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Generating spatially and statistically representative maps of environmental variables to test the efficiency of alternative sampling protocols

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Abstract

Accurate assessment of environmental variables is vital to understanding the global issues of land-use change and climate change, but is hindered by their high spatial and temporal heterogeneity. Extensive surveys are needed to model such large-scale problems, with their success dependent on adequate sampling protocols. We present a robust method for designing efficient sampling protocols for environmental variables. The SIMAP method involves the following steps: 1) Selecting sites that cover a representative range of spatial variability, 2) Intensive and spatially-accurate surveys within sites, 3) construction of continuous Maps that replicate the spatial and statistical variation of the surveys, 4) Accuracy simulations based on sampling of these maps and 5) determining a sampling Protocol for subsequent broader surveys. To illustrate the method, we used estimation of soil C stocks in mixed-species tree plantings and pastures to estimate carbon sequestration following reforestation. Soil C was surveyed intensively from these two land uses at several farms that covered a large rainfall gradient to provide contrasting datasets. In this example, sampling simulations showed that a systematic design generally required one less sample than a restricted-random design to achieve the same accuracy, while a simple-random design required substantially more samples. We found taking a minimum of 30 soil samples was needed for both bulk density and C concentration to accurately estimate soil C content within a 1-ha plot in a pasture or tree planting, which suggests many previous surveys of soil C were sampled inadequately. The SIMAP method could be readily applied to a range of abiotic and biotic variables, with the construction of maps allowing most sampling intensities and designs to be tested. Adequate sampling intensities differ widely among environmental variables, so the SIMAP method enables researchers to determine which variables require more investment. For many variables, costs may be minimised while maintaining a high accuracy of the sampling design via bulking of well-mixed samples prior to analysis.

Keywords: Carbon sequestration; Reforestation; Sampling design; Sampling intensity; Soil mapping; Spatial heterogeneity
1. Introduction

Accurate assessment of environmental variables is vital to understanding and managing the global issues of land-use change and climate change. For many environmental variables, designing efficient and robust sampling protocols (i.e. balancing the need for sufficient sites to be representative, but with also adequate sampling within a site) is crucial for providing accurate information at scales appropriate to understanding these issues. Land-use change and climate change, however, most environmental variables exhibit a high degree of spatial and temporal heterogeneity, presenting a significant challenge to their accurate measurement (Vasseur and McCann, 2007). Environmental variables change spatially with topography, geology and climate, and temporally with the seasons, development and disturbance. To ensure adequate spatial sampling of biological processes, several designs have been used (De Grujter et al., 2006). Without prior knowledge of a system, ecologists typically use simple-random or systematic (i.e. based on a regular grid) sampling (Fig. 1a, b). When the spatial pattern of potential predictors is known, samples can be taken randomly within strata of the predictor using a stratified random design (Fig. 1c). In the absence of such knowledge, a restricted-random design can be used by randomly sampling within cells of a regular grid (De Grujter et al., 2006). Samples that are representative of the variation and spatially balanced can be collected with more complex designs, such as generalised random tessellation stratified sampling (Stevens and Olsen, 2004).

Sampling requirements for statistical accuracy often are not determined prior to the main survey or from the resultant data set (Caughlan and Oakley, 2001). An understanding of variability in an environmental variable is necessary to statistically guide the required sampling intensity based on either choosing a target variance (e.g. 80% chance of being within 10% of the population mean, Vasconcelos et al., 2014), or a target power of a statistical test to be applied to the survey data set (e.g. 80% chance of detecting a difference of a certain magnitude, Franco et al., 2015). Sampling intensity routinely is based on the expense and time involved in data collection, with some assumptions about the expected variability. However, effective science is achieved by avoiding under-sampling, which generally produces inconclusive or misleading results, and over-sampling.
that provides inefficient use of resources highly accurate estimates for individual sites at the expense of gathering a representative sample of sites. High sampling intensities may be achieved while minimising resources required for analysis via bulking a number of samples together. Although care would need to be taken to ensure samples are well mixed and that the process of mixing does not affect the properties of interest (e.g. Giesler and Lundstrom, 1993).

Soil is an example of a highly heterogeneous environment where adequate sampling is vital to acquiring accurate estimates of its properties (e.g. Yuan et al., 2013). At regional scales or across multiple sites, soil heterogeneity affects the distribution and productivity of native and production systems (van der Maarel and Franklin, 2012). At the local or site scales, interactions between plants and soil further increases soil heterogeneity (Hutchings et al., 2003). Coefficients of variation for nutrients in surface soils often are >50% (e.g. Cambardella et al., 1994), so soils provide an ideal test for developing a method to design efficient sampling protocols.

Accurate assessments of soil properties are required to determine the productivity, hydrology and biology of soil under different ecosystems. In particular, soil C content (Mg C ha⁻¹) is considered an important indicator of soil quality, including fertility, structure and hydraulics (Manlay et al., 2007; Raiesi and Kabiri 2016) and forest productivity (Seely et al., 2010). A poignant example of the impact of land-use change on soils is reforestation of agricultural land to sequester C in soil to reduce atmospheric C and potentially mitigating climate change (Mackey et al., 2013). Estimation of soil C storage at regional and national scales is infeasible economically using labour-intensive soil sampling alone (e.g. Dai et al., 2014). Soil C stocks across regions have been estimated by kriging soil surveys (Liu et al., 2011), relating surveys to maps of known drivers such as relief, land cover and geology (Ceddia et al., 2015) or both (e.g. Ungar et al., 2010). Many process models have been built to estimate C sequestration in forest soils, which inform national C accounting (e.g. 'Century', Parton et al., 1994). Whether large-scale estimation of soil C stocks is based on pattern or process models, its success relies on accurate site-level measurements.

Although theoretical relationships between spatial variability and the required sampling intensity for accurate estimates are well known (Muller, 2001), like most environmental variables, the
adequacy of sampling for soil C is rarely quantified. At the plot scale (< 0.05 ha), accurate estimation (within 10% of the mean) of soil C stocks can require around five samples in both tree plantings and pastures, and more cores are needed with increasing depth (Allen et al., 2010; Cunningham et al., 2012). An order of magnitude more samples can be needed for accurate estimates of soil C for a whole tree planting (approx. 1 ha, Chaudhuri et al., 2011) or a grazed field (100 ha, Pringle et al., 2011). Differences in soil C following reforestation of pasture are often small relative to the total stock (e.g. 3%, Laganière et al., 2010). Consequently, detecting a 10% change in soil C stocks in cultivated fields and forests can require tens of samples (e.g. Conant et al., 2003).

Here, we present the SIMAP method that involves the following steps, which could be adapted to field measurements of most environmental variables in production and native systems (Fig. 12). The generation of continuous variable maps provides the versatility to test a range of sampling designs beyond that used to collect the data. {[i]}

1. Selecting sites that cover a representative range of spatial variability. {[ii]}
2. Intensive and spatially accurate surveys within sites. {[iii]}
3. Building continuous Maps of sites. {[iv]}
4. Accuracy simulations based on these maps, and {[v]}
5. Establishing a sampling Protocol

As an illustration, we present the development of a sampling protocol that provides accurate but efficient estimation of soil C in environmental plantings and grazed pastures, which was subsequently used in a national survey across temperate Australia (England et al., 2016; Paul et al., 2017). Environmental plantings are defined here as plantings of several native tree and shrub species that are established for environmental benefits (e.g. biodiversity potential) and not for harvesting. We selected these land uses because forested soils generally are substantially more heterogeneous than agricultural soils (e.g. Conant et al., 2003), providing a strong contrast for the method. Environmental plantings are an increasing land use in temperate Australia because of their relatively high potential to sequester C, provide habitat for native species and improve environmental conditions compared with other uses of agricultural land (Cunningham et al., 2015b; Paul et al.,
Environmental plantings and their adjacent pastures were surveyed intensively at contrasting farms that covered a large rainfall gradient (approx. 400-1100 mm yr⁻¹ rainfall). Model simulations based on surveys of these contrasting sites allowed us to answer the following questions needed to develop a national protocol for sampling soil C in environmental plantings and pastures:

1. What sampling intensity is required for accurate estimation?
2. What are the most efficient sampling designs?

### 2. Material and methods

#### 2.1. Site selection

The first step of the SIMAP method involves selecting sites with a range of variation in the target variable (Fig. 1.2). Environmental plantings and their adjacent pastures were chosen at three farms to provide strong contrasts in potential soil C sequestration for exploring sampling protocols. For this reason, farms were selected across a large rainfall gradient (400-1100 mm yr⁻¹) in Victoria, Australia (Table 1). These sites were typical of where environmental plantings are established in temperate Australia. This region has been extensively cleared of their Eucalyptus-dominated woodlands since European settlement in the 1840s for dryland agriculture. The regional climate is temperate with seasonal changes in mean monthly maximum temperature (12-30 °C) and mean monthly minimum temperature (3-12 °C), and a winter-dominant rainfall (1961-1990, BOM, 2009). There were large differences in tree density and basal area among the plantings (Table 1), which should have increased the desired differences in spatial variability of soil C among farms.

A search of farms was conducted to find appropriate plantings with the following selection criteria. Tree plantings had to be > 2 ha to allow establishment of a 1 ha plot, > 30 m wide to minimise edge effects in the plot and approximately 15 years old to allow sufficient time for differences in soil C to have developed between the planting and adjacent pasture. Tree plantings older than 15 years are uncommon and are not representative of current establishment practices (i.e. eucalypts only). To provide a valid comparison, the planting needed to be on land previously part of the same field as the...
pasture to ensure they had received the same management pasture prior to the establishment of the planting. None of the farms were irrigated, and areas with soil salinity or erosion were avoided. Soils at the farms included the cracking clay of a vertosol and two sodosols, which are sodic (Table 1, Isbell, 2002). Tree plantings were established by ripping the soil into furrows, fencing to exclude stock and hand planting tubestock seedlings into the furrows at 3 m spacing, with no subsequent management. Sites were planted with a mixture of 12-18 regionally endemic woody species, including 3-7 tree species, with species predominantly from the genera Acacia Mill., Allocasaurina L.A.S. Johnson and Eucalyptus L’Hér. Pastures were planted with perennial grasses, continued to be grazed by stock and had fertilizer added.

2.2. Field survey

The next step of the SIMAP method was to conduct intensive surveys of soil C at the sites to inform the mapping (Fig. 12). The farms at Glenrowan, Minyip and Archies Creek were surveyed in February, July and September 2013, respectively. At each farm, a 1-ha plot was randomly selected in the planting and in the adjacent pasture. We used a paired-sited design, which is the most common design used in surveys of C stocks following reforestation (see Paul et al., 2002; Laganière et al., 2010). The adjacent pastures were located approximately 50 m from the planting to limit the influence of the trees. But the pasture and planting sites were within the same original field to minimise differences in previous land-use history, and were also at the same topographic position to avoid changes in soil type. Pastures are sampled in C sequestration studies to determine differences in soil organic C between land uses, and to indicate likely conditions at the reforestation plot if trees had not been planted, but do not provide an estimate of conditions prior to establishment. Planting and pasture plots at Glenrowan and Minyip were 50 m × 200 m, whereas the narrower planting at Archies Creek was sampled using an irregular plot of 30 m × 320 m that extended to 40 m wide for a length of 40 m at one end to make a total area of one hectare. Each plot was divided into 100 cells of 10 m × 10 m for sampling.
Soils were sampled using a restricted-random design. Within planting plots, a sample was taken within 10 cm of a randomly-selected point inside each of the 10 m × 10 m cells. To ensure variation at distances < 10 m was sampled adequately, additional samples were taken randomly within ten cells at a point 1 m from the first sample point and within another ten cells at a point 3 m from the first sample point. A total of 120 soil samples were taken within planting plots. Within pasture plots, samples were taken at a randomly-selected point within 56 randomly-selected cells. A reduced sampling effort was taken in pasture plots due to the expected lower variability in soil C, and because the focus was environmental plantings. Intact soil cores were collected manually from two depths (0-10 cm, 10-30 cm) using a corer with an internal diameter of 44 mm.

2.3. Sample processing and analysis

All soil samples were air-dried for two weeks and then weighed. Air-dried soil was crushed to a 2 mm diameter using a Retsch Jaw Crusher (Retsch, Haan, Germany) jaw crusher to ensure that soil aggregates were not retained in the coarse (> 2 mm) fraction. Soil was passed through a 2-mm sieve, roots removed from the coarse fraction and both fractions weighed. A 40 g subsample from the fine (< 2 mm fraction) fraction was weighed, oven-dried at 105 °C for a week and then reweighed. The ratio of initial and final mass of these subsamples was used to convert the total mass of the fine fraction to an oven-dried equivalent. Bulk density was calculated from each core sample using the total mass of the oven-dry fine fraction and the sample volume. A riffle box (13 mm x 12 slots, Civilab, Geelong, Australia) was used to split the fine fraction down to a well-mixed 40 g subsample for chemical analysis. Each subsample was ground to a fine powder using a mill. Total C concentration (%) was determined from 0.4 g subsamples using dry combustion (Trumac CNS Analyser, LECO, Michigan, USA).

2.4. Variation in survey data

The survey data set included 120 samples of soil (total C concentration, total C content and bulk density at 0-10 cm, 10-30 cm and 0-30 cm) from the plantings and 56 equivalent samples of soil from the adjacent pasture. Total C concentrations were converted to contents (t ha⁻¹) based on the ground
area and volume sampled by the corer, and the bulk density of a soil core. Basic statistics (mean, standard deviation and coefficient of variation) were calculated for all these variables to describe differences in statistical variability.

2.5. Soil maps

The next step of the SIMAP method was to build maps of these soil variables from the survey data to allow different sampling designs to be assessed (Figs. 12 & 13). Maps were produced for each soil variable (bulk density, total C concentration and total C content) x farm x land-use x soil depth combination. Initial krigged maps were created in ArcGIS 10 (ESRI, California, USA) for planting plots from the 100 samples collected across the grid of 10 m x 10 m cells, and for pasture plots from the 56 samples collected randomly across the grid (Fig. 23a). Ordinary kriging was used with an exponential semivariogram model, a search radius of 12 points and output resolution of 1 m. Kriging maintained the form of the semivariogram of the survey data but substantially reduced the magnitude of spatial variance (Fig. 23a). To create realistic variation among sampled points, the following approach was used: a) the frequency distribution of the survey data was approximated by fitting an appropriate probability distribution function, with a log-normal distribution used here, b) the required number of 1 m x 1 m cell values was drawn at random from this distribution to provide pseudo-observations at that scale, and c) pseudo-observations replaced the krigged values in rank order to maintain the spatial autocorrelation of the krigged map.

Whilst this improved the representation of overall spatial variability and ensured the distribution of values in pseudo-observed maps was similar to the surveyed data, it failed to adequately capture variability at the finest-scales (lag < 10 m). This was rectified by spatially disrupting individual cells within the krigged surface prior to the replacement of values. A normal deviate with a mean of zero and a global, user-defined coefficient of variation (CV) was added independently to each cell within the krigged map. Values of global CV were obtained by numerically minimising the sum-of-squares between the semivariograms for the surveyed data and for the pseudo-observed map, and varied among sites and soil variables (0.08 - 0.35). This local disruption based on a global CV yielded maps
that replicated the statistical (frequency distribution) and spatial (semivariogram) variation of the survey data (Fig. 23). The statistical and spatial fit of each map was assessed by comparing random samples from the maps with the survey data. One hundred random samples of 100 samples from planting maps and 56 samples from pasture maps were taken using the raster package in R (R Development Core Team, 2010; Hijmans et al., 2014). Frequency distributions of maps were assessed by comparing the distribution of the surveyed data with each of the random samples using the Kolmogorov-Smirnov test (ks.test) from the stats package in R. A mean probability of the frequency distribution of the survey data and map being different was calculated from these 100 comparisons. To assess spatial variation in a map, 100 bootstrapped samples (response, x and y coordinates) were taken without replacement from the surveyed data and the 100 random samples from a map (a total of 10,100 samples). The more powerful and precise bootstrapping approach was used over the alternative method of jackknifing (i.e. leave out samples and recalculate, Wolter, 2007). Each bootstrapped sample contained either 30 from 56 samples of pasture data or 50 from 100 samples of planting data. Semivariograms were calculated for each of these bootstrapped samples using the geoR package in R (Diggle and Ribeiro Jr, 2007). The sum-of-squares difference between the overall semivariogram for a map and the semivariogram of each bootstrapped sample was calculated. From these bootstrapped estimates, a mean sum-of-squares was calculated for the surveyed data and each of the original 100 random samples. The probability of a map having less variation than the surveyed data was determined from the rank-order of these mean values of sum-of-squares. An optimal map would have a probability of 0.5 (i.e. half of the mapped samples have more variation than the surveyed data and half have less variation) while an adequate map would have a probability of 0.25 < Pr < 0.75.

2.6. Accuracy simulations

The soil maps generated from the survey data facilitated accuracy simulations (Fig. 12) to explore:

a) sampling intensity required for an accurate estimate, and b) the most efficient sampling design.
Three sampling designs were included (simple-random, restricted-random and systematic) that are commonly used when there is no prior knowledge regarding the pattern of spatial variation for a location. Bootstrapped resampling with replacement from the survey data was performed for comparison with the map simulations. For simple-random sampling, the desired number of sample points was located independently and at random within the plot. For systematic sampling, a regular grid of the desired sampling intensity was superimposed over the plot, with a random starting position and orientation for each realization. Restricted-random sampling was based on a grid of the same construction, but with sample points located randomly within the grid-cells.

For a given sampling experiment, sampling intensity was varied from 2 to 200 samples ha\(^{-1}\), with 1000 replicate samplings per sampling intensity. Overall accuracy of sampling can be decomposed into two components: bias and precision. Because random sampling was used for all experiments, bias, measured as the difference in sample mean and the true underlying population mean, was zero in all cases. Precision was quantified as the CV of replicate samplings at a given sampling intensity, with the relationship between sample CV and sampling intensity following a power function (Roxburgh et al., 2015). We used the probable limit of error (PLE) as the index of precision instead of CV, which are related as follows:

\[
PLE = \left(\frac{SD}{\bar{x}}\right) \times t = CV \times t
\]

where SD = standard deviation of mean values across the 1000 replicate samplings, \(\bar{x}\) is the sample mean and \(t\) = \(t\)-value with degrees of freedom = \(N\) - 1 sample points. We estimated the sampling intensity required to achieve a PLE of 10% with a probability of 95%. This sampling intensity was estimated from the simulation results by interpolation of the relationship between sampling precision (i.e. PLE) and sampling intensity.

Precise measurement of bulk density in the field is time consuming due to the difficulty of excavating bulk density rings at depth. Many scientists take less bulk density samples than nutrient samples to reduce costs, with the belief that it is unnecessary. We used the above simulations to explore the trade-off between differing numbers of bulk density samples and C analysis samples on the accuracy of estimates of total C content in 0-30 cm layer. The sampling intensity for bulk density
and C concentration were varied from 2 to 75 samples, with 5,000 replicate samplings for each possible combination.
3. Results

3.1. Variation in survey data

The CV ranged from 0.11 to 0.46 for the soil properties (Table 2). For most land-use × site combinations, bulk density was the least variable soil property and soil C concentration was the most variable. Soil C content, which is calculated from soil C concentration and bulk density, was generally equally or less variable to soil C concentration (Table 2). Generally, the variability of soil C concentration and content was higher in the lower soil layer (10-30 cm) than in the upper layer (0-10 cm). The variability of bulk density decreased with depth at the Glenrowan and Archies Creek farms while it increased with depth at Minyip farm (Table 2). There was a trend for soil properties (bulk density, C concentration and C content) to be more variable under the plantings than under the adjacent pastures (Table 2) within a given soil layer on a farm. The Glenrowan farm had the most variable soil C content while the Minyip and Archies Creek farms had similar variability. Previous work provides more a comprehensive analysis of site factors attributable to differences in soil C content (e.g. England et al., 2016; Paul et al., 2017).

3.2 Soil maps

All maps provided accurate representations of the frequency distribution and spatial variation of the surveyed data (Table 3). Random samples from the maps were found to have similar frequency distributions to the surveyed data ($P > 0.3$). Spatial variation of the surveyed data sets was represented adequately by the maps [$Pr(\text{mapped SS} < \text{surveyed SS} = 0.28-0.71)$. Although there was variation in the spatial fit of the maps, there were no consistent trends among soil variables or sites.

3.3 Accuracy simulations

The sampling simulations for soil properties showed consistently that systematic sampling required the fewest samples to achieve a target probable limit of error (PLE, Fig. 34). Restricted-random sampling produced very similar results to that of systematic sampling, on average requiring just one additional sample. Simple-random sampling required substantially more samples to the
other designs and produced similar results to the bootstrapped resampling of the surveyed data. For soil C content in the 0-30 cm layer, more samples were required for a specific PLE at the Glenrowan farm, regardless of the sampling design, than at the other farms, which required similar sample numbers (Fig. 3). For a given PLE, estimating soil C content in the 0-30 cm layer required more samples for plantings than the pastures at all farms. Similar trends among farms and between land-use types were shown for bulk density, C concentration and C content in the 0-10 cm and 0-30 cm soil layers.

The sampling simulations consistently showed that systematic sampling was the most or one of the most efficient designs for achieving the target PLE (i.e. 95% probability of being within 10% of the true mean) for all soil properties (Tables 4). For 39% of the sampling combinations, restricted-random sampling was equally efficient as systematic sampling for soil properties (Table 4). Soil properties at the Glenrowan farm required the most samples for accurate estimation (Table 4), which reflects the higher variability of soil properties at that farm (Table 2). A total of 30 and 25 cores were need for accurate estimates of soil C content at the Glenrowan farm in the 0-10 cm and 0-30 cm layers, respectively. In contrast, soil properties at the Archies Creek farm required the least number of samples for most sampling combinations (Table 4). For most soil properties, more samples were needed to accurately estimate values in the planting than the adjacent pasture. Estimating bulk density and total C content for the 0-30 cm layer required fewer cores than the 0-10 layer whereas C concentration did not show consistent trends between the soil layers (Table 4).

The simulations based on the surveyed data showed that the target PLE for soil C content was achieved most efficiently by taking equal numbers of bulk density and C concentration samples (Fig. 4). At a given sampling intensity for bulk density, there were minor increases in the accuracy of soil C content estimates when a larger number of C concentration samples than bulk density samples were used. Conversely, there were negligible increases in the accuracy of soil C content estimates when a larger number of bulk density samples than C concentration samples were used. For example at Minyip, soil C content can be estimated with the same precision (i.e. 95% probability of being within 10% of the true mean) from measurements of (i) C concentration and bulk densities from the
same 10 cores, (ii) C concentration from 10 cores and bulk densities from up to 50 cores without any improvement or (iii) C concentration from 20 cores and bulk densities from eight cores (Fig. 4a).

This trend was consistent among farms and between land-use types (Fig. 45).

4. Discussion

The SIMAP method developed here (Site selection-Intensive survey-Mapping-Accuracy simulations-Protocol, Fig. 12) provides a systematic way for determining sampling protocols for environmental variables, which are commonly highly heterogeneous. We used the estimation of soil C stocks under different land uses as an illustration of how the SIMAP method can be applied. Our survey of environmental plantings and adjacent pastures at three contrasting farms quantified the spatial variability of soil properties (Table 2). This intensive survey of 1-ha plots allowed the generation of maps that replicated the statistical and spatial variability of soil properties (Fig. 23, Table 3). Simulations of simple-random, restricted-random and systematic sampling were possible using these maps, which showed the most efficient sampling intensities and designs for accurate estimates of soil properties (Figs. 45, Table 4). From the simulation results, an efficient but conservative sampling protocol for soil C content was determined for a national survey of environmental plantings and their adjacent pastures (England et al. 2016).

4.1. Surveying a representative range of variability

We choose tree plantings and pastures on farms with distinct environmental and structural characteristics (Table 1), which provided contrasting examples of statistical and spatial variation in soil properties (Table 2). As case studies, these farms provided valuable insight into the sampling intensities and designs needed to accurately measure soil C following reforestation, but they should not be considered representative of environmental plantings across temperate Australia. Soil C and bulk density were highly variable (Tables 2), with more variability under the plantings than under the adjacent pastures. This is consistent with previous surveys of reforestation (Conant et al., 2003; Cunningham et al., 2012) and reflects the more heterogeneous distribution of plant biomass in
forests compared with agricultural fields. The higher complexity and consequently variability of environmental variables in native ecosystems than production systems is a common finding (Vasseur and McCann, 2007). Spatial variability in soil C differed more among farms than between land uses on a farm (Fig. 24), which is consistent with our previous survey of environmental plantings (Cunningham et al., 2012). This suggests spatial variability in soil C under developing (approx. 15 year-old) tree plantings is dominated by the legacy of soil variability in the original pasture.

4.2. Producing an accurate map

Soil maps commonly are produced using krigging to predict soil property values between survey points (e.g. Piccini et al., 2014). As demonstrated here (Fig. 23), krigging interpolates between data points producing a ‘smoothed’ data set that has substantially less spatial variation than the surveyed data and would result in a substantial underestimation of the required sampling intensity. Instead, using the frequency distribution of the surveyed data to replace the krigged values provided realistic spatial and statistical variation in a map (Fig. 22). We used statistical tests of map accuracy based on how well the map matched the surveyed data (Table 3). A more rigorous test would be to conduct a future survey of a site, stratified by predicted values of soil properties, to see how well the map predicted soil properties in the field.

4.3. Determining sampling intensity

Sampling intensity for environmental variables is generally based on previous studies. Soil C stocks are often estimated from 10-20 samples per site (Smith, 2004). Our simulations suggest that many surveys of soil C have been under-sampling and that a minimum of 30 samples per hectare are required to accurately estimate soil C stocks in pastures and tree plantings (Table 4). Soil surveys of a lower intensity are likely to produce highly uncertain estimates, and be unable to detect differences between land uses and management treatments. Studies of reforestation have often taken less than 10 samples per site to estimate soil C stocks (e.g. Harper et al., 2012; Cunningham et al., 2015a), which is likely to be substantial under-sampling. Previous surveys of forests and agricultural fields have found the sample size to estimate soil C to our target probable limit of error (i.e. 95% probability of
Cunningham et al. 17

being within 10% of the true mean) ranged from 6-42 cores (Garten and Ashwood, 2002; Allen et al., 2010; Chaudhuri et al., 2011; Cunningham et al., 2012; Kristensen et al., 2015).

For most soil properties, more samples were needed to accurately estimate values in the planting than the adjacent pasture (Table 4), which follows the difference in their variability between these land-uses. Similarly, detecting change in soil C at a site scale required substantially more samples in a temperate-deciduous forest than a nearby field (Conant et al., 2003). At the landscape scale (14,000 ha), temperate-deciduous forests and pastures required similar sampling intensities to achieve our target probable limit of error (Garten and Ashwood, 2002).

Estimates of soil C content require measurements of bulk density and C concentration in the soil. Carbon concentration is often analysed from substantially more soil samples than bulk density, which is difficult to acquire precise estimates in the field, making it time consuming and expensive (Allen et al., 2010). The simulation results showed that having more C concentration samples than bulk density samples provided little improvement in estimates of soil C content (Fig. 45). Several studies have found that C concentration explains more variation in soil C content estimates than bulk density and, therefore, support taking less bulk density samples (e.g. Don et al., 2007). Besides being from different locations and soil types, these other simulations were based on far fewer field samples (N < 25). Therefore, the cautious approach would be to take an equal number of bulk density and C concentration samples, and to measure both from the same samples.

High sampling intensities may be achieved while also minimising resources required for soil preparation if is often reduced by bulking a number of samples together for subsequent analysis. But even when soil samples are thoroughly mixed using a riffle box, or roughly by hand, introduces errors to the estimate of soil properties. For example, there is evidence that the nutrient availability measured from a bulked sample is higher than the mean from the individual samples due to destruction of aggregates during bulking (e.g. Giesler and Lundstrom, 1993). The effect of bulking on soil C estimates would need to be tested from field samples and not using the SIMAP method, which assumes statistically perfect bulking.
The simulations showed that systematic sampling was the most efficient design for sampling soil properties (Table 4). Systematic designs are relatively easy to implement in the field, require little prior knowledge and provide consistent coverage. Although systematic designs cannot provide a valid estimate of error in the mean because of a lack of statistical independence among samples, the conservative approach of assuming the samples were independent is usually applied. Furthermore, the regular nature of the grid means properties may align with the grid (e.g., planting rows), leading to a possible bias in the mean estimate. Random sampling has the advantages of removing selection bias, easy implementation and statistical independence in analyses. As demonstrated here, sample sizes for random designs often have to be large to ensure representativeness because by chance they may not be dispersed evenly in space (Muller, 2001). There was little difference in efficiency between restricted-random and systematic sampling, and with restricted-random sampling generally requiring one extra sample to achieve the same level of precision (Table 4). Restricted-random sampling provides a useful compromise between random and regular designs, which provides a good coverage of independent samples. However, stratified-random sampling is often better when there is prior knowledge of potential correlates of the target environmental variable (De Gruijter et al., 2006).

An important strength of the SIMAP method is that the maps allow numerous sampling designs to be tested beyond that used to collect the underlying data. Besides random and systematic sampling, there are many variations of stratified sampling that ensure efficient and representative sampling of environmental variables (De Gruijter et al., 2006). With no prior knowledge of an area, there are several methods to acquire a random but spatially-balanced design including restricted-random, compact geographic stratification (Brus et al., 1999) and general randomized tessellation stratified sampling (Stevens and Olsen, 2004). The maps can be combined with other spatial data sets of potential predictors such as topographic, vegetation and climate maps to allow equal or proportional stratified sampling within these strata. Hierarchical random sampling generally is used to survey
large areas. For example, freshwater taxa may be sampled using the hierarchy of watersheds, reaches and channels (Townsend et al., 1997). In these designs, the SIMAP method could determine an efficient sampling design of the tertiary unit within the secondary unit (e.g. channels within reaches). The SIMAP method provides a tool to explore the utility of different sampling designs for various environmental variables (e.g. taxa abundance and chemical concentrations).

4.5. Determining the sampling protocol

The simulations suggested the following sampling protocol may be efficient for a broad survey of soil C stocks in environmental plantings and their adjacent pastures in the low to medium rainfall areas (400-1100 mm yr⁻¹) of temperate Australia. Samples should be collected using a design that ensures a representative spatial distribution of sample points, such as a restricted-random design. A minimum of 30 soil samples across a 1-ha plot should be taken, with bulk density and C concentration measured from all samples, to estimate soil C content in the planting and adjacent pasture. This is a less intensive but conservative sampling intensity based on the requirements for the most heterogeneous farm at Glenrowan (Table 4). Our findings have been applied in an Australian study and subsequently used to inform a national survey and model study of soil C sequestration under environmental plantings across temperate Australia (England et al., 2016; Paul et al., 2017).

The suggested sampling intensity is appropriate for a 1-ha plot. How sampling intensity scales to smaller or larger plots has yet to be determined. Variation in soil C is expected to increase with sample area, which was shown from the county to national scale for grassland soils of the United States (Conant and Paustian, 2002). Our previous survey of tree plantings showed that only nine cores were required to estimate soil C content to the target PLE in a smaller 0.04 ha plot (Cunningham et al., 2012). The maps generated here could be used to determine sampling intensity required in areas smaller than that surveyed.

The protocol for estimating soil C stocks in environmental plantings could be refined with intensive surveys at more farms, but we note that even low-intensity surveys of soil C rarely exceed
10 sites (e.g. Hoogmoed et al., 2012). With a targeted survey, it may be possible to relate the variability in soil properties, and consequently sampling intensity, to easier-measured environmental variables such as topography, soil type and planting structure. This would allow a sampling protocol to be tailored to context-specific variability in soil properties. Such a rigorous sampling protocol would increase the efficiency of sampling the numerous plantings required to inform an accurate model for C sequestration in environmental plantings.

4.6. Versatile method

Construction of continuous maps of environmental variables allow the researcher to go beyond the commonly used tests of a target variance or power based on a single survey design (e.g. Keizer-Vlek et al., 2012). The maps allow numerous sampling intensities and most spatial designs to be tested, with only a few standard designs tested here. The SIMAP method could be applied to a wide range of environmental variables (e.g. soil nutrients, vegetation structure, animal behaviour, and pollutants of air or water). We used a similar approach to determine the number of trees to harvest to develop precise allometric relationships for estimating tree biomass within environmental plantings (Roxburgh et al., 2015). Repeated sampling would be required to gain a representative sample of more temporally-variable environmental properties, such as estimating the abundance of animal species or probabilities of water quality exceeding a threshold. We presented two-dimensional maps but the SIMAP method could be adapted to three dimensions for simulations of terrain, oceanic or atmospheric sampling with appropriate field sampling. Adequate sampling intensities differ widely among environmental variables and the SIMAP method enables the researcher to determine which variables need more investment. For many variables of interest (e.g. soil C content), there may be opportunities to minimise costs while maintaining a high accuracy of the sampling design via bulking of well-mixed samples prior to analysis.

Acknowledgements
This study was funded by the Australian Government’s ‘Filling the Research Gap’ program (Project 01203.052). We acknowledge the Australian Research Council for financial support of SCC (LP0990038) and TRC (FT120100463). We thank the land holders (John and Felicia Di Stefano, Glenn and Glenice Foster, and David Tepper) for providing access to and history of their farms. Thanks to Phil De Zylva and Jarrod Hodgson for cheerfully managing the fieldwork and processing the samples. Thanks to the whole field team: James Bull, Greg Horrocks, Jessica MacKay, Gerardo Martinez, Katherine Selwood, Justine Smith and Nancy Van Nieuwenhove.

References


Fig. 1. Sampling designs commonly used to measure environmental variables, including those used in the simulations to determine an efficient design for accurate estimation of soil properties (a, b, d). Systematic sampling takes samples at a fixed interval (b), stratified-random takes samples randomly within strata of a potential predictor (c), while restricted-random sampling provides a random pattern while ensuring a consistent density of samples across the area (d).

Fig. 2. Steps involved in the SIMAP method (Site selection, Intensive surveys, Map build, Accuracy simulations and Protocol choice) for determining sampling protocols. Five areas are presented with increasing spatial variability from left to right. PLE = probable limit of error.

Fig. 3. Method used to generate maps of variables from the survey data to inform the accuracy simulations. Sample points used to produce the map are shown (N = 100 cores), with their size relative to the magnitude of the observed values. Darker areas of the krigged maps indicate areas of higher total soil C content. ArcGIS was used to create krigged maps (a) and then realistic random variation was introduced between samples by producing pseudo-observations that replicated the semivariogram and frequency distribution of the surveyed data (b). Frequency distributions and semivariograms of the surveyed data (red) and the map (blue) are shown. See Methods for more detail.

Fig. 4. Relationships between the probable limit of error and sampling size for total soil C (0-30 cm) under plantings and pastures at the three farms. Sampling designs are indicated by different lines: simple-random (orange), restricted-random (blue) and systematic (red). Bootstrapped resampling of the survey data (black) is provided for comparison.

Fig. 5. Effect of the sampling intensity for bulk density and carbon concentration on the accuracy of soil C content (0-30 cm) estimates under environmental plantings (a, c, e) and pastures (b, d, f) at the three farms. Contours indicate a 95% probability of being within the stated percentage of the mean, given the sampling intensity for bulk density and C concentration.
Fig. 1. Sampling designs commonly used to measure environmental variables, including those used in the simulations to determine an efficient design for accurate estimation of soil properties (a, b, d). Systematic sampling takes samples at a fixed interval (b), stratified random takes samples randomly within strata of a potential predictor (c), while restricted random sampling provides a random pattern while ensuring a consistent density of samples across the area (d).
Fig. 2. Steps involved in the SIMAP method (Site selection, Intensive surveys, Map build, Accuracy simulations and Protocol choice) for determining sampling protocols. Five areas are presented with increasing spatial variability from left to right. PLE = probable limit of error.
Fig. 3. An example (Glenrowan) of how methods used to generate maps were generated: (a) using ArcGIS and kriging, and (b) by introducing more realistic random variation by producing pseudo-observations that replicated the semivariogram and frequency distribution of the surveyed data. Sample points used to produce the map are shown ($N = 100$ cores), with their size relative to the magnitude of the observed values. Darker areas of the krigeed maps indicate areas of higher total soil C content. ArcGIS was used to create krigeed maps (a) and then realistic random variation was introduced between samples by producing pseudo-observations that replicated the semivariogram and frequency distribution of the surveyed data (b). Frequency distributions and semivariograms of the surveyed data (red) and the map (blue) are shown. See Methods for more detail.
Fig. 4.3. Relationships between the probable limit of error and sampling size for total soil C (0-30 cm) under plantings and pastures at the three farms. Sampling designs are indicated by different lines: simple-random (orange), restricted-random (blue) and systematic (red). Bootstrapped resampling of the survey data (black) is provided for comparison.
Fig. 5. Effect of the sampling intensity for bulk density and carbon concentration on the accuracy of soil C content (0-30 cm) estimates under environmental plantings (a, c, e) and pastures (b, d, f) at the three farms. Contours indicate a 95% probability of being within the stated percentage of the mean, given the sampling intensity for bulk density and C concentration.
Table 1 Location and environmental conditions of the three farms studied.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Minyip</th>
<th>Glenrowan</th>
<th>Archies Creek</th>
</tr>
</thead>
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<tr>
<td>Location</td>
<td>36.54°S 142.62°E</td>
<td>36.50°S 146.14°E</td>
<td>38.50°S 145.57°E</td>
</tr>
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<td>Mean rainfall (mm yr⁻¹) †</td>
<td>392</td>
<td>663</td>
<td>1095</td>
</tr>
<tr>
<td>Max. temp hottest month (°C) †</td>
<td>30.9</td>
<td>31.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Min. temp coldest month (°C) †</td>
<td>4.0</td>
<td>2.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Planting age (yr)</td>
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<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Planting size (ha)</td>
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<td>4.9</td>
<td>2.0</td>
</tr>
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<td>Sodosol</td>
<td>Sodosol</td>
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<td>312</td>
<td>690</td>
</tr>
<tr>
<td>Basal area (m² ha⁻¹)</td>
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<td>11.6</td>
<td>23.8</td>
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<td>Dominant eucalypts</td>
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<td><em>E. sideroxylon</em></td>
<td><em>E. globulus spp.</em></td>
</tr>
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<td><em>E. bridgesiana</em></td>
<td><em>globulus</em></td>
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<td></td>
<td><em>E. polyanthemos</em></td>
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<td></td>
</tr>
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<td>perennial grass</td>
<td>perennial grass</td>
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<tr>
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<td>sheep</td>
<td>cattle</td>
<td>cattle and sheep</td>
</tr>
<tr>
<td>Fertilizer addition</td>
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<td>none</td>
<td>none</td>
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† Calculated over the life time of the planting
Table 2 Summary statistics for soil properties under different land-uses at each farm. Values are means ($N = 120$ cores for plantings, $N = 56$ cores for pastures) followed by standard deviations and coefficients of variation in brackets.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Land-use</th>
<th>Soil depth (cm)</th>
<th>Bulk density ($g \text{ cm}^{-3}$)</th>
<th>Total C conc (%)</th>
<th>Total C content (Mg ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minyip</td>
<td>planting</td>
<td>0-10</td>
<td>1.06±0.17 (0.16)</td>
<td>2.08±0.50 (0.24)</td>
<td>21.6±4.4 (0.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-30</td>
<td>1.26±0.21 (0.17)</td>
<td>1.09±0.30 (0.28)</td>
<td>26.8±5.9 (0.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-30</td>
<td>1.19±0.18 (0.15)</td>
<td>1.42±0.32 (0.22)</td>
<td>48.5±8.1 (0.17)</td>
</tr>
<tr>
<td></td>
<td>pasture</td>
<td>0-10</td>
<td>0.90±0.12 (0.14)</td>
<td>1.77±0.32 (0.18)</td>
<td>15.8±2.8 (0.18)</td>
</tr>
<tr>
<td></td>
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<td>10-30</td>
<td>1.02±0.15 (0.15)</td>
<td>1.06±0.19 (0.18)</td>
<td>21.3±3.8 (0.18)</td>
</tr>
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<td>0-30</td>
<td>0.98±0.12 (0.12)</td>
<td>1.29±0.20 (0.15)</td>
<td>37.1±5.1 (0.14)</td>
</tr>
<tr>
<td>Glenrowan</td>
<td>planting</td>
<td>0-10</td>
<td>1.04±0.26 (0.25)</td>
<td>3.32±0.68 (0.20)</td>
<td>34.2±10.5 (0.31)</td>
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<td></td>
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<td>10-30</td>
<td>1.18±0.22 (0.19)</td>
<td>1.01±0.46 (0.46)</td>
<td>23.1±9.3 (0.40)</td>
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<td>0-30</td>
<td>1.13±0.19 (0.16)</td>
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<td>57.3±15.1 (0.26)</td>
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<td>0-10</td>
<td>0.97±0.26 (0.27)</td>
<td>3.07±0.65 (0.21)</td>
<td>29.5±8.6 (0.29)</td>
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<td>10-30</td>
<td>1.16±0.22 (0.19)</td>
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<td>0-30</td>
<td>1.09±0.19 (0.17)</td>
<td>1.56±0.36 (0.23)</td>
<td>47.6±12.1 (0.25)</td>
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<td>Archies Creek</td>
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<td>0-10</td>
<td>0.75±0.12 (0.16)</td>
<td>4.75±1.07 (0.23)</td>
<td>34.9±5.9 (0.17)</td>
</tr>
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<td>10-30</td>
<td>1.09±0.13 (0.11)</td>
<td>2.50±0.52 (0.21)</td>
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<td>0-30</td>
<td>0.90±0.11 (0.11)</td>
<td>3.25±0.64 (0.20)</td>
<td>88.9±14.3 (0.16)</td>
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<td>32.8±4.7 (0.14)</td>
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<td>1.05±0.13 (0.13)</td>
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<td>0.98±0.11 (0.11)</td>
<td>2.78±0.59 (0.21)</td>
<td>77.8±12.2 (0.16)</td>
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Table 3  Fit of mapped values to the frequency distribution and semivariance of surveyed data for soil properties under different land-uses at each farm. Probabilities for the frequency distributions are the mean $P$ values ($N = 100$) of Kolmogorov-Smirnov tests comparing the surveyed data with 100 random samples from the map. Probabilities for semivariance were based on the sum-of-squares difference between the overall semivariogram for the map and random samples of the map ($N = 100$) and the surveyed data. See Methods for more detail.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Land-use</th>
<th>Soil depth (cm)</th>
<th>Frequency distribution (mean $P$)</th>
<th>Semivariance [Pr(mapped SS &lt; surveyed SS)]</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>BD [C] Cont</td>
<td>BD [C] Cont</td>
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<tr>
<td>Minyip</td>
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<td>0.67 0.67 0.67</td>
<td>0.56 0.32 0.36</td>
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<td></td>
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<td>0.57 0.64 0.70</td>
<td>0.29 0.70 0.71</td>
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<td>0-10</td>
<td>0.67 0.68 0.76</td>
<td>0.62 0.36 0.39</td>
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<td>0.32 0.63 0.62</td>
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<td>0.71 0.68 0.58</td>
<td>0.42 0.61 0.71</td>
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Table 4 Simulation results showing the sampling intensity (cores ha$^{-1}$) required to have a 95% probability of getting within 10% of the mean (PLE) for soil properties. Results are given for bootstrapped resampling of the survey data (BS) and simple-random (SR), restricted-random (RR) and systematic (SM) sampling of the maps. The most efficient sampling design for each variable × land-use × farm combination is indicated in bold.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Land-use</th>
<th>Soil depth</th>
<th>Number of cores to attain PLE</th>
<th>Bulk density</th>
<th>Total C conc</th>
<th>Total C content</th>
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