Title:

Changes to C stocks in Scottish soils due to afforestation

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1 Introduction

One of the most significant land use changes in Scotland in the second half of the 20th Century was that of converting semi-natural habitats to woodland, especially to conifer plantations. This afforestation is set to continue with the Scottish Forestry Strategy and Land Use Strategy proposing an increase in woodland cover by 10 000 ha per year over the next 10 years. Both native woodlands and non-native coniferous species will contribute to this target, with different proportions in different parts of the country. While projects such as BioSoil (Cools and De Vos, 2010; Vanguelova et al., 2013) undertaken by Forest Research and the National Soil Inventory of Scotland (NSIS) resampling done by the James Hutton Institute (Lilly *et al*., 2013a) allow some predictions of changes in the amount of soil carbon sequestered to be made, there is still insufficient data to address some key questions regarding the response to afforestation on different soil types and with different tree species.

A recent review of soil morphological and analytical data held in the Scottish Soils Database identified 40 sites on land owned by the Forestry Commission (FC) that had been afforested subsequent to the original description and sampling of the soil. These sites provide an opportunity to assess the extent of changes in soil carbon over time for a range of soil types and under different tree species.

The aim of the work was to relocate these sites, collect new soil morphological data, sample the soils by horizon and by fixed depth, analyse the samples for a range of soil properties but primarily soil organic carbon concentration, and assess the changes in soil carbon. The fixed depth sampling alongside the traditional sampling by horizon (layers) was included as a way of attempting to integrate the BioSoil and NSIS sampling schemes in order to develop a more substantial database of afforested soils in Scotland. Subsequently the data will be used as input into models and site assessments in collaboration with Forest Research (FR) to assess the amount of above- and below-ground carbon that is stored within the soils and trees.

2 Background information

The James Hutton Institute holds the Scottish Soils Database which has approximately 14, 000 spatially referenced soil profiles dating from the 1930s to the present day including profile descriptions, horizon samples, contextual information including vegetation community type and analytical data. A large number of these profiles were sampled between 1960 and 1980 which also coincides with a period of extensive woodland expansion. The individual horizons were sampled rather than sampling at fixed, predetermined depths and excess soil not used for analyses was retained within the National Soil Archive.

The Forestry Commission National Forest Inventory (NFI) has information on the extent and type of all woodland within Great Britain. Additionally, the FC holds more detailed information (including planting dates, previous cultivations and tree species) within its Sub-Compartment Database (SCDB) for all of the FC managed public forest estate. This FC-wide SCDB was accessed by FR staff to help identify locations used within this study.

In a previous study (Lilly *et al*., 2013b & Appendix 4), the soils and woodland data from the NFI were combined to identify those sites and soils that have undergone a land use change to forestry since the date of the original sampling. A rigorous selection process was applied to identify suitable sites where all horizons including surface organic layers had been sampled and where the soils had sufficient archived soil material available to re-run analyses. This last step was crucial as previous work (Chapman *et al*., 2013) had shown an 11% difference between C concentrations of samples measured around 19-30 years ago and those measured between 2007 and 2009, therefore, it was necessary to re-analyse the archived samples alongside the new soil samples.

Lilly *et al*. (2013b) had identified a total of 20 sites where all horizons in the profile had been sampled and where there was more than 80g of soil remaining in the archive. The limit of 80g was set to allow further analyses without entirely depleting the sample. Of these 20 sites two were previously sampled as part of the NSIS10k sampling scheme, two were from the NSIS5k, one post 1979 and the remaining 15 from selected profiles sampled between 1960 and 1979. A second set of 20 soils were identified where the archival material remaining was less than 80g and here, careful consideration was made as to whether there was sufficient sample remaining to allow at least the measurement of soil carbon.

3 Methods

Methods were developed to locate and resample as many of the 40 sites (Table 1) as possible using a combination of the NSIS 20km soil sampling protocols (Lilly *et al*., 2013a) and the BioSoil protocols (Cools and De Vos, 2010); to undertake analyses of the sampled soil material to determine carbon stocks; to reanalyse the archived soil material to determine carbon concentration; develop and apply pedotransfer functions based on NIR spectroscopy to predict soil bulk density of the original soil profile and so determine if there has been any significant change in soil carbon stocks at these sites. Those soils sampled between1961–1988 (the original sampled profiles) were denoted as 'archive' and those sampled in 2013 as 'recent'.

3.1 Site location

The NSIS 2007-2009 protocols (Lilly *et al*., 2013a) for relocation and resampling of the soil profile were used as these were tested in the field for the resampling of 183 sites throughout Scotland and so that these additional sites could be added to the data for those NSIS sites that were also under forestry to enhance the data on changes to the soil due to afforestation.

At each of the 40 sites the original grid reference which was assessed from maps, was used to locate the site to within 100m, and then a more precise re-location was made using the site characteristics (aspect, slope degree and form, rockiness, boulders and flushing) recorded during the first visit to the site. For those NSIS sites originally sampled between 1978 and 1988, aerial photographs (where available) on which the site location was marked were used to locate the site. However, due to one of the original sites having an inaccurate grid reference in the database, when revisited, the site (Altimeg 1) was found not to have been afforested and so was not resampled.

At each of the remaining 39 sites, small trial soil pits or a soil auger were used to match the major soil subgroup and the horizon sequence at the site with the original soil profile description as closely as possible. The national grid reference, as indicated by Global Positioning Satellite (Garmin GPS map62 or Garmin 12), was recorded to help relocate the sites in the future if required.

Table 1: Profiles, land cover and locations on FC land where all horizons were sampled and archived soil material remained (Soils classified by Soil Survey of Scotland Staff (1984) classification system).

PROFILE_DATE	SITE_NGR	SITE_NAME	MAJOR SOIL SUBGROUP	SAMPLE FRAME	LAND COVER
03/10/1979	NC300100	Glen Oykel 1	Dystrophic peat	NSIS10	Bog heather moor
1969	ND270485	Achairn 1	Dystrophic peat	60_79	Rush pasture
08/06/1978	NG703162	Kinloch 2	Peaty gley	60_79	Heather moorland
1965	NH589534	Monadh Mor	Dystrophic peat	60_79	Bog heather moor
1962	NH705773	Cnoc-an-t-Sabh. 2	Humus-iron podzol	60_79	Heather moorland
1968	NH711343	Lairgs 1	Peaty gley	60_79	Heather moorland
1961	NH740754	The Wilderness 1	Humus podzol	60_79	Heather moorland
1965	NH746814	Tain Quarries	Humus-iron podzol	60_79	Heather moorland
1968	NH843323	Glenkirk 2	Peaty gley	60_79	Bog heather moor
1968	NH845323	Glenkirk 4	Peaty podzol	60_79	Heather moorland
1968	NH845324	Glenkirk 3	Peaty gleyed podzol	60_79	Heather moorland
1966	NH991642	Buckie-Loch 4	Noncalcareous regosol	60_79	Rough grassland
1965	NJ289548	Teindland Forest 1	Peaty gleyed podzol	60_79	Heather moorland
1967	NJ322337	Glen Fiddich 2	Peaty gley	60_79	Rush pasture
1967	NJ423291	Greenknowe 1	Humic gley	60_79	Rough grassland
16/08/1966	NJ424295	Greenknowe 2	Brown Magnesian soil	60_79	Rough grassland
1961	NJ552224	Suie 1	Peaty podzol	60_79	Heather moorland
20/09/1988	NJ5530022200	Suie B	Humus-iron podzol	Post79	Heather moorland
1961	NJ556220	Suie 3	Iron podzol	60_79	Rough grassland
17/05/1983	NM500450	Mull 8	Brown ranker	NSIS5	Heather moorland
20/04/1983	NM824613	Tom an T Sidhein 11	Humus-iron podzol	post79	Rough grassland
15/07/1982	NM850700‡	Ardgour 7	Peaty gleyed podzol	NSIS5	Bog heather moor
07/05/1980	NM900100	Mid Lorn 41	Mesotrophic peat	NSIS10	Rush pasture
12/04/1979	NN100900	North Lochaber 52	Dystrophic peat	NSIS10	Heather moorland
05/08/1978	NN400500	North Lorn 23	Dystrophic peat	NSIS10	Heather moorland
1964	NO501256	Tentsmuir 3	Calcareous regosol	60_79	Rough grassland
1962	NO763883	Fetteresso Forest	Peaty podzol	60_79	Heather moorland
1963	NS336009	Knockinculloch	Gleyed brown earth	60_79	Rough grassland
1965	NT061602	Camilty Moss	Peaty gley	60_79	Heather moorland
1970	NT350336	Minchmoor	Humus-iron podzol	60_79	Heather moorland
1968	NX135557	Mid Torrs 3	Noncalcareous gley	60_79	Rough grassland
1961	NX224793	Dochroyle 2	Peaty gley	60_79	Bog heather moor
1961	NX320853	Clauchrieskaig 1	Peaty gleyed podzol	60_79	Heather moorland
1961	NX322855	Clauchrieskaig 2	Brown earth	60_79	Rough grassland
1961	NX326884‡	Fardin 1	Peaty gleyed podzol	60_79	Heather moorland
1961	NX335785	Creebank 1	Peaty gley	60_79	Rough grassland
29/04/1987	NX400950	Lodge Craiglure	Peaty ranker	NSIS5	Rough grassland
1968	NX569682	Cullendoch 3	Peaty gley	60_79	Heather moorland
01/07/1982	NX600700	Glengainoch 1	Peaty gley	NSIS10	Rough grassland
1962	NX605765*	Altimeg 1	Gleyed brown earth	60_79	Rush pasture

* Site not under woodland when revisited
‡ Profiles sampled in clearing within the forest

3.2 Soil profile description and sampling

The sites were sampled following the protocols established for the re-sampling of the National Soil Inventory of Scotland 2007-2009 programme on a 20 km x 20 km grid (Lilly *et al.,* 2013). These protocols contain details of the attributes recorded in the field. The following is a summary of the main aspects of the sampling that was carried out as part of this project (see also Appendix 1).

A soil profile pit was excavated at each location in an area as undisturbed as possible within the forest to a depth in excess of 80cm wherever possible and certainly to within the soil parent material. Once excavated, the profile was examined, the main horizons identified and a full pedological description of the profile was made following the NSIS protocols. The boundaries of the horizons to be sampled were marked with a knife on the pit face and where possible, with the exception of iron pans (Bf horizon), each horizon in the profile was sampled. In particular, every effort was made to sample surface organic horizons (L, F, H and O horizons), but it remained up to the surveyor to assess the practicality and relative importance of taking such samples. In circumstances where surface organic horizons were not thick enough, or the delineation too indistinct, to allow sampling horizons individually, composite samples were taken to allow a satisfactory volume of sample to be obtained. Approximately 1 kg of relatively stone free soil was collected from each horizon with as many stones as possible removed from the sample in the field.

Bulk, disturbed soil samples were generally taken from a 10 cm thick band centred around middle of the horizon or at depths thought to be more appropriate, either for comparative purposes with archive samples or where the thickness of the horizon was judged to warrant more than one sample. In some situations where the thickness of the horizon was less than 10 cm, the top and bottom sample depths were selected to allow a representative and pure sample (that is, not containing any material from the horizons above or below) to be collected from the horizon.

The soil material was loosened and extracted by a clean trowel or knife, collected in a sampling tray held level with the lower boundary of the sample depth, and placed in a bag. Excess air was removed from the sample bag and it was sealed as soon as possible to avoid contamination.

In addition to these profile horizon bulk samples, bulk density samples were also collected from the main horizons within the profile. Food-grade stainless steel rings of 7.6 cm diameter (7.2 cm internal diameter) x 5 cm height were inserted either vertically or horizontally into the soil horizon by applying an even pressure. A knife, scraping tool or other sharp instrument was used to cut the soil and large roots below the core depth and to push small stones to one side to improve insertion.

Triplicate bulk density samples (each 210 cm^3) were taken from each horizon wherever possible and in a manner that avoided compression or compaction. The samples were carefully extracted using a trowel or knife and carefully trimmed to ensure that there was no extruding material or stones. Small gaps were repacked with aggregates and extruding stones were removed with the gap again filled with an appropriately sized uncompressed aggregate. The sample from each core was then extruded into individual mini-grip bags. Where horizons were thinner than 5cm, the sample ring was partly filled, excavated, moved to a fresh area and re-inserted until full. Care was taken not to compress the material.

In order to make the soil data generated in this project compatible with data collected during the BioSoil forest soil sampling programme (European Union, 2006) as well as with the data generated during the NSIS sampling programme, fixed depth samples from below the organic horizons at 0-5, 5-10, 10-20, 20-40 and 40-80 cm (or to the base of the profile pit if soil thickness < 80cm) were collected from the soil profile at each site where 0 (zero) was taken as the upper boundary of the first mineral layer below the organic surface horizons (see Figure 1). These were taken by scrapping a column of soil from the pit face over the full sample depth. In addition, the surface organic horizons were sampled over their full thickness. Where the soil was classified as organic (peaty surface layers >50cm thick), the

Oa horizon (amorphous peat) was taken as zero.

The BioSoil protocols suggest that the bulk density for the upper mineral sample (0-5 cm) can be estimated using pedotransfer functions, however, cores taken to determine the bulk density of the mineral horizon following the NSIS protocols could be used instead. The determination of bulk density of mineral layers deeper than 10cm was not mandatory within the BioSoil protocols (European Union, 2006) and therefore was not measured. Also, it is difficult to get a representative bulk density for mixed subsoil layers over 40-80 cm layer. Appendix 2 shows a summary of the main differences between the NSIS and BioSoil sampling protocols and the methods used to harmonize the data collection.

Figure 1: Example and stylised horizon sequence fixed depth sampling depths

		Mineral Soil	Organo-mineral Soil		Organic Soil
			Aerobic	Anaerobic	
Organic layers	*-x - -y*	L		L	L
	-y - -z	F	L	Of	Of
	-z - 0	LF	F	Os	Os
Zero					
Soil	0-5	Ah	H	Oa	Oa 1
	5-10	A	H	Oa	Oa 1
	10-20	A	E	E	Oa 1
	20-40	Bs	Bs/Bg	Bs/Bg	Oa 1
	40-80	BC/C	C/Cg	C/Cg	Oa 2/Oa 3

3.3 Soil analytical data

3.3.1 Soil carbon

The soil samples were returned to the laboratory where they were air-dried at 30°C, sieved to remove stones (>2mm fraction) and large roots and subsampled. Subsamples for C content were further dried at 50°C and ball-milled to a fine powder before being analysed using a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a DeltaPlus XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen, Germany). The C contents were calculated from the area output of the mass spectrometer calibrated against National Institute of Standards and Technology (NIST) standard reference material 1547 peach leaves. Long term precision for a quality control standard (milled flour) was: total carbon 40.24 ± 0.29 % (mean ± SD, n=200).

3.3.2 Soil pH and Loss on Ignition (LOI)

The soil pH in both water and in 0.01M CaCl was determined by introducing a pH electrode to soil suspensions and the loss on ignition was determined by burning subsamples at 450 and 900°C.

3.3.3 Soil bulk density

Triplicate (where possible) 210cm^3 cores were taken from each of the main soil horizons (mineral and organic) for the determination of soil dry bulk density (g cm^{-3}) and averaged. Where it was not possible to take a whole core sample in some organic horizons less than 5cm thick these were sampled by either partially filling and then moving the ring (see Appendix 1) or by sampling an area of 25 cm x 25cm over the whole thickness of the horizon and the bulk density determined from this calculated volume.

The individual core samples were bagged separately in the field and returned to the laboratory where they were weighed, dried at 105°C for 48 hours, weighed and then sieved to removed stones (>2mm size fraction) and large woody roots. The dry bulk density was calculated as dry weight (including stones and roots) divided by the soil volume (210cm^3) and the bulk density of the fine earth (<2mm size fraction) was calculated as dry weight minus stones and large roots divided by the soil core volume minus the volume of stones and large woody roots.

3.3.4 Profile carbon stocks

Carbon stocks of the soil profiles sample were calculated by multiplying the carbon concentration (g/g) by the predicted dry bulk density (see below) of the fine earth (<2mm) fraction and then by the thickness (cm) of the soil horizon from which the sample was taken making a correction for stone content and then summing this to 1m (assuming that the lowest horizon sampled continues to this depth). Where the soil profile was <1m thick, this calculation was terminated at the depth of the rock or boulder. The final result is soil C stocks to 1 m (t ha^{-1}).

3.4 Archive soil samples

Re-analysing archived soil samples is crucial when measuring changes in C stocks at the same site over time as recent work (Chapman *et al*., 2013) found that there was an 11% difference in C concentration of samples when re-analysed. A key part of this project was that soil samples from the original sampling were still available in the National Soils Archive and in sufficient quantities that the analyses could be repeated. In some cases where there was <80g of archived soil sample remaining, it was decided not to measure soil pH on these as this requires a minimum of 15g of soil. Carbon concentration was measured on all archived soil samples.

3.5 Spectroscopy

Bulk density is a key component of the calculation of C stocks in soils, however, cores were not taken during the original soil sampling and so, bulk density was not measured. Chapman *et al*. (2013) devised a method to predict soil bulk density for archived soil material when analysing changes in C stocks for the NSIS sites that used near infrared reflectance spectroscopy (NIRS). We applied the same methodology to the archived soil samples used in this project.

Near infrared reflectance spectroscopy (NIRS) methodology is based on measuring the light in the near infrared region of the spectrum (1100 – 2500 nm) reflected from the air-dried soil and then correlating the spectra generated to one or more properties of interest, in this case, to dry bulk density of the fine earth (<2mm) fraction.

Specifically, near infrared spectroscopy is a vibrational spectroscopic technique, that is, signals in the NIR spectra of soil samples occur as a consequence of molecular vibrations when a soil sample is irradiated with a source of light emitting in the near infrared range. The chemical bonds of the component parts of the soil sample (organic and mineral) stretch and bend causing a wave movement that is characteristic of each functional group (Figure 2). It is possible to record the amount of light that has been absorbed by a sample and to mathematically correlate it, for example by regression equations, with known chemical or physical attributes of the sample (i.e. reference values), determined by traditional laboratory analyses. In this case, the spectra were correlated with the measured bulk density samples from the resampled (recent) soils using partial least squares regression.

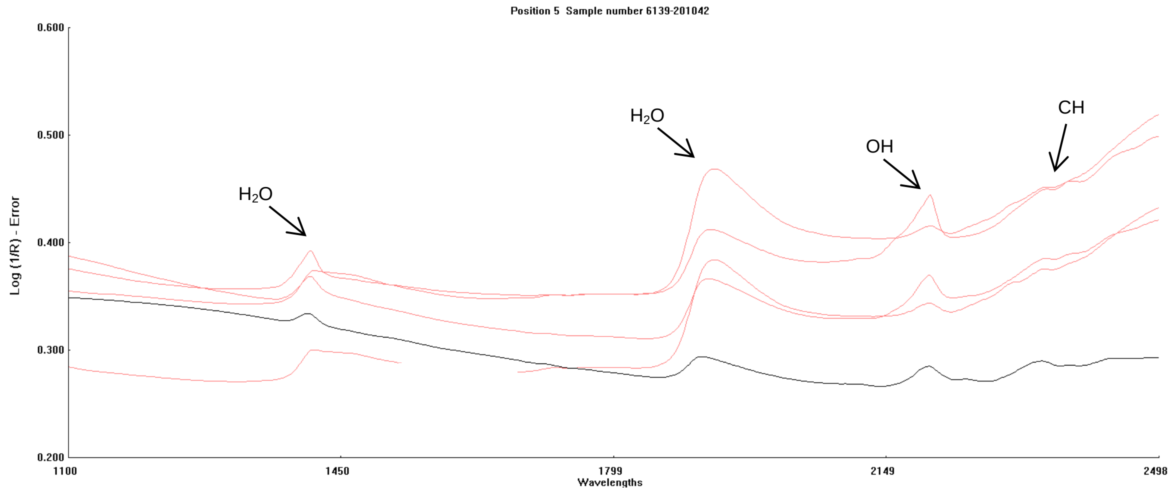

Figure 2. Example of NIR spectra of forest soil samples and some functional groups identified according to the pattern of the absorption spectra.

3.5.1 Prediction of bulk density using NIRS

Firstly, NIR spectra of the dried and milled (2 mm) soil samples were recorded in the range from 1100 to 2500 nm, at 2-nm intervals, on a FOSS NIRS Systems 5000 spectrophotometer (FOSS NIRSystems, Silver Springs, MD,USA), using a transport module sampling attachment and a quartercup sample holder (Figure 3). Reflectance mode spectra were collected using Infrasoft International ISIscan Software, Version 2.85.3 (FOSS Analytical AB, Hoganas, Sweden).

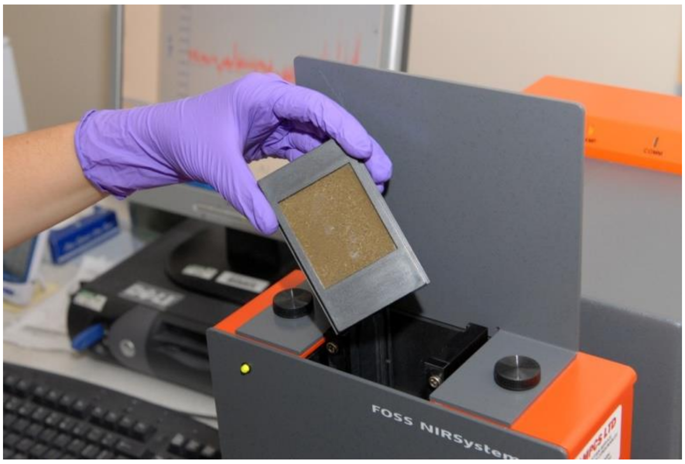

Figure 3. Scanning of a soil sample in a NIR spectrophotometer

Infrasoft International WINISI III Software, Version 1.50 E (FOSS Tecator AB, Hoganas, Sweden), was used for the development of calibration equations to predict the bulk densities. Initial calibrations were developed by regressing laboratory derived bulk density values against spectral data of 118 recent soil samples from the resampled forest soil profiles using partial least squares regression. Prior to developing the calibrations, some pre-processing of the raw spectral data was carried out (standard normal variate with detrend (SNVD) scatter correction and a second derivative).

Assessment of the initial calibration using a test set of 86 resampled forest soil samples showed that NIR spectra contained information that correlated to the bulk density (squared correlation coefficient between predicted and measured values of 0.81 and standard error of prediction of 0.26 g cm^{-3}). Although these parameters predicted by the equation developed for the forest soil dataset were acceptable, to avoid the potential bias of having measured values for one set (recent) and predicted values for the other (archive) the bulk density was predicted for both sets. The predictions were based on a calibration previously developed for the National Soils Inventory of Scotland (NSIS) which encompassed a wider range of soils and meant that both recent and archived samples were predicted using an equation not solely derived from one of the afforested soil datasets. This calibration equation gave a squared correlation coefficient between spectra and measured values of 0.91and standard error of calibration of 0.14 g cm^{-3} (Chapman *et al*., 2013) for the NSIS dataset.

Ideally, a calibration set should encompass as much of the variability found in the population under study as possible. Figure 4 represents a 3D plot resulted from Principal Components analysis (PCA) and shows that the spectral variability of the forest soils fits well within the variability found in the NSIS soils population. In other words, the population of NSIS soils contains samples that have a similar nature to the forest soils population and therefore it could be expected that calibrations developed using the NSIS soils would be representative and robust enough to predict bulk densities of the resampled afforested soils.

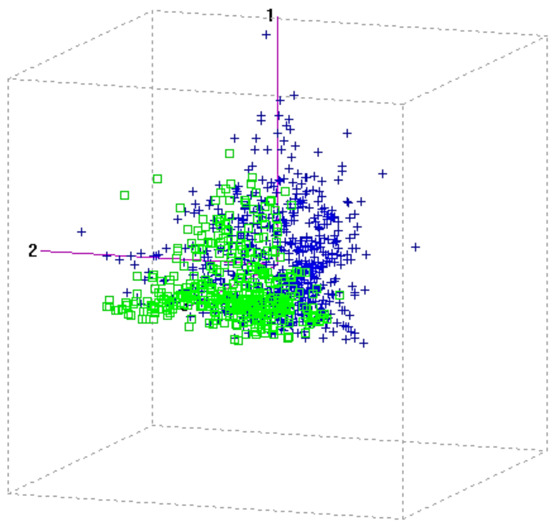

Figure 4. 3D plot showing distribution of PCA scores of the NIR spectra of NSIS soil (blue) and forest soil (green) along the first three principal components. Each point describes an individual sample spectrum as a point in space, and its distance from the centre of the box is known as its global H (GH) value. Samples with a GH greater than 3 are regarded as not typical of the materials being examined.

Initial predictions using the NSIS soils equation were obtained for the resampled, afforested soils; however, the predicted bulk density values of the afforested showed that the equation was not performing uniformly; in particular, residuals of the comparison were greater for those samples with greater carbon content. Therefore, as with the NSIS dataset (Chapman *et al*., 2013) the dataset split on the basis of whether carbon content of the soils was greater or less than 370 g kg^{-1} C (which corresponds approximately to an organic matter content of 75% and around the threshold above which a soil is considered to be peat).

Predictions for the forest soils set with carbon content greater than 370 g kg^{-1}, were more accurate when applying a calibration equation developed using NSIS soil samples using the same carbon content threshold (partial least squares regression, 2nd order derivative and SNVD scatter correction were applied) and achieved a standard error of prediction of 0.034 g cm^{-3} as opposed to an error of 0.092 g cm^{-3} achieved using the NSIS equation for all soil samples. For the forest soils that contained less than 370 g kg^{-1} carbon, the best predictions were achieved when the calibration equation was derived from all NSIS samples (partial least squares regression, 1st derivative and SNVD scatter correction) achieving a standard error of prediction of 0.284 g cm^{-3} as opposed to an error of 0.92 g cm^{-3} obtained for the equation developed using only the NSIS soils with a carbon content below 370 g kg^{-1}. While this seems to be a relatively high error, it is likely to apply equally to both the recent and archived samples.

A small number of predicted samples within each set presented a spectra that differed significantly from the mean spectra of the NSIS calibration population (a global H value > 3 see figure 4 and footnote[1]) and were highlighted as outliers. These samples were from top horizons, which have high organic matter contents but low bulk density (i.e. Litter layers) and also those rare soils with specific characteristics that were underrepresented within the NSIS population. Two samples which had carbon content just below 370 g kg^{-1} were predicted to have negative bulk densities so the predictions were repeated using the equation applied to the set of samples with carbon content greater than 370 g kg^{-1}.

3.6 Carbon stock calculation

The soil profile sampling methodology essentially followed that used for the National Soil Inventory of Scotland (NSIS) as described by Lilly *et al*. (2013) and Chapman *et al*. (2013). Those sampled 1961–1988 were denoted as ‘archive’ and those sampled in 2013 as ‘recent’. Accordingly, carbon stocks were calculated on the same basis as previously described (Chapman *et al*., 2013) with minor differences. Briefly, the profile C stock to 100 cm was calculated as the sum of the separate soil horizon carbon stocks as calculated from the product of horizon depth, fine earth bulk density and carbon content. A correction was applied for stone content. Unfortunately for some archive samples the description of the stone content was incomplete and did not follow the six-point scale in two size classes (Lilly *et al*., 2010). Hence stone content for both archive and recent samples was based only on the recent sampling values. While this approach may introduce some errors in the C stock calculation, it provides consistency across all the sampled profiles and care was taken to site the profile pit in the least disturbed, unploughed area of the forest.

A second difference in calculation procedure was designed to account for the appearance of a significant litter layer under the growing forest, which was evident in the majority of recent samples. Previously, the surface was taken as the top of the soil, including any litter layer. However, measuring 100 cm from the top of the developing litter layer would effectively discount an equivalent depth of soil from the base of the profile and result in a reduced C stock, i.e. we would not be comparing C stocks to the same original depth. Hence, we also calculated carbon stocks for the recent samples to (100 + h) cm where h is the thickness of the new litter layer or the increase in the thickness of the litter layer. Generally, the litter layer was identified as L, LF or LFH but in a few cases was seen as an increase in F, FH or H.

[1] The Global H (GH) value is the square of the Mahalanobis distance divided by the number of dimensions. As a rule of thumb samples GH values greater than 3 are commonly regarded as outliers.

Bulk density values were measured for the recent samples but were not determined for the archive samples. As previously described (Chapman *et al.*, 2013), bulk density for both archive and recent sample sets were predicted from their NIR spectra using calibrations from the NSIS (National Soil Inventory of Scotland) dataset. However, as described above, comparison of measured and predicted bulk density values for litter layers (L only) indicated that the NIR predicted values were being over-predicted (see results below). Hence for recent L layers the measured values were used. Ten recent sites with L layers (all under conifers) were missing measured data so for these the mean value from the other sites (0.0591 g cm^{-3}) was used. There was only one archive moorland site with a L layer; again the mean value was used for this.

4 Results

4.1 Changes in C stock

In total, 39 profiles (sites) were sampled from the 40 proposed. For the archive samples this gave 190 soil horizons (a mean of approximately 5 per profile) and for the recent samples there were 239 horizons (a mean of approximately 6 per profile). In a few cases these numbers include horizons that had been sampled at 2 or 3 different depths. Of the archive profiles, only 8 had a litter layer while for the recent sampling 37 had a litter layer. At the archive sampling, 26 profiles were organo-mineral, 5 were deep peat (peat ≥100 cm) and 8 were mineral. More specifically, 15 were podzols, 11 were gleys, 6 were peats (includes one shallow peat, between 50 and 100 cm); there were two rankers, two regosols, two brown earths and one brown magnesian soil (see Table 1).

In terms of vegetation, two sites (Ardgour and Fardin 1) were listed as heather moor as, for operational reasons, they were sampled within open areas within the woodland; these were those without a litter layer. While they may have had some tree root impacts, they were lacking tree litter inputs and so were omitted from further analysis. One site (Monadh Mor) had no planting date and, following inspection of photographs and maps, it was concluded to be a woodland generation area. Since this makes the time and intensity of afforestation uncertain, this site was omitted. A further site (Minchmoor) had been felled and the original planting date was uncertain; current photographs showed brash but some regeneration. In view of these uncertainties, this site was also omitted. This left 35 sites; 14 had Sitka spruce, 10 were Larch, four had Scots pine, two had pine (not specified), four had Lodgepole pine and there was one of Norway spruce. Of these 35 remaining sites, 23 profiles were organo-mineral, 4 were peat and 8 were mineral.

The mean sampling depths were 97.0 (range 40–156) and 96.0 (range 70–110) cm for the archive and recent samplings, respectively, excluding those profiles where rock, boulders, flooding or an indurated layer were present. In the majority of cases, sampling was down to the C horizon, excluding the deep peats.

The time interval between planting and either the recent sampling or the felling varied between 21.4 and 57.0 years with a mean of 37.5 years (FR *pers. comm.*). For one felled site (North Lochaber) the felling date was not available so it was taken as the mean of the other felled sites (2009). Altogether four sites had been felled (North Lochaber, Fetteresso Forest, Dochroyle and Creebank) and one further site had been both felled and put into second rotation (Cullendoch). The time in second rotation (<5 years) was not considered long enough to have any significant impact on C stocks. However the processes of felling and replanting may have impacted surficial horizons such that results from these sites should be regarded with some circumspection.

The carbon stock data (expressed as t C ha^{-1}) for the individual sites at the two sampling times is given in Appendix 3. The recent sampling stock is calculated with and without adding the increase in litter layer thickness to the calculation of soil profile depth. In a number of cases there is no difference between these two calculations; this is because the sampling depth was limited by rock or other obstruction such that it would be unrealistic to extrapolate to 100 cm. All the subsequent calculations utilize the values that account for the increased litter thickness. Besides calculating the change in carbon stock, we have also calculated the annual change in carbon stock. This is on the assumption that the longer the soil is under trees, the greater the impact, and also to give a figure that may be comparable with other studies. However, it should be recognised that the impact is unlikely to be linear with time and probably follows more of a sigmoid curve. In practice there was no significant correlation between time and the measured changes including thickness of the litter layer.

The increase in thickness of the litter layer varied between -5 and 11 cm with a mean of 5.0 ± 0.6 cm (where ± is the standard error and n=35). Eight sites had a litter layer (L, LF or LFH) at the archive sampling. In two cases there was an apparent loss of litter layer (at Lairgs 1 and Glenkirk 3). The litter layer recorded at the recent sampling was taken as LFH (or some combination of LFH). However, careful inspection of the developed horizons suggested that in some cases an Of, Oa or Os horizon should also be included as part of the new litter. As might be expected, the increase in litter depth was highly significant (t_{35} = 8.60, P<0.001).

The increase in carbon stock within the litter layer and the annual change in litter layer (with the same caveats as for the overall change in carbon stock) are also given in Appendix 3. We have also calculated a change in soil carbon stock (and the annual change); this is the difference between the overall change in carbon stock and the change in the litter carbon stock. The intention was to see if there were any significant impacts of afforestation on the soil carbon stock, apart from the formation of a litter layer.

A summary of the individual site data is given in Table 2. It should be noted that some of the carbon stock values are heavily influenced by the sites on deep peat which tended to have rather large C stock values but also exhibit large, and variable, changes in C stock. For reasons previously discussed (Chapman *et al*., 2013), it is not possible to include deep peat soils (defined as >1m thick) in total carbon stock calculations. As the carbon content of peat is relatively uniform, the only way to establish losses or gains in C stock is through measurement of overall depth but this is often difficult to establish where the peat layer extends beyond 1m in deep peats. Also, it is more difficult to obtain bulk density samples at depths greater than 1 m. However, it is perfectly possible to include them when solely looking at litter layer changes.

Table 3 gives the results of testing whether the changes in carbon stock are significantly different from zero. Where the deep peat soils are included there is no significant change in total carbon stock or in the soil carbon stock. However, the increase in litter carbon stock of -18.7 t C ha^{-1} and the annual increase of 0.53 t C ha^{-1} a^{-1} in litter stock are both highly significant. Also the increase in depth of the litter layer of 5.0 cm is also highly significant. Table 4 gives the results with the deep peat soils excluded. In this case, in addition to the increase in litter stock and annual increase in litter stock being highly significant, the annual change in total carbon stock to 1 m of 0.48 t C ha^{-1} a^{-1} approaches significance (P=0.07). It is worth noting that removing the deep peat soils from the dataset does not alter the mean value for the change in litter carbon stock as the mean for the deep peat sites is quite similar to the other non-deep peat sites. Additionally, the change in litter carbon stock fully accounts for the overall change in carbon stock. The value for the change in soil carbon stock (i.e. that below the litter layer) suggests a loss but it is not significantly different from zero.

Table 5 gives the results with the clear fell sites omitted; these were similar to the previous tables. Table 6 gives the results with both deep peats and clear fell sites removed from the dataset. In this case, besides the changes in litter being highly significant, the overall change in carbon (litter plus soil) was significant (P=0.031) at 21.4 t C ha^{-1} and when expressed as the annual change it was again significant (P=0.026) at 0.65 t C ha^{-1} a^{-1}. Notably, 81% of the total carbon gain was attributable to the increase in thickness of the litter layer. Table 7 shows the data for the clear fell sites only; here there was significant gains in the litter though the number of sites (5) was really too small for reliable analysis. However, it does suggest that despite the felling operations (and in one site the added second planting operations) the accumulated litter was still identifiable.

Where there were sufficient numbers for ANOVA, the litter accumulation under different tree stands were compared. Litter accumulation under Larch (n=10), Pine (n=10) and Spruce (n=15) were 11.7, 17.7 and 24.1 t C ha^{-1}, respectively, but the differences just failed to be statistically significant (P=0.060). More specifically, Sitka spruce (24.2 t C ha^{-1}, n=14) accumulated significantly (P=0.024) more litter carbon than larch (11.7 t C ha^{-1}, n=10). This was also true on an annual basis (P=0.045): Sitka spruce with 0.65 t C ha^{-1} a^{-1}, and Larch with 0.33 t C ha^{-1} a^{-1}. Litter depths were 3.4, 5.3 and 5.9 cm under Larch, Pine and Spruce, respectively, with the difference between Larch (3.4 cm) and Sitka spruce (5.9 cm) being statistically significant (P=0.042).

A similar process was applied to the different soil types although the sample numbers for most soil types were too small to test. However, there were no significant differences between podzols (n=12) and gleys (n=11). There was perhaps a weak trend (P=0.208) for podzols to lose soil carbon below the litter layer (-13.1 t C ha^{-1}) and gleys to gain carbon (17.7 t C ha^{-1}). Soils were also divided into mineral (n=7), organo-mineral (n=23) and organic (peat) (n=5) but there were no statistically significant differences between these groupings.

An examination of the purely organic soil horizons, designated either ‘O’ or ‘H’ as the first letter in the horizon description was undertaken. The deep peats were omitted since the horizons carried on below the sampling point. Eleven sites had no ‘O’ or ‘H’ horizons at the first sampling time and so were also omitted, giving a total of 20 sites. Carbon stocks were then calculated for these organic horizons and compared between the two time points. There was a mean loss of 7.93 t C ha^{-1} but this was not statistically different from zero (95% CI -37.02 to 21.16; P=0.575). There was a trend for Pine (n=3) to gain (51.26 t C ha^{-1}) and Spruce (n=12) to lose (-25.48 t C ha^{-1}), with Larch (n=5) almost neutral (1.32 t C ha^{-1}) but these were not statistically significant (P=0.156). There was no significant difference in the change in the organic horizons between soil types. However, Figure 5 shows the combination of soil type and tree type; numbers are very small but there is a tendency for gleys to gain carbon and for the podzols and rankers to lose carbon, which is more marked under Spruce. All three Pine examples gain carbon. As the sample numbers in these groupings were low, it is difficult to ascertain if these trends are real and the results should be treated with caution until a larger dataset can be derived.

Table 2. Summary of carbon stock data for afforested sites (n=35). Note that minus values indicate carbon loss.

Parameter	Units	Min	Mean	Max	Standard Error
Archive stock	t C ha^{-1}	5.6	240.5	735.6	31.4
Recent stock	t C ha^{-1}	40.2	245.1	648.0	26.5
Change	t C ha^{-1}	-428.1	4.5	185.6	17.2
Time interval	years	21.4	37.5	57.0	1.7
Annual change	t C ha^{-1} a^{-1}	-10.59	0.23	5.10	0.45
Change in litter	t C ha^{-1}	-6.4	18.7	52.8	2.2
Annual change in litter	t C ha^{-1} a^{-1}	-0.16	0.53	1.49	0.07
Change in soil	t C ha^{-1}	-448.0	-14.2	161.0	17.3
Annual change in soil	t C ha^{-1} a^{-1}	-11.08	-0.30	4.42	0.45
Litter depth	cm	-5.0	5.0	11.0	0.6

Table 3. Results of t-tests on carbon stock parameters.

Parameter	Units	Mean	95% confidence interval	t_{34}	P
Change (litter + soil)	t C ha^{-1}	4.54	-30.46 to 39.53	0.26	0.794
Annual change (litter + soil)	t C ha^{-1} a^{-1}	0.23	-0.70 to 1.15	0.50	0.620
Change in litter	t C ha^{-1}	18.7	14.3 to 23.2	8.55	**<0.001**
Annual change in litter	t C ha^{-1} a^{-1}	0.53	0.40 to 0.66	8.21	**<0.001**
Change in soil	t C ha^{-1}	-14.2	-49.3 to 20.9	-0.82	0.416
Annual change in soil	t C ha^{-1} a^{-1}	-0.30	-1.22 to 0.61	-0.67	0.508
Litter depth	cm	5.00	3.82 to 6.18	8.60	**<0.001**

Table 4. Results of t-tests on carbon stock parameters (sites with deep peat excluded).

Parameter	Units	Mean	95% confidence interval	t_{30}	P
Change (litter + soil)	t C ha^{-1}	15.04	-4.45 to 34.53	1.58	0.125
Annual change (litter + soil)	t C ha^{-1} a^{-1}	0.48	-0.05 to 1.02	1.87	0.072
Change in litter	t C ha^{-1}	18.7	13.7 to 23.7	7.61	**<0.001**
Annual change in litter	t C ha^{-1} a^{-1}	0.53	0.38 to 0.67	7.31	**<0.001**
Change in soil	t C ha^{-1}	-3.6	-23.8 to 16.5	-0.37	0.714
Annual change in soil	t C ha^{-1} a^{-1}	-0.04	-0.57 to 0.48	-0.16	0.872
Litter depth	cm	4.9	3.6 to 6.2	7.58	**<0.001**

Table 5. Results of t-tests on carbon stock parameters (sites with clear fell excluded).

Parameter	Units	Mean	95% confidence interval	t_{29}	P
Change (litter + soil)	t C ha^{-1}	8.33	-31.44 to 48.10	0.43	0.672
Annual change (litter + soil	t C ha^{-1} a^{-1}	0.32	-0.74 to 1.38	0.62	0.537
Change in litter	t C ha^{-1}	17.5	13.0 to 22.1	7.82	**<0.001**
Annual change in litter	t C ha^{-1} a^{-1}	0.52	0.37 to 0.66	7.31	**<0.001**
Change in soil	t C ha^{-1}	-9.2	-48.8 to 30.4	0.48	0.638
Annual change in soil	t C ha^{-1} a^{-1}	-0.19	-1.24 to 0.85	0.38	0.707
Litter depth	cm	4.6	3.4 to 5.9	7.68	**<0.001**

Table 6. Results of t-tests on carbon stock parameters (sites with both deep peat and clear fell excluded).

Parameter	Units	Mean	95% confidence interval	t_{25}	P
Change (litter + soil)	t C ha^{-1}	21.4	2.1 to 40.8	2.29	**0.031**
Annual change (litter + soil)	t C ha^{-1} a^{-1}	0.65	0.08 to 1.21	2.37	**0.026**
Change in litter	t C ha^{-1}	17.3	12.0 to 22.6	6.76	**<0.001**
Annual change in litter	t C ha^{-1} a^{-1}	0.51	0.35 to 0.68	6.34	**<0.001**
Change in soil	t C ha^{-1}	4.1	-15.3 to 23.5	0.44	0.664
Annual change in soil	t C ha^{-1} a^{-1}	0.13	-0.42 to 0.68	0.49	0.629
Litter depth	cm	4.5	3.1 to 5.9	6.56	**<0.001**

Table 7. Results of t-tests on carbon stock parameters (clear fell sites only).

Parameter	Units	Mean	95% confidence interval	t_4	P
Change in litter	t C ha^{-1}	25.9	5.9 to 45.9	3.59	**0.023**
Annual change in litter	t C ha^{-1} a^{-1}	0.60	0.14 to 1.06	3.62	**0.022**
Litter depth	cm	7.2	2.5 to 11.9	4.27	**0.013**

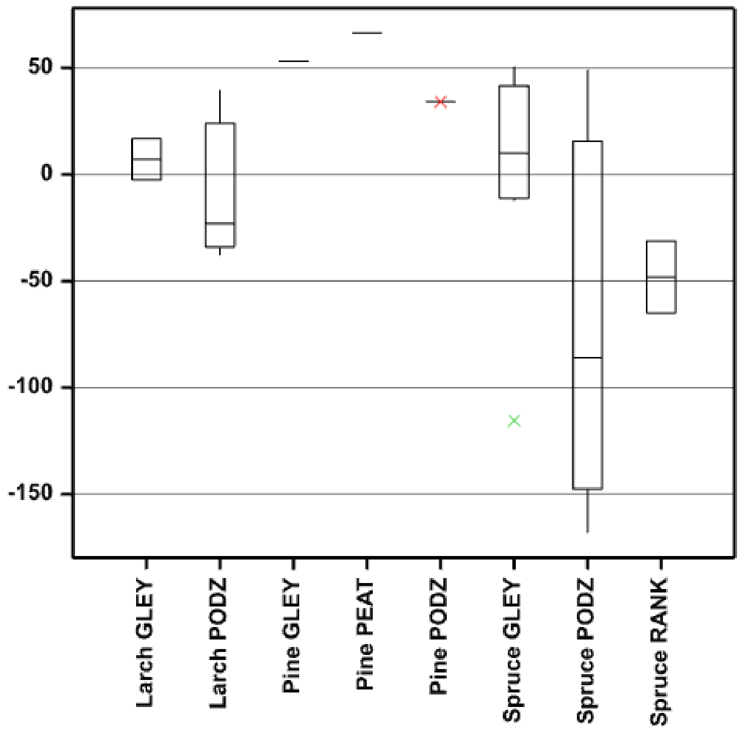

Figure 5. Box plot of change in carbon (t C ha^{-1}) within organic (O/H) horizons by tree and soil type. Negative values are showing carbon loss.

4.2 Inter laboratory comparison of C concentration

As well as sampling the soil profile following the NSIS 2007-9 protocols, we also sampled the soil profile following the BioSoil protocols (by fixed depth). We subdivided these samples and had them analysed for carbon concentration both at the James Hutton Institute and at Forest Research's Alice Holt laboratory with the aim of merging both the NSIS data from woodland sites with that of the BioSoil sample sites to increase the sample size of woodland soils in Scotland. Initial results showed that there was a systematic difference in C concentration between the two laboratories with organic horizons (those with C concentration > 37%) to be on average 5.1± 1.06% (mean ± SD, n=111) greater than the measurement from one laboratory while, for mineral horizons, they were greater by 1.3 ± 1.96% (mean ± SD, n=152) at the other and the overall mean difference for all horizons was 2.9 ± 2.48% C (n=263). Some of the difference may be due to differences in the reference material used by each laboratory and this will be explored in the future.

5 Discussion

There is little evidence of any significant changes in the soil carbon below the surface litter horizon. There was a trend for organic layers to decrease under Spruce in podzols and rankers but this was not statistically significant. The overall extent of carbon accumulation is similar to that seen in for woodland vegetation during the NSIS resampling (Chapman *et al*., 2013). In that study, woodlands showed a mean increase of 23 t ha^{-1} compared to the 21.4 t C ha^{-1} reported here. On an annual basis Chapman *et al*. (2013) reported an increase of 0.54 t C ha^{-1} a^{-1} in the top 15 cm (which would include the litter layer), which is similar to the

0.53 t C ha^{-1} a^{-1} found here for the increase in litter stock. However, they reported only a 0.9 cm increase in the litter layer thickness compared with the 5.0 cm found here though it is important to note that the majority of the woodland sites in the NSIS study (25/30) were already woodland at the first sampling. In terms of the gain in carbon (soil plus litter), it might be suggested that Sitka spruce is superior to larch.

6 Conclusion

We have demonstrated that long-term afforestation of soils previously under moorland vegetation generally leads to an increase in soil carbon, expressed either on a total change or annual change basis and that this increase can largely be accounted for by the increase in thickness and carbon content of the litter layer. It is possible that some of the larger changes are due to differences in soil horizon thickness (particularly of the organic layers) between the two sampling periods. These differences are principally due to the inherent natural variability in soils but it means that direct comparison between pre- and –post planting at individual sites is not advised and that a large number of sample sites are needed to identify real changes.

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8 Appendix 1: Field sampling protocols (Afforested soils resampling, 2013)

These protocols were given to each survey team prior to the start of the sampling phase.

A soil profile pit should be excavated at each location to a depth in excess of 80 cm wherever possible and certainly to within the soil parent material and to allow a fixed depth sample from 40 to 80 cm to be taken. Once excavated, the profile should be examined and the main horizons identified.

Profile horizon Samples (PB)

The limits of the horizons to be sampled should first be marked with a knife on the pit face and where possible, with the exception of iron pans (Bf horizon), each horizon in the profile should be sampled. The surface organic horizons (L, F, H and O horizons) should be sampled even if it means that material has to be taken from a wide area bearing in mind these horizons will also need to be sampled to determine their bulk densities. In circumstances where surface organic horizons are not thick enough, or the delineation so indistinct, to allow sampling horizons individually, composite samples may have to be taken to allow a satisfactory volume of sample to be obtained. The suggested composite samples are FH with the litter layer (L) sampled separately. Approximately 1 to 1.5 kg of relatively stone free soil should be collected from each horizon (1 for organic horizons). As many stones should be removed from the sample as possible.

Samples should be taken from a 10 cm depth band, approximately in the middle of the horizon or at depths thought to be appropriate, either for comparative purposes with archive samples or where the thickness of the horizon is judged to warrant more than one sample. In some situations where the horizon thickness is less than 10 cm, the top and bottom sample depths will be set to allow a representative and pure sample to be collected from the horizon. Thin transition zones up to 6 cm thick can be excluded where boundaries are gradual or diffuse and where they were not sampled previously.

The soil material is loosened and extracted by a clean trowel or knife, collected in a sampling tray held level with the lower boundary of the sample depth, and placed in a bag. Two labels are prepared with profile name, NGR, horizon symbol, depth of sample, type of sample (MARK AS PB), date of sampling and surveyor initials written legibly. Excess air should be removed from the sample bag and sealed as soon as possible to avoid contamination. It should be made air tight by folding over the top few centimetres twice. If not to be double bagged, a sample label should be slotted under the fold and another attached to the outside before stapling shut. This bag can be placed inside another if required, in which case, a label can be placed between both bags. This second bag is then stapled with the second label attached to it.

Bulk Density Samples (DB)

Food-grade stainless steel rings of 7.6 cm diameter (7.2 cm internal diameter) x 5 cm height are inserted either vertically or horizontally into the appropriate horizon by applying an even pressure. A knife, scraping tool or other sharp instrument can be used to cut the soil and large roots below the core depth and to push small stones to one side to improve insertion, however, it is best to attempt to avoid stones. Ensure there is no compression or compaction sustained in the sampling process. Bulk density samples will be taken in triplicate from each horizon whenever possible. The rings are carefully extracted using a trowel or knife and carefully trimmed to ensure that there is no extruding material or stones. Small gaps can be repacked with aggregates provided they are not compressed and extruding stones should be removed with the gap again filled with an appropriately sized aggregate.

Where horizons are thinner than 5 cm, it is legitimate to partially fill the ring, excavate, move to a fresh area and insert the partially filled ring until full. Take care not to compress the material. All the material is then extruded into individual mini-grip bags which are labelled using a marker pen with sample number 1-3. These bags can then be placed into a large soil sample bag for ease of sorting later. This bag is then labelled with profile name, NGR, horizon symbol, depth of sample, type of sample (MARK AS DB), date of sampling and surveyor initials and then stapled.

For litter layers (L) thinner than 5 cm an alternative method to determine bulk density which matches the BioSoil protocols is to sample a volume comprising an area and the full horizon thickness and record the dimensions. This volume may exceed that of the mini-grip bags so the material should be placed in a normal soil sample bag, labelled with profile name, NGR, horizon symbol, depth and area of sample, type of sample (MARK AS DB), date of sampling and surveyor initials and then stapled.

A guide to achieving equivalent sample volumes by this method is given below compared with 3 ring samples each 210 cm^3:

Thickness L layer (cm)	***Sample area***	***Volume cm^3***
1	*25 x 25*	*625*
2	*18 x 18*	*578*
3	*15 x 15*	*675*
4	*13 x 13*	*676*

Fixed depth sampling (FD)

As one of the objectives of the resampling programme is to compare sampling by pedology with fixed depth sampling (eg according to BioSoil protocols), samples at depths of 0-5, 5-10, 10-20, 20-40, and 40-80 cm will be taken from the right hand side of the profile pit or where the horizon depths and sequences are similar to the depths where the Profile Bulk samples were taken. **Zero** is taken as the top of the mineral or amorphous peat layer **NOT the soil surface**. Care is required to differentiate between loose litter and weakly decomposing layers (forest floor) and the top of the upper soil layer. See figure below.

Mineral soils: the top of the mineral layer is taken as zero and any litter above is recorded as a negative depth. The mineral soil is sampled 0-5, 5-10,10-20, 20-40, 40-80cm (where appropriate and feasible). The litter (L) is sampled by area (25x25 cm minimum) and by depth. The full thickness of any loose or weakly decomposed organic **layer** above the original mineral surface layer should be sampled. Where an organic-rich layer has developed within the original mineral surface layer, this should be treated as pedological development and the zero datum will be the surface of this layer.

Organo-mineral soils: as above 'organic layers' are considered to be loose or only partly decomposed forest litter, therefore the zero datum is the top of the amorphous H (for HIP) and O (for PG/PGP/PP) and the soil is sampled 0-5, 5-10,10-20, 20-40, 40-80cm (where appropriate and feasible). The litter (L) is sampled by area (25cm x 25 cm min) and by depth. The full thickness of any organic layer above the zero datum for these soils should be sampled and will be recorded as negative depths.

Organic soils: where the soil has >40 cm of peat (excluding live sphagnum and loose litter or partly decomposing material which are considered as ‘forest floor’ deposits) then the zero datum is taken as the top of the amorphous layer. In this case the zero is the top of the amorphous peat and the soil is sampled 0-5, 5-10, 10-20, 20-40, 40-80cm (where appropriate and feasible). The litter (L) is sampled by area (25 cm x 25 cm min) and by depth. The full thickness of any organic layer above the zero datum for these soils should be sampled and will be recorded as negative depth.

Fixed depth samples should be labelled with profile name, NGR, horizon symbol for organic layers above Zero, depth of sample (negative for organic layers above zero), type of sample

(MARK AS FD), date of sampling and surveyor initials. For samples taken below the Zero datum, use S05, S10, S20, S40, S80 for fixed depths 0-5, 5-10,10-20, 20-40, 40-80cm

Figure 1: Example and stylised horizon sequence sampling depths

		Mineral Soil	Organo-mineral Soil		Organic Soil
			Aerobic	Anaerobic	
Organic layers	*-x - -y*	L		L	L
	-y - -z	F	L	Of	Of
	-z - 0	LF	F	Os	Os
Zero					
Soil	0-5	Ah	H	Oa	Oa 1
	5-10	A	H	Oa	Oa 1
	10-20	A	E	E	Oa 1
	20-40	Bs	Bs/Bg	Bs/Bg	Oa 1
	40-80	BC/C	C/Cg	C/Cg	Oa 2/Oa 3

10 Appendix 2: Comparison of sampling approaches.

NSIS/BioSoil and notes on FC sites, 2013

	NSIS sampling	**BioSoil**	**Notes**	**FC sites sampling 2013 outcome**
1	Profile bulk sampling by horizon (volume 1.5-2 kg)	Fixed depth sampling (0-10, 10-20, 20-40, 40-80 cm) Min 500g	Possibly could do some fixed depth sampling in addition to the horizon samples – note: BioSoil horizon depths measured from top of mineral unless the soil is a peat	Sample by horizon to be comparable to baseline (approx.1 kg)
2	Bulk density by horizon x 3 replicates	From mineral topsoil (0-10 cm) X 5 replicates	Can use pedo-transfer functions (PTFs) but measurement preferred	By horizon x 3 and use data in validation of PTFs
3	Profile bulk and bulk density variability samples, based on 4 satellite pits	Differences between Level I and Level II in terms of numbers of sub-samples and composite samples	Variability sampling not part of baseline sites from 1960s	No variability sampling to be included
4	Sampling generally away from tree stems	Not within 1 m of tree stems	May affect site location towards open areas/rides which is not appropriate	Field test and always sample >1 m from tree stems
5	NGR record	GPS record	Use GPS	Use GPS
6	Site description	Site description	Obtain records from database	Use baseline description in relocation
7	Profile description	Profile description	Beware different organic horizon nomenclature	Use baseline description in relocation
8	Soil classification, Soil Survey of Scotland/JHI	WRB	Can be translated	Compatible and will be described in both terms
9	Soil texture - BSTC	USDA	Can be translated from analyses	BSTC in field and both BSTC and USDA from lab analyses
10	Organic horizons, sampled same as mineral horizons	Sample known volume and fresh/dried weight	Weighed and sampled for moisture content	Samples for both NSIS and BioSoil type determinations
11	Peatland soils are defined as >50 cm	Peatland soils are defined as >40 cm	Note difference	Record as found in field
12	Samples for analysis – labelled as per protocols and measured from surface (0cm) down	‘0 cm’ starts at top of mineral	If samples were being analysed by FC as well, need to be labelled differently and with different depths	To be decided and taken note of in the field sampling

11 Appendix 3 Carbon stocks and changes for individual sites

SITE	Archive Carbon Stock ($t\ C\ ha^{-1}$)	Recent Carbon Stock[a] ($t\ C\ ha^{-1}$)	Recent Carbon Stock[b] ($t\ C\ ha^{-1}$)	Change in Carbon Stock[c] ($t\ C\ ha^{-1}$)	Time interval[d] (years)	Annual Change ($t\ C\ ha^{-1}\ a^{-1}$)	Change in Litter ($t\ C\ ha^{-1}$)	Annual Change in Litter ($t\ C\ ha^{-1}\ a^{-1}$)	Change in Soil[e] ($t\ C\ ha^{-1}$)	Annual Change in Soil ($t\ C\ ha^{-1}\ a^{-1}$)
Glen Oykel	495.8	561.4	608.3	112.5	31.5	3.57	21.1	0.67	91.4	2.90
Achairn 1	697.7	513.8	520.1	-177.6	34.5	-5.15	10.7	0.31	-188.3	-5.46
Kinloch	83.8	174.4	174.4	90.6	32.4	2.79	26.3	0.81	64.3	1.98
Cnoc-an-t-Sabh. 2	79.8	77.2	77.8	-2.0	27.5	-0.07	16.0	0.58	-18.0	-0.66
Lairgs 1	157.4	208.4	207.5	50.1	41.5	1.21	-6.4	-0.16	56.5	1.36
The Wilderness 1	115.5	146.0	146.8	31.3	56.5	0.55	14.4	0.25	17.0	0.30
Tain Quarries	76.8	94.5	94.5	17.7	53.5	0.33	26.4	0.49	-8.7	-0.16
Glenkirk 2	328.9	279.2	279.2	-49.7	27.4	-1.81	2.8	0.10	-52.5	-1.92
Glenkirk 4	234.8	285.1	286.2	51.4	27.4	1.88	14.7	0.54	36.7	1.34
Glenkirk 3	341.3	304.5	304.1	-37.2	27.4	-1.36	-2.8	-0.10	-34.4	-1.26
Buckie-Loch 4	5.6	39.7	40.2	34.6	29.4	1.17	33.8	1.15	0.8	0.03
Teindland Forest 1	169.8	105.4	105.5	-64.3	48.5	-1.33	2.7	0.06	-67.1	-1.38
Glen Fiddich 2	219.7	303.6	304.3	84.6	25.5	3.32	5.2	0.20	79.4	3.11
Green Knowe 1	102.5	136.5	136.5	34.0	33.4	1.02	12.8	0.38	21.3	0.64
Greenknowe 2	108.5	110.0	110.0	1.5	33.4	0.05	9.2	0.28	-7.7	-0.23
Suie 1	172.6	202.8	203.2	30.7	35.4	0.86	52.8	1.49	-22.2	-0.63
Suie B	189.6	231.5	231.5	41.8	35.4	1.18	8.6	0.24	33.3	0.94
Suie 3	124.3	125.4	125.9	1.6	36.4	0.04	11.7	0.32	-10.1	-0.28
Mull	143.2	196.6	196.6	53.3	31.3	1.70	23.6	0.75	29.8	0.95
Tom an t Sidhein	198.7	208.7	214.9	16.2	24.3	0.67	31.0	1.28	-14.8	-0.61
Mid Lorn	735.6	298.9	307.6	-428.1	40.4	-10.59	20.0	0.49	-448.0	-11.08
North Lochaber	629.9	544.8	565.7	-64.2	34.0	-1.89	27.0	0.80	-91.3	-2.68
North Lorn	462.5	598.4	648.0	185.6	36.4	5.10	24.6	0.67	161.0	4.42
Tentsmuir 3	6.5	49.0	50.7	44.1	43.4	1.02	31.7	0.73	12.4	0.29
Fetteresso Forest	471.0	356.7	357.4	-113.6	57.0	-1.99	26.0	0.46	-139.6	-2.45
Knockinculloch	145.8	143.8	143.8	-2.0	21.4	-0.09	22.3	1.04	-24.3	-1.13
Camilty Moss	288.0	220.4	225.9	-62.0	41.4	-1.50	31.5	0.76	-93.5	-2.26
Mid Torrs 3	37.8	62.1	63.0	25.2	52.4	0.48	6.6	0.13	18.5	0.35
Dochroyle 2	309.1	378.9	380.3	71.2	39.0	1.82	26.7	0.68	44.5	1.14
Clauchrieskaig 1	220.4	307.4	311.6	91.2	29.4	3.11	20.7	0.71	70.5	2.40
Clauchrieskaig 2	52.4	85.2	85.2	32.8	29.4	1.12	15.7	0.53	17.1	0.58
Creebank 1	250.3	243.9	245.4	-4.9	47.0	-0.10	47.5	1.01	-52.4	-1.11
Lodge Craiglure	246.5	182.3	182.3	-64.2	44.4	-1.45	22.4	0.50	-86.5	-1.95
Cullendoch 3	222.0	242.0	242.4	20.4	48.0	0.43	2.1	0.04	18.3	0.38
Glengainoch	294.9	401.0	401.0	106.1	56.4	1.88	16.4	0.29	89.7	1.59

[a] To 100 cm, unless constrained by rock, weathered rock, boulders, induration, or flooding on both samplings
[b] To 100 cm + increase in litter depth, unless constrained as above
[c] Minus value indicates C loss from soil
[d] From planting to either recent sampling or felling
[e] Difference between change in total C stock and change in litter C stock

12 Appendix 4: Phase 1 feasibility study (Jan 2013)

Identification of suitable sites for resampling to assess the role of afforestation on soil carbon contents.

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Context: The most significant land use change in Scotland in the second half of the 20th Century was that of converting semi-natural habitats to woodland, especially to conifer plantations. This afforestation is set to continue with the Scottish Forestry Strategy and Land Use Strategy proposing an increase in woodland cover by 10 000 ha per year over the next 10 years. Both native woodlands and non-native coniferous species will contribute to this target, with different proportions in different parts of the country. While projects such as Biosoil (FR) and NSIS resampling (JHI) allow some predictions of soil C sequestration to be made, there is still insufficient data to address some key questions regarding the response to afforestation on different soil types and with different tree species.

This short-term research project investigates existing data to explore the feasibility of a field sampling campaign to secure new soil organic carbon data to address these questions by bringing together information on soils and forestry from James Hutton Institute and Forestry Commission datasets.

Data and Integration

The James Hutton Institute soil database has approximately 13, 000 spatially referenced soil profiles from the 1940s to the present day including profile descriptions, horizon samples, contextual information including vegetation community type and analytical data. A large number of these profiles were sampled between 1960 and 1980 which also coincides with a period of extensive woodland expansion. The individual layers (horizons) were normally sampled rather than sampling at fixed, predetermined depths.

The Forestry Commission National Forest Inventory (NFI) has now published a new woodland area map for Scotland (http://www.forestry.gov.uk/forestry/INFD-8EYJWF). As the NFI ground survey continues, more detail on the woodland areas will be added to the previous National Inventory of Woodlands and Trees (NIWT) survey database. There is already detailed information in the FC's Sub-Compartment Database (SCDB) on the publicly owned forest estate, which contains information on the smallest (mapped) management area within the forests and includes date of planting, previous cultivation and, in many cases, soil type classified according to the FC soil classification system (Kennedy, 2002). A single Sub-compartment may still contain smaller areas within it (components), such as different species (perhaps planted at different times) or unplanted areas. It is therefore possible to have more than one crop within a Sub-compartment.

New plantings undertaken by the private forestry sector are recorded under the Scottish Forestry Grant Scheme (SFGS) which contains information on planting year and possible records of native broadleaved woodland. This information was extracted from the SFGS database but not used further in this analysis of potential sampling sites as it was felt that the initial selection should concentrate on those sites that were within land owned by the Forestry Commission as access for any resampling could be more readily obtained.

When the soils and woodland data are combined, those sites and soils that have undergone a land use change to forestry since the date of the original sampling can be identified. These sites are possible candidates for a future resampling campaign to determine changes in soil carbon stock resulting from land use change between the two dates. The methods employed to identify these sites are detailed below.

Methods

Profile locations that were not under woodland and where the soil horizons had been sampled were selected from the Scottish Soils Database. This selection was made using 5 selection criteria:

1) The 10km National Soil Inventory of Scotland sampled profiles,
2) The 5km National Inventory points of Scotland sampled profiles,
3) The remaining soil profiles sampled since 1979 (which coincides with the start of the National Soil Inventory of Scotland sampling),
4) Soil profiles sampled between 1960 and 1979 and,
5) Soil profiles sampled prior to 1960 (these profiles dated from 1947).

Those 10km National Soil Inventory of Scotland (NSIS) profiles that were sampled during the 2007-09 resampling campaign were omitted from subsequent analyses as the change in C stocks at these sites is already known. Profiles that were not part of the NSIS were subject to additional selection criteria and those that were part of a closely spaced grid or transect sampling scheme, were taken to characterise specific experimental sites, those soils unlikely to be planted with trees (e.g. Alpine podzols), profiles that were sampled to characterise archaeological sites or had 'forest' or 'wood' in the profile name, were not considered as suitable for resampling.

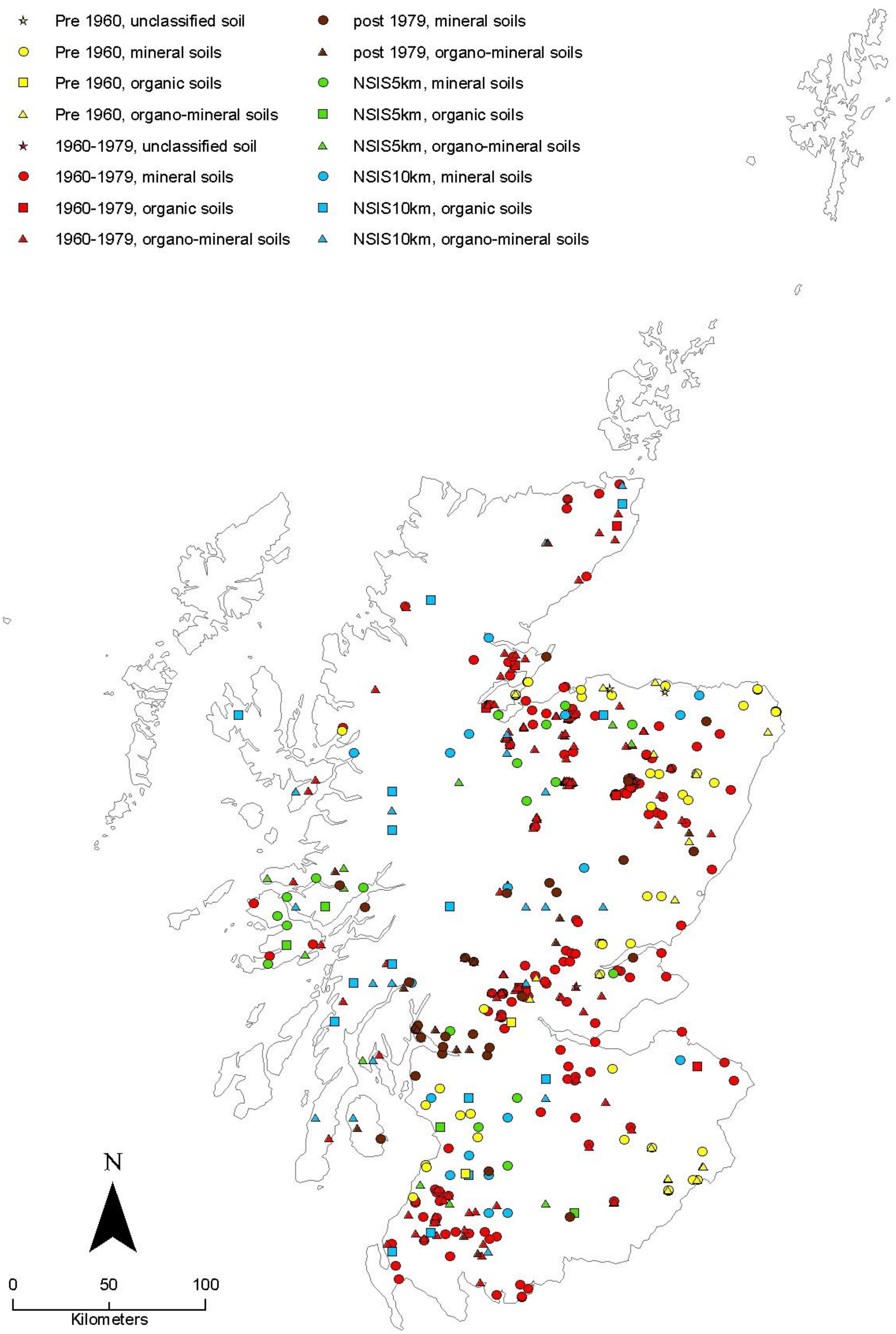
N.B. One of the closely spaced grid sampling schemes was at the JHI Glensaugh farm prior to the installation of an agroforestry experiment. This site offers considerable potential for examining the effect of different species and different tree densities on agricultural land as an additional to national scale profile sampling.

The georeferences for the remaining profiles were then overlain with a national map of the National Forest Inventory spatial dataset (which details all woodland over 0.5ha in extent) and those that lay within these woodland polygons were selected as potential sites for resampling as it appeared that the original land use had changed to forest. The numbers of these profiles are shown in Table 1 and their spatial distribution in Figure 1. The soil profiles in Figure 1 were grouped by broad soil type (mineral, organo-mineral and organic) and by the five sampling periods outlined above. The locations of the profiles are primarily in lowland Scotland which reflects the sampling bias of the Soil Survey of Scotland towards cultivated lowland agricultural soils in the years preceding the National Soil Inventory sampling.

Table 1: The number of soil profiles (per soil type) occurring from each sampling regime which intersected the NFI data during an initial GIS analysis.

	Pre 1960	**1960-79**	**post 1979**	**5km NSIS**	**10km NSIS**	**Total**	**comments**
Alpine podzol		1				1	Unlikely to be planted
Archaeological site		10				10	Not relevant
No class	1	1				2	Not relevant
Brown calcareous soil		1				1	
Brown earth with gleying	8	23	4	1	1	37	
Brown earth	6	38	4	7	2	57	
Brown magnesian soil		4	1			5	
Brown podzolic soil	1	7	2		4	14	
Brown rendzina	1					1	
Brown ranker			1	1	1	3	Unlikely to be planted
Calcareous gley		1				1	
Calcareous regosol		1				1	
Dystrophic peat	2	5		4	13	24	
Humic gley	1	6	3		3	13	
Humus podzol	1	1				2	
Humic ranker				1		1	Unlikely to be planted
Humus iron podzol	16	60	9	6	10	101	
Iron podzol	9	4				13	
Magnesian gley		2				2	
Mesotrophic peat					2	2	
Mineral alluvial soil	2	7	2	1	2	14	
Noncalcareous gley		31	9	2	1	43	
Noncalcareous regosol	14	2				15	
Peat (unclassified)		1				1	
Peaty gleyed podzol	1	16	4	1	1	23	
Peaty gley	7	41	6	7	10	71	
Peaty podzol	5	39	1	1	1	47	
Peaty ranker		1	1	1	1	4	Unlikely to be planted
Podzol (unclassified)		1				1	
Regosol		1				1	
Subalpine podzol		1				1	Unlikely to be planted
Total	75	306	47	33	52	513	

Figure 1: Distribution of soil profiles which intersected the NFI data during an initial GIS analysis.



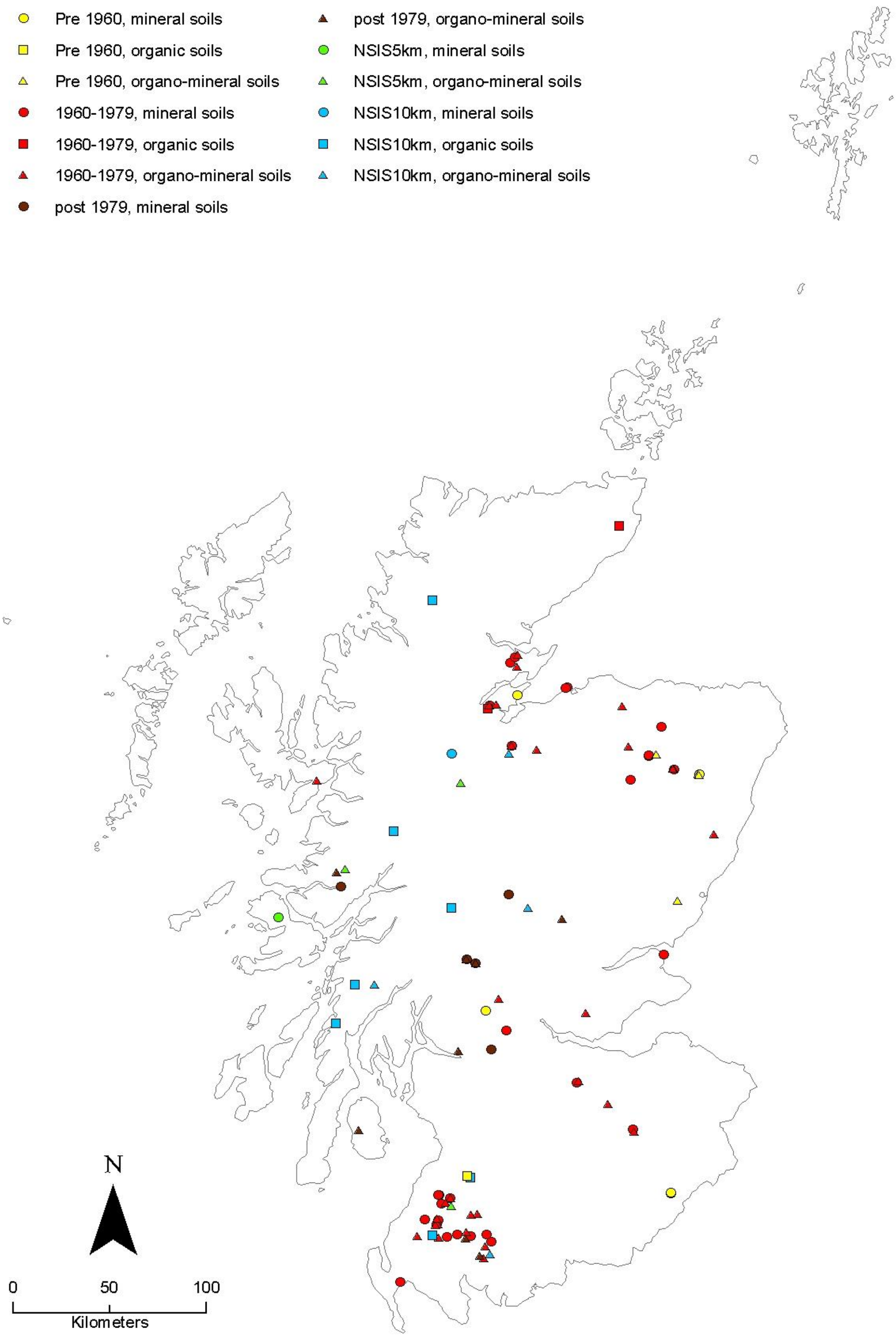
The next stage in the selection process was to identify which of these profiles were located in land now managed by the Forestry Commission and where information on the forest crop are contained within the SCDB. It was felt that this initial selection should concentrate on those sites that were well characterised with information on planting dates and species and were contained within land owned by the Forestry Commission as access for any resampling could be more readily obtained.

This selection produced 104 possible sites where JHI have soil profile information (Table 2) As some sub-compartments comprise more than one tree species or planting date, it was not possible to determine exactly what the species or planting year was at the precise location of the soil profile pit. However, this more detailed information can be obtained during the soil sampling. Figure 2 shows the locations of these profiles, again grouped by broad soil type and sampling period. From Table 2, it can be seen that the most common soil types in Forestry Commission-owned woodland are Humus-iron podzols, Peaty podzols and Peaty gleys (Organo-mineral soils). There is a cluster of sample points in the south west of Scotland (Figure 2) which were sampled in the 1960 to 1979 period. Almost all of these sites were sampled between 1961 and 1964 and reflect the soil mapping programme in that area during this time.

Table 2: JHI profiles at locations where FC have species information at a sub-compartment level.

	Pre 1960	**1960-79**	**post 1979**	**5km NSIS**	**10km NSIS**	**Total**	**comments**
Brown earth with gleying		2	1			3	
Brown earth	1	5	1			7	
Brown magnesian soil		2				2	
Brown podzolic soil		2			1	3	
Brown ranker				1		1	Unlikely to be planted
Calcareous regosol		1				1	
Dystrophic peat	1	2			6	9	
Humic gley		4	1			5	
Humus podzol		1				1	
Humus iron podzol	6	11	5		1	22	
Iron podzol		1				1	
Mesotrophic peat					1	1	
Mineral alluvial soil		1				1	
Noncalcareous gley	2	4	1			7	
Noncalcareous regosol		2				1	
Peaty gleyed podzol		4	1	1	1	7	
Peaty gley	1	13	3		1	18	
Peaty podzol		8		1	1	10	
Peaty ranker			1	1		2	Unlikely to be planted
Podzol (unclassified)		1				1	
Total	11	63	14	4	12	104	

Figure 2: locations of soil profiles where FC has species information at a sub-compartment level



From Table 2, it can be seen that the most common soil types in Forestry Commission-owned woodland are Humus-iron podzols, Peaty podzols and Peaty gleys and, from Figure 2, it can be seen that a number of sample locations are clustered in the south west of Scotland, which introduces a bias in the spatial distribution of the potential sampling sites.

The primary reason for potentially resampling these afforested sites is to determine any changes in soil organic carbon (SOC) content and consequently C stock. Although data on C contents already exist for these soils, from previous soil sampling, it was necessary to establish if all the horizons identified in the soil profiles were indeed sampled. This is crucial to calculate carbon stocks for the profile. Another key aspect is the availability and the amount of archival soil material from those sites on which new analyses can be performed. This is also crucial as previous studies have shown that the measured carbon contents can vary depending on the method and equipment. Therefore, in order to establish change in SOC, it is good practice to reanalyse the archival soil material alongside the newly sampled soil thus eliminating any potential differences between methods or equipment. Therefore, the next stage in the selection process was to establish if all soil horizons had been sampled by comparing the information held within the soil analytical table of the Scottish Soil Database with that from the soil morphology table. A number of soil profile descriptions (61) have not yet been entered into the soil morphology table of the database so the information on the horizon type and thickness had to be collated from paper records.

Once this was completed, the information for the 104 profiles was reviewed and sites with soil profiles where horizons had not been sampled were rejected. If these missing horizons were mineral layers with low C contents then perhaps the rejection criteria could have been relaxed but many of the horizons were highly organic surface horizons (for example, LF horizons) that would be crucial in determining C stocks. This has led to the rejection of a considerable number of sites and only 40 sites remained (Table 3 and Figure 3).

Since it was also important that there was sufficient archived soil material available for analyses, the weight of soil material for each of the horizons in these 40 profiles was assessed. Around half of these profiles (20) were deemed to have a weight of soil below the recommended limit of 80g, below which there is a presumption against further use of this material unless there is a very strong scientific case. Table 3 shows the number and soil types in these final selections while Figures 3 and 4 show the distribution of the 40 potential sampling locations and the 20 which are on FC land, have a full horizon sequence sampled and where there is >80g of archived soil remaining. While there is a presumption against reanalysing archived soil material, one possible way of retaining a potentially larger sample size is to compare the carbon contents of reanalysed freshly sampled soils with the previous measurements of soil carbon from that location. However, during the reanalyses of the archived material and the new soil samples from the NSIS20km sample locations, it was found that current measurements of C concentrations were approximately 11.5% lower than the previous measurements. Thus, although this correction factor could be applied, which reduces the need to reanalyse archived material, this will also increase the uncertainty in the analysis of changes in carbon stocks.

Table 3: JHI profiles at locations where FC has information at a sub-compartment level and where each identified horizon has been sampled. Numbers in brackets are the number of profiles where a horizon has > 80g of material remaining.

	Pre 1960	**1960-79**	**post 1979**	**5km NSIS**	**10km NSIS**	**Total**	**comments**
Brown earth with gleying		2(2)				2(2)	
Brown earth		1(1)				1(1)	
Brown magnesian soil		1(1)				1(1)	
Brown ranker				1(1)		1(1)	Unlikely to be planted
Calcareous regosol		1(1)				1(1)	
Dystrophic peat		2(0)			3(1)	5(1)	
Humic gley		1(0)				1(0)	
Humus podzol		1(0)				1(0)	
Humus iron podzol		5(4)	1(1)			6(5)	
Mesotrophic peat					1(1)	1(1)	
Noncalcareous gley		1(1)				1(1)	
Noncalcareous regosol		1(1)				1(1)	
Peaty gleyed podzol		4(2)		1(0)		5(2)	
Peaty gley		8(1)			1(0)	9(1)	
Peaty podzol		3(1)				3(1)	
Peaty ranker				1(1)		1(1)	Unlikely to be planted
Total		31(15)	1(1)	3(2)	5(2)	40(20)	

Figure 3: JHI soil profiles at locations where FC has information at a sub-compartment level and where each identified horizon has been sampled *(Note; some locations have multiple soil profiles that cannot be shown separately at this scale)*.

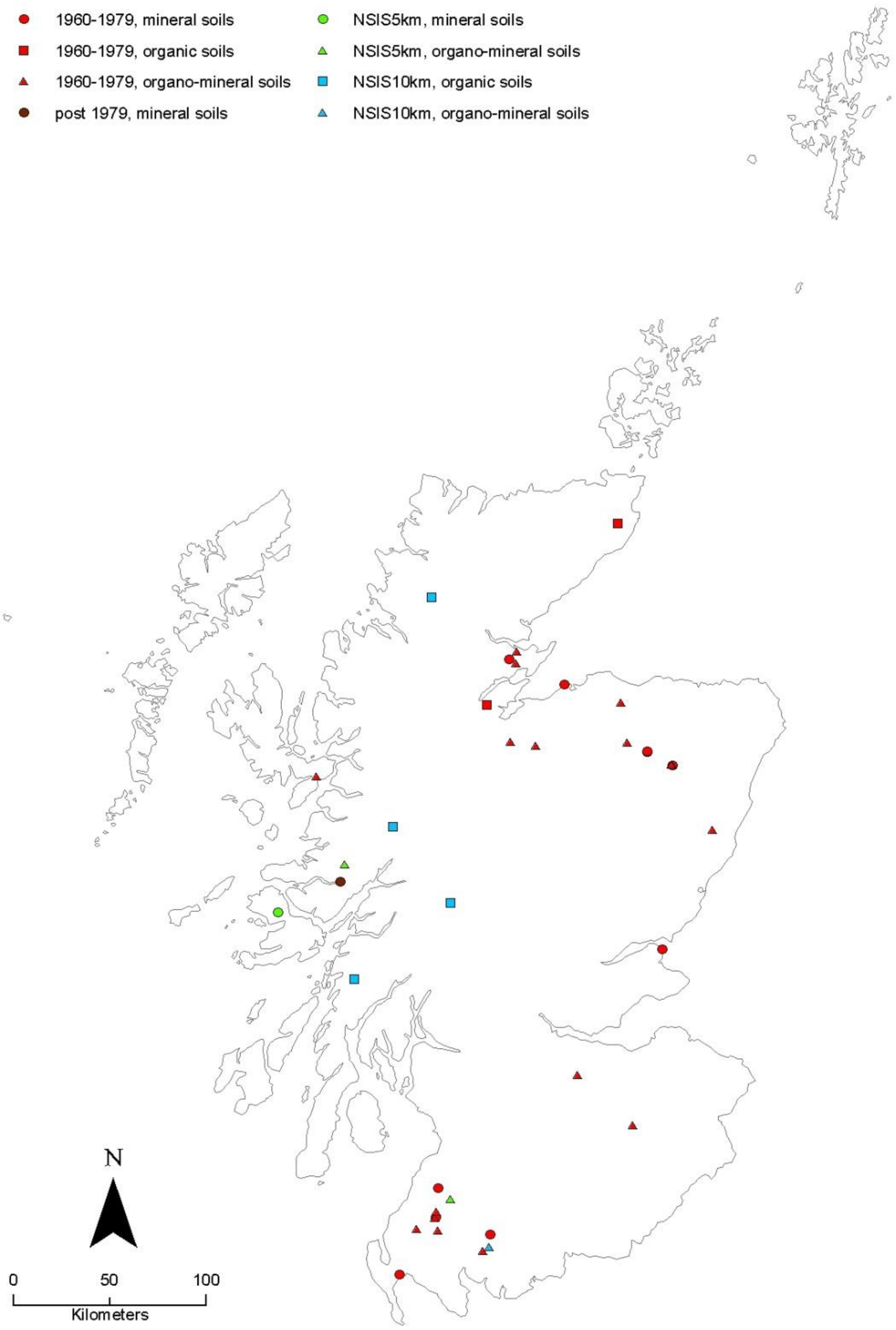
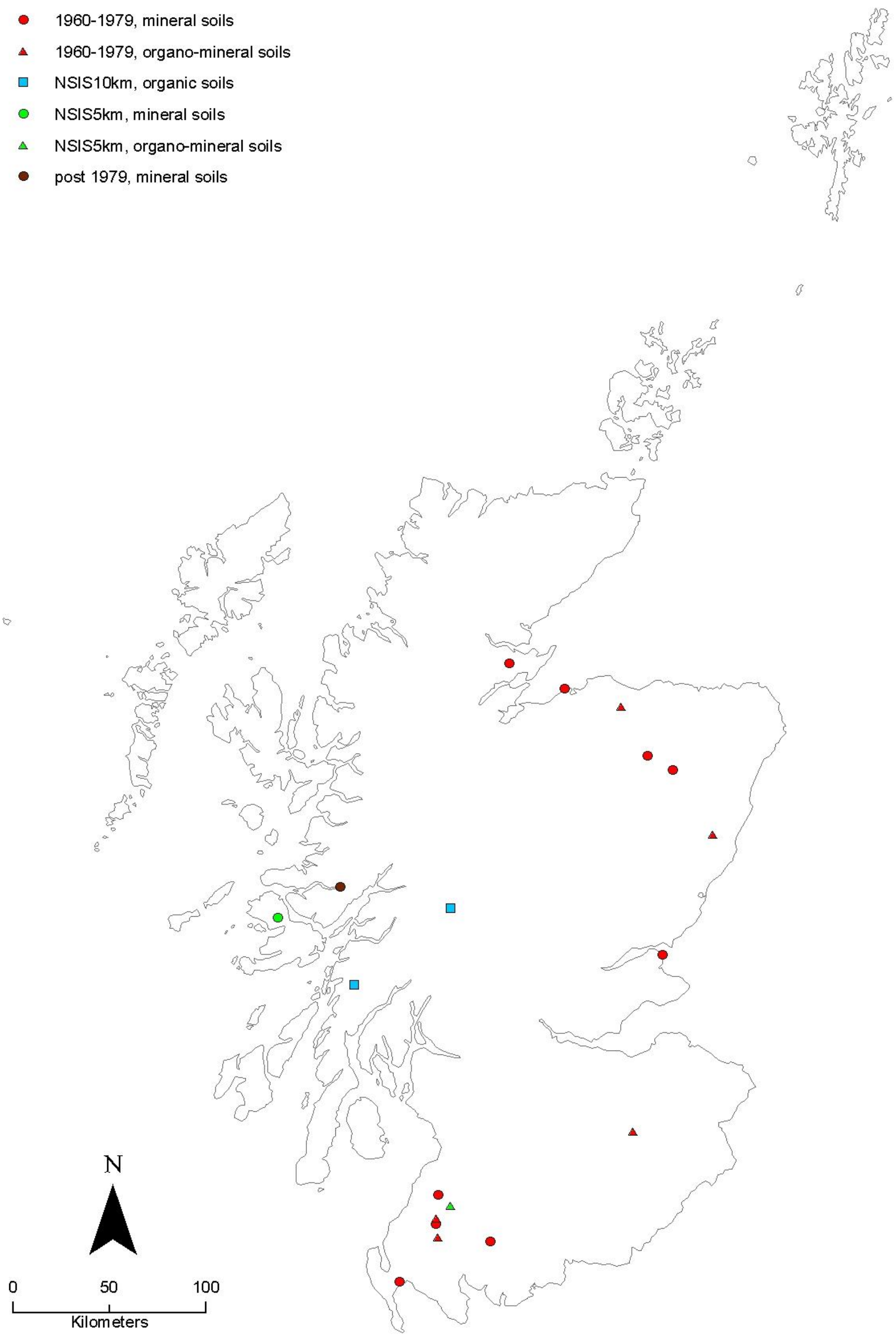


Figure 4: JHI soil profiles at locations where FC has information at a sub-compartment level and where each identified horizon has been sampled and there is more than 80g or archived soil material available for reanalyses *(Note; some locations have multiple soil profiles that cannot be shown separately at this scale)*.



Discussion

From a large selection of 513 potential resampling sites which undergone land use change to forestry, various selection criteria has reduced the number to 40 potential sites, the majority of which were sampled between 1960 and 1979. These sites would be difficult to locate accurately as the georeference given would only locate the site to within 100m (resampling of the NSIS was possible as there were air photographs with the location clearly marked). Contextual information such as slope and aspect may be of assistance. There may be soil mapping field sheets available with the location of the profile marked which would aid in location but this would need to be investigated further and would require additional time to undertake.

In order to increase the potential sample size, it would be useful to undertake the same exercise for those locations under privately owned forestry that have been extracted from the SFGS database as there are around 5 times more potential sites in this dataset than in the Sub-compartment Database.

Options

The selection criteria used (sites only on Forestry commission owned land, sub-compartment data being available, analytical data available for all soil horizons in the profiles and archive material weight greater than 80g) has led to the rejection of a considerable number of sites from 513 to 20.

If we can make the scientific case for using the archival material below this 80g threshold, the sample size will increase to 40 sites. Alternatively, a correction factor could be applied to the original measurements of carbon concentration although this will increase the level of uncertainty in any assessment of changes in carbon stocks. However, there are logistical problems in relocating these 40 sites and additional work will be required in order to assess in they can be accurately located from the surveyors' paper field maps as locations were generally only indicated to within 100m.

Assumptions could be made regarding the C contents of those horizons which were not sampled (largely LF horizons) based on an analysis of data from the 2007-2009 NSIS_2 resampling campaign but that would increase the level of uncertainty regarding change in C content and stock. This would increase the potential sample size to 104.

The potential sample size could be increased if those locations under privately owned forestry were included as there are around 5 times more potential sites in this dataset than in the FC Sub-compartment Database. Although that would almost certainly increase the sample size, the logistics of undertaking a sampling campaign on privately owned land should not be underestimated, for example, having to identify and contact multiple owners.

Potential way forward

In the first instance we recommend that we clarify if we can use the archive soil material where the total weight is less than 80g to determine their C content. If this is approved, we should then identify if we can locate the 40 sites where we have analyses for all horizons in the profile. It should be borne in mind that the relocation of the NSIS sites was greatly aided by the existence of air photographs with the location clearly marked.

Another potential way forward to increase the sample size is to identify the number of sites within the privately owned forestry that were sampled during the NSIS 5 and 10km grid sampling campaign from the more recent 2012 NFI dataset. These sites could be more accurately located as the profile sampling locations are marked on existing air photography. This should not preclude the inclusion of earlier profiles but these will have the same issues regarding locational accuracy as those under Forestry Commission-owned land.

Although the sampling of a mixture of NSIS sites and older, subjective sampled profiles would allow a better examination of change over a greater period of time, there would be a potential loss of accuracy due to relocation problems for the older profiles.

Acknowledgements

JHI data extraction and production of the maps of sample sites were performed by Malcolm Coull and the capture of soil profile morphology from profile description cards was done by John Bell. Caroline Thomson and Gillian Green assessed the weight of archived soil material. Peter Crow has carried out the FC GIS analysis.

Amended Jan 2013