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

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26 **Abstract**

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

49
50 *Keywords:* Carbon sequestration; Reforestation; Sampling design; Sampling intensity; Soil
51 mapping; Spatial heterogeneity

52 **1. Introduction**

53 ~~Accurate assessment of environmental variables is vital to understanding and managing the global~~
54 ~~issues of land use change and climate change.~~ For many environmental variables, designing
55 efficient and robust sampling protocols (i.e. balancing the need for sufficient sites to be
56 representative, but with also adequate sampling within a sites) is crucial for providing accurate
57 information at scales appropriate to understanding ~~these issues~~ land-use change and climate change.
58 However, most environmental variables exhibit a high degree of spatial and temporal heterogeneity,
59 presenting a significant challenge to their accurate measurement (Vasseur and McCann, 2007).

60 Environmental variables change spatially with topography, geology and climate, and temporally
61 with the seasons, development and disturbance. To ensure adequate spatial sampling of biological
62 processes, several designs have been used (De Gruijter *et al.*, 2006). Without prior knowledge of a
63 system, ecologists typically use *simple-random* or *systematic* (i.e. based on a regular grid) sampling
64 (Fig. 1a, b). When the spatial pattern of potential predictors is known, samples can be taken randomly
65 within strata of the predictor using a *stratified random* design (Fig. 1c). In the absence of such
66 knowledge, a *restricted-random* design can be used by randomly sampling within cells of a regular
67 grid (De Gruijter *et al.*, 2006). Samples that are representative of the variation and spatially balanced
68 can be collected with more complex designs, such as generalised random tessellation stratified
69 sampling (Stevens and Olsen, 2004).

70 Sampling requirements for statistical accuracy often are not determined prior to the main survey
71 or from the resultant data set (Caughlan and Oakley, 2001). An understanding of variability in an
72 environmental variable is necessary to statistically guide the required sampling intensity based on
73 either choosing a target variance (e.g. 80% chance of being within 10% of the population mean,
74 Vasconcelos *et al.*, 2014), or a target power of a statistical test to be applied to the survey data set
75 (e.g. 80% chance of detecting a difference of a certain magnitude, Franco *et al.*, 2015). Sampling
76 intensity routinely is based on the expense and time involved in data collection, with some
77 assumptions about the expected variability. However, effective science is achieved by avoiding
78 under-sampling, which generally produces inconclusive or misleading results, and over-sampling

79 that provides ~~inefficient use of resources~~ ~~highly accurate estimates for individual sites at the expense~~
80 ~~of gathering a representative sample of sites. High sampling intensities may be achieved while~~
81 ~~minimising resources required for analysis via bulking a number of samples together. Although care~~
82 ~~would need to be taken to ensure samples are well mixed and that the process of mixing does not~~
83 ~~affect the properties of interest (e.g. Giesler and Lundstrom, 1993).~~

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

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

104 Although theoretical relationships between spatial variability and the required sampling intensity
105 for accurate estimates are well known (Muller, 2001), like most environmental variables, the

106 adequacy of sampling for soil C is rarely quantified. At the plot scale (< 0.05 ha), accurate estimation
 107 (within 10% of the mean) of soil C stocks can require around five samples in both tree plantings and
 108 pastures, and more cores are needed with increasing depth (Allen *et al.*, 2010; Cunningham *et al.*,
 109 2012). An order of magnitude more samples can be needed for accurate estimates of soil C for a whole
 110 tree planting (approx. 1 ha, Chaudhuri *et al.*, 2011) or a grazed field (100 ha, Pringle *et al.*, 2011).
 111 Differences in soil C following reforestation of pasture are often small relative to the total stock (e.g.
 112 3%, Laganière *et al.*, 2010). Consequently, detecting a 10% change in soil C stocks in cultivated fields
 113 and forests can require tens of samples (e.g. Conant *et al.*, 2003).

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

- 118 1. Selecting sites that cover a representative range of spatial variability: (ii).
- 119 2. Intensive and spatially accurate surveys within sites: (iii).
- 120 3. Building continuous Maps of sites: (iv).
- 121 4. Accuracy simulations based on these maps, and: (v).
- 122 5. Establishing a sampling Protocol

123 As an illustration, we present the development of a sampling protocol that provides accurate but
 124 efficient estimation of soil C in environmental plantings and grazed pastures, which was
 125 subsequently used in a national survey across temperate Australia (England *et al.*, 2016; Paul *et al.*
 126 2017). Environmental plantings are defined here as plantings of several native tree and shrub species
 127 that are established for environmental benefits (e.g. biodiversity potential) and not for harvesting.
 128 We selected these land uses because forested soils generally are substantially more heterogeneous
 129 than agricultural soils (e.g. Conant *et al.*, 2003), providing a strong contrast for the method.
 130 Environmental plantings are an increasing land use in temperate Australia because of their relatively
 131 high potential to sequester C, provide habitat for native species and improve environmental
 132 conditions compared with other uses of agricultural land (Cunningham *et al.*, 2015b; Paul *et al.*,

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133 2016). Environmental plantings and their adjacent pastures were surveyed intensively at contrasting
134 farms that covered a large rainfall gradient (approx. 400-1100 mm yr⁻¹ rainfall). Model simulations
135 based on surveys of these contrasting sites allowed us to answer the following questions needed to
136 develop a national protocol for sampling soil C in environmental plantings and pastures: (i)

137 1. — What sampling intensity is required for accurate estimation? and: (ii)

138 2. What are the most efficient sampling designs?

139

140 2. Material and methods

141 2.1. Site selection

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

153 A search of farms was conducted to find appropriate plantings with the following selection
154 criteria. Tree plantings had to be » 2 ha to allow establishment of a 1 ha plot, » 30 m wide to minimise
155 edge effects in the plot and approximately 15 years old to allow sufficient time for differences in soil
156 C to have developed between the planting and adjacent pasture. Tree plantings older than 15 years
157 are uncommon and are not representative of current establishment practices (i.e. eucalypts only). To
158 provide a valid comparison, the planting needed to be on land previously part of the same field as the

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159 pasture to ensure they had received the same management pasture prior to the establishment of the
160 planting. None of the farms were irrigated, and areas with soil salinity or erosion were avoided.

161 Soils at the farms included the cracking clay of a vertosol and two sodosols, which are sodic (Table
162 1, Isbell, 2002). Tree plantings were established by ripping the soil into furrows, fencing to exclude
163 stock and hand planting tubestock seedlings into the furrows at 3 m spacing, with no subsequent
164 management. Sites were planted with a mixture of 12-18 regionally endemic woody species,
165 including 3-7 tree species, with species predominantly from the genera *Acacia* Mill., *Allocasaurina*
166 L.A.S. Johnson and *Eucalyptus* L'Hér. Pastures were planted with perennial grasses, continued to be
167 grazed by stock and had fertilizer added.

168 2.2. Field survey

169 The next step of the SIMAP method was to conduct intensive surveys of soil C at the sites to inform
170 the mapping (Fig. 12). The farms at Glenrowan, Minyip and Archies Creek were surveyed in February,
171 July and September 2013, respectively. At each farm, a 1-ha plot was [randomly selected established](#)
172 in the planting and in the adjacent pasture. We used a paired-sited design, which is the most common
173 design used in surveys of C stocks following reforestation (see Paul *et al.*, 2002; Laganière *et al.*,
174 2010). The adjacent pastures were located approximately 50 m from the planting to limit the
175 influence of the trees. But the pasture and planting sites were within the same original field to
176 minimise differences in previous land-use history, and were also at the same topographic position to
177 avoid changes in soil type. Pastures are sampled in C sequestration studies to determine differences
178 in soil organic C between land uses, and to indicate likely conditions at the reforestation plot if trees
179 had not been planted, but do not provide an estimate of conditions prior to establishment. Planting
180 and pasture plots at Glenrowan and Minyip were 50 m × 200 m, whereas the narrower planting at
181 Archies Creek was sampled using an irregular plot of 30 m × 320 m that extended to 40 m wide for a
182 length of 40 m at one end to make a total area of one hectare. Each plot was divided into 100 cells of
183 10 m × 10 m for sampling.

184 Soils were sampled using a restricted-random design. Within planting plots, a sample was taken
185 within 10 cm of a randomly-selected point inside each of the 10 m × 10 m cells. To ensure variation
186 at distances < 10 m was sampled adequately, additional samples were taken randomly within ten
187 cells at a point 1 m from the first sample point and within another ten cells at a point 3 m from the
188 first sample point. A total of 120 soil samples were taken within planting plots. Within pasture plots,
189 samples were taken at a randomly-selected point within 56 randomly-selected cells. A reduced
190 sampling effort was taken in pasture plots due to the expected lower variability in soil C, and because
191 the focus was environmental plantings. Intact soil cores were collected manually from two depths
192 (0-10 cm, 10-30 cm) using a corer with an internal diameter of 44 mm.

193 2.3. Sample processing and analysis

194 All soil samples were air-dried for two weeks and then weighed. Air-dried soil was crushed to a 2
195 mm diameter using a [Retsch Jaw Crusher \(Retsch, Haan, Germany\) jaw crusher](#) to ensure that soil
196 aggregates were not retained in the coarse (> 2 mm) fraction. Soil was passed through a 2-mm sieve,
197 roots removed from the coarse fraction and both fractions weighed. A 40 g subsample from the fine
198 (< 2 mm fraction) fraction was weighed, oven-dried at 105 °C for a week and then reweighed. The
199 ratio of initial and final mass of these subsamples was used to convert the total mass of the fine
200 fraction to an oven-dried equivalent. Bulk density was calculated from each core sample using the
201 total mass of the [oven-dry](#) fine fraction and the sample volume. A riffle box (13 mm x 12 slots, Civilab,
202 Geelong, Australia) was used to split the fine fraction down to a well-mixed 40 g subsample for
203 chemical analysis. Each subsample was ground to a fine powder using a mill. Total C concentration
204 (%) was determined from 0.4 g subsamples using dry combustion (Trumac CNS Analyser, LECO,
205 Michigan, USA).

206 2.4. Variation in survey data

207 The survey data set included 120 samples of soil (total C concentration, total C content and bulk
208 density at 0-10 cm, 10-30 cm and 0-30 cm) from the plantings and 56 equivalent samples of soil from
209 the adjacent pasture. Total C concentrations were converted to contents (t ha⁻¹) based on the ground

210 area and volume sampled by the corer, and the bulk density of a soil core. Basic statistics (mean,
211 standard deviation and coefficient of variation) were calculated for all these variables to describe
212 differences in statistical variability.

213

214 2.5. Soil maps

215 The next step of the SIMAP method was to build maps of these soil variables from the survey data
216 to allow different sampling designs to be assessed (Figs. 12 & 23). Maps were produced for each soil
217 variable (bulk density, total C concentration and total C content) × farm × land-use × soil depth
218 combination. Initial krigged maps were created in ArcGIS 10 (ESRI, California, USA) for planting plots
219 from the 100 samples collected across the grid of 10 m × 10 m cells, and for pasture plots from the
220 56 samples collected randomly across the grid (Fig. 23a). Ordinary kriging was used with an
221 exponential semivariogram model, a search radius of 12 points and output resolution of 1 m. Kriging
222 maintained the form of the semivariogram of the survey data but substantially reduced the
223 magnitude of spatial variance (Fig. 23a). To create realistic variation among sampled points, the
224 following approach was used: a) the frequency distribution of the survey data was approximated by
225 fitting an appropriate probability distribution function, with a log-normal distribution used here, b)
226 the required number of 1 m × 1 m cell values was drawn at random from this distribution to provide
227 pseudo-observations at that scale, and c) pseudo-observations replaced the krigged values in rank
228 order to maintain the spatial autocorrelation of the krigged map.

229 Whilst this improved the representation of overall spatial variability and ensured the distribution
230 of values in pseudo-observed maps was similar to the surveyed data, it failed to adequately capture
231 variability at the finest-scales (lag < 10 m). This was rectified by spatially disrupting individual cells
232 within the krigged surface prior to the replacement of values. A normal deviate with a mean of zero
233 and a global, user-defined coefficient of variation (CV) was added independently to each cell within
234 the krigged map. Values of global CV were obtained by numerically minimising the sum-of-squares
235 between the semivariograms for the surveyed data and for the pseudo-observed map, and varied
236 among sites and soil variables (0.08 - 0.35). This local disruption based on a global CV yielded maps

237 that replicated the statistical (frequency distribution) and spatial (semivariogram) variation of the
238 survey data (Fig. 23).

239 The statistical and spatial fit of each map was assessed by comparing random samples from the
240 maps with the survey data. One hundred random samples of 100 samples from planting maps and
241 56 samples from pasture maps were taken using the *raster* package in R (R Development Core Team,
242 2010; Hijmans *et al.*, 2014). Frequency distributions of maps were assessed by comparing the
243 distribution of the surveyed data with each of the random samples using the Kolmogorov-Smirnov
244 test (*ks.test*) from the *stats* package in R. A mean probability of the frequency distribution of the
245 survey data and map being different was calculated from these 100 comparisons.

246 To assess spatial variation in a map, 100 bootstrapped samples (response, x and y coordinates)
247 were taken without replacement from the surveyed data and the 100 random samples from a map (a
248 total of 10,100 samples). The more powerful and precise bootstrapping approach was used over the
249 alternative method of jackknifing (i.e. leave out samples and recalculate, Wolter, 2007). Each
250 bootstrapped sample contained either 30 from 56 samples of pasture data or 50 from 100 samples
251 of planting data. Semivariograms were calculated for each of these bootstrapped samples using the
252 *geoR* package in R (Diggle and Ribeiro Jr, 2007). The sum-of-squares difference between the overall
253 semivariogram for a map and the semivariogram of each bootstrapped sample was calculated. From
254 these bootstrapped estimates, a mean sum-of-squares was calculated for the surveyed data and each
255 of the original 100 random samples. The probability of a map having less variation than the surveyed
256 data was determined from the rank-order of these mean values of sum-of-squares. An optimal map
257 would have a probability of 0.5 (i.e. half of the mapped samples have more variation than the
258 surveyed data and half have less variation) while an adequate map would have a probability of 0.25
259 $< Pr < 0.75$.

260 2.6. Accuracy simulations

261 The soil maps generated from the survey data facilitated accuracy simulations (Fig. 12) to explore:
262 a) sampling intensity required for an accurate estimate, and b) the most efficient sampling design.

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

271 For a given sampling experiment, sampling intensity was varied from 2 to 200 samples ha⁻¹, with
272 1000 replicate samplings per sampling intensity. Overall accuracy of sampling can be decomposed
273 into two components: bias and precision. Because random sampling was used for all experiments,
274 bias, measured as the difference in sample mean and the true underlying population mean, was zero
275 in all cases. Precision was quantified as the CV of replicate samplings at a given sampling intensity,
276 with the relationship between sample CV and sampling intensity following a power function
277 (Roxburgh *et al.*, 2015). We used the probable limit of error (PLE) as the index of precision instead
278 of CV, which are related as follows:

$$279 \text{ PLE} = (SD / \bar{x}) * t = CV * t$$

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

285 Precise measurement of bulk density in the field is time consuming due to the difficulty of
286 excavating bulk density rings at depth. ~~Many scientists take less bulk density samples than nutrient~~
287 ~~samples to reduce costs, with the belief that it is unnecessary.~~ We used the above simulations to
288 explore the trade-off between differing numbers of bulk density samples and C analysis samples on
289 the accuracy of estimates of total C content in 0-30 cm layer. The sampling intensity for bulk density

290 and C concentration were varied from 2 to 75 samples, with 5,000 replicate samplings for each
291 possible combination.

292 3. Results

293 3.1. Variation in survey data

294 The CV ranged from 0.11 to 0.46 for the soil properties (Table 2). For most land-use × site
295 combinations, bulk density was the least variable soil property and soil C concentration was the most
296 variable. Soil C content, which is calculated from soil C concentration and bulk density, was generally
297 equally or less variable to soil C concentration (Table 2). Generally, the variability of soil C
298 concentration and content was higher in the lower soil layer (10-30 cm) than in the upper layer (0-
299 10 cm). The variability of bulk density decreased with depth at the Glenrowan and Archies Creek
300 farms while it increased with depth at Minyip farm (Table 2). There was a trend for soil properties
301 (bulk density, C concentration and C content) to be more variable under the plantings than under the
302 adjacent pastures (Table 2) within a given soil layer on a farm. The Glenrowan farm had the most
303 variable soil C content while the Minyip and Archies Creek farms had similar variability. [Previous](#)
304 [work provides more a comprehensive analysis of site factors attributable to differences in soil C](#)
305 [content \(e.g. England *et al.*, 2016; Paul *et al.*, 2017\).](#)

306

307 3.2 Soil maps

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

313 3.3 Accuracy simulations

314 The sampling simulations for soil properties showed consistently that systematic sampling
315 required the fewest samples to achieve a target probable limit of error (PLE, Fig. 34). Restricted-
316 random sampling produced very similar results to that of systematic sampling, on average requiring
317 just one additional sample. Simple-random sampling required substantially more samples to the

318 other designs and produced similar results to the bootstrapped resampling of the surveyed data. For
319 soil C content in the 0-30 cm layer, more samples were required for a specific PLE at the Glenrowan
320 farm, regardless of the sampling design, than at the other farms, which required similar sample
321 numbers (Fig. 34). For a given PLE, estimating soil C content in the 0-30 cm layer required more
322 samples for plantings than the pastures at all farms. Similar trends among farms and between land-
323 use types were shown for bulk density, C concentration and C content in the 0-10 cm and 0-30 cm
324 soil layers.

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

337 The simulations based on the surveyed data showed that the target PLE for soil C content was
338 achieved most efficiently by taking equal numbers of bulk density and C concentration samples (Fig.
339 45). At a given sampling intensity for bulk density, there were minor increases in the accuracy of soil
340 C content estimates when a larger number of C concentration samples than bulk density samples
341 were used. Conversely, there were negligible increases in the accuracy of soil C content estimates
342 when a larger number of bulk density samples than C concentration samples were used. For example
343 at Minyip, soil C content can be estimated with the same precision (i.e. 95% probability of being
344 within 10% of the true mean) from measurements of (i) C concentration and bulk densities from the

345 same 10 cores, (ii) C concentration from 10 cores and bulk densities from up to 50 cores without any
346 improvement or (iii) C concentration from 20 cores and bulk densities from eight cores (Fig. 45a).
347 This trend was consistent among farms and between land-use types (Fig. 45).
348

349 4. Discussion

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

362 4.1. Surveying a representative range of variability

363 We choose tree plantings and pastures on farms with distinct environmental and structural
364 characteristics (Table 1), which provided contrasting examples of statistical and spatial variation in
365 soil properties (Table 2). As case studies, these farms provided valuable insight into the sampling
366 intensities and designs needed to accurately measure soil C following reforestation, but they should
367 not be considered representative of environmental plantings across temperate Australia. Soil C and
368 bulk density were highly variable (Tables 2), with more variability under the plantings than under
369 the adjacent pastures. This is consistent with previous surveys of reforestation (Conant *et al.*, 2003;
370 Cunningham *et al.*, 2012) and reflects the more heterogeneous distribution of plant biomass in

371 forests compared with agricultural fields. The higher complexity and consequently variability of
372 environmental variables in native ecosystems than production systems is a common finding (Vasseur
373 and McCann, 2007). Spatial variability in soil C differed more among farms than between land uses
374 on a farm (Fig. 34), which is consistent with our previous survey of environmental plantings
375 (Cunningham *et al.*, 2012). This suggests spatial variability in soil C under developing (approx. 15
376 year-old) tree plantings is dominated by the legacy of soil variability in the original pasture.

377 4.2. Producing an accurate map

378 Soil maps commonly are produced using krigging to predict soil property values between survey
379 points (e.g. Piccini *et al.*, 2014). As demonstrated here (Fig. 23), krigging interpolates between data
380 points producing a 'smoothed' data set that has substantially less spatial variation than the surveyed
381 data and would result in a substantial underestimation of the required sampling intensity. Instead,
382 using the frequency distribution of the surveyed data to replace the krigged values provided realistic
383 spatial and statistical variation in a map (Fig. 32). We used statistical tests of map accuracy based on
384 how well the map matched the surveyed data (Table 3). A more rigorous test would be to conduct a
385 future survey of a site, stratified by predicted values of soil properties, to see how well the map
386 predicted soil properties in the field.

387 4.3. Determining sampling intensity

388 Sampling intensity for environmental variables is generally based on previous studies. Soil C
389 stocks are often estimated from 10-20 samples per site (Smith, 2004). Our simulations suggest that
390 many surveys of soil C have been under-sampling and that a minimum of 30 samples per hectare are
391 required to accurately estimate soil C stocks in pastures and tree plantings (Table 4). Soil surveys of
392 a lower intensity are likely to produce highly uncertain estimates, and be unable to detect differences
393 between land uses and management treatments. Studies of reforestation have often taken less than
394 10 samples per site to estimate soil C stocks (e.g. Harper *et al.*, 2012; Cunningham *et al.*, 2015a), which
395 is likely to be substantial under-sampling. Previous surveys of forests and agricultural fields have
396 found the sample size to estimate soil C to our target probable limit of error (i.e. 95% probability of

397 being within 10% of the true mean) ranged from 6-42 cores (Garten and Ashwood, 2002; Allen *et al.*,
398 2010; Chaudhuri *et al.*, 2011; Cunningham *et al.*, 2012; Kristensen *et al.*, 2015).

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

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

415 ~~High sampling intensities may be achieved while also minimising resources required for soil~~
416 ~~preparation y is often reduced~~ by bulking a number of samples together ~~for subsequent analysis.~~
417 ~~Bulking soils, But even when soil samples are ther-~~ mixed thoroughly using a riffle box, ~~or roughly by~~
418 ~~hand, introduces errors are introduced~~ to the estimate of soil properties. ~~For example, t~~here is
419 evidence that the nutrient availability measured from a bulked sample is higher than the mean from
420 the individual samples due to destruction of aggregates during bulking (e.g. Giesler and Lundstrom,
421 1993). The effect of bulking on soil C estimates would need to be tested from field samples and not
422 using the SIMAP method, which assumes statistically perfect bulking.

423

424 4.4. Determining the sampling design

425 The simulations showed that systematic sampling was the most efficient design for sampling soil
426 properties (Table 4). Systematic designs are relatively easy to implement in the field, require little
427 prior knowledge and provide consistent coverage. Although systematic designs cannot provide a
428 valid estimate of error in the mean because of a lack of statistical independence among samples, the
429 conservative approach of assuming the samples were independent is usually applied. Furthermore,
430 the regular nature of the grid means properties may align with the grid (e.g. planting rows), leading
431 to a possible bias in the mean estimate. Random sampling has the advantages of removing selection
432 bias, easy implementation and statistical independence in analyses. As demonstrated here, sample
433 sizes for random designs often have to be large to ensure representativeness because by chance they
434 may not be dispersed evenly in space (Muller, 2001). There was little difference in efficiency between
435 restricted-random and systematic sampling, and with restricted-random sampling generally
436 requiring one extra sample to achieve the same level of precision (Table 4). Restricted-random
437 sampling provides a useful compromise between random and regular designs, which provides a good
438 coverage of independent samples. However, stratified-random sampling is often better when there
439 is prior knowledge of potential correlates of the target environmental variable (De Gruijter *et al.*,
440 2006).

441 An important strength of the SIMAP method is that the maps allow numerous sampling designs to
442 be tested beyond that used to collect the underlying data. Besides random and systematic sampling,
443 there are many variations of stratified sampling that ensure efficient and representative sampling of
444 environmental variables (De Gruijter *et al.*, 2006). With no prior knowledge of an area, there are
445 several methods to acquire a random but spatially-balanced design including restricted-random,
446 compact geographic stratification (Brus *et al.*, 1999) and general randomized tessellation stratified
447 sampling (Stevens and Olsen, 2004). The maps can be combined with other spatial data sets of
448 potential predictors such as topographic, vegetation and climate maps to allow equal or proportional
449 stratified sampling within these strata. Hierarchical random sampling generally is used to survey

450 large areas. For example, freshwater taxa may be sampled using the hierarchy of watersheds, reaches
451 and channels (Townsend *et al.*, 1997). In these designs, the SIMAP method could determine an
452 efficient sampling design of the tertiary unit within the secondary unit (e.g. channels within reaches).
453 The SIMAP method provides a tool to explore the utility of different sampling designs for various
454 environmental variables (e.g. taxa abundance and chemical concentrations).

455

456 4.5. Determining the sampling protocol

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

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

475 The protocol for estimating soil C stocks in environmental plantings could be refined with
476 intensive surveys at more farms, but we note that even low-intensity surveys of soil C rarely exceed

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477 10 sites (e.g. Hoogmoed *et al.*, 2012). With a targeted survey, it may be possible to relate the
478 variability in soil properties, and consequently sampling intensity, to easier-measured
479 environmental variables such as topography, soil type and planting structure. This would allow a
480 sampling protocol to be tailored to context-specific variability in soil properties. Such a rigorous
481 sampling protocol would increase the efficiency of sampling the numerous plantings required to
482 inform an accurate model for C sequestration in environmental plantings.

483 4.6. Versatile method

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

500

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509

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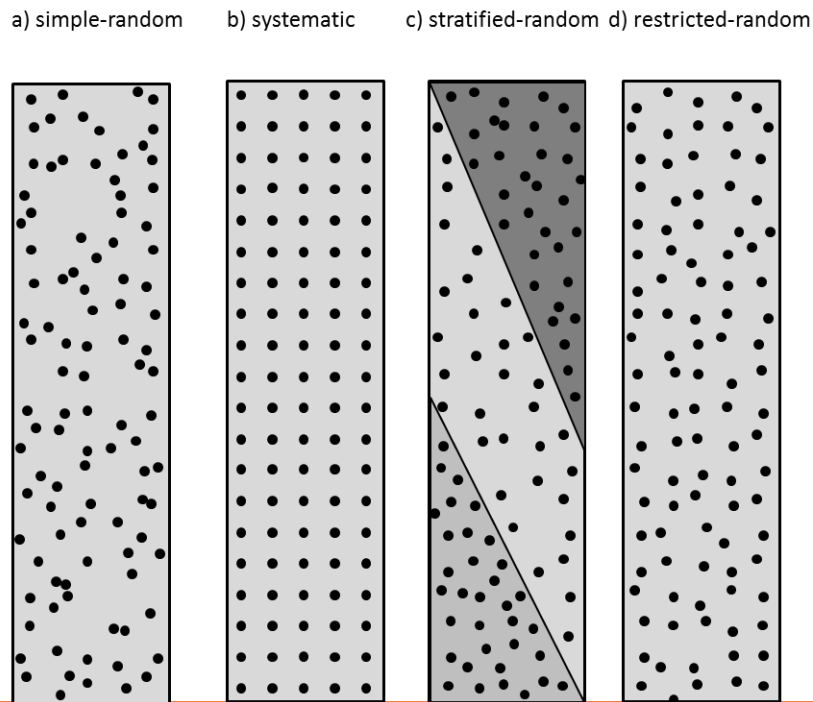
647 **Fig. 1.** Sampling designs commonly used to measure environmental variables, including those used
648 in the simulations to determine an efficient design for accurate estimation of soil properties (a, b, d).
649 Systematic sampling takes samples at a fixed interval (b), stratified random takes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

652 **Fig. 21.** Steps involved in the SIMAP method (Site selection, Intensive surveys, Map build, Accuracy
653 simulations and Protocol choice) for determining sampling protocols. Five areas are presented with
654 increasing spatial variability from left to right. PLE = probable limit of error.

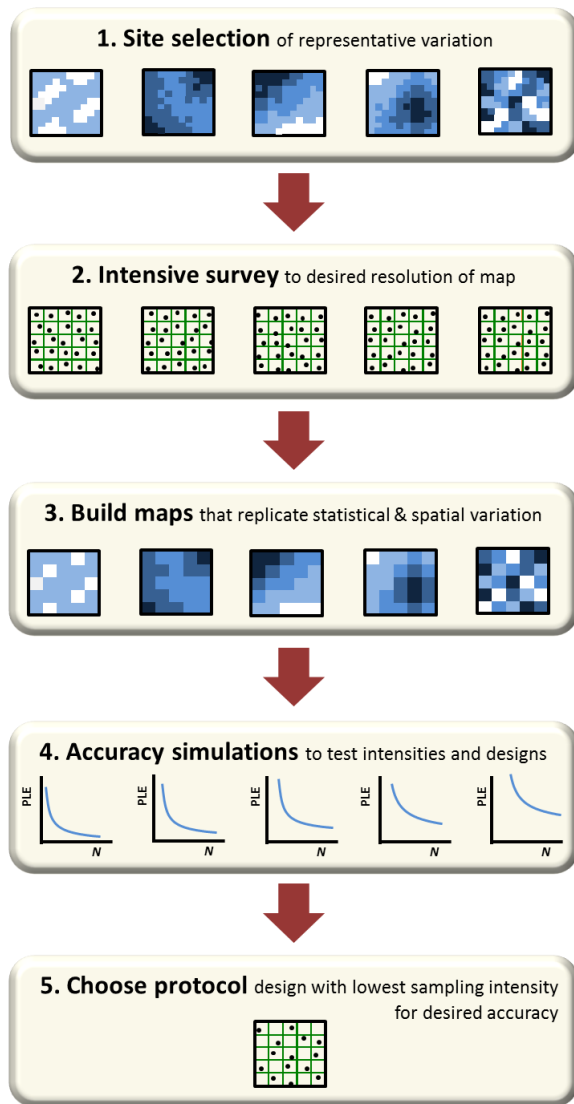
655 **Fig. 32.** Method used to generate maps of variables from the survey data to inform the accuracy
656 simulations. Sample points used to produce the map are shown ($N = 100$ cores), with their size
657 relative to the magnitude of the observed values. Darker areas of the krigged maps indicate areas of
658 higher total soil C content. ArcGIS was used to create krigged maps (a) and then realistic random
659 variation was introduced between samples by producing pseudo-observations that replicated the
660 semivariogram and frequency distribution of the surveyed data (b). Frequency distributions and
661 semivariograms of the surveyed data (red) and the map (blue) are shown. See Methods for more
662 detail.

663 **Fig. 43.** Relationships between the probable limit of error and sampling size for total soil C (0-30
664 cm) under plantings and pastures at the three farms. Sampling designs are indicated by different
665 lines: simple-random (orange), restricted-random (blue) and systematic (red). Bootstrapped
666 resampling of the survey data (black) is provided for comparison.

667 **Fig. 54.** Effect of the sampling intensity for bulk density and carbon concentration on the accuracy of
668 soil C content (0-30 cm) estimates under environmental plantings (a, c, e) and pastures (b, d, f) at the
669 three farms. Contours indicate a 95% probability of being within the stated percentage of the mean,
670 given the sampling intensity for bulk density and C concentration.

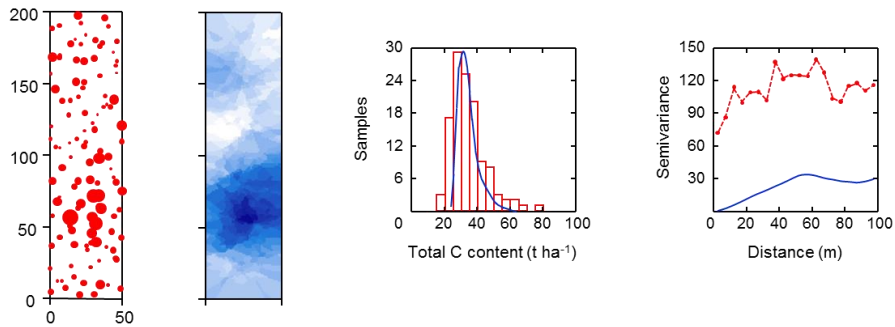


671 **Fig. 1.** Sampling designs commonly used to measure environmental variables, including those used
672 in the simulations to determine an efficient design for accurate estimation of soil properties (a, b, d).
673 Systematic sampling takes samples at a fixed interval (b), stratified random takes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

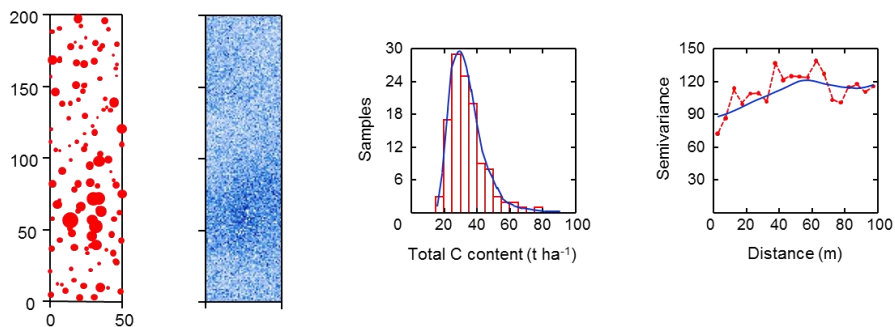


676 **Fig. 21.** Steps involved in the SIMAP method (Site selection, Intensive surveys, Map build, Accuracy
 677 simulations and Protocol choice) for determining sampling protocols. Five areas are presented with
 678 increasing spatial variability from left to right. PLE = probable limit of error.

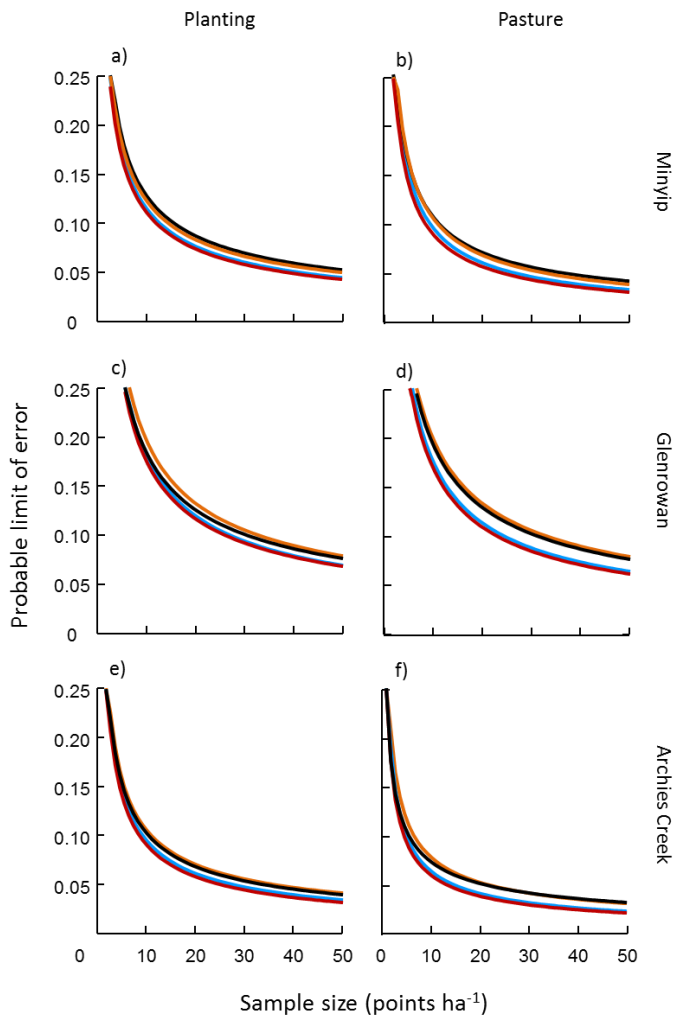
a) Krigged map of total soil C (0-10 cm) at Glenrowan planting



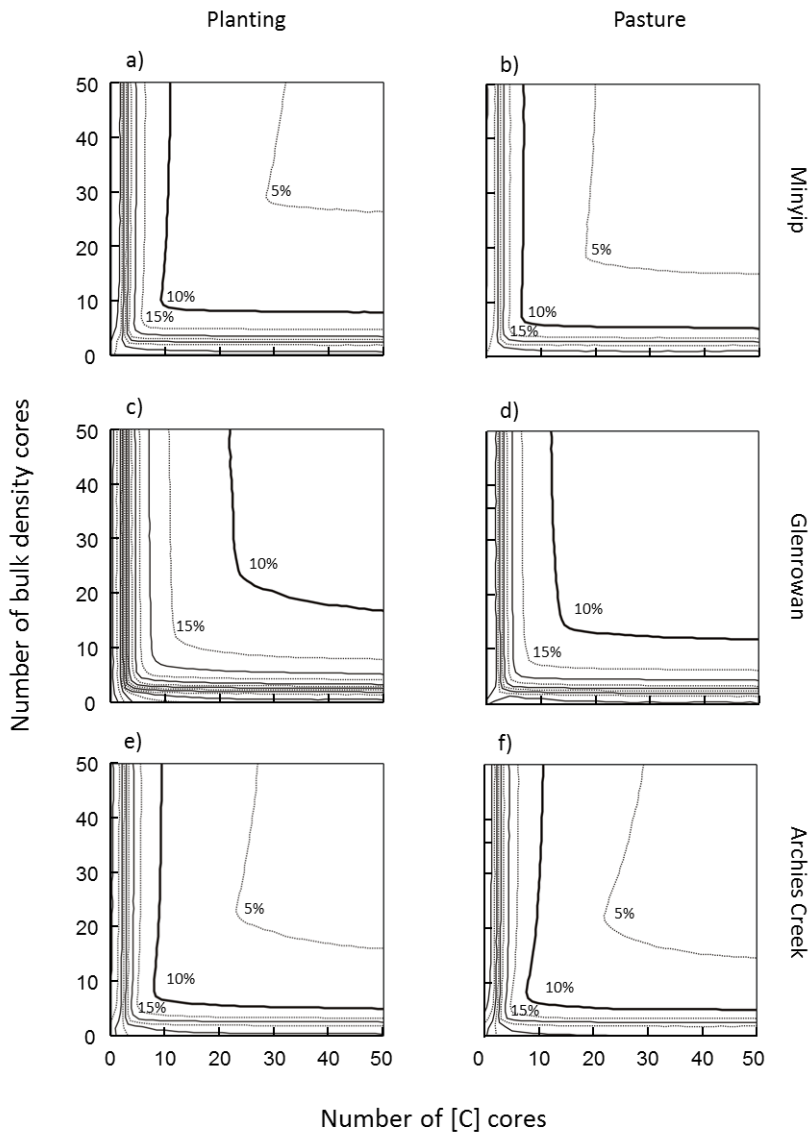
b) Map with realistic variation among sample points added



679 **Fig. 32.** An example (Glenrowan) of how Method used to generate maps were generated: (a) using
 680 ArcGIS and kriging, and (b) by introducing more realistic random variation by producing pseudo-
 681 observations that replicated the semivariogram and frequency distribution of the surveyed data
 682 generations of variables from the survey data to inform the accuracy simulations. Sample points used
 683 to produce the map are shown ($N = 100$ cores), with their size relative to the magnitude of the
 684 observed values. Darker areas of the krigged maps indicate areas of higher total soil C content. ArcGIS
 685 was used to create krigged maps (a) and then realistic random variation was introduced between
 686 samples by producing pseudo observations that replicated the semivariogram and frequency
 687 distribution of the surveyed data (b). Frequency distributions and semivariograms of the surveyed
 688 data (red) and the map (blue) are shown. See Methods for more detail.



689 **Fig. 43.** Relationships between the probable limit of error and sampling size for total soil C (0-30
 690 cm) under plantings and pastures at the three farms. Sampling designs are indicated by different
 691 lines: simple-random (orange), restricted-random (blue) and systematic (red). Bootstrapped
 692 resampling of the survey data (black) is provided for comparison.



693 **Fig. 54.** Effect of the sampling intensity for bulk density and carbon concentration on the accuracy of
 694 soil C content (0-30 cm) estimates under environmental plantings (a, c, e) and pastures (b, d, f) at the
 695 three farms. Contours indicate a 95% probability of being within the stated percentage of the mean,
 696 given the sampling intensity for bulk density and C concentration.

697 **Table 1** Location and environmental conditions of the three farms studied.

Variables	Farm		
	Minyip	Glenrowan	Archies Creek
Location	36.54 °S 142.62 °E	36.50 °S 146.14 °E	38.50 °S 145.57 °E
Mean rainfall (mm yr ⁻¹) †	392	663	1095
Max. temp hottest month (°C) †	30.9	31.5	23.4
Min. temp coldest month (°C) †	4.0	2.6	5.9
Planting age (yr)	14	16	15
Planting size (ha)	4.2	4.9	2.0
Soil type	Vertosol	Sodosol	Sodosol
Tree density (trees ha ⁻¹)	360	312	690
Basal area (m ² ha ⁻¹)	4.5	11.6	23.8
Dominant eucalypts	<i>E. largiflorens</i> <i>E. melliodora</i>	<i>E. sideroxylon</i> <i>E. bridgesiana</i> <i>E. polyanthemos</i>	<i>E. globulus</i> spp. <i>globulus</i> <i>E. obliqua</i>
Field	perennial grass pasture	perennial grass pasture	perennial grass pasture
Stock	sheep	cattle	cattle and sheep
Fertilizer addition	none	none	none

698 † Calculated over the life time of the planting

699 **Table 2** Summary statistics for soil properties under different land-uses at each farm. Values are
 700 means ($N = 120$ cores for plantings, $N = 56$ cores for pastures) followed by standard deviations and
 701 coefficients of variation in brackets.

Farm	Land-use	Soil depth (cm)	Bulk density (g cm ⁻³)	Total C conc (%)	Total C content (Mg ha ⁻¹)
Minyip	planting	0-10	1.06±0.17 (0.16)	2.08±0.50 (0.24)	21.6±4.4 (0.20)
		10-30	1.26±0.21 (0.17)	1.09±0.30 (0.28)	26.8±5.9 (0.22)
		0-30	1.19±0.18 (0.15)	1.42±0.32 (0.22)	48.5±8.1 (0.17)
	pasture	0-10	0.90±0.12 (0.14)	1.77±0.32 (0.18)	15.8±2.8 (0.18)
		10-30	1.02±0.15 (0.15)	1.06±0.19 (0.18)	21.3±3.8 (0.18)
		0-30	0.98±0.12 (0.12)	1.29±0.20 (0.15)	37.1±5.1 (0.14)
Glenrowan	planting	0-10	1.04±0.26 (0.25)	3.32±0.68 (0.20)	34.2±10.5 (0.31)
		10-30	1.18±0.22 (0.19)	1.01±0.46 (0.46)	23.1±9.3 (0.40)
		0-30	1.13±0.19 (0.16)	1.78±0.43 (0.24)	57.3±15.1 (0.26)
	pasture	0-10	0.97±0.26 (0.27)	3.07±0.65 (0.21)	29.5±8.6 (0.29)
		10-30	1.16±0.22 (0.19)	0.80±0.29 (0.36)	18.1±6.5 (0.36)
		0-30	1.09±0.19 (0.17)	1.56±0.36 (0.23)	47.6±12.1 (0.25)
Archies Creek	planting	0-10	0.75±0.12 (0.16)	4.75±1.07 (0.23)	34.9±5.9 (0.17)
		10-30	1.09±0.13 (0.11)	2.50±0.52 (0.21)	54.0±10.1 (0.19)
		0-30	0.98±0.11 (0.11)	3.25±0.64 (0.20)	88.9±14.3 (0.16)
	pasture	0-10	0.84±0.12 (0.14)	4.00±0.81 (0.20)	32.8±4.7 (0.14)
		10-30	1.05±0.13 (0.13)	2.18±0.53 (0.25)	45.0±8.9 (0.20)
		0-30	0.98±0.11 (0.11)	2.78±0.59 (0.21)	77.8±12.2 (0.16)

702 **Table 3** Fit of mapped values to the frequency distribution and semivariance of surveyed data for soil properties under different land-uses at each
 703 farm. Probabilities for the frequency distributions are the mean P values ($N = 100$) of Kolmogorov-Smirnov tests comparing the surveyed data with
 704 100 random samples from the map. Probabilities for semivariance were based on the sum-of-squares difference between the overall semivariogram
 705 for the map and random samples of the map ($N = 100$) and the surveyed data. See Methods for more detail.

Farm	Land-use	Soil depth (cm)	Frequency distribution (mean P)			Semivariance [Pr(mapped SS < surveyed SS)]		
			BD	[C]	Ccont	BD	[C]	Ccont
Minyip	planting	0-10	0.67	0.67	0.67	0.56	0.32	0.36
		0-30	0.57	0.64	0.70	0.29	0.70	0.71
	pasture	0-10	0.67	0.68	0.76	0.62	0.36	0.39
		0-30	0.63	0.62	0.61	0.61	0.38	0.66
Glenrowan	planting	0-10	0.32	0.63	0.62	0.63	0.32	0.39
		0-30	0.59	0.53	0.55	0.51	0.33	0.28
	pasture	0-10	0.68	0.75	0.72	0.55	0.28	0.28
		0-30	0.62	0.70	0.60	0.59	0.44	0.31
Archies Creek	planting	0-10	0.59	0.66	0.70	0.56	0.69	0.49
		0-30	0.69	0.58	0.59	0.69	0.68	0.34
	pasture	0-10	0.69	0.60	0.50	0.41	0.61	0.63
		0-30	0.71	0.68	0.58	0.42	0.61	0.71

706 **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)
 707 for soil properties. Results are given for bootstrapped resampling of the survey data (BS) and simple-random (SR), restricted-random (RR) and
 708 systematic (SM) sampling of the maps. The most efficient sampling design for each variable × land-use × farm combination is indicated in bold.

Farm	Land-use	Soil depth (cm)	Number of cores to attain PLE											
			Bulk density				Total C conc				Total C content			
			BS	SR	RR	SM	BS	SR	RR	SM	BS	SR	RR	SM
Minyip	planting	0-10	12	12	10	10	27	26	17	14	21	19	14	13
		0-30	11	11	10	9	24	20	16	15	15	14	12	12
	pasture	0-10	10	10	10	9	16	16	11	10	15	16	11	10
		0-30	8	8	7	6	11	12	11	11	10	10	9	8
Glenrowan	planting	0-10	25	27	26	25	19	20	16	15	43	41	31	30
		0-30	12	13	11	11	23	26	23	22	30	32	26	25
	pasture	0-10	31	31	30	29	22	25	16	14	40	44	29	28
		0-30	15	15	15	15	26	26	15	12	30	32	23	22
Archies Creek	planting	0-10	11	11	10	10	20	20	16	15	12	12	11	10
		0-30	7	6	6	6	15	15	13	12	11	11	9	9
	pasture	0-10	8	8	7	7	14	15	10	9	9	9	7	7
		0-30	6	6	6	5	16	16	11	10	7	7	6	6