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Review
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# A review of sampling designs for the measurement of soil organic carbon in Australian grazing lands

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**Abstract.** The accurate measurement of the soil organic carbon (SOC) stock in Australian grazing lands is important due to the major role that SOC plays in soil productivity and the potential influence of soil C cycling on Australia's greenhouse gas emissions. However, the current sampling methodologies for SOC stock are varied and potentially conflicting. It was the objective of this paper to review the nature of, and reasons for, SOC variability; the sampling methodologies commonly used; and to identify knowledge gaps for SOC measurement in grazing lands. Soil C consists of a range of biological materials, in various SOC pools such as dissolved organic C, micro- and meso-fauna (microbial biomass), fungal hyphae and fresh plant residues in or on the soil (particulate organic C, light-fraction C), the products of decomposition (humus, slow pool C) and complexed organic C, and char and phytoliths (inert, passive or resistant C); and soil inorganic C (carbonates and bicarbonates). Microbial biomass and particulate or light-fraction organic C are most sensitive to management or land-use change; resistant organic C and soil carbonates are least sensitive. The SOC present at any location is influenced by a series of complex interactions between plant growth, climate, soil type or parent material, topography and site management. Because of this, SOC stock and SOC pools are highly variable on both spatial and temporal scales. This creates a challenge for efficient sampling. Sampling methods are predominantly based on design-based (classical) statistical techniques, crucial to which is a randomised sampling pattern that negates bias. Alternatively a model-based (geostatistical) analysis can be used, which does not require randomisation. Each approach is equally valid to characterise SOC in the rangelands. However, given that SOC reporting in the rangelands will almost certainly rely on average values for some aggregated scale (such as a paddock or property), we contend that the design-based approach might be preferred. We also challenge soil surveyors and their sponsors to realise that: (i) paired sites are the most efficient way of detecting a temporal change in SOC stock, but destructive sampling and cumulative measurement errors decrease our ability to detect change; (ii) due to (i), an efficient sampling scheme to estimate baseline status is not likely to be an efficient sampling scheme to estimate temporal change; (iii) samples should be collected as widely as possible within the area of interest; (iv) replicate of laboratory analyses is a critical step in being able to characterise temporal change. Sampling requirements for SOC stock in Australian grazing lands are yet to be explicitly quantified and an examination of a range of these ecosystems is required in order to assess the sampling densities and techniques necessary to detect specified changes in SOC stock and SOC pools. An examination of techniques that can help reduce sampling requirements (such as measurement of the SOC fractions that are most sensitive to management changes and/or measurement at specific times of the year – preferably before rapid plant growth – to decrease temporal variability), and new technologies for *in situ* SOC measurement is also required.

## Introduction

For the purpose of this review, rangelands are defined as relatively undisturbed ecosystems containing savannas, woodlands, and shrublands, where rainfall is too low or unreliable and soils too poor to support regular cropping (Beeton *et al*. 2006; Bastin 2008). One of the major threats to the sustainability of Australian grazing lands, and particularly rangelands where inputs such as fertiliser are not economically feasible, is the depletion of soil organic carbon (SOC). Soil organic matter contains ~58% SOC and is made up of a range of biological materials, living organisms (micro- and meso-fauna), fresh plant residues in or on the soil, particulate organic matter, the products of decomposition (humus), and inert (humic and char) substances (Gregorich *et al*. 1994), and silica-occluded plant C or phytoliths (Parr and Sullivan 2005). It plays an important role in maintaining the sustainability of grazing lands due to the function it plays within the soil. For example, it provides a primary source of many plant nutrients, improves the water-holding capacity of the soil, is responsible for the formation of stable aggregates that protect the soil from erosion, and provides a habitat for soil microbial bioversity (Weil and Magdoff 2004).

In addition to its role in maintaining soil productivity, in recent years there has been a focus on the ability of SOC to act as a $CO_2$ 'sink', and thus assist in the reduction of atmospheric greenhouse gases (Follett 2001). Changes to grazing management practices that increase SOC storage may have the potential to


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reduce Australia's net greenhouse gas emissions, and thus contribute towards Australia achieving its greenhouse gas emissions targets.

Because of its important role, the effect of management practices on SOC stock (i.e. the product of SOC concentration and soil bulk density and sampling depth) in grazing lands has been studied extensively for many years. However, one issue that has continually been encountered by researchers is the high spatial and temporal variability of SOC stock and the difficulties that this creates when using statistical techniques to measure relatively small differences between land uses or management treatments. It is desirable to be able to detect relatively small changes in SOC stock, since across large areas of rangelands these can represent a substantial sequestration or loss of C. In addition, being able to detect small SOC changes enables the effect of management practices to be assessed within shorter timescales.

Numerous and varied approaches have been used when sampling for SOC stock, and we are faced with what can be a confusing array of choices when selecting sampling methodology. Thus, there is a need for greater understanding of the sampling methodologies available to estimate SOC stock in grazing lands. It is the objective of this review to: (i) examine the nature of, and reasons for, SOC variability; (ii) examine the strengths and weaknesses of the sampling methods commonly used for SOC stock; and, (iii) identify knowledge gaps and areas for future research for SOC measurement in grazing lands.

## Characteristics of soil C

Soil C consists of organic C as organic matter containing a range of organic materials and inorganic C as carbonates and bicarbonates. Organic C stocks are ~1500 Gt ($10^{15}$ g) C and inorganic C stocks are ~720 Gt C in the top 1 m of soil depth (Batjes 1996).

Soil organic C is heterogeneous in nature and consists of several SOC pools, which can be broadly grouped based on their turnover rates in soil. For example, Parton *et al*. (1987) postulated 3 SOC pools: (i) the active or labile C pool; (ii) the slow C pool; and, (iii) the resistant or passive pool. These have turnover periods of, respectively, <10 years, 10–200 years, and >100 years (Table 1).

The labile pool consists of soluble fresh plant residues including fine roots, living organisms (microbial biomass), particulate organic C and/or light fraction, in varying proportions of <5, <10 and up to 30–40% of SOC, respectively. These are measured as soluble and microbial products and root exudates, microbial biomass, and <53 µm organic C fraction (particulate organic C) and/or light fraction (<1.6–2 g/cm$^3$ density) of soil, respectively, (Parton *et al*. 1987; Cambardella and Elliott 1992; Baldock and Skjemstad 1999; Dalal and Chan 2001; Franzluebbers and Stuedemann 2003).

Dissolved organic C, usually <1% from root exudates, microbial products and plant materials forms the most labile fraction in soil. It is transported within the soil profile and in run-off waters to streams and eventually to oceans (Hopkinson and Vallino 2005). Within the soil profile, it is immediately available to soil microbes and is rapidly mineralised within hours to days; otherwise it enters into or forms soil microaggregates (Smucker *et al*. 2007).

Microbial biomass C comprises <5% of the SOC (Dalal 1998). It is considered as a sensitive indicator of SOC changes due to land-use change and/or management (Sparling 1992). In most soils, light-fraction C is essentially similar to particulate organic C, and rarely exceeds 30% of the total SOC, with turnover periods of <10 years in mesic subhumid and semi-arid environments (Dalal and Chan 2001).

Humus or clay-sorbed C forms the slow C pool, which varies from 30 to 60%, depending on soil type, clay content, clay mineralogy and iron and aluminium oxides, with turnover periods from 10 years to 200 years or more, depending on climatic conditions (Parton *et al*. 1987). Three possible mechanisms have been suggested for slow turnover rates of this C pool. These are: chemical nature of SOC, with increasing aromaticity; increasing spatial inaccessibility to microorganisms and extracellular enzymes due to microaggregation and physical separation; and sorption of C on mineral surfaces and/or interaction with mineral particles (Sollins *et al*. 1996; von Lutzow *et al*. 2007).

The resistant or passive SOC pool comprises primarily charcoal C, up to 30% (Skjemstad *et al*. 1999), with turnover periods generally >100 years, depending on the charcoal source and quality although charcoal C dynamics in soil is not known. A proportion of organo-mineral-metal complexed SOC also

**Table 1. Soil carbon (C) pools, soil C fractions, forms measured and sensitivity to management change**

| Soil C pool | Soil C fraction | Pool C/total C (%) | Form measured | Turnover period (year) | Sensitivity to management change |
|---|---|---|---|---|---|
| Labile (active) C[A] | Soluble fresh residues | 0.5–5 | Microbial and root exudates | <0.1 | Very rapid[A] |
| | Living micro- and meso-flora and fauna | 1–10 | Microbial biomass | <5 | Rapid[A,C] |
| | Particulate organic C | 1–40 | >53 µm | <10 | Rapid[B,D,E] |
| | Light fraction | 1–30 | <1.6–2 g/cm$^3$ | <10 | Rapid[C] |
| Slow C[A] | Humus | 30–50 | Total organic C – particulate organic C | 10–200 | Medium[B] |
| | Clay-complexed C | 30–60 | <2 µm | 10–100 | Medium[C] |
| Resistant (passive) C[A] | Charcoal C | 1–30 | Resistant to chemical oxidation | >100 | Slow[B] |
| | Phytoliths | 1–30 | Oxidised at ~1300°C | Millenia? | Very slow[F,G] |
| | Carbonates | 0–30 | Release of $CO_2$ by acid treatment | >1000 | Very slow[H,I,J] |

[A]Parton *et al*. (1987). [B]Baldock and Skjemstad (1999). [C]Dalal and Chan (2001). [D]Cambardella and Elliott (1992). [E]Franzluebbers and Stuedemann (2003). [F]Drees *et al*. (1989). [G]Parr and Sullivan (2005). [H]Cerling (1984). [I]Dalal and Mayer (1986*b*). [J]Knowles and Singh (2003).

forms the passive SOC pool (Kögel-Knabner *et al*. 2008). The phytolith C pool is generally small, up to 3% (Drees *et al*. 1989) although some reports suggest much higher amounts, up to 60% in the soil profile (Parr and Sullivan 2005). Since phytoliths are very stable in soil, these have been used to reconstruct the paleovegetation during the Holocene and/or Pleistocene periods (Piperno and Becker 1996). However, the role of phytoliths in SOC dynamics in response to land use and management change is little understood.

Soil carbonates and bicarbonates are the primary pools of soil inorganic C and comprise a substantial total C pool (>30%) in mesic, semi-arid and arid regions (Knowles and Singh 2003). These are derived both from parent material as well as formed *in situ* as pedogenic carbonates. Similar to phytolith C and charcoal C, pedogenic carbonate C is used to reconstruct region-wide paleoenvironments (Zhou and Chafetz 2010). Since pedogenic carbonates are relatively stable, with turnover periods >1000 years (Cerling 1984; Amundson *et al*. 1994; Zhou and Chafetz 2010), the carbonate stocks in soil are generally similar following land-use and management changes. For example, Dalal and Mayer (1986*a*) and Knowles and Singh (2003) found similar carbonate stocks in soil after 60 years of agriculture following conversion of land use from native vegetation even though initial SOC stocks declined by up to 60% during this period. Therefore, carbonate C is not considered in this review although further studies may elucidate the role of carbonate C in the rangeland soils, especially if the legume component of the vegetation increases, which may enhance the acidification of the soil profile and dissolution of carbonate C.

## Depth distribution of SOC

Spain *et al*. (1983) summarised SOC concentrations of several Australian soils. They noted that SOC stocks generally decreased exponentially with soil depth, but the magnitude of this change depends on soil type and vegetation, and generally follows root density distribution of the dominant vegetation. For example, in Fig. 1, SOC stocks under Queensland blue grass (*Dicanthium sericeum* L.) vegetation changed less with depth as compared with that under Brigalow (*Acacia harpophylla* L.) vegetation, where SOC stocks were higher in the top 0.3-m depths although total SOC stocks in the top 1.2-m depths were similar (104 t/ha) under both vegetations (Dalal and Carter 2000). Generally, IPCC (2006) recommends the sampling of the top 0.3-m depth of soil for SOC stock measurement or estimation since changes in SOC stock due to land-use change or management are primarily confined to the top 0.1- or 0.3-m depths in most soils (Dalal and Mayer 1986*b*; Knowles and Singh 2003; Dalal *et al*. 2005).

The depth distribution of SOC pools generally follow the total SOC trends except that in the top 0.1-m depth, labile organic C may be proportionally greater than the total SOC, mainly due to the mixing of litter with the soil in the top layer (Dalal *et al*. 2005).

## Soil bulk density

Soil bulk density, expressed as mass per unit volume of soil (units of g/cm$^3$ or t/m$^3$), is used to calculate soil C density or SOC stock for a given depth (kg/m$^2$ or t/ha) from SOC concentration (%), bulk density and soil depth. For measurement of soil bulk density in the field, including soils containing coarse fragments such as gravel, refer to McKenzie *et al*. (2000) and Cresswell and Hamilton (2002). Precise measurement of bulk density in the field is time consuming and expensive and remains a challenge, although recent developments in the *in situ* measurement of bulk density using gamma-ray attenuation, and electromagnetic induction appears to be promising across a range of landscapes (Tyler *et al*. 2001; Pires *et al*. 2009).

Soil bulk density is affected by farming systems such as cropping (through tillage, residue management, vehicular traffic) and grazing (through pasture type, grazing intensity, compaction,

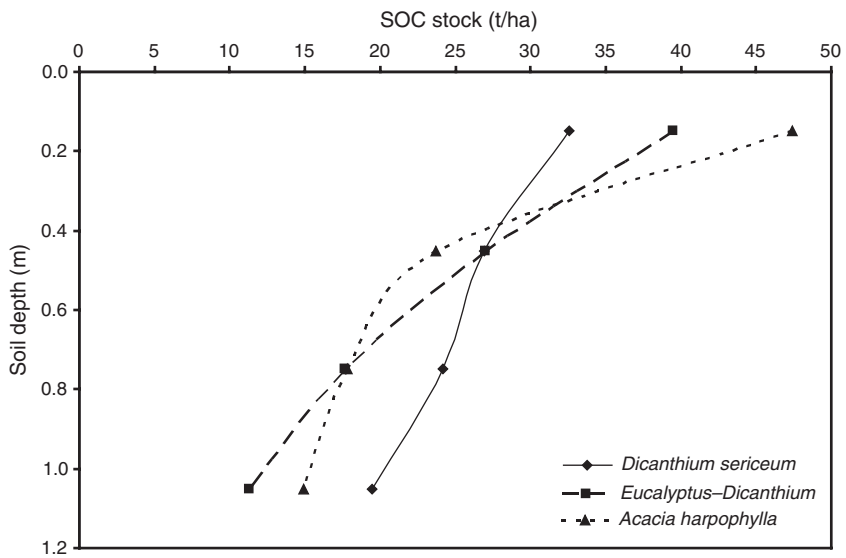


**Fig. 1.** Depth distribution of soil organic carbon under three native vegetations, *Dicanthium sericeum* (Queensland blue grass) on black Vertosol, *Eucalyptus populnea–Dicanthium sericeum* (Eucalypt woodland-savanna) on grey Vertosol, *Acacia harpophylla* (Brigalow) on grey Vertosol in southern Queensland, Australia (data from Dalal and Carter 2000).

fire, drought) and forestry systems (through land preparation for plantation and harvesting operations), besides the natural factors such as soil characteristics, climate and vegetation. In shrink-swell soils such as Vertosols, bulk density is also affected by soil water content. This means that, in these soils, the moisture content needs to be explicitly considered when attempting to compare SOC stocks of paired-sites, or for chronosequence-sampled sites or treatments with variable moisture content and bulk density on an equivalent soil mass basis to assess management effects on SOC stocks (Dalal and Mayer 1986*b*; Gifford and Roderick 2003; Wuest 2009), as shown in Fig. 2 (Dalal *et al*. 2005).

## Spatial and temporal variability of SOC and SOC pools in Australian grazing lands

The SOC that is present at any particular location is a result of the balance between (i) inputs of SOC from plant growth or material deposited during erosion, and (ii) losses of SOC due to soil respiration or export of SOC offsite due to erosion and leaching. This balance is affected by a series of complex interactions between plant growth, climate, soil type, topography and site management (Baldock and Skjemstad 1999). These processes affect SOC concentrations and stocks on a range of different temporal and spatial scales ranging from plant/pedon scales (mm–200 m), to community scales (20 m–km), and to landscape and regional scales (>km) (Bird *et al*. 2001) (Fig. 3).

### *Spatial variability*

#### *Plant/pedon scale (up to 200 m)*

At the plant/pedon scale, the main contributors to the spatial variability of SOC are vegetative patterns and plant community dynamics. Plant material provides the main source of SOC through litter drop, the production of root exudates, and root mortality (Bird *et al*. 2001). Consequently, the size, morphology (e.g. tree, shrub, grass) and spatial distribution of plants affects the areas where C is input into the soil (Jackson and Caldwell 1993; Hook and Burke 2000).

In grazing areas and particularly rangelands, strong heterogeneity at the plant/pedon scale is often a characteristic of sites with sparsely distributed plants. In these sites, C enrichment tends to occur in the area surrounding plants, with areas of lower C content in interplant areas (Hook and Burke 2000; Lechmere-Oertel *et al*. 2005). In semi-arid woodland areas, for example, the spatial distribution of resources may operate on several levels. At the largest scale (~100 $m^2$), distinct groves of trees separated by open intergroves may be responsible for a concentration of organic C. In addition, at a plant level, individual trees, shrubs and grasses create distinct areas where organic C will accumulate, interspersed with areas that are relatively nutrient-poor (Ludwig and Tongway 1995). This occurs not only due to the direct inputs of organic C from plants, but also due to the entrapment of organic material that is moved across the landscape by wind and water (Ludwig and Tongway 1995). Thus, Jackson and Caldwell (1993) found that SOC stocks varied by as much as 5-fold within a 120-$m^2$ area of sagebrush-steppe vegetation.

The pattern of plant growth also affects the location of other sources of SOC such as the soil microbial biomass and soil fauna. These components will tend to congregate around areas already high in organic C content, further contributing to SOC heterogeneity (Bird *et al*. 2001). Soil macro- and micro-fauna also contribute to SOC heterogeneity due to the soil mixing or bioturbation they cause (VandenBygaart 2006).

#### *Community scale (20 m–km)*

At the community scale, spatial variability is primarily affected by soil type, and site management. Soil type is influential due to the effect that soil nutrition can have on biomass production, with soil types higher in clay content generally able to provide more nutrients and higher moisture retention, and thus act as a better substrate for plant growth (Burke *et al*. 1995).

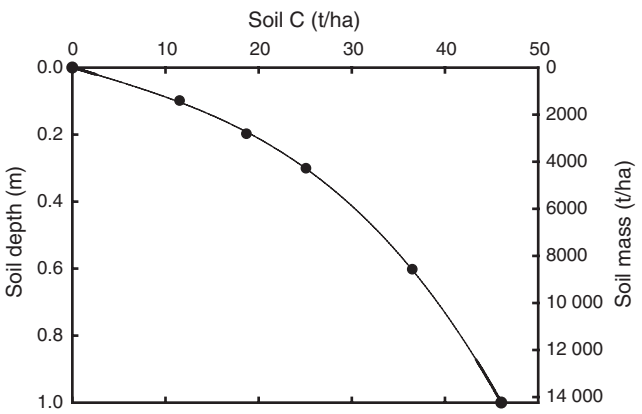


**Fig. 2.** Relationship between soil carbon and soil depth and soil mass (soil depth × bulk density) on Kandosol at a mulga (*Acacia aneura* L.) site for estimation of soil organic carbon stocks on equivalent soil mass, using polynomial equation (from Dalal *et al*. 2005).

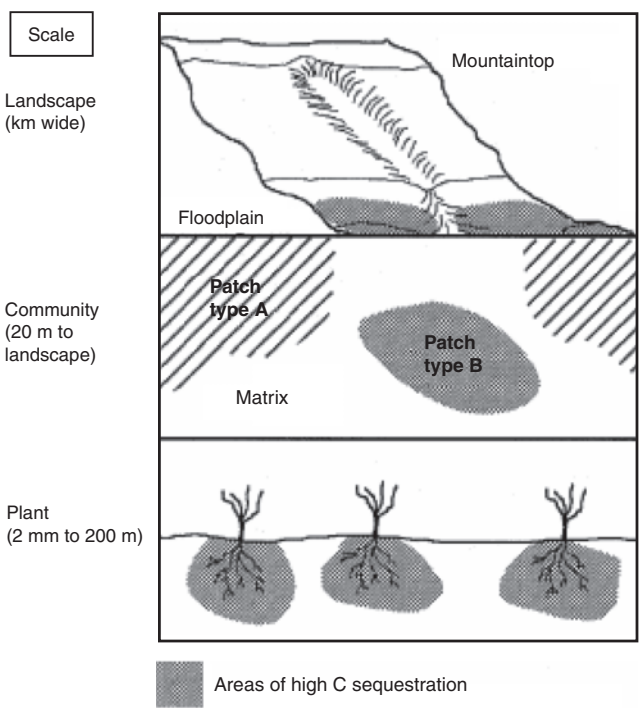


**Fig. 3.** Schematic representation of soil carbon features at the pedon/plant, community and landscape scale, as affected by vegetation, soil type, environment and management (adapted from Bird *et al*. 2001).

In addition, the sorption of SOC to clay, its isolation in micropores and its physical protection within stable macro- and microaggregates can reduce SOC availability and hence decomposition rates. Consequently, SOC is generally lower in coarse textured and poorly structured soils and numerous authors have observed positive correlations between SOC and clay content (Burke *et al*. 1989; Hook and Burke 2000; VandenBygaart and Kay 2004; Don *et al*. 2007). This trend has also been observed in Australian grazing environments. For example, in Queensland rangeland environments, sites with fine textured soils had significantly greater SOC stocks than coarse textured soils (Harms and Dalal 2003).

At the community scale, land management also starts to play a role in SOC variability. In grazing lands, management practices that increase yield such as fertiliser use, lime application or the use of more productive species, can increase SOC, particularly where soil has inherently low soil fertility (Chan 1997; Schnabel *et al*. 2001). However, such strategies can also decrease SOC in certain situations due to increases in the decomposability of the organic material, or decreases in root biomass sometimes associated with increases in fertility (Schnabel *et al*. 2001). The activities of grazing animals will also influence SOC variability. Hoof action creates surface disturbances that can both increase erosion (and thus decrease SOC) and help incorporate surface litter into the soil (potentially increasing SOC) (Dormaar *et al*. 1977; Schuman *et al*. 2009). Consequently, grazing intensity can alter SOC concentrations, with the direction of change a result of the balance between SOC loss due to overgrazing and the SOC gains due to incorporation of litter into the soil (Schnabel *et al*. 2001). The accumulation of SOC from animal excreta around animal camps or watering places also contributes substantially to SOC variability (Schnabel *et al*. 2001; Bisigato *et al*. 2008).

Although natural bushfires occur infrequently in semi-arid grazing lands, fire as a grazing management tool is used regularly in large areas of northern rangelands (Bradstock 2010). Up to 40% of tropical rangelands are burned every year (Bastin 2008). Fire frequency in semi-arid woodlands affects the woodland thickening (Bastin 2008) and vegetation communities and grass composition (Rossiter *et al*. 2003), and increases landscape spatial variability (Ludwig and Tongway 1995), and hence SOC variability. For example, Coetsee *et al*. (2010) found that frequent fires in savannas over a 50-year period changed the distribution of SOC (and N) under canopies and away from canopies but had no significant effect on total SOC stocks. However, Williams *et al*. (2004) surmised that frequent and extensive fires reduced the potential net ecosystem productivity by ~2 t C/ha by decreasing both SOC stocks and aboveground C stocks in mesic savanna in the Northern Territory, Australia. Actual data on SOC stocks and SOC pools (labile C, charcoal C) and transfer between them in this region are currently not available to verify their assessment.

### *Regional and landscape scales (>km)*

On a regional or landscape scale, topography and climate are the main factors responsible for SOC variability. Topography is particularly influential due to the effects that slope and aspect can have on soil moisture and depth, and hence biomass production and C input. Steeper slopes have been found to have lower SOC and down-slope positions higher SOC due to erosion (Burke *et al*. 1995; Hook and Burke 2000; Jia and Akiyama 2005; Liu *et al*. 2006). The higher moisture contents, and hence biomass production, in down-slope position also contribute to the higher SOC concentrations (VandenBygaart 2006) and stocks.

In addition to water erosion, wind erosion can also be responsible for moving soil and its associated organic C around in the landscape and thus contribute to SOC variability (Zuo *et al*. 2008). Grazing areas are particularly susceptible to wind erosion (Webb *et al*. 2009) during periods of low vegetation cover such as after fire, during drought or due to overgrazing (Bastin 2008; Zuo *et al*. 2008).

Climate also plays a large role in SOC variability, particularly on regional scales. Temperature and rainfall effects have a large influence on both plant biomass production and soil respiration, and generally SOC tends to be higher in cold, wet climates and lower in warm, dry ones (Amundson 2001). With increasing temperature, both plant biomass production and soil respiration rates tend to be higher. Adequate moisture will also increase biomass production and decomposition rates. However, excessively high moisture contents will lead to anaerobic conditions within the soil and a decrease in decomposition rates, thus increasing SOC storage (Amundson 2001).

### *Temporal variability*

Where distinct growing seasons exist due to the seasonality of temperature or rainfall, plant biomass production and the activity of the soil microbial biomass can vary throughout the year and potentially impact on SOC concentrations (Dormaar *et al*. 1977; Saggar and Hedley 2001; Jacobs *et al*. 2007) and stock. In areas where distinct warm and cold periods exist, pasture production and root growth is often observed to vary during the year, being highest in spring or summer and lowest in winter (Saggar and Hedley 2001; Jacobs *et al*. 2007). In addition, where plants have distinct growing periods, litter fall is often higher at certain times of the year (Wilson and Thompson 2005). Similarly, microbial growth and soil respiration also show distinct seasonality, being influenced by temperature, the availability of organic substrates (e.g. root exudates), and moisture availability (Kaiser *et al*. 1995; Corre *et al*. 2002). One feature of Australian grazing lands particularly relevant to variation on a temporal scale is the inherent climatic variability. Cycles of drought and rainfall are common, and contribute to periods of low followed by high organic matter input (Bastin 2008). Thus, rangelands are subject to SOC losses during drought and this should be considered when comparing long-term SOC stocks from different grazing managements (Schuman *et al*. 2009).

While the seasonality of C input into, and cycling through, soil systems is generally acknowledged, there are currently very few studies that have quantified the subsequent changes to SOC and SOC pools in Australian grazing systems and the effect that this could potentially have on the measurement of SOC. In overseas grazing systems, however, total SOC has been observed to increase by over 60% in the upper humus layer (Ah) between summer and winter sampling times (Dormaar *et al*. 1977), possibly due to increase mostly in particulate organic C or labile pool. A corollary to these studies from cropping soils in Australia shows that SOC stocks in the top 0.1-m depth decreased by 10% during the fallow period, primarily as a result of substantial decomposition of labile organic C and lack of fresh plant C input

(Wang *et al*. 2004). This emphasises the importance of sampling the soil for SOC stock at the same time each year (preferably before rapid plant growth) to minimise the temporal (seasonal) variation to discern the land-use and management effects on SOC stock.

### *Spatial variability of SOC in grazing lands compared with other land uses*

Grazing lands may encompass a wide range of different ecosystems, ranging from the intensively managed and high input dairy pastures in eastern Australia, through to the grazing of rangeland ecosystems in central and northern Australia. Varied grazing management was noted as a possible explanation for spatial variability in SOC found in Australia's uncleared landscapes in some of the National Carbon Accounting System studies (Griffin *et al*. 2003; Harms and Dalal 2003; Murphy *et al*. 2003). However, there is very little published information on the variability of SOC in Australian grazing lands compared with other land uses. In addition, from the international literature it is difficult to draw conclusions regarding the variability of SOC in one type of land use compared with another, as this often depends on the characteristics of the area in question. However, as a generalised statement, SOC in grazing areas is often found to be more heterogeneous than in cropped locations (Miao *et al*. 2000; Bird *et al*. 2001), particularly where mixing and homogenisation by cultivation occurs.

Comparison of grazing and forest lands suggests the degree of spatial variability in SOC tends to depend more on the type of forest and the characteristic of the grazing land. For example, some studies have observed greater spatial heterogeneity in ungrazed rangeland soils compared with protected forested areas (oak) (Nael *et al*. 2004). This observation was explained by the fact that the protected forests were relatively homogeneous over- and understorey, creating a more homogeneous input of organic C. The rangeland area, however, had a significant amount of shrub vegetation and also grass tussocks (Ludwig and Tongway 1995), resulting in the concentration of organic C under shrubs and tussocks and thus, a greater heterogeneity of SOC. Other studies of more typical grassland pastures, however, have been observed to have a lower degree of spatial heterogeneity under pasture compared to forested sites (Conant and Paustian 2002). Most likely, comparison of the extent of SOC spatial variability between different ecosystems is complicated by variation in soil type, landscape and topographic position, and vegetation type and distribution, among other factors such as seasonality, temperature and rainfall amount and distribution.

The degree of spatial variability of SOC observed in grazing lands may also depend on grazing management. Where stocking rates are too high and not sustainable, grazing can change the nature of the surface vegetation, for example, leading to an increase in the invasion of shrub species (Schlesinger *et al*. 1990), species composition (annual *v.* perennial grasses, slow-rooted *v.* deep-rooted vegetation) (Schuman *et al*. 2009) or decreasing plant cover so that plant growth becomes 'patchy' and is characterised by areas of greater fertility interspersed with bare, infertile soil (Schlesinger *et al*. 1990; Ludwig and Tongway 1995; Su *et al*. 2006). In such cases an increase in the spatial heterogeneity of SOC can be expected. In other instances grazing pressures may reduce vegetation cover to such an extent that SOC distribution starts to become more homogeneous due to the limited input of organic material and the compaction and homogenisation of soil due to hoof action (Nael *et al*. 2004; Zhao *et al*. 2007). In instances where ecosystems are naturally characterised by shrub vegetation and overgrazing leads to the replacement of shrubs by grassland, decreased spatial variability has also been observed (Lechmere-Oertel *et al*. 2005).

The above discussion indicates that the spatial variability of SOC in grazed areas, and particularly rangeland areas with shrub vegetation, is likely to be high in all but the most degraded areas. Consequently, sampling methodologies need to be designed in order to adequately characterise this variation, and must be capable of doing so at a variety of spatial scales. This is important for C accounting purposes, since SOC stock is usually expressed at larger spatial scales and estimated according to relative land-use area.

## Sampling designs to characterise SOC

Without an appropriate sampling design, the ability for inference about SOC is compromised. de Gruijter *et al*. (2006) note that there are three ways to choose where to sample: (i) choosing by convenience; (ii) choosing at random; or, (iii) choosing those locations thought to be the most informative (i.e. choosing purposively). The advantages of choosing locations by convenience are self-evident – soil sampling by the roadside is a typical example – but its statistical properties are questionable, and we will not deal with it further. Choosing locations at random or purposively give rise to, respectively, two contrasting philosophies of statistical investigation: the design-based approach, and the model-based approach. Papritz and Webster (1995*a*) summarised the essential difference between the two: '. . .the random character of an observation arises in the design-based approach from randomising the selection of the sampling positions. In model-based estimation, in contrast, each observed value *per se* is considered to be the outcome of a random variable postulated for a given position in space'. Neither approach is 'best' to characterise SOC, although, depending on the motivation for sampling, one is usually more appropriate than the other. de Gruijter *et al*. (2006) note that the suitability of the two approaches to a particular task changes with the spatial resolution of interest: for example, the design-based approach might be favoured to estimate the mean SOC stock for a paddock ('global estimation'), but the model-based approach might be favoured to map SOC stock within a paddock ('local estimation'). But it is misleading to classify their roles so crisply: the design-based approach can be used for local estimation, just as the model-based approach can be used for global estimation. A summary of the advantages and disadvantages of each approach are presented in Table 2.

### *Design-based approach*

The design-based approach evolved in the first half of the 20th Century, largely through the pioneering ideas of R. A. Fisher (1890–1962). For illustrative purposes, let us say that our variable of interest is SOC stock. In the simplest case of sampling for this variable, where all locations in an area of interest have equal probability of being chosen, the sample mean, sample variance,

**Table 2. A summary of the two sampling approaches to characterise soil organic carbon stock**

| Approach | Site selection | Advantages | Disadvantages | Ideal use[A] | Inference |
|---|---|---|---|---|---|
| Design-based | Random | Unbiased[A] | In its simplest form, provides poor spatial coverage[A] | Non-spatial summary of a study area | Analysis of variance[A] |
| Model-based | Purposive | Optimises the spatial coverage[A] | Not a safeguard against bias[A] | Mapping of a study area | Linear mixed model[A] |
| | | | Obtaining the 'model' can be difficult[A] | | |
| | | | Analysis is complex[A] | | |

[A]Ideal, though not exclusive.

and estimation variance of the sample mean [$\mu_s$, $\sigma_s^2$, and $\sigma_s^2(\mu_s)$, respectively], are computed, without bias, by (after de Gruijter *et al*. 2006):

$$\mu_s = \frac{1}{n}\sum_{i=1}^{n} y_i \quad (1)$$

$$\sigma_s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(y_i - \mu_s)^2 \quad (2)$$

$$\sigma_s^2(\mu_s) = \frac{\sigma_s^2}{n} \quad (3)$$

where $y_i$ is the $i$th of $n$ observations of SOC stock. More information on these quantities can be found in standard statistical texts, such as Snedecor and Cochran (1989), and Zar (1999). Through randomisation we ensure that the deviations about the mean (the errors) form an independent random variable (i.e. one sample has no relation to another), a necessary assumption for design-based inference. The most familiar application of the design-based approach is ANOVA (Snedecor and Cochran 1989), due to Fisher. In part, the design-based approach was an attempt to overcome historical constraints on the gathering and processing of information: contemporaries of Fisher needed a way to interpret and extrapolate results from what would now be considered relatively small sample sizes. Fisher's techniques were tremendously successful, and have since become convention. Besides unbiasedness, the advantage of the design-based approach is that, because it is convention, many of its accompanying statistical analyses have been packaged in commercial software as 'one-click' procedures. The disadvantage of simple random sampling is that it tends to cluster the samples, which can result in undesirably large parts of the study area remaining unsampled (Fig. 4*a*).

To circumvent the clustering effect of simple random sampling, the study area can be stratified, i.e. split into strata that are, ideally, as homogeneous as possible. Two samples (at least) are then selected, at random, from each stratum. An unbiased estimate of the sample mean of SOC stock through stratified random sampling, $\mu_{st}$, is computed by (after de Gruijter *et al*. 2006):

$$\mu_{st} = \sum_{h=1}^{H} a_h \mu_h \quad (4)$$

where $H$ is the number of strata, and $a_h$ and $\mu_h$ are, respectively, the proportion of the study area and the mean SOC stock associated with the $h$th stratum. Equation 1 is used to estimate $\mu_h$; the variance and estimation variance of the $h$th stratum, $\sigma_h^2$ and $\sigma_h^2(\mu_h)$, are estimated according to Eqns 2 and 3, respectively. The estimation variance of $\mu_{st}$ is computed, without bias, by:

$$\sigma_{st}^2(\mu_{st}) = \sum_{h=1}^{H} a_h^2 \sigma_h^2(\mu_h) \quad (5)$$

and an unbiased estimate of the sample variance, $\sigma_{st}^2$, is given by:

$$\sigma_{st}^2 = \left(\frac{1}{n}\sum_{i=1}^{n} {y_i}^2\right) - \mu_{st}^2 + \sigma_{st}^2(\mu_{st}) \quad (6)$$

There are two ways in which strata can be delineated (de Gruijter *et al*. 2006): (i) through geographic coordinates (Fig. 4*b*); or, (ii) through ancillary data (Fig. 4*c*). de Gruijter *et al*. (2006) recommend the use of a $k$-means classifier (e.g. Hartigan and Wong 1979) to derive the strata, which we have followed here. In Fig. 4*b*, we see that stratification by geographic coordinates has dispersed the sampling locations about the study area more than simple random sampling. When stratifying by an ancillary variable, the ancillary variable should have a plausible correlation with the target variable; we used here an estimate of the long-term mean vegetative cover of the ground surface (%) (Scarth *et al*. 2006), derived from 20 years of Landsat satellite imagery, under the hypothesis that SOC will increase proportionately. The resulting sample locations are not guaranteed to disperse spatially; however, they are dispersed over the range of variation of the ancillary variable.

A systematic grid can be used as part of a design-based sampling approach, but only on the condition that the initial location of the grid is chosen randomly (de Gruijter *et al*. 2006). It may be necessary to permute the initial location and the grid spacing many times before the desired number of samples fit in the area of interest. In the example in Fig. 4*d*, the samples are spread adequately through the study area with a spacing of 247 m, but the choice of initialising location has meant that one sample is very close to the field boundary. To move this sample away from the boundary would introduce bias. Under systematic random sampling, as this design is known, the user can estimate the sample mean without bias according to Eqn 1, but there is no unbiased estimate of sample variance. de Gruijter *et al*. (2006) note that Eqn 2 can be used but it will generally overestimate. However, if there is periodic variation in the study area that occurs at a wavelength coincident to the sampling interval (e.g. water drains, hedge rows) then Eqn 2 will severely underestimate. An alternative, less-biased approximation is the method of balanced differences (Yates 1981; Papritz and Webster 1995*a*). The

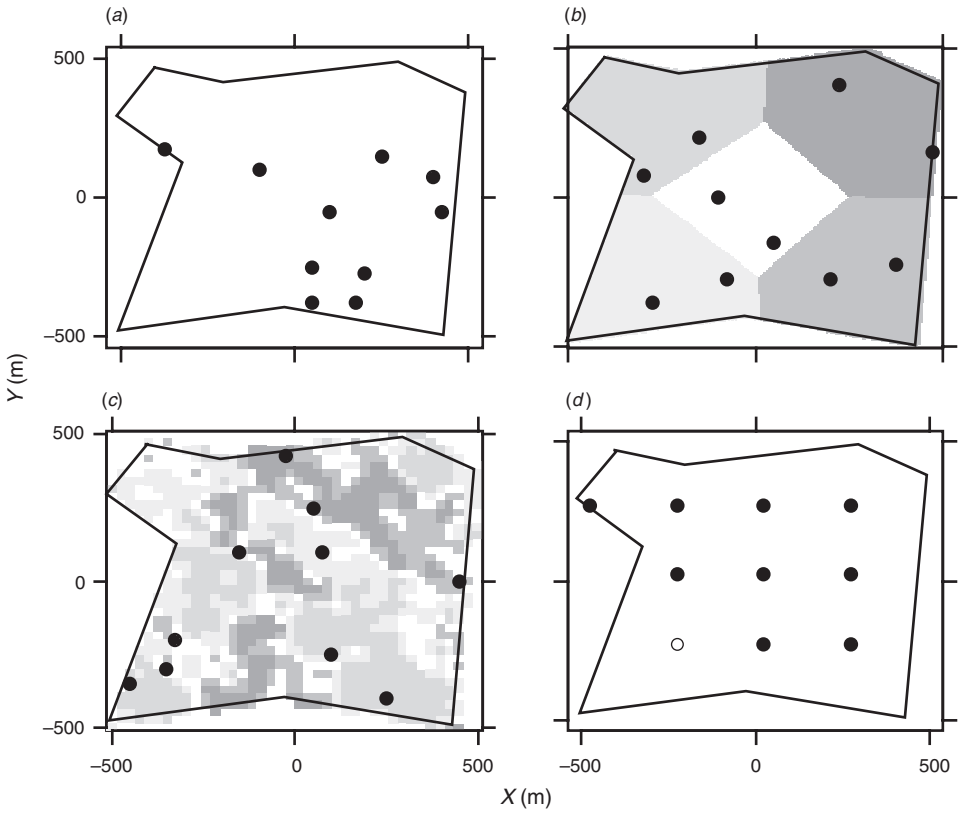


**Fig. 4.** Examples of four different kinds of design-based sampling (each with $n = 10$) for a hypothetical paddock: (*a*) simple random sampling; (*b*) random sampling stratified by classified geographical coordinates (shown in the background shading); (*c*) random sampling stratified by a classified ancillary variable (in this case, an estimate of long-term mean ground cover, shown in the background shading); (*d*) systematic random sampling (with the initial location shown as an open circle).

technique is related closely to signal processing, where engineers commonly filter the informative component of data ('signal') from background variation ('noise'). In the context of estimating the sample variance of a systematic design, it is the 'noise' that is relevant. Webster and Oliver (2001) describe a filter of the form:

$$\begin{array}{cccc} -0.25 & +0.50 & -0.50 & +0.25 \\ +0.50 & -1.00 & +1.00 & -0.50 \\ -0.50 & +1.00 & -1.00 & +0.50 \\ +0.25 & -0.50 & +0.50 & -0.25 \end{array}$$

Note that each row and column of the filter sum to zero. This filter moves over the systematic grid in $J$ steps (where $J < n$; there can be some overlap between the steps). At the $j$th location the values of the 16 nearest sample locations are convolved with the filter coefficients to yield a single value, $d_j$. The sample variance is then computed as:

$$\sigma^2_{sy} = \frac{1}{J \times 6.25} \sum_{j=1}^{J} d_j^2 \qquad (7)$$

where '6.25' is the sum of the squared coefficients in the filter given above. The method may be less biased than Eqn 2, but has its own problems, such as how to handle the data at the edge of the study area, and the arbitrary choice of the dimension of the filter.

Another commonly used design-based strategy is nested sampling. Webster and Oliver (2001) provide an overview of the technique. The simplest form of nested sampling involves selecting a set of $n_1$ locations separated by distance $d_1$. These are called 'first-stage' samples. At a distance $d_2$ (where $d_2 < d_1$) from each first-stage sample, with random orientation, one sample is taken to form the collection of $n_2$ samples. Then, at a distance $d_3$ (where $d_3 < d_2$) from each first- and second-stage sample, with random orientation, one sample is taken to form the collection of $n_3$ samples. The process is repeated for any number of stages, although the total sample number quickly becomes large. This basic scheme forms a 'balanced' hierarchy, which means that there is full replication at each stage. For three stages, labelled $a$, $b$, and $c$, respectively, the model of variation is:

$$z_{ijk} = \mu + a_i + b_{ij} + c_{ijk} \qquad (8)$$

where: $z_{ijk}$ is the value of the $k$th unit in the $c$th stage, in the $j$th unit of the $b$th stage, in the $i$th unit of the $a$th stage; $\mu$ is the overall mean; $a_i$ is the difference between $\mu$ and the mean of the $a$th stage; $b_{ij}$ is the difference between the mean of the first stage and the mean of the $j$th subclass in class $i$; and, $c_{ijk}$ is the difference between the observed value and its class mean at the third stage.

The quantities $a_i$, $b_{ij}$, and $c_{ijk}$ are independent random variables associated with the three stages. Each stage has zero mean and the respective variance components $\sigma_a^2, \sigma_b^2, \sigma_c^2$. The overall variance of $z$ is:

$$\sigma^2 = \sigma_a^2 + \sigma_b^2 + \sigma_c^2 \quad (9)$$

Analysis of a balanced hierarchy is relatively straightforward, through ANOVA. However, full replication is wasteful of resources, and a user might prefer to concentrate resources at particular stages. This creates an unbalanced hierarchy. Pettitt and McBratney (1993) proposed a form of unbalanced nested design for soil sampling, suited to situations where the variability of the target process is not known. To summarise their method, the study area is divided into strata, and within each stratum a randomly oriented transect is placed. Individual samples are then collected at exponential spacings along the transect. However, analysis of an unbalanced hierarchy is complex. Garrett and Goss (1980) provided a computer program to tackle the task. Unfortunately, the method suffers the possibility of returning negative estimates for some variance components. Spijker *et al*. (2005) circumvented the issue by substituting zeros for the negative estimates. A more elegant way to ensure valid estimates of the variance components is through residual maximum likelihood (e.g. Pettitt and McBratney 1993), although this technically makes the scheme a hybrid of the design-based and model-based sampling approaches.

There are other types of design-based sampling scheme besides those we have outlined above. We refer the reader to de Gruijter *et al*. (2006) for a comprehensive treatment.

### *Model-based approach*

The model-based approach evolved through advances in computing, and the ability to collect and process large amounts of information quickly. Choosing sampling locations purposively necessitates the existence of prior knowledge, in the form of a model. In its least tractable form this model might reside in the mind of expert. More commonly, we will derive the model through statistical procedures. For SOC stock arguably the most relevant model is born of geostatistical theory, which is discussed in detail below.

The advantage of the model-based approach is that samples can be spread optimally throughout the area of interest (although this does assume that the model is sensible and can be extrapolated). Compared with design-based sampling, the disadvantages of the model-based approach are: (i) the latter is not as secure a safeguard against bias; and, (ii) the statistical analyses that accompany the model-based approach are relatively complicated, and less prevalent in commercial software. de Gruijter *et al*. (2006) note that to gain advantage over the design-based approach, the model-based approach must satisfy three conditions: (i) there must be many samples; (ii) the target variable must display spatial autocorrelation; and, (iii) a large proportion of the samples must be taken at spatial intervals much smaller than the range of the variogram. The concepts of 'autocorrelation', 'variogram' and 'range' are introduced below.

The basic tenet of geostatistical theory is that if you observe SOC stock at location $\mathbf{x}$ in a paddock, then step $\mathbf{h} = 1$ m (in some arbitrary direction) and make another observation, the pair of recorded values will probably be quite similar. However, if you walk $\mathbf{h} = 100$ m from $\mathbf{x}$ and make an observation of SOC stock, you will probably find that the recorded value is quite dissimilar to the value at $\mathbf{x}$. This is the concept of autocorrelation. Over many pairs of observations we can compute the average dissimilarity between each pair (based on half their squared difference) as a function of $\mathbf{h}$, which is known as the experimental (semi)-variogram (Webster and Oliver 2001):

$$\bar{\gamma}(\mathbf{h}) = \frac{1}{2n(\mathbf{h})} \sum_{i=1}^{n(\mathbf{h})} \{z(\mathbf{x}_i) - z(\mathbf{x}_i + \mathbf{h})\}^2 \quad (10)$$

where $\bar{\gamma}(\mathbf{h})$ denotes the average semi-variance as a function of $\mathbf{h}$; $n(\mathbf{h})$ is the number of pairs as a function of $\mathbf{h}$; $z(\mathbf{x}_i)$ is the $i$th value of the observed variable; and, $z(\mathbf{x}_i + \mathbf{h})$ is another observation of $z$, located $\mathbf{h}$ units from $z(\mathbf{x}_i)$. The experimental variogram is usually quite noisy, and, to be useful, has to be idealised with what is known as an 'authorised function', to form the theoretical variogram. It is the theoretical variogram that lends itself so readily to the 'model' of model-based sampling, in that it summarises the available knowledge about the spatial variability of SOC stock in an area of interest. Webster and Oliver (2001) describe the various authorised functions, how to fit them to the experimental variogram, and then choose the best theoretical model. Figure 5 illustrates how the theoretical variogram summarises the spatial variability of observations under different amounts of autocorrelation. In each case the authorised function is a spherical model (Webster and Oliver 2001). When a process is autocorrelated strongly, as in the top row of Fig. 5, the observations show a distinct spatial pattern. The variogram of this process shows that the 'nugget' variance component – the $y$-intercept – is relatively small. Nugget variance describes uncorrelated variation, and is due to the combined effects of measurement error and fluctuations in the process that occur over intervals smaller than the minimum sampling distance. As the proportion of nugget variance increases, uncorrelated fluctuations supersede the autocorrelated fluctuations (the middle and bottom rows of Fig. 5). In each of the three cases, the variogram rises from the nugget variance to a maximum known as the 'sill' variance. If the sample mean and sample variance of the observations can be assumed constant within the area of interest, the sill variance theoretically equals the sample variance; when this is not the case, it indicates that the spatial variability of the process is complex, and the user might wish to consult a statistician for advice on how to proceed. The separation distance at which the sill is reached is called the 'range'. Samples separated by distances larger than the range can be considered independent under the model-based approach. For simplicity we have ensured that the range of the three variograms in Fig. 5 is constant at a 30-m distance.

The theoretical variogram relates to purposive sampling through the interpolation method known as kriging. Kriging is a type of moving average that interpolates estimates at unsampled locations, conditional on the values observed at sampled locations (Webster and Oliver 2001). The moving average is weighted inversely by the semi-variances between observed locations, which, as we have seen, are a function of $\mathbf{h}$ according to the model of spatial variation. The uncertainty associated with a kriging estimate – the kriging variance, analogous to the estimation variance of the sample mean in design-based

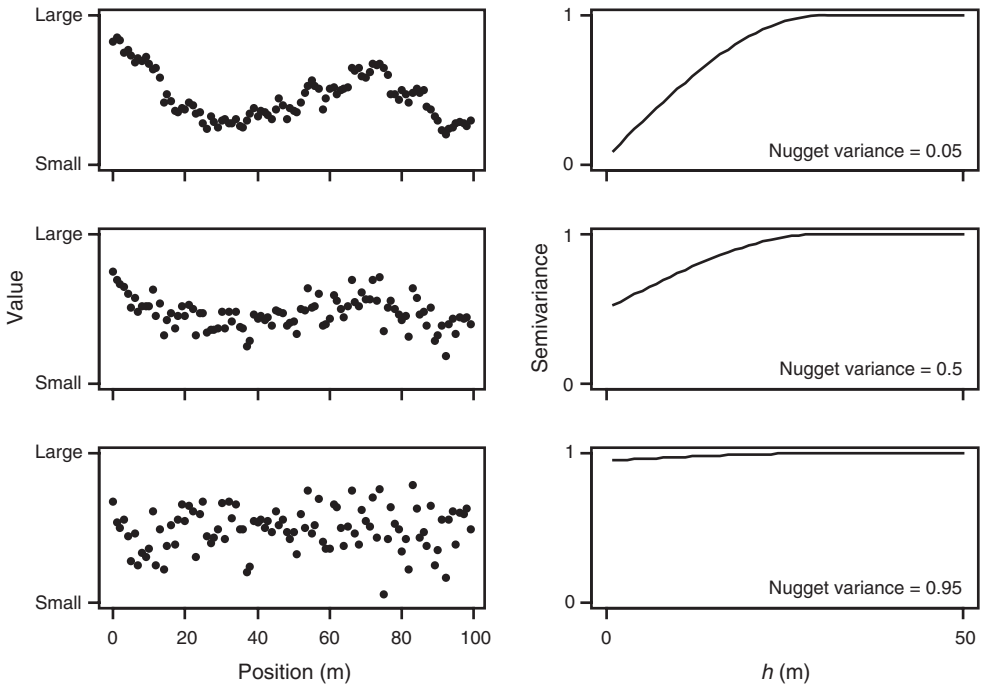


**Fig. 5.** Spatial variability described by the variogram. Panels on the left show hypothetical observations of a variable at 100 locations along a transect. Panels on the right show the associated theoretical (standardised) variogram. The process is autocorrelated strongly in the first row, moderately in the second row, and weakly in the third row. The range parameter of variogram is 30 m in each case.

statistics – depends, not on the values of the observations, but on the theoretical variogram and on the spatial arrangement of the sample locations. Therefore, if one is lucky enough to know the variogram in advance, a set of locations for purposive sampling can be proposed, and kriging used to ensure that the kriging variance for the entire area of interest is smaller than some nominated threshold (McBratney *et al*. 1981). Often the variogram is not known in advance of sampling, and must be gleaned from a meta-analysis, or estimated with a reconnaissance survey. McBratney and Pringle (1999) surveyed the published literature for variograms of topsoil attributes, and created averages that could serve as an initial guess about the spatial variability of an attribute, before sampling. Pringle and Lark (2008) updated the averages, and placed those for SOC concentration (%) and bulk density (g/cm$^3$)—as noted above, both variables are needed to estimate SOC stock on a mass-per-area basis—in the context of a 'linear model of coregionalisation' (LMCR) (Journel and Huijbregts 1978) (Table 3). A LMCR is a construct that describes how the theoretical variogram of one attribute relates to another, through their cross-variogram. Of the two variables, bulk density has the largest proportion of nugget variance to sill variance, at 0.25. This reflects the inherent randomness of bulk density at the scale of a soil core. The LMCR of SOC concentration and bulk density enables optimisation of a model-based sampling strategy for both variables simultaneously. McBratney and Webster (1983*a*) explored this idea in the context of the components of soil texture.

In regard to reconnaissance survey, Marchant and Lark (2006) developed an adaptive method. An initial theoretical variogram is computed from a bare minimum of samples in the first phase, and used to propose a set of optimum sample locations for the second phase. Following their collection, the second-phase samples are used to update the variogram, which then optimises the sample locations for the third phase, and so on. The method could be extended to cater for more than one variable, but cannot escape the fact that it is suited ideally to variables that can be measured *in situ*, or to variables not expected to vary substantially between one phase of sampling and the next. SOC concentration fails to meet both of these criteria, as the variable: (i) has to be estimated through laboratory analysis; and, (ii) has been shown to change seasonally (Leinweber *et al*. 1994; Saggar and Hedley 2001). In

**Table 3. Coregionalisation matrices of topsoil organic carbon concentration (SOC, units of %$^2$) and bulk density [BD, units of (g/cm$^3$)$^2$]**
The authorised function that links the three matrices is a double-spherical model (Webster and Oliver 2001)

| | | SOC | BD |
|---|---|---|---|
| | *(a) Nugget structure* | | |
| $d_0 = 0$ m | SOC | 0.009 | –0.005 |
| | BD | – | 0.010 |
| | *(b) 1st autocorrelated structure* | | |
| $d_1 = 30$ m | SOC | 0.009 | –0.005 |
| | BD | – | 0.010 |
| | *(c) 2nd autocorrelated structure* | | |
| $d_2 = 300$ m | SOC | 0.090 | –0.023 |
| | BD | – | 0.020 |

regard to the former, Gehl and Rice (2007) reviewed the ability of proximal sensing to measure SOC content *in situ*, and concluded that laboratory techniques will be needed for some time yet.

An alternative reconnaissance method for estimating the variogram is nested sampling, introduced above. The variance components of Eqn 9 can be plotted as a function of separation distance, thus approximating the variogram (Oliver and Webster 1987). Unbalanced schemes are generally favoured because they allow the concentration of sampling resources at the finest spatial scales, which are crucial for variogram estimation (de Gruijter *et al*. 2006). The studies of Schöning *et al*. (2006) and Rossi *et al*. (2009) both used unbalanced nested schemes to create variograms of SOC stock, which were then used to propose optimum model-based sampling schemes. Corstanje *et al*. (2007) used the variograms obtained from an unbalanced nested ANOVA to examine how SOC concentration correlated with the activity of the urease enzyme, an important component of N-cycling. They found that, for a pasture site, the variables were correlated only weakly, and that the correlation did not change significantly with spatial scale.

We mentioned above how the LMCR of SOC concentration and bulk density (Table 3) enables optimisation of a model-based strategy for simultaneous sampling of both variables. Figure 6 presents an example. Because there are two variables, we are dealing with cokriging variance rather than kriging variance (Webster and Oliver 2001). Central to optimisation is the definition of an objective function, i.e. a quantity that we want to minimise (or perhaps maximise) through the action of sampling. In a univariate context, van Groenigen *et al*. (1999) used the mean kriging variance across the study site as an objective function, on the basis that kriging variance represents uncertainty, which, obviously, we want to minimise. A more rigorous objective function might be conceived (e.g. Lark 2002), but we have chosen here to follow the idea of van Groenigen *et al*. (1999). At any one location in the study area there will be two values of cokriging variance, one for each variable. The objective function must be a single value. To integrate the two variables we computed the proportion of cokriging variance to sill variance, averaged over both variables, over the study site. The optimisation procedure involved: (i) proposing a set of $n = 20$ initial sampling locations for both variables, based on stratified random sampling by geographic coordinates (Fig. 4*b*) (n.b. it is not necessary to specify the same number of samples for both variables, but we have done so here for illustrative purposes); (ii) calculating the objective function based on the LMCR and the proposed sampling locations; and (iii) using spatial simulated annealing (van Groenigen *et al*. 1999) to minimise the objective function. The initial proposed locations returned mean cokriging variances of $0.080\%^2$ and $0.037\ (g/cm^3)^2$ for SOC concentration and bulk density, respectively. The initial value of the objective function was 0.828. Following optimisation the samples are spread evenly throughout the study site (Fig. 6), on a roughly triangular grid. van Groenigen *et al*. (1999) demonstrated the same effect, which reflects the fact that a triangular grid is the most efficient way to implement systematic sampling (McBratney *et al*. 1981). The mean cokriging variances reduced to $0.068\%^2$ and $0.034\ (g/cm^3)^2$, for SOC concentration and bulk density, respectively, while the final value of the objective function was 0.745.

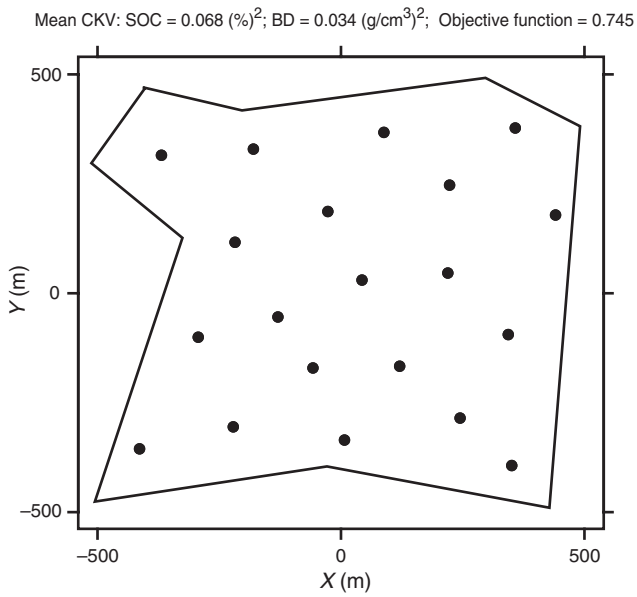


**Fig. 6.** Model-based sampling scheme that minimises the mean cokriging variance for soil organic carbon concentration and bulk density. We have assumed that both variables will be collected at the same locations, but this is not essential.

## Number of samples needed to estimate SOC

We have seen above the basic sampling arrangements that might be used by the design-based approach. We have also seen how the model-based approach might be used to optimise a sampling arrangement for a study area. But how many samples do we need to take? We must ensure we have adequate samples for inference, but we do not want to be wasteful of resources. Both sampling approaches provide answers to this question.

### *Design-based approach*

Let us say that we need to estimate the mean SOC stock for an area of interest. The optimum number of samples to collect will depend on the sample variance of SOC stock, and on what sort of uncertainty we can tolerate. Usually we know in advance, at least roughly, the tolerable uncertainty. Against the uncertain background we need to sample enough locations to ensure that we can detect a case of SOC stock departing from the mean. The probability of correct detection is known as 'statistical power' (Snedecor and Cochran 1989). Statistical power, in this context, is a function of four quantities: (i) the number of samples; (ii) the tolerable uncertainty, scaled by the square-root of the sample variance; (iii) the significance level ($\alpha$); and (iv) whether the test is one-tailed or two-tailed. For a given dataset, the only variable will be the number of samples; the rest are constant. The choice of (iii) and (iv) are somewhat arbitrary, although two tailed-tests are generally used at $\alpha = 0.05$.

Consider the simple random sampling scheme in Fig. 4*a*. In implementing this strategy we would observe SOC concentration and bulk density ($g/cm^3$) at all 10 locations, then make the necessary conversion to SOC stock. In our experience this latter variable has a log-Normal distribution, but statistical power

requires a Normal distribution. Therefore, we transform the data to SOC [log(Mg/ha)]. We used the LMCR in Table 3, in conjunction with the method of Lark (2002), to generate a realisation of SOC [log(Mg/ha)] for the paddock in Fig. 2. The mean and variance of the realisation were $\mu = 3.762$ log(Mg/ha), and $\sigma^2 = 0.141$ [log(Mg/ha)]$^2$, respectively, although we could never know this in reality. We then sampled the area according to the scheme in Fig. 4*a*, where we found that the sample mean was $\mu_s = 3.840$ log(Mg/ha), and the sample variance was $\sigma_s^2 = 0.099$ [log(Mg/ha)]$^2$. Following Larsen *et al.* (2001), we used tolerable uncertainty of 10% above and below the sample mean. Snedecor and Cochran (1989) present the computational steps for statistical power. For a particular number of samples we start by finding the values of $\mu_s$ that constitute a significant deviation: at $\alpha = 0.05$, a significant deviation lies outside the interval $\mu_s \pm 1.96\sqrt{\sigma_s^2(\mu_s)}$, which corresponds to $\mu_s < 3.645$ and $\mu_s > 4.035$ in our example. We then calculate the probability of $\mu_s$ being outside this interval when the mean is at the limit of tolerance, $\mu_s + 0.1\mu_s = 4.224$. For $\mu_s = 3.645$ the normal deviate is $z_l = (3.645 - 4.224)/\sqrt{\sigma_s^2(\mu_s)} = -5.807$. For $\mu_s = 4.035$ the normal deviate is $z_u = (4.035 - 4.224)/\sqrt{\sigma_s^2(\mu_s)} = -1.896$. The probabilities associated with these deviates are $P(z_l) < z_l \approx 0$ and $P(z_u) > z_u = 0.971$, respectively. These quantities are summed to yield the statistical power to detect a deviation greater than the tolerable uncertainty with 95% confidence (two-tailed test). We repeated this process for various sample sizes, and plotted the results (Fig. 7). Also shown in Fig. 7 are the statistical power functions for the stratified and systematic sampling schemes shown in Fig. 4*b* and *d*, respectively, where we observed $\mu_{st} = 3.683$ log(Mg/ha), $\sigma_{st}^2 = 0.087$ [log(Mg/ha)]$^2$, $\mu_{sy} = 3.748$ log(Mg/ha), and $\sigma_{sy}^2 = 0.156$ [log(Mg/ha)]$^2$ (where, for simplicity, the latter was calculated according to Eqn 2). A statistical power of 0.8 is used conventionally as a benchmark for minimum sampling effort (Lenth 2001). Simple random sampling and stratified random sampling both require 5 samples to estimate mean SOC stock with a statistical power of 0.8, while systematic random sampling requires 8 samples. These numbers serve only as an illustrative example, and should not be construed as a recommendation.

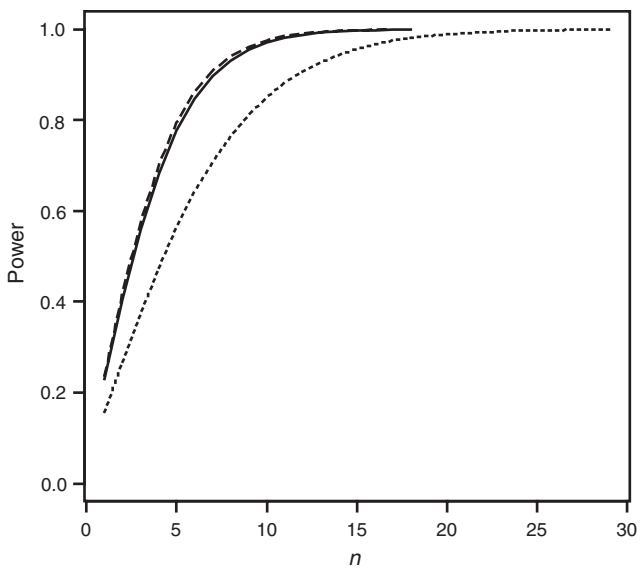


**Fig. 7.** Statistical power to detect a deviation within 10% of the sample mean, with 95% confidence (two-tailed test): solid line = simple random sampling (Fig. 4*a*); dashed line = random sampling stratified by geographical coordinates (Fig. 4*b*); dotted line = systematic random sampling (Fig. 4*d*).

An alternative expression of statistical power is the minimum detectable difference (MDD), which, to paraphrase Garten and Wullschleger (1999), is defined as the smallest difference that can be detected between means with a certain amount of confidence. The conventional formula for determining the optimum sample size to estimate a mean is (Cline 1944; Snedecor and Cochran 1989; Zar 1999):

$$n = \frac{\sigma^2 t^2_{\alpha/2,(n-1)}}{d^2} \tag{11}$$

where $\sigma^2$ is the sample variance; $t^2_{\alpha/2,(n-1)}$ is Student's *t*-value at a confidence level of $\alpha$ (two-sided), with $n-1$ degrees of freedom; and, $d$ is half the width of the desired confidence interval. The value of $n$ has to be determined iteratively because it appears on both sides of the equation (Zar 1999). Equation 11 can be reformulated in terms of statistical power (Zar 1999):

$$n = \frac{\sigma^2}{d^2}\left(t_{\alpha/2,(n-1)} + t_{(1-\beta),(n-1)}\right)^2 \tag{12}$$

where $1-\beta$ is the desired statistical power (say, 0.8). The MDD is obtained by rearranging Eqn 12 (Zar 1999):

$$\text{MDD} = \sqrt{\frac{\sigma^2}{n}}\left(t_{\alpha/2,(n-1)} + t_{(1-\beta),(n-1)}\right)^2 \tag{13}$$

The equation needs to be modified if computing the MDD for multiple treatments; see Zar (1999) for details. The study of Garten and Wullschleger (1999), who examined SOC stocks under different types of plant cover, is often cited for its use of MDD. They showed how the MDD decreases asymptotically with increasing sample size.

Finally, it is possible to derive a model-based solution for optimal design-based sampling, provided that a variogram of the target variable can be used as prior knowledge (de Gruijter *et al.* 2006). The first step is to compute the mean semi-variance for the (two-dimensional) area of interest, $B$ (Webster and Oliver 2001):

$$\bar{\gamma}(B,B) = \frac{1}{|B^2|}\int_B\int_B \gamma(\mathbf{x}-\mathbf{x}')\mathrm{d}\mathbf{x}\mathrm{d}\mathbf{x}' \tag{14}$$

where $\gamma(\mathbf{x}-\mathbf{x}')$ represents the theoretical semi-variance at the separation distance between a pair of locations $\mathbf{x}$ and $\mathbf{x}'$. The quantity $\bar{\gamma}(B, B)$ is known as the 'dispersion variance' in geostatistical terminology. The integration is usually done numerically over a large number of pairs. If simple random sampling is the desired arrangement, the estimation variance for the observations will be (after de Gruijter *et al.* 2006):

$$\sigma_s^2(\mu_s) = \frac{\bar{\gamma}(B,B)}{n} \tag{15}$$

If stratified random sampling is desired, the estimation variance will be:

$$\sigma_{st}^2(\mu_{st}) = \sum_{h=1}^{H} a_h^2\,\bar{\gamma}(b,b) \tag{16}$$

where $\bar{\gamma}(b, b)$ denotes the dispersion variance for the $h$th stratum. Under systematic random sampling the estimation variance will be:

$$\sigma^2_{sy}(\mu_{sy}) = \bar{\gamma}(B, B) - \bar{\gamma}(b, b) \quad (17)$$

where $\bar{\gamma}(b, b)$ denotes here the dispersion variance of the proposed locations in the grid. (Actually, multiplying $\sigma^2_{sy}(\mu_{sy})$ by $n$ yields another way of approximating the sample variance of a systematic random design, besides Eqn 2 and the method of balanced differences.)

### *Model-based approach*

The method of McBratney *et al*. (1981) for optimising a model-based sampling scheme uses a pre-specified maximum kriging variance to propose $n$, arranged in a grid pattern. Rossi *et al*. (2009) recently used this method to derive optimum sampling schemes for SOC stocks in Tanzanian forests. But because many soil surveyors know in advance roughly how many samples they can afford to collect, it is arguably better to reverse the method of McBratney *et al*. (1981). This was done by McBratney and Webster (1983*b*), who showed how, under the model-based approach, a geostatistical analogue of the classical estimator in Eqn 11 returned relatively efficient estimates of the optimum $n$ for a study area. The approach was adopted recently by Worsham *et al*. (2010) to describe SOC stocks associated with different types of land cover in a forest in Georgia, USA. Mooney *et al*. (2007) proposed a method to optimise sampling for SOC stock by reducing the estimate of variance, while circumventing the need for a formal geostatistical analysis. They argued that this was necessary on the (rather flimsy) basis that geostatistics requires specialised software. To reduce variance they first had to infer a value for the range of spatial correlation. In our opinion the work-around needed to avoid computing a variogram is not worth the trouble.

Power analysis can also be used with the model-based approach. Schöning *et al*. (2006) examined variograms of SOC stock, then substituted estimates of semi-variance at particular lag distances to compute the MDD. Stroup (2002) detailed a method for power analysis under a model-based approach, based on the output of a linear mixed model (Lark and Cullis 2004). It is not our intention to describe the details of a linear mixed model, other than to say that it is a regression model that accounts explicitly for spatial variability by simultaneously estimating regression parameters and the parameters of the theoretical variogram. The key advantage of a linear mixed model is that, as under the design-based approach, one can test hypotheses about the regression coefficients. The disadvantages of the linear mixed model are that the method is relatively complex, computationally intense, and relies on iterative fitting that, if done without care, might return a suboptimal solution. Kravchenko *et al*. (2006) followed the method of Stroup (2002) to find the optimum sample size to describe total soil C content. They found that, for a particular sample size, MDD decreased as the range of the variogram increased.

We have seen in Fig. 6 how a theoretical variogram can be used in conjunction with simulated annealing to optimise a model-based sampling scheme. In that example, we assumed from simplicity that $n = 20$ for both variables but noted that this did not have to be the case. To find the optimum number of samples for each variable, the method we outlined above could be run for different combinations of $n$ for both SOC concentration and bulk density, and the most satisfactory outcome chosen. A more elegant approach would be to explicitly incorporate $n$ as a parameter to optimise.

## Sampling in space and time

In the above discussion of adequate sample sizes we have purposefully conveyed only the 'spatial' component of sampling. But what about the 'time' component? Assuming that we sample adequately to establish the baseline status of SOC stock, we will ultimately want to sample adequately to detect a change over time.

Papritz and Webster (1995*a*) provided a theoretically rigorous treatment of how to estimate change under both the design-based approach and the model-based approach, and provided some pointers on how to choose the right approach for a certain situation. de Gruijter *et al*. (2006) discuss four basic arrangements for space-time sampling: (i) static (spatial positions are fixed, but temporal positions change); (ii) synchronous (temporal positions are fixed, but the spatial positions change such that they might never be revisited); (iii) static-synchronous (a combination, most easily related as a space-time grid); and, (iv) rotational (a compromise, where the spatial positions sampled at a particular time are partially replaced at the next sampling time). A static design, in the strictest sense, cannot be implemented for SOC stocks because of destructive sampling, i.e. we can never sample the same location twice. For general flexibility and ease of statistical inference, de Gruijter *et al*. (2006) recommend the synchronous pattern; however, each pattern has its own advantages and disadvantages, which depend somewhat on the nature of the variable being monitored, and on the objective of the monitoring programme. We refer the reader to de Gruijter *et al*. (2006) for further details about calculating sample means, sample variances, and estimation variances under each arrangement.

### *Design-based approach*

In the context of design-based analysis, it is recommended that, when revisiting an already sampled study area with the intention of detecting SOC change, one should sample as close as possible to the original baseline locations. This approximates the 'static' approach described above, but might also be termed 'temporally paired sites' (Conteh 1999). Such a scheme increases the precision of the estimates of change (Papritz and Webster 1995*b*; Lark 2009). Note the words: *estimates of change*. What makes change detection particularly difficult is that we are interested in neither the baseline observations nor the revisit observations; rather, we are interested in their difference, $Y$, which is distributed as (Lark 2009):

$$Y \sim (\mu_Y, \sigma^2_Y + 2\sigma^2_e + \sigma^2_L) \quad (18)$$

where $\mu_Y$ is the mean difference, and $\sigma^2_Y + 2\sigma^2_e + \sigma^2_L$ represents the total variance of $Y$. The variance component $\sigma^2_Y$ is the sample variance of $Y$; the variance component $\sigma^2_e$ represents sampling and measurement errors accrued in the baseline and revisit observations (multiplied by 2 because they are independent in

space and time); and, the variance component $\sigma_L^2$ represents location error (necessary due to destructive sampling). Note that estimation of $\sigma_e^2$ entails a proportion of replicated laboratory analysis for both the baseline survey and the revisit. It is unlikely (in the extreme) that the term $\sigma_Y^2 + 2\sigma_e^2 + \sigma_L^2$ will equal the sample variance of the baseline observations. The importance of this cannot be overstated: to quote Lark (2009), 'Soil scientists and the administrators who sponsor surveys should not fall into the trap of assuming that a survey planned to estimate status will also suffice for estimating change or that the requirements for estimating change can be computed in a simple way from data on status alone'. Similar sentiments were echoed by de Gruijter *et al.* (2006), who noted that rotational sampling patterns are relatively efficient for estimating the current mean of a target variable, but static synchronous patterns are relatively efficient for detecting change in the mean. Lark (2009) recommended adopting stratified reconnaissance sampling, where only a proportion of baseline sites are initially revisited in any one stratum. Strata that show a large change could then sampled more intensively and *vice versa*.

At this point it is worth returning briefly to MDD (Eqn 13). Garten and Wullschleger (1999) applied the method to assess SOC stock spatially, but speculated that it could be used to detect a change in SOC stock. Many studies have since promoted MDD for the elucidation of change (for example, Conant *et al.* 2003; Kucharik *et al.* 2003; Poussart *et al.* 2004; Homann *et al.* 2008; Heim *et al.* 2009). Schöning *et al.* (2006) did the same in terms of a model-based analysis. But as none of these studies considered the implications of Eqn 18 they have used an incorrect estimate of variance, and so their conclusions are flawed.

We refer the reader to Stewart-Oaten *et al.* (1992) for further discussion of the statistical pitfalls of estimating change in paired samples, collected under the design-based approach.

### Model-based approach

Papritz and Flühler (1994) presented a model-based method for estimating the change of a spatially autocorrelated target quantity between two dates, say baseline sampling and a revisit. The method is a modification of cokriging. As such it requires that the observed variables associated with each date are described by an LMCR (Table 3). However, due to the necessity for destructive sampling that accompanies estimates of SOC stock, the standard cross-variogram in the LMCR will have to be replaced with the pseudo-cross-variogram (Myers 1991). There are three advantages to the method of Papritz and Flühler (1994). First, because the temporally paired samples are not collocated, cokriging will always return a more precise estimate of the change than kriging the two variables independently and subtracting one interpolated surface from another (de Gruijter *et al.* 2006). The second advantage is that the LMCR can be used to optimise a model-based sampling scheme to detect further change in SOC stock. The third advantage relates to the concept of geostatistical blocking (Webster and Oliver 2001). Geostatistical blocking should not be confused with the blocking that is applied to experimental designs. Through geostatistical blocking the user can effectively scale the predictions of the method of Papritz and Flühler (1994) to represent global estimates of the change in the mean (as opposed to the local estimates that would usually be returned). This is an example of the model-based approach lending itself to a task that many would associate readily with the design-based approach.

### Krige's relation

Soil monitoring programmes are expensive to maintain. An obvious way to reduce costs is to limit the amount of time spent travelling from site to site. Thus, the financial benefit of sampling in a relatively small area, say 25 × 25 m, is self-evident. This has the added advantage of being easily communicated, which increases its adoptability as a standard procedure. Advocates of this approach argue that, by minimising spatial effects, the chance of detecting a change over time is increased. Unfortunately, due to the geostatistical principle of Krige's relation, the method is flawed if wishing to describe temporal change over an area larger than 25 × 25 m.

Let us say that we have been asked to estimate the baseline mean SOC stock for a paddock. This paddock is part of a larger property. From a random part of the paddock, we choose a site for the 25 × 25-m area, from which we will collect 10 samples. We denote the property, paddock and sample area as $R$, $B$, and $b$, respectively. In Eqn 14 we described how to compute the dispersion variance for an area of interest, $B$. The same formula applies for the dispersion variance associated with each of $R$ and $b$. Krige's relation describes how the dispersion variance is partitioned according to the spatial scale of interest (after Webster and Oliver 2001), that is:

$$\sigma^2(b \in R) = \sigma^2(b \in B) + \sigma^2(B \in R) \qquad (19)$$

where $\sigma^2(b \in R) = \bar{\gamma}(R, R) - \bar{\gamma}(b, b)$, $\sigma^2(b \in B) = \bar{\gamma}(B, B) - \bar{\gamma}(b, b)$, and $\sigma^2(B \in R) = \bar{\gamma}(R, R) - \bar{\gamma}(B, B)$. The term $\sigma^2(b \in B)$ is the key here, because it relates the uncertainty of the sample mean of the paddock, estimated under a particular sampling pattern.

What is the implication of Krige's relation? For the hypothetical paddock of Fig. 4 we demonstrate in Table 4 how the $\sigma^2(b \in B)$ component of Eqn 19 changes according to two contrasting sampling arrangements: (i) selecting $n = 10$ locations from within a $b = 25 \times 25$-m area within the paddock; and, (ii) selecting $n = 10$ locations from the entire paddock (i.e. $b = B \approx 1000 \times 1000$-m area). We assume that the theoretical variogram is described by a spherical function with a sill of 1 unit and a nugget variance of 0.5 units, but alter the range parameter to take values of $a = \{5, 10, 30, 50, 100, 1000\}$ (in units of m). For simplicity we have used simple random sampling (Fig. 4*a*) to

**Table 4. Dispersion variance (uncertainty) of a hypothetical variable, when estimated from samples spread over an area *b*, within a paddock of $B \approx 1000 \times 1000$ m**

| Range (m) | *b* | |
|---|---|---|
| | 25 × 25 m | 1000 × 1000 m |
| 5 | 0.115 | 0.098 |
| 10 | 0.143 | 0.098 |
| 30 | 0.310 | 0.098 |
| 50 | 0.397 | 0.097 |
| 100 | 0.468 | 0.094 |
| 1000 | 0.347 | 0.085 |

derive the estimates of $\sigma^2$ ($b \in B$), although the same principle applies to any design-based or model-based arrangement. There is some fluctuation in the values of $\sigma^2$ ($b \in B$) (Table 4), inherited from the process of random site selection in $b$, but the general pattern is clear: Krige's relation implies that we obtain relatively precise estimates of the paddock mean by sampling as widely as possible within the paddock. This is a simple and sensible message, but it is easily supplanted by the desire for convenience, which, as noted by de Gruijter *et al*. (2006), creates samples with weak statistical properties.

## Further considerations for optimum sampling

In addition to statistical issues, two important considerations relate to: (i) sample compositing; and, (ii) the temporal variability of SOC and its constituent pools. Sample compositing ('bulking') has been used by numerous authors in order to reduce lateral variability (e.g. Webster and Burgess 1984; Dalal and Mayer 1986*b*; Studdert *et al*. 1997; Brus *et al*. 1999; Conant *et al*. 2003; Harms and Dalal 2003). Compositing involves collecting several soil cores in close proximity, then mixing the cores together to form a single sample. The effect of compositing is to smooth short-range fluctuations, which increases the chance of detecting longer-range treatment differences. A variogram can be used as prior knowledge to help decide the optimum way in which samples should be composited (Webster and Burgess 1984). The principal reason for compositing is that analytical costs are reduced. A user must be aware of the implicit assumption that the composite sample, upon analysis, must yield the same value as the mean of the individual cores that comprise the composite (disregarding sampling and measurement errors) (de Gruijter *et al*. 2006). Fortunately, this is the case for both SOC concentration and bulk density. (A notable soil attribute to be affected by this assumption is pH: due to the log. transform applied to the activity of the $H^+$ ions a composite sample of soil pH will not equal the mean of individual observations.)

In regard to temporal variability, we expect that a management-induced change to SOC stock will, in general, manifest itself slowly over several years. Some may consider this to be too long. Encouragingly, in several studies it has been found that the particulate fraction of SOC or light-fraction C (labile C) is lost preferentially under a change in management (Chan 1997; Franzluebbers and Stuedemann 2003; Dalal *et al*. 2005). Research is needed to verify whether this applies to northern Australian rangeland conditions. If so, then concentrating analytical effort on this fraction of SOC might expedite the process of detecting change.

Following Conteh (1999), VandenBygaart (2006), and Goidts *et al*. (2009), some additional considerations for sampling for SOC stock include: (i) whether it is better to sample by fixed depth intervals (IPCC default value is a 0–0.3-m depth) or sample by horizon; (ii) the sampling process cannot be streamlined by assuming that bulk densities or SOC pools such as labile C are temporally constant (consider sampling at the same time of the year); (iii) clay particles play an important role in C cycling (Sollins *et al*. 1996), and their concentration should not be assumed temporally stable, particularly in areas prone to erosion; (iv) plant litter and roots are important C sinks (contribute to labile C pool) and should be sampled concomitantly with soil; (v) rocky soil is a major source of uncertainty due to its influence on bulk density – affected areas may require estimation by spatial interpolation or by a calibrated pedotransfer function; and (vi) the more background information one collects about a site, the better (two of the more obvious for rangelands are historical stocking rates and rainfall). In addition, the background information gathered from electromagnetic surveys (clay content, salinity etc.), biomass and yield maps (Dang *et al*. 2009) and remote sensing (Fisher *et al*. 2009) can be employed to stratify soil sampling. An example of utilising the variability in long-term ground cover for designing a sampling scheme is shown in Fig. 4.

Sampling and analytical costs and time required to estimate SOC stocks (and other soil properties) may be reduced by employing emerging technologies for *in situ* estimation (surrogate measures) of soil C (Gehl and Rice 2007). These techniques include laser-induced breakdown spectroscopy (Ebinger *et al*. 2003), inelastic neutron scattering (Wielopolski *et al*. 2001), visible-near infrared spectroscopy (Morgan *et al*. 2009), and remote sensing for surface cover and plant biomass, normalised difference vegetation index (NDVI), and hence potential C input (Chen *et al*. 2000).

## Conclusion

We have discussed the nature and the causes of spatial and temporal variability in SOC stock and SOC pools, and the statistical issues that arise when they are sampled. At the very least we hope that we have shown how statistical considerations pervade every aspect of sampling, and enlightened the reader to the contrast between the design-based approach and the model-based approach, and how they might apply to his or her own research.

McKenzie *et al*. (2000) proposed a broad soil sampling strategy for terrestrial C accounting to support the National Carbon Accounting System. This strategy recommended a stratified random sampling scheme, with a minimum of 4 replicates per strata. Due to a dearth of information, it is not yet known whether this is too few or too many for Australian rangelands. Ultimately, we should be able to detect, with the confidence afforded by statistical rigour, whether the SOC stock and SOC pools at a certain location have increased or decreased due to management effect or following land-use change in comparison with a baseline value. MDD is an appropriate tool by which this can be achieved. However, before we arrive at this goal, the discussion above has highlighted several important issues:

(i) At the outset, which approach do we want to follow, design-based or model-based? The ultimate objective is to determine a change in the mean SOC stock over some aggregated area (e.g. a paddock, a farm, a region). This implies that we are interested in global estimation. Consequently, the design-based approach might be preferred, provided of course that the principle of random site selection can be adhered to strictly. We have seen that a model-based approach can also lend itself to global estimation of change. If random site selection cannot be guaranteed then the model-based approach is the sole option, although it will require a greater sampling intensity than the design-based approach.

(ii) Sample as widely as possible with the unit of interest (respecting, of course, the principles of the particular approach that is being used). This principle will ensure that the mean is estimated precisely, and applies to intensively grazed dairy pasture paddocks as well as extensively grazed rangeland paddocks. If working under the model-based approach, we recommend that, at a proportion of sampling sites, an additional, adjacent sample is collected. These additional samples will help improve the accuracy of the variogram of the target variable, which will, in turn, improve future sampling schemes.

(iii) A proportion of the samples must be replicated during laboratory analysis, in order to quantify laboratory measurement error. It is easy to overlook this consideration but it is critically important when making inference about change.

(iv) Temporally paired sites are the most efficient way of detecting a change in SOC stock, but destructive sampling and cumulative measurement errors decrease our ability to detect change. To minimise the effect of seasonal variation, especially for labile C pools, we recommend that revisit samples are collected at the same time of year as the baseline samples.

(v) Research is needed to establish an appropriate MDD for Australian rangelands.

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