table depth (cm)				

Field notes