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METHODS PAPER

A five-stage protocol for systematic measuring soil carbon and greenhouse gas fluxes in complex estates

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Abstract
Background and Aims Plant-soil interactions are critical in governing soil carbon (C) stocks and greenhouse gas (GHG) fluxes, but they vary significantly across land uses, soil types, and soil management practices. Finding a potential intervention that could enhance soil C and GHG fluxes relies on reliable baseline data that can capture these variations. Complex estates, characterised by such heterogeneous conditions, require standardised protocols to ensure reproducibility and comparability across sites.
Methods This study introduces a five-stage protocol for systematically measuring and potentially monitoring soil C stocks (including organic and inorganic forms) and GHG fluxes. The protocol is exclusively designed for "Time-Zero" (T = 0) baseline assessments and the strategic selection of sampling sites. However, it also offers a consistent and robust adjustment of the protocol for long-term soil sampling and GHG flux measurements (i.e. monitoring purposes). The approach was tested at RAF Leeming, a Royal Air Force base (500 ha) located in Yorkshire, UK, with varied land uses, soil types, and management practices.
Results The protocol provided a rigorous, reproducible and adaptable framework for obtaining robust baseline data. It also facilitated the quantification of soil C and GHG fluxes, demonstrating the value of a standardised approach to avoid potential under- or overestimation. Additionally, the proposed protocol proved to be useful to guide site-specific interventions by ensuring that relevant factors, such as plant and soil interactions and environmental covariates, are integrated to enhance comparability across space and time. The results also reinforce the scalability of the protocol, with potential applications across a range of complex estates, including urban areas, military installations, airports, and other managed landscapes.
Conclusions The proposed protocol enables standardised, transparent soil C and GHG flux monitoring to meet internationally accepted standards. We advocate for its broad implementation across estates with varying land uses and soil characteristics to support sustainable soil management and climate mitigation efforts.

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Introduction

The increase of greenhouse gas (GHG) concentrations in the atmosphere since the Industrial Revolution has brought about global climate change concerns. According to the Intergovernmental Panel on Climate Change (IPCC), the Earth's surface temperature has already increased by approximately 1.1 °C above pre-industrial levels. Projections indicate that, without emission reductions, this warming could reach or even exceed 1.5 °C by 2040, and approach 2 °C by 2100 under current emissions trends (IPCC 2021). This increase in GHG concentration in the atmosphere can cause sea level rise, extreme weather events, loss of biodiversity, and ocean acidification, etc. (IPCC 2014).

To mitigate the effects of GHG fluxes, the Paris Agreement, which aims to keep the global average temperature increase to "well below" 2 °C above pre-industrial levels and to pursue efforts to limit it to 1.5 °C, was signed in 2015 by 195 countries (UNFCCC 2015). To achieve the goals of the Paris Agreement, countries have committed to reduce their GHG fluxes and implement adaptation strategies to lessen the extent and impact of climate change. However, recent research has shown that GHG mitigation (i.e. reducing emissions) and adaptation alone will not be enough, also requiring efforts to promote carbon (C) removals (also known as negative emissions) from the atmosphere (Anderson & Peters 2016).

Arguably, soil could play an important role in the C capture goal as it has the largest dynamic reservoir of C on Earth, with figures suggesting a capacity of 2500 Pg (Batjes 1996; Lal 2004; Moinet et al. 2023). The absolute quantity of C held within a soil (i.e. the soil C stock) consists of two major components: soil inorganic C (SIC) and soil organic C (SOC). Soil inorganic C, the smaller portion of C in soils (approx. 950 Pg), is represented mainly by carbonates derived from pedogenic processes as well as geologic or soil parent material sources while soil organic C, the most abundant terrestrial C pool (approx. 1550 Pg), comprises soil organic matter (SOM) components (Trumper et al. 2009). According to Lal (2018), the potential for soils to sequester atmospheric C globally is between 1.4 and 3.4 Pg C year^{-1}. As a practical example, and only considering SIC, in urban soils (Technosols), the presence of materials derived from demolition leads to the potentially rapid formation of pedogenic carbonates. Washbourne et al. (2015) found that calcium carbonate had accumulated in an urban soil at a rate equivalent to the removal of 85 t CO_2 per hectare per year, across a 12-ha city-centre site. The carbonate was confirmed to be of pedogenic origin through the analysis of stable C and oxygen isotopes, as well as radiocarbon (^{14}C), which indicated the presence of modern C in the mineral. In addition to C capture and potential climate regulation, soil provides essential ecosystem services, such as food, fibre and fuel production, water filtration, and nutrient cycling, all of which are fundamental to human survival and sustainable development.

Immediate actions are required across all sectors, including, but not limited to: energy, transport, agriculture, industry, and the military (IPCC 2022). For military operations, particularly aviation, the implications of reducing reliance on fossil fuels are particularly serious (NATO 2021). Currently, in the UK, it is estimated that the Ministry of Defence (MoD) contributes to around 50% of all government departmental emissions (TEAM Defence 2020), highlighting the need for the military sector to play a key role in decarbonisation (Rajaeifar et al. 2022). Additionally, since the MoD is one of the largest landowners in the country, with an estate (433,000 ha) nearly equal to 2% of the UK's land mass (National Statistics, 2022), the opportunity to manage and enhance C capture in soils is a strategy not yet explored by the defence sector.

Despite soil's large C storage capacity, factors such as land use, agricultural systems, and management practices influence soil and plant interactions and can cause soils to act either as a sink or a source of C, with substantial variations in both magnitude and rate (Lal 2004; Smith et al. 2007, 2008). Hence, it is critical to consider these, along with key soil-forming factors such as vegetation, topography, and climate, when planning a reliable and robust soil sampling campaign for baseline measurement and long term monitoring of soil C and GHG fluxes (Smith et al. 2008; Minasny et al. 2017; Lal et al. 2018; Batjes 2019). However, a standardised protocol for baseline measuring and monitoring SOC/SIC changes and GHG fluxes is still lacking.

For single land management practices (such as farming or forestry), there have been notable advancements in the formulation of guidelines for measuring and monitoring, reporting, and verification (MRV) of SOC/SIC baseline and changes, as well as GHG fluxes (FAO 2020; World Bank 2021; Puro 2022; VERRA 2023). However, these advancements have primarily centred on the field level, with occasional attention extended to the farm level or even the national level. There is, therefore, still a need to elucidate strategies for soil sampling and GHG measurements for estates that combine different land uses, soil types, and soil management practices, and which span over large areas. This is particularly challenging as it must also be cost-effective and easily understood, as well as simple and broadly applicable in practice. The standardisation of strategies for baseline measuring and monitoring SOC/SIC and GHG fluxes is critical as it will provide the basis for where soil samples and GHG measurements must be undertaken.

The overall aim of this study is to establish a standardised protocol for baseline measuring and monitoring soil C (accounting for both SOC and SIC) and soil GHG fluxes in estates with different land uses, soil types, and soil management practices. The five-stage protocol has been designed to offer a unified approach that is cost-effective, repeatable, and easy-to-use across any sector, allowing SOC and SIC, as well as soil GHG fluxes, to be rigorously and systematically measured and monitored.

Material and Methods

While this five-stage protocol represents a unique approach to baseline measuring and monitoring SOC/SIC and soil GHG fluxes, it is important to highlight that this also encompasses elements of a series of international protocols previously published by different public and private institutions (including, but not limited to: Alberta Government 2012; Australian Government, 2018; Gold Standard 2019; USDA-NRCS-CSU 2019; FAO 2020; World Bank 2021; Puro 2022; VERRA 2023). For instance, the stratification of land units and the emphasis on representative sampling design draw upon methodologies outlined in the FAO (2020) and USDA-NRCS-CSU (2019) guidelines. The importance of time-zero (T = 0) baseline assessment and the application of quality control principles are informed by approaches in the Australian Government (2018) and VERRA (2023) protocols. Recommendations for long-term monitoring intervals, rotation of sample locations, and ensuring data transparency are adapted from Gold Standard (2019) and World Bank (2021) frameworks. Additionally, MRV flexibility and C market alignment are consistent with principles established by Puro (2022) and the Alberta Government (2012).

The guidelines were deliberately designed to be rigorous and systematic, but elements of simplicity, repeatability, and feasibility were thoroughly considered. In this sense, it is expected that it can be applied by any individual with basic computer knowledge and skills, who wishes to assess soil C stocks and soil GHG fluxes in an estate with different land uses, soil types, and soil management practices.

Although the stages described below have been developed and deployed at a military base (RAF Leeming, Yorkshire, UK; 54.2927° N, 1.5317° W), it is expected that they could also be adopted at any other location. Figure 1 presents a schematic overview diagram of the five-stage protocol, including brief explanations and examples for each step, using RAF Leeming as a case study.

Planning and developing a soil sampling design for measuring soil C stocks at T = 0

This protocol recommends the use of the SCORPAN framework (McBratney et al. 2003) as a basis for the compilation of relevant data/information, hereafter referred to as covariates, for designing the soil sampling programme. The SCORPAN framework is a concept that highlights that soil formation and/or properties are highly dependent on their position in the landscape, i.e. affected by several environmental factors (including plant and soil interactions), which also apply to SOC/SIC storage, and thus potential C capture. As such, most of the elements/covariates needed for planning and developing a soil sampling design are primarily based on the SCORPAN function (Eq. 1):

$$S = f(s, c, o, r, p, a, n) \tag{1}$$

where S is soil classes or attributes to be focussed, “s” refers to the soil (other or previously measured

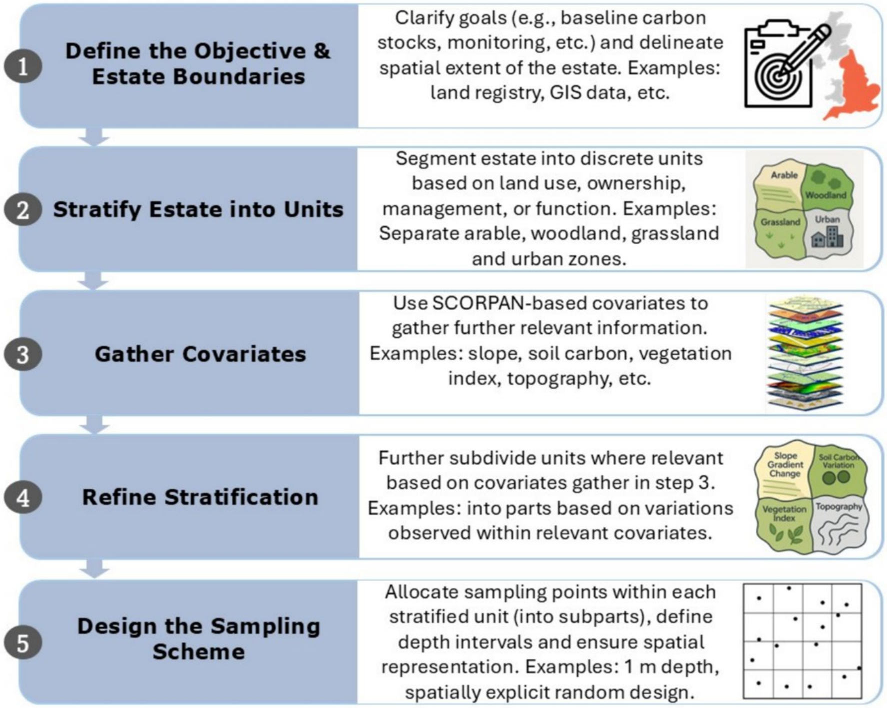


Fig. 1 Schematic overview of the five-stage protocol for measuring soil carbon and GHG fluxes. Each stage builds on the previous one to enable robust baseline assessments and potentially long-term monitoring. The protocol integrates spatial data, expert input, and field measurements to ensure scientifically rigorous and policy-relevant outcomes

properties of the soil at a point), "*c*" is climatic properties of the environment at the point of interest, "*o*" refers to organisms, including land cover and natural vegetation or fauna or human activity (plant and soil interactions), "*r*" is the relief, topography, landscape attributes, "*p*" is the parent material/lithology, "*a*" refers to the age, i.e. the time factor and finally, "*n*" is the spatial or geographic position.

Stage 1 – Defining overall boundary The first step is to identify, delineate, and map the spatial boundaries of the target estate, which relates to the *"o"* in the SCORPAN function. This can be done by consulting the landowner(s) and requesting a simple drawing of the estate boundaries using, for example, Google Earth maps ("Google Earth Pro," 2023) or any other mapping platform. Alternatively, other methods, rather than satellite images and tools, can be used, e.g. land records or hard copy maps.

At the end of this stage, the output should be a geospatial map/satellite image with the total spatial boundary of the target estate. Figure 2 shows an example of the spatial boundary for the RAF Leeming base, taken from ArcGIS (Environmental Systems Research Institute, Inc., Redlands, CA, USA) (Esri 2023).

Stage 2 – Target estate stratification Still considering the *"o"* in the SCORPAN function, it is also important to identify and delineate current different land uses within the total area (i.e. high-level stratification of the target estate into discrete units). Examples include: farmland, paved areas (including runways in this case), urban/recreation, native vegetation, etc. If within one of these (or other) land uses, there is a different management system these should be considered as two different target intervention areas for soil sampling, i.e. management zones. Examples at this location of the same land use but different management systems, include but are not limited to the following: a farm that is partly conventionally managed and partly organically managed, land designed solely for pasture, or for crops, or for woodland, or native vegetation (or other distinctive management systems), a recreation area solely designed for gardening, or recreation, or football/sport pitch, etc. The easiest way of finding out such information is by discussing it with landowners and/or tenants, but some tools such as DIGIMAP (Digimap 2023) (only for UK-located target estates)

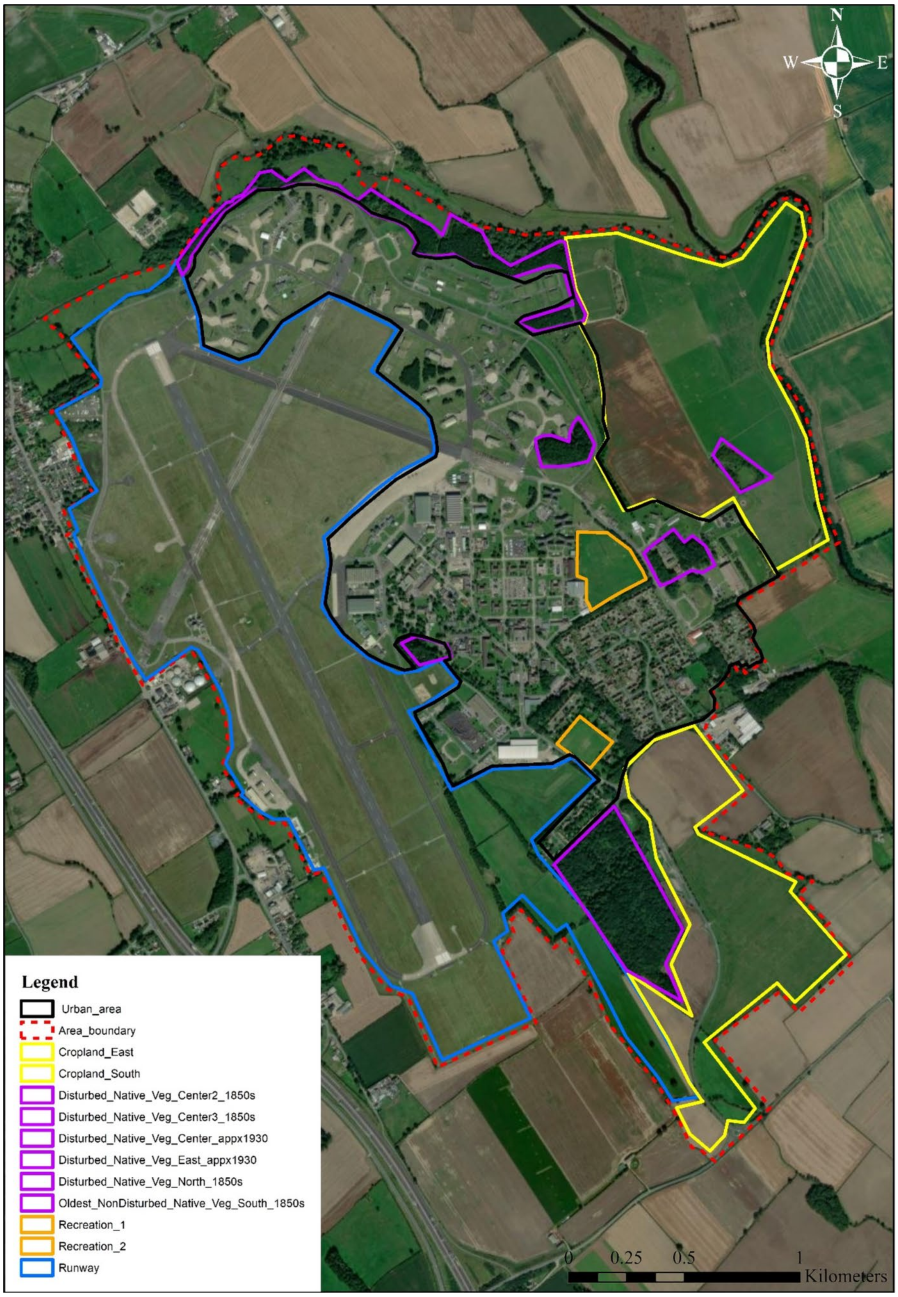
Fig. 2 Spatial representation and delineated map for the total boundary of the RAF base

can also be used to gather such information. For the RAF Leeming base, we have used both approaches, i.e., we talked to landowners and tenants, as well as using DIGIMAP for gathering land use and management system information.

At the end of this stage, the product should be a geospatial map/satellite image of the target estate that includes stratification (i.e. units) concerning different land uses and management systems. Figure 3 shows an example of the stratification of RAF Leeming base considering differences in current land uses and management systems.

Stage 3 – Collecting covariates Once the total boundary, land uses, and management systems/zones

Fig. 3 Spatial representation and delineated map considering differences in current land uses and management systems within the total boundary of the RAF base



are delineated, it is important to gather covariates related to potential material differences within the target estate, as well as in each identified unit. This step relates to *"s"*, *"c"*, *"r"*, *"p"*, and *"a"* in the SCORPAN function, and therefore must be thoroughly considered.

Material differences include potential discrepancies in previously measured soil properties within the target estate that might affect SOC/SIC and soil GHG fluxes (e.g. nutrient content, soil bulk density, texture, pH, SOM, microbial abundance/diversity, etc.), soil type and underlying geology, land use history, landform, and climate (depending on the size of the target estate). In this protocol, we particularly highlight the use of the following covariates, which should be prioritised where available accordingly:

1. Previously measured soil properties (e.g., texture, pH and SOM)
2. Topography (e.g., slope, aspect, elevation, curvature), which influences erosion, water flow, and organic matter accumulation
3. Hydrology (e.g., flow accumulation, topographic wetness index, distance to drainage), which affects moisture regimes and potential for anaerobic C preservation
4. Land use and land cover (e.g., arable, grassland, forest, urban), which strongly impacts inputs and disturbance regimes
5. Soil type, which can inform inherent mineralogy, texture, and C stabilisation potential
6. Vegetation indices (e.g., NDVI), which serve as proxies for biomass productivity and thus C inputs
7. Soil depth. Although only addressed later in the sampling design of this protocol, prior knowledge of expected depth variability at this stage can guide stratification by identifying zones with shallow soils (e.g., due to bedrock) or deep organic horizons

Functions on how to calculate TWI, TPI and all other aforementioned landform covariates are available in Moore et al. (1993) and ArcGIS (Environmental Systems Research Institute, Inc., Redlands, CA, USA) (Esri 2023). Other mapping and analysing tools are also available and can be used to perform such analysis and derive the recommended landform covariates (e.g. QGIS, Maptitude, Python, R studio, etc.). There are no restrictions on what mapping and analysis tool to use in this step, but specific knowledge of how to operate such software is required. For the RAF Leeming base, soil type, past land uses, landform, and climate covariates were all collected using DIGIMAP and/or derived from them by using geostatistical approaches on ArcGIS. If the target area is outside the UK and/or DIGIMAP is not available, we recommend talking to the landowner/tenant(s) of the target estate to collect as much material information as possible from them. If the data are still not available or limited, some can be obtained from global data sources, but local data are always preferred. Table 1 provides global databases and web links that can be used at this stage.

At the end of this stage, the output should be one or more geospatial maps/satellite images of each unit and/or from the whole target estate with material differences that might affect soil C storage and soil GHG fluxes. Examples are given for the RAF Leeming base in supporting information figures A1-A25. Table 2 shows how such covariates are related to the SCORPAN framework and their description.

Stage 4 – Division into discrete parts and subparts This step refers to further stratification of the units (as designed in *Stage 2*) into discrete parts and subparts, which will be the target sampling areas, based on material differences found in *Stage 3.* Unfortunately, there is no set-in-stone procedure to be followed in this phase as it will depend on the availability, as well as the amount, of data gathered in the previous steps. However, it is recommended to carry out an in-depth evaluation of the project scope, such as present land uses, soil types, and management practices, as well as the availability of covariates, in order to gain a better insight into the priorities for the target estate. In addition, it is highly advisable to carefully study all the maps, as well as other relevant information available to design the best strategy for soil sampling at the target estate. The following approach, developed for the RAF Leeming base, is particularly recommended for target estates that present these three elements, i.e. different land uses, soil types, and soil management practices.

First, choose one of the material differences found in *Stage 3* as a basis for the division of the units into discrete parts. We recommend using soil type and/or soil texture maps, if available, as they are closely related to high/low soil C potential. Divide each unit (i.e., each land use/management system/historic use, delineated in *Stage 2*) into corresponding soil type parts (or use other information that can characterise the unit's high-level variability). Figure 4 shows an example of how the runway unit for the RAF Leeming base was divided into discrete parts based on soil type.

Subsequently, divide each discrete part of the unit (in this example, based on soil type) into four further subparts (or more if needed) based on another covariate collected with a fine resolution variability (e.g. long-term average normalised difference vegetation index, soil electrical conductivity, yield maps, elevation, etc.). Any covariate with a fine-resolution variability can be used. However, if available, this protocol recommends elevation as the covariate to

Table 1 Global databases available for spatial information (adapted from FAO 2020)

Type	Source	Web address	Resolution
Range of datasets including historic, geology, marine, environmental, elevation across the UK	Digimap	https://digimap.edina.ac.uk/	Many
Range datasets including historic, geology, marine, environmental, across the UK	Magic	https://magic.defra.gov.uk/	Many
Monthly climatic data	CRU – Climate Research Unit, University of East Anglia	https://crudata.uea.ac.uk/cru/data/hrg/cru_ts_4.03/cruts.1905011326.v4.03/	50 km × 50 km
National and regional climate for the UK	Meteorological Office for climate averages	www.metoffice.gov.uk/weather/uk/climate.html	Local
Geology across the UK	British Geological Survey	http://www.bgs.ac.uk/discoveringGeology/geologyOfBritain/viewer.html	Variable depending on location
SOC stocks 0–30 cm	GSOC Map—FAO-ITPS	http://54.229.242.119/GSOCmap/	1 × 1 km
SOC stocks and SOC concentration; profiles	International Soil Carbon Network	https://iscn.fluxdata.org/	Different resolutions
Soil texture 0–30 cm	ISRIC Soil Grids	https://soilgrids.org and at global level from https://data.isric.org/)):	250 × 250 m 500 × 500 m 1 × 1 km
Soil types for England and Wales	Landis Soilscapes viewer	https://www.landis.org.uk/soilscapes/	1 × 1 km 5 × 5 km
NDVI- Historic images (2001–2020) every 16 days	MODIS—MOD13A2 datasets	https://lpdaac.usgs.gov/products/mod13a2v006/	1 × 1 km
Land Cover – Land Use	MODIS Land Cover Dynamics MCD12Q2	https://modis.gsfc.nasa.gov/data/dataprod/mod12.php	500 × 500 m 1 × 1 km
Land Cover – Land Use	European Space Agency (ESA) Climate Change Initiative (CCI)- Copernicus Climate Change Service (C3S)	https://www.esa-landcover-cci.org/	300 × 300 m
Land Cover – Land Use	IMAGE Integrated Model to Assess the Global Environment PBL Netherlands Environmental Assessment Agency	https://models.pbl.nl/image/index.php/Land_cover_and_land_use	10 × 10 km
Land Cover – Land Use	FAO. Global Land Cover SHARE	http://www.fao.org/land-water/land/land-governance/landresources-planning-toolbox/category/details/en/c/1036355/	~ 25 × 25 km
Land Cover – Land Use	USGS Global Land Survey	https://lta.cr.usgs.gov/GLS	30 × 30 m
Land Cover – Land Use	CORINE land cover (Europe only)	https://land.copernicus.eu/paneuropean/corine-land-cover	100 × 100 m

be used in this step, as this is known to have a close relationship with spatially implicit soil-factors (Behrens et al. 2010). If elevation data are unavailable, the protocol recommends, preferably, utilising a relevant covariate with a high-resolution that may contribute to the variability within the unit's part. For the RAF Leeming base, elevation data was available at a 5 m resolution for the entire area (Fig. A14) and was selected as the covariate for subdividing the discrete unit's part into subparts (Fig. 5). If high-resolution data/information or covariates are not available, it is recommended to subdivide each discrete unit part

Table 2 Covariates collected and their relationship with SCORPAN framework and description

Covariate	Scorpan Factor	Description
Elevation	R	The height of a location above the Earth's sea level
Slope	R	The inclination of the land surface from the horizontal
Flow Direction	R	Direction of water flow in a given cell based on its steepest descent drop
Flow Accumulation	R	Accumulated flow determined by accumulating the weight for all cells that flow into each downslope cell
Basin	N	Connected cells belonging to the same drainage basin defined by the flow direction
Aspect	R, N	The direction in which a land surface slope face
Curvature	R	The shape or curvature of the slope i.e. concave or convex
Hillshade	C	Representation of the surface considering the sun position for shading
Topographic Wetness Index (TWI)	C, R	The relative wetness within moist catchments, but is more commonly used as a measure of position on the slope with larger values indicating a lower slope position
Topographic Position Index (TPI)	R	Topographic position classification identifying upper, middle and lower parts of the landscape
Agricultural Systems	O	Organic system in accordance with the Soil Association Organic Standards or Conventional system (UK best practices recommendations)
Land uses	O	Runways, Urban, Native vegetation, farmland, recreation, etc

into a minimum of equal four subparts. This minimum ensures adequate spatial coverage and helps to capture within-unit variability, which is particularly important in heterogeneous estates (Fig. 6). Additionally, having at least four subparts supports statistical robustness by enabling the collection of replicates and allowing for flexibility in future monitoring or stratified analysis (Carter and Gregorich 2007; Lawrence et al. 2020). The primary objective of this stage is to ascertain that the sampling points (which will be designed in the next stage) exhibit greater homogeneity within the specific subunit than across the entire estate.

At the end of this stage, the output should consist of geospatial maps/satellite images of each unit (as designed in *Stage 2*) further stratified into discrete parts and subparts according to the chosen material differences and high-resolution covariate, respectively. This stratification should consider both high-level and high-resolution covariates, using soil type as a high-level covariate and elevation as a high-resolution covariate, for example (Figs. 5 and 6). It is important to highlight that this step must be repeated for all units identified in *Stage 2* of this protocol.

Stage 5 – Designing sampling points In this step, the target sampling points are chosen. The protocol recommends a minimum of three (for statistical purposes) random locations within each of the subparts designed at the end of *Stage 4* for the extraction of soil cores. However, the larger the area and the expected or known variability within the subpart's unit, the more samples must be taken within that subpart. The use of statistical software (R, JMP, Minitab, SPSS, etc.) for the selection of the random sampling locations is highly advised. While the sampling locations should be randomly assigned, it is important to ensure that they adhere to the following constraints:

- Locate each sampling point at least 50 m away from each other within the subpart; this is to achieve a balance between spatial autocorrelation and redundancy (Radočaj et al. 2021; Zani et al. 2022). The sampling distance can be adjusted by conducting preliminary spatial analysis, such as variograms, which is advisable for accuracy and cost purposes. For instance, in highly heterogeneous areas, where the range of spatial correlation is usually greater, the minimum distance can be decreased to account for this increased variability.

- Avoid locating the sampling point near the field border (> 20 m from a field boundary); this is to avoid edge effects.

- If known, avoid locations that are likely to be disproportionately affected by compaction from either

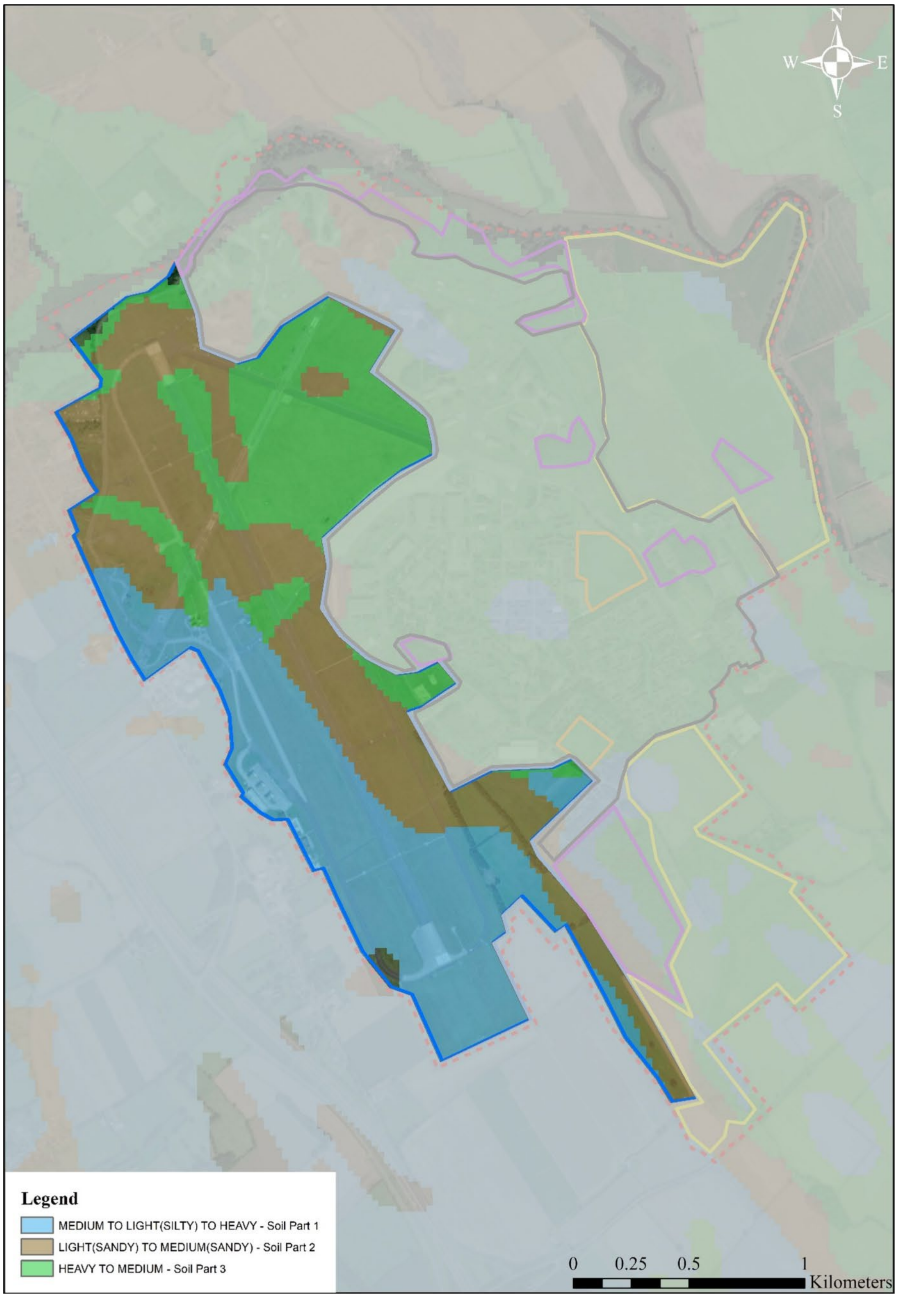


Fig. 4 Division of the runway unit into three discrete parts according to correspondents' soil types at the RAF base

machinery and/or animal activity and/or chemical or other types of disposals or spillage.

In addition to stratified random allocation of sampling points, further sampling in locations expected to have high or low soil C potential may be considered, but only as a complementary strategy to better capture the spatial heterogeneity of the target area. This should not replace random sampling and must be carefully implemented to avoid introducing bias. Where uncertainty or variability is high, increasing the number of random sampling points is preferred. Such a step can be done by using the other covariate maps gathered in the previous stages (e.g. slope, flow

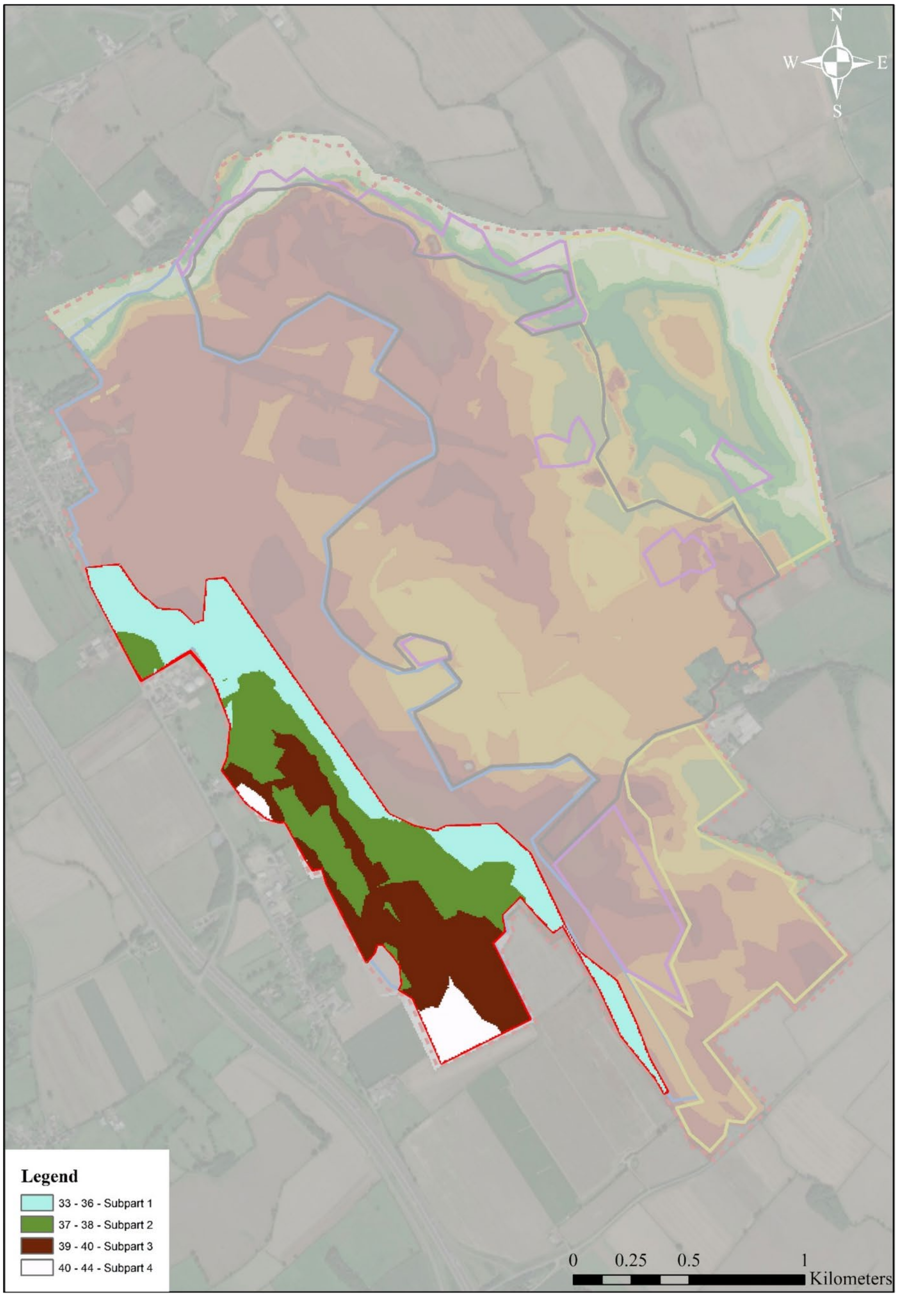
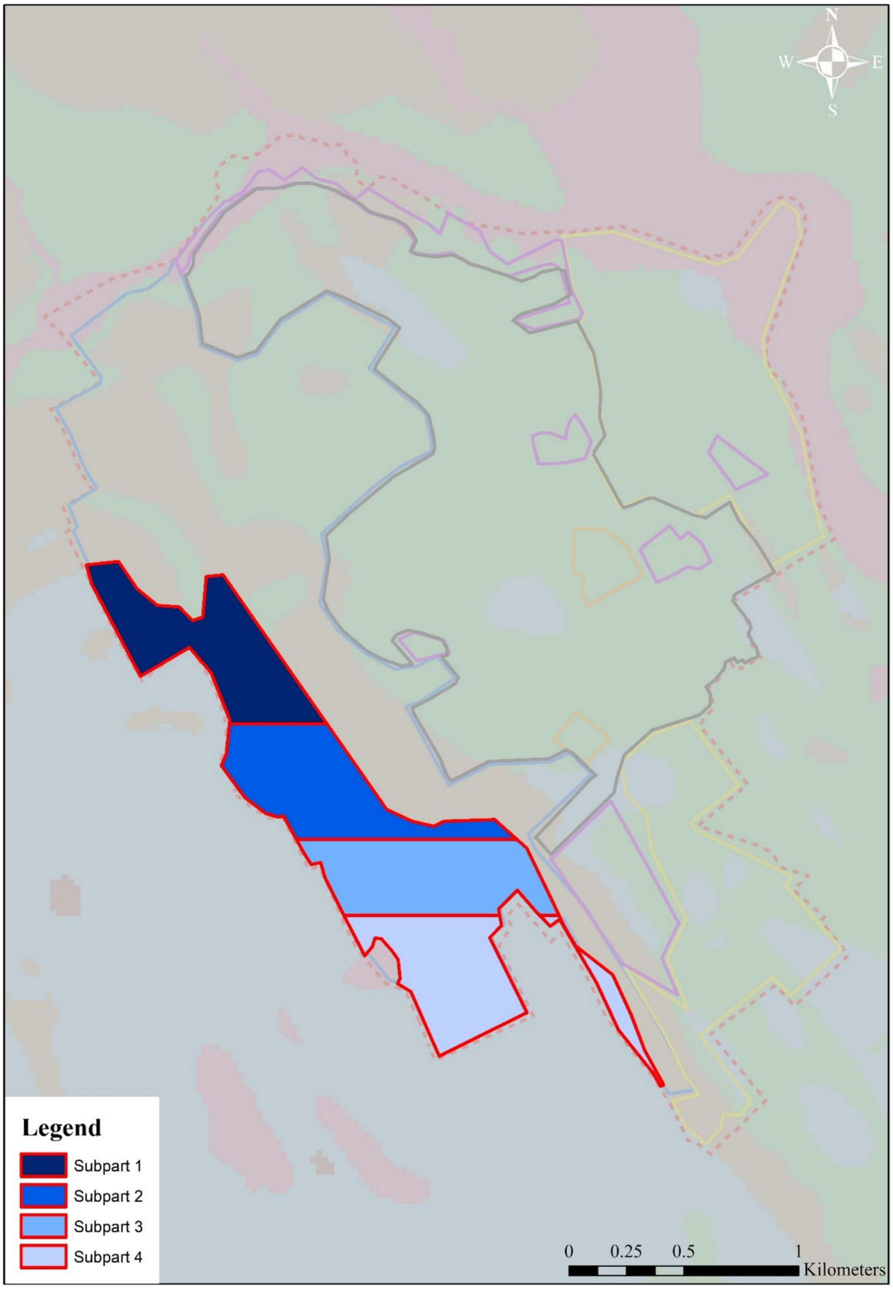


Fig. 5 Division of the runway unit, soil part 1 (only), into four subparts based on observed variation in the elevation at the RAF base

direction, flow accumulation, basin, aspect, curvature, hillshade, TWI, TPI, or others), or high-level global maps, for example, the FAO-GSOC map (Global Soil Organic Carbon map; available at http://54.229.242.119/GSOCmap/), which gives a rough estimation of the current soil C stock (t C ha^{-1} at 30 cm) expected at the location. Please note that the estimated soil C stock will not always be in line with the measurement of the lab. This potential discrepancy arises for several reasons:(i) soil C is highly variable both spatially and with depth, and may vary by up to 50% within short distances; (ii) global datasets such as FAO-GSOC rely on interpolated values from sparse or generalised data sources, which may not capture local-scale heterogeneity; and (iii) differences in spatial resolution between the global maps and the actual

Fig. 6 Division of the runway unit, soil part 1 (only), into four subparts with no high-resolution data available



sample plot size can lead to misalignment (Batjes 2019; Minasny et al. 2017; FAO 2020).

As a general rule of thumb, the more parts the unit is divided into and the greater the number of sampling points within each one of the subparts, the better the capacity to reliably measure soil C stocks baseline, as well as to detect changes in soil C storage and GHG fluxes over time. A power analysis can be used to calculate the ideal number of sampling points (Eq. 2 and Eq. 3).

$$MDD \geq \frac{S}{\sqrt{n}} \times \left(t_{\alpha,u} + t_{\beta,u}\right) \tag{2}$$

$$n \geq \left(\frac{S \times \left(t_{\alpha} + t_{\beta}\right)}{MDD}\right)^2 \tag{3}$$

where *MDD* is the minimum detectable difference, *"S"* is the standard deviation of the difference in SOC stocks between t_0 and t_1, *"n"* is the number of samples, "t_α" refers to two-sided critical value of the t-distribution at a given significance level (α) frequently taken as 0.05 (5 percent), "t_β" is the one-sided quartile of the t-distribution corresponding to a probability of type II error β (e.g., 90 percent).

While power analysis is without doubt useful for determining the number of samples required to detect statistically significant changes in soil C over time, here, we considered it as an optional approach. This is because it requires prior knowledge of variance in soil C, which may not be available, particularly during the initial baseline phase.

After randomly (and arbitrarily for some specific points, if needed) choosing the sampling point locations, if a deep soil sampling is going to be carried out, it is necessary to take into account information on underground services (water pipes, gas pipes, electrical wires, fibre optics, cables, etc.) and then manually adjust any sampling point that might be in a "restricted" location of this type. The final target locations (coordinates, latitude, and longitude) are then recorded (Fig. 7).

For a full inventory of soil C, soil core collections to a minimum of 1 m, with recommended distinctive soil depth intervals of 0–0.10, 0.10–0.20, 0.20–0.30, 0.30–0.60 and 0.60–0.90 m depths are required. This ensures that vertical variability in both SOC and SIC is fully captured. Exceptions to the 1 m depth should only be made where prior site-specific information justifies shallower sampling (e.g. in sites with shallow bedrock). Furthermore, if field observations or available data suggest a substantial amount of C at depth greater than 1 m, this protocol recommends sampling beyond 1 m where possible, while balancing cost, feasibility and safety conditions. For all soil depth sampling, the use of equipment that allows for soil bulk density determination (e.g. a cylinder of a known volume) and/or soil mass is required.

Adapting the soil sampling design for monitoring soil C stocks and soil GHG post baseline (T = 0) measurements

The steps described earlier provide a systematic protocol for measuring soil C stocks at the baseline (T = 0). However, it is recognised that repeating full-scale sampling campaigns for soil C stocks or implementing soil GHG measurements across all baseline locations may not be practical or feasible in long-term monitoring programs. Therefore, a modified yet robust approach is recommended to monitor both components, soil C stocks and GHG fluxes, over time, while maintaining data quality and minimising resource demands.

We recommend the following monitoring protocol, which builds on the T = 0 sampling strategy but adapts it for repeated measurements:

1. Start with the full baseline soil sampling design, including all soil sampling locations identified (T = 0)
2. Select a reduced subset of sampling points for monitoring, based on stratification (Maillard et al. 2017):
   (a) For each soil type within each intervention area, select at least one sampling location per subpart (e.g., for four subparts, minimum of four points per soil type per land use/management system)
   (b) Attempt to select points that will cover the range of covariates used in the sampling design (in the example of the RAF Leeming base, elevation) within the soil type
   (c) Include additional locations if the selected subset does not adequately represent the range of key covariates used
3. Apply the same subset of locations for soil C stock re sampling and GHG measurements, thus ensuring integration and efficiency in monitoring C budgets
4. Rotate the sampling locations over successive monitoring periods to improve representativeness and avoid site specific bias (critical when results are used for C crediting or policy reporting)

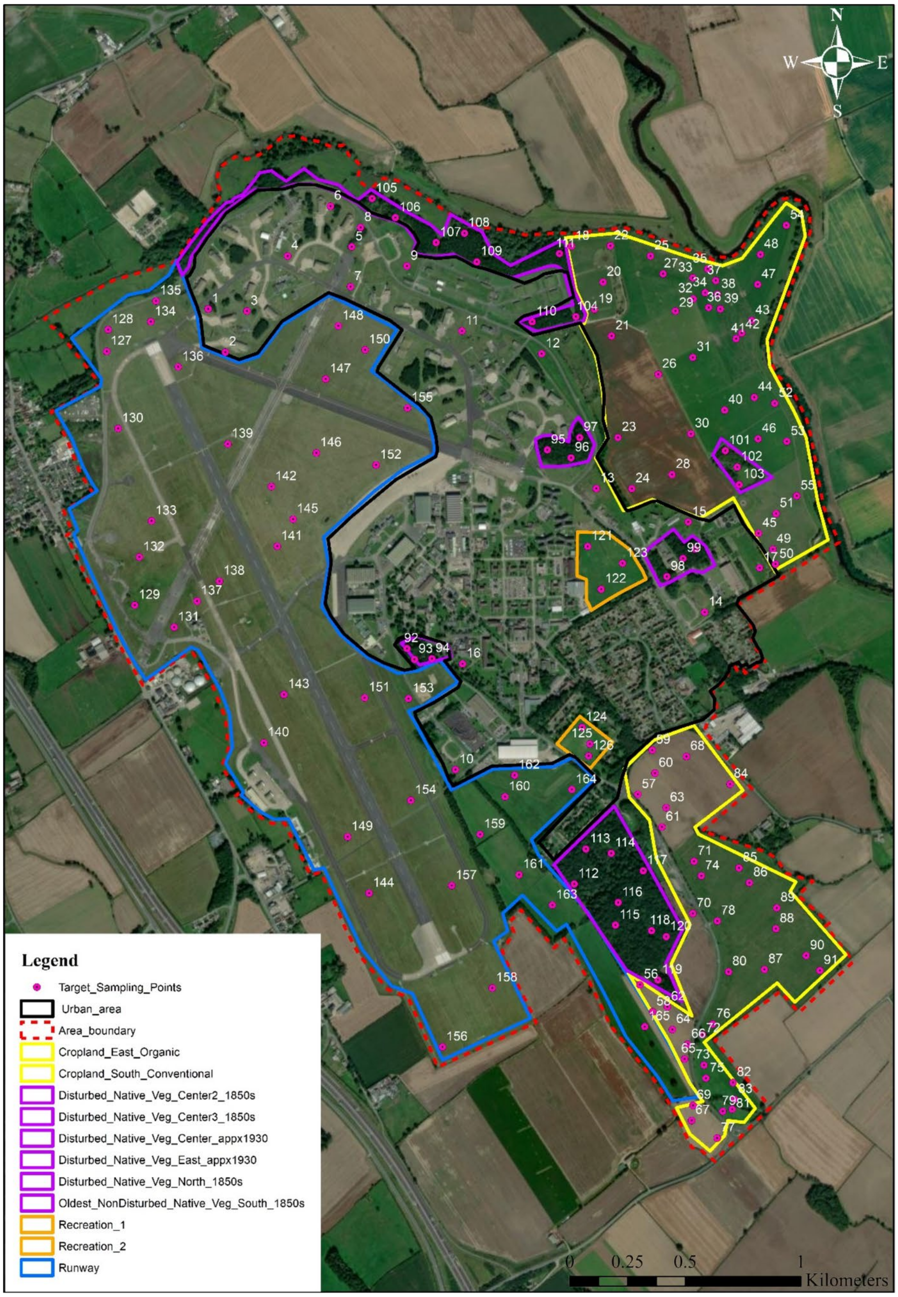


Fig. 7 Final target soil sampling locations applying the guidelines proposed in this report across the RAF base

5. For cases where full re-sampling is feasible, the original t = 0 design can be reused, but, to reduce laboratory costs, samples with small intra-unit variation (identified during the baseline) can be pooled before analysis during the monitoring stage

Statistical analysis for ensuring the protocol is fit for purpose

To ensure the rigour of the proposed protocol and the validity of the selected sampling locations for soil C and soil GHG measurement, we recommend a supporting quantitative approach that highlights the statistical robustness and representativeness of the design. For this, two key aspects should be assessed: stratification validation and sampling adequacy. For the former, after initial stratification of the target estate based on physical, ecological, and/or management criteria, we recommend evaluating within- and between-stratum variability. This can be done using exploratory data analysis techniques (e.g., boxplots, variograms) and statistical tests such as analysis of variance (anova) or permutational multivariate analysis of variance (permanova), to ensure that strata represent meaningfully distinct groups in terms of their key covariates. For the latter, we recommend that sampling density may be guided through power analysis or by estimating the coefficient of variation (CV) within strata. A $CV < 20\%$ is generally considered acceptable for estimating means with reasonable confidence in environmental and soil data. This step helps ensure that the number and distribution of sampling points are sufficient to capture heterogeneity across the estate while maintaining statistical confidence.

Results

The results presented in this section pertain exclusively to the implementation of Stages 1–5 of the proposed five-stage protocol, corresponding to the"Time-Zero"($T = 0$) baseline measurement. The present study focuses on the methodological application of the protocol, therefore, specific soil C stocks and GHG flux data are not presented.

Deployment of the protocol at RAF Leeming, a 500 hectare estate, led to the delineation of 165 soil sampling points equating approximately one sampling point for every three hectares within the target part of the unit. These sampling points were stratified across a diverse range of land uses (e.g. arable fields, runways, recreational zones, woodland areas, etc.) and soil types, ensuring adequate representation of the estate's heterogeneity (Fig. 7).

The stratification process, informed by SCORPAN-based covariates, revealed substantial spatial variability in soil properties. For example, within runway area alone, preliminary soil C stock results (0–10 cm depth) indicate up to 200% difference between high and low C zones (Figs. 4 and 5 for the runway area, and Figs. A1 to A25 for the whole estate). These differences were associated with variations in soil type, historic land use, and elevation data, underscoring the importance of integrating such covariates in the sampling design. The protocol's stratified sampling approach ensured statistical robustness while minimising user bias. Sampling locations were distributed using a quasi-random sampling approach within defined units and subparts (Fig. 7), providing comprehensive coverage of the target estate. The random component within each stratified layer improves the generalisability of results and supports the defensibility of C estimates used in future monitoring or verification processes.

Figures A1 to A25 illustrate the estate's spatial heterogeneity, including variations in topography (elevation), soil characteristics, and land use/management practices. This spatial complexity was effectively captured by the random aspect of the proposed protocol, demonstrating its capacity to represent C relevant gradients. Out of the 165 sampling points identified using the protocol, 159 were set randomly within specific units and subparts, whilst only six were set manually based on the chosen covariates'variability. These six samples were only selected to ensure that adequate spatial coverage was used to be able to capture within-unit variability (Fig. 7).

Lastly, by using a stratified approach with random sampling locations, the design ensured unbiased measurements that are statistically valid at a 90% confidence level. The inclusion of multiple soil depths, (0–0.10 m, 0.10–0.20 m, 0.20–0.30 m, 0.30–0.60 m, and 0.60–0.90 m), while optional, is strongly recommended. Stratified depth sampling enables more detailed vertical soil C distribution across profiles, particularly in landscapes with variable C distribution by depth. In summary, the implementation of the protocol at RAF Leeming provided a scalable, statistically sound baseline design that can be adapted to other complex estates with similar or different land-use characteristics. The results confirm that the protocol ensures both representativeness and rigour, laying a reliable foundation for future monitoring and site-specific interventions.

Discussion

The potential for C capture in soils and the concurrent reduction of GHG fluxes is widely acknowledged within the scientific community (Batjes 1996; Lal 2004). This consensus extends well beyond the primary goal of climate change mitigation and encompasses a range of vital ecosystem services (Moinet et al. 2023). However, despite its undisputable importance, no scientific consensus exists when it comes to measuring and monitoring soil C (accounting for both SOC and SIC) and soil GHG fluxes, particularly in estates with different land uses, soil types, and soil management practices (Smith et al. 2020). Here, we outlined a comprehensive five-stage protocol for encouraging the standardisation of the soil C baseline measurement and subsequently monitoring C changes as well as GHG fluxes. We strongly advocate that this is of ultimate importance as it will steer the process towards results that are unbiased, reliable, and comparable.

Alongside skilled workers and expensive pieces of equipment, the number of samples needed (i.e. sample size) is often deemed a challenging aspect for high-accuracy soil C results (Izaurralde et al. 2013). As pointed out by Lohr (2010), the determination of the sample size entails a balance between augmenting accuracy and managing the associated costs and complexities. This becomes even more important when it comes to the spatially dependent nature of soil C (Lawrence et al. 2020). The results obtained here (i.e. the sampling approach) show that it is possible to have a reasonable sample size even in large estates with mixed land uses, soil types and/or soil management practices (165 sampling points in a 500 hectare estate). Yet, it was able to cover the range of soil types, elevation levels, and variation on other covariates, found across all land uses and management practices in the entire base (Figs. A1-A25). For the sake of comparison, the MRV protocol outlined by FAO (2020) for agricultural landscapes, recommends collecting a composite sample every 10 hectares within target areas exceeding 50 hectares in size.

The quasi-random stratified soil sampling design with individual samples across units is acknowledged as a robust way of identifying even small variations across the target site (Carter and Gregorich 2007). Besides that, it should meet statistical requirements for 10% uncertainty under a 90% confidence level (Oldeman 1992), providing a good coverage of the target estate while ensuring no bias through the introduction of the random aspect of the design. Commonly, guidelines suggest the use of systematic sampling using transects or a grid approach with composite samples sent for analysis. However, depending on the size of the target area and its heterogeneity (especially if it is an estate with different land uses, soil types and soil management practices), such an approach might lead to skewed results (i.e. under- or overestimation; Lawrence et al. 2020) with a potential increase in uncertainties and scepticism particularly with soil C programs that carry out/suggest such an approach. Based on the maps generated by our approach alone (Figs. 3 and 4 and Figs. A1-A25), it is already possible to predict a potential large variation in soil C across this estate, which could lead to misleading results when using different methods than the one suggested here for soil sampling and measurement of the soil C baseline.

It is evident that the larger the sample size, the smaller the errors and uncertainties might be. However, another frequently emphasised challenge pertains to the selection of sampling points, i.e. the actual locations where soil samples should be taken. For soil C measurement, the selection of sampling points should be accurate and precise, which will ensure faithful assessment of the actual C stocks and minimal error intervals in the estimation, respectively (World Bank 2021). If the selection of sampling points is not well designed (importantly: this is dependent on the soil variable of interest) it can result in either inaccurate and imprecise, inaccurate but precise, or accurate but imprecise estimation, which could lead to bias or systematic error and the presence of random errors (Pearson et al. 2007). In this present protocol, we highlight that having prior knowledge concerning variates that affect soil C variability, as suggested in the example of RAF Leeming base, is critical for meeting accuracy and precision while reducing sample size to the bare minimum. The rationale behind it, is that many topographic/terrain attributes, particularly those derived from elevation (e.g., slope, curvature, water flow, TWI, etc., Figs. A15-A22), will play a key role when it comes to soil C storage (organic and inorganic), as these factors can directly affect plant and soil interactions that govern the quantity and quality of SOM inputs, and decomposition rates under uncultivated soils (Minasny et al.

2013), as well as mineral dissolution and fine-scale temperatures (Puro 2022). Combining it with land use (current and historic), soil map units, and management zones (as suggested in *Stage 3* of this protocol) for stratification and sampling design, is expected to address potential limitations such as enhanced quantification compared to a simple random sampling approach (Mueller et al. 2001) and potentially inconsistent temporal results (Franzen et al. 1998). It is also pertinent to note that many chemical and biological factors can affect soil C storage (Vicca et al. 2022). In this context, the current protocol is anticipated to encompass the capacity to account for variations in spatial distribution linked to these factors. Selection of sampling points without prior knowledge will always lead to bias (even if it is carried out randomly) and, therefore, should be avoided at all costs, especially in estates with different land uses, soil types and soil management practices.

Current knowledge and techniques being performed for the purpose of measuring and monitoring soil C are still too vague. For instance, in the Puro. Earth protocol (Puro 2022), there is a reference to the requirement for in-field soil C measurements by participants engaged in C crediting programs (related to C inorganic forms). However, there was no explicit stipulation regarding sampling design for both baseline and/or monitoring assessments. Similarly, in the VERRA methodology designed for SOC credits (VERRA 2023), the need for direct measuring at $t=0$ (i.e. baseline) and subsequent monitoring at intervals (e.g. approximately every 5 years) is acknowledged, but no explicit method is provided in this regard. The methodology, however, stresses the need for procedures to be unbiased and representative, citing, for instance, the use (or adaptation) of published handbooks/protocols such as those provided in the FAO Soils Portal (FAO 2020), and/or the ISO standards on soil sampling (ISO 2018), and/or the IPCC Good Practice Guidance LULUCF (IPCC 2003). While those methodologies offer valuable guidance on how, what, and why soil sampling should be conducted, they are often either more generic or focussed only on agricultural landscapes, rather than being tailored specifically for soil C assessments in estates with different land uses, soil types, and soil management practices. Furthermore, the potential for methodological adaptation may introduce biases into the results, as previously discussed. Similarly, recent research has introduced innovative and technically advanced methodologies for measuring and monitoring soil C storage (such as geostatiscal approaches and model-assisted sampling), but, again, they have predominantly concentrated on agricultural lands and, most importantly, not considered SIC (de Gruijter et al. 2016; Manning et al. 2024).

The rather simple rationale outlined in this five-stage protocol could improve our current knowledge and techniques, particularly those proposed in methodologies such as FAO (2020) and World Bank (2021), without bringing an extra layer of complexity. In addition, whilst this protocol aims to standardise the measurement and monitoring of soil C (SOC and SIC) and GHG fluxes, we underscore that the same samples could potentially be used for the measurement of other soil variables. Ultimately, we highlight the significance of understanding spatial heterogeneity, particularly with regard to plant and soil interactions and soil physicochemical characteristics, which has already been heavily emphasised in the advancement of sustainable strategies for agricultural crop cultivation (AbdelRahman and Arafat 2020). Therefore, it is imperative to consider this aspect when measuring/assessing soil C and/or GHG fluxes. We also advocate for an ongoing enhancement of this protocol through collaboration with fellow researchers, institutions, organisations, and practitioners (e.g. farmers, technicians, and soil analysis laboratories) who are actively engaged in soil C programs.

Conclusions

This study presents a robust, scalable, and adaptable protocol for baseline soil C and GHG flux assessment in complex estates. Its structured approach supports consistent and comparable data collection across diverse land uses and soil types, ranging from agricultural and semi-natural areas to highly managed sites like military bases and airports. By integrating stratification and random sampling, the protocol ensures statistical representativeness while accommodating site-specific variability. Moreover, it emphasises the importance of including both SOC and SIC across multiple depths, a component often overlooked in existing methodologies. The proposed framework supports both measurement and future monitoring efforts, offering a foundational tool for C accounting, climate mitigation planning, and informed land management. In the case study reported here, within an area of 500 ha overall, the systematic five-stage approach led to the designation of 165 sampling locations that represent variability in land use type and natural conditions. Following this protocol, a robust strategy for soil C stock baseline measuring and monitoring (SOC and SIC) and GHG fluxes can be set into motion and spatial–temporal variations accurately assessed, especially when interventions are deployed. Whilst we encourage the use of such a protocol, it is crucial to underscore that it should remain a dynamic and evolving framework. Inputs from fellow researchers, institutions, entities, and practitioners (including farmers, technicians, and soil analysis laboratories), must be actively incorporated into its development.

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Estates — Large, complex land holdings, often comprising multiple land uses, soil types, ownership types, and administrative units, such as military bases, institutional campuses, or public land portfolios.

GHG Fluxes — The emission or uptake of greenhouse gases (e.g., CO2, CH4, N2O) from soils to the atmosphere, typically measured using static chambers or micrometeorological techniques.

SCORPAN — A conceptual framework used in digital soil mapping that identifies key soil-forming factors: soil (s), climate (c), organisms (o), relief (r), parent material (p), age (a), and spatial position (n).

Interventions — Any land management practice or ecological restoration activity intended to alter or improve soil carbon stocks and/or greenhouse gas fluxes (e.g., reforestation, reduced tillage, compost application, enhanced rock weathering, etc.).

Baseline Assessment — The initial, systematic measurement of soil carbon stocks and related covariates before any intervention or monitoring takes place, used as a reference point for future comparisons.

Monitoring — The repeated measurement of soil carbon and/or GHG fluxes over time to detect changes associated with management practices or environmental change.

Covariates — Environmental or land use variables (e.g., slope, aspect, vegetation cover, soil type) that influence soil carbon distribution and are used to inform sampling design.

Material Differences — Substantial variations in previously measured soil properties within a target estate that could meaningfully affect carbon organic and/or inorganic stock estimates, carbon accounting, greenhouse gas fluxes, and /or the design of monitoring strategies.

Units — The high-level stratification of the target estate in different land uses and/or management systems/zones and/or historic use.

Discrete Parts — Further stratification of the units into more detailed parts based on high-level material differences (with enough resolution) that have a direct impact on soil carbon stocks and/or greenhouse gas fluxes. Examples of material differences to be used for stratification of the units into discrete parts are: soil type and/or soil texture and/or any other measured soil property closely related to high/low soil C potential.

Subparts Subunits within each of the discrete parts, which are defined by either an available covariate with a fine resolution or equal-size subunits. The subparts are used to distribute soil sampling points systematically, ensuring adequate spatial coverage and helping capture within-unit variability, which is particularly important in heterogeneous estates.

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