Quantifying soil organic carbon in complex landscapes: an example of grassland undergoing encroachment of woody plants

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Abstract

The invasion of woody plants into grass-dominated ecosystems has occurred worldwide during the past century with potentially significant impacts on soil organic carbon (SOC) storage, ecosystem carbon sequestration and global climate warming. To date, most studies of tree and shrub encroachment impacts on SOC have been conducted at small scales and results are equivocal. To quantify the effects of woody plant proliferation on SOC at broad spatial scales and to potentially resolve inconsistencies reported from studies conducted at fine spatial scales, information regarding spatial variability and uncertainty of SOC is essential. We used sequential indicator simulation (SIS) to quantify spatial uncertainty of SOC in a grassland undergoing shrub encroachment in the Southern Great Plains, USA. Results showed that both SOC pool size and its spatial uncertainty increased with the development of woody communities in grasslands. Higher uncertainty of SOC in new shrub-dominated communities may be the result of their relatively recent development, their more complex above- and belowground architecture, stronger within-community gradients, and a greater degree of faunal disturbance. Simulations of alternative sampling designs demonstrated the effects of spatial uncertainty on the accuracy of SOC estimates and enabled us to evaluate the efficiency of sampling strategies aimed at quantifying landscape-scale SOC pools. An approach combining stratified random sampling with unequal point densities and transect sampling of landscape elements exhibiting strong internal gradients yielded the best estimates. Complete random sampling was less effective and required much higher sampling densities. Results provide novel insights into spatial uncertainty of SOC and its effects on estimates of carbon sequestration in terrestrial ecosystem and suggest effective protocol for the estimating of soil attributes in landscapes with complex vegetation patterns.

Introduction

Woody encroachment has occurred in grass-dominated ecosystems around the world (Van Auken, 2000; Archer *et al.*, 2001; Asner *et al.*, 2004; Tape *et al.*, 2006). Causes of this change appear to involve some combination of climate change, livestock grazing, suppression of fire, and atmosphere CO_2 enrichment (Bond & Midgley, 2000; Archer *et al.*, 2001; Polley *et al.*, 2003). The shift from grass to woody domination has important impacts on ecosystem structure, microclimate, and key ecosys-

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tem processes such as productivity, hydrology, soil erosion, and the storage and turnover of soil nutrients (Archer, 2009). Since a large portion of the world's land surface is covered by grass-dominated ecosystems (Daly *et al.*, 2000), woody invasion into these ecosystems can have important implications for regional and global biogeochemistry and climate (Boutton *et al.*, 1999; Wessman *et al.*, 2004; Asner & Archer, 2009). The Southern Great Plains of the USA are a case in point. Studies using historical aerial photos, tree rings, carbon isotopes, and ecosystem models have independently shown extensive woody plant invasion into areas once characterized as grassland over the past 100 + years (as summarized in Archer, 1995).

Given the global extent of woody plant invasion into grasslands across the world, there is growing interest in determining how changes from grass to woody plant dominance might influence global biogeochemical

cycles and the climate system via impacts on soil carbon storage and dynamics (Houghton *et al.*, 1999; Asner *et al.*, 2004; CCSP, 2007; Archer, 2009). Studies have found increased soil organic carbon (SOC) storage after woody invasion in some ecosystems (Schlesinger *et al.*, 1996; McKinley & Blair, 2008; Boutton *et al.*, 2009), but no net change, or decreased SOC storage, in others (Gill & Burke, 1999; Jackson *et al.*, 2002). Reasons for these discrepancies still remain unclear. It is our contention that the ability to quantify the impacts of woody encroachment on the carbon cycle is currently hindered due to the lack of appropriate methodology for estimating SOC storage in arid and semiarid landscapes with complex vegetation patterns across multiple spatial scales.

Understanding spatial variability and uncertainty of soil attributes is necessary for their accurate estimation (Conant & Paustian, 2002; Legendre *et al.*, 2004). Direct measurements of SOC are time-consuming and expensive, both in the laboratory and in the field, particularly when attempting to characterize SOC at broad spatial scales. A better understanding of the spatial uncertainty and variability of SOC would help optimize field sampling designs to accurately estimate SOC storage with minimum sampling effort. Many studies have quantified spatial heterogeneity of soil properties after woody plant invasion (Jackson & Caldwell, 1993; Schlesinger *et al.*, 1996; Bekele & Hudnall, 2006; Throop & Archer, 2008), but none have explored the spatial variability and uncertainty in SOC and how they may affect SOC storage estimates.

We refer spatial variability as the variation in SOC across spatial extent; and spatial uncertainty as the degree of uncertainty in estimating SOC at a given location based on known sample data. In this distinction, a variable at a given extent with high spatial variability may have low spatial uncertainty if the variable has a strong and consistent spatial pattern. In such instances, accurate predictions at unsampled locations can be made based on neighboring samples. Conditional stochastic simulations, including sequential Gaussian simulation (SGS) and sequential indicator simulation (SIS), (Rossi *et al.*, 1993; Zhao *et al.*, 2005), have been used to assess spatial uncertainty by generating a large number of realization maps based on known sample data. The conditional probability distribution of the variable of interest at a given location, which quantifies the amount of uncertainty, can be obtained from these realizations (Isaaks & Srivastava, 1989; Goovaerts, 1999). Spatial uncertainty obtained from simulations can then be used to help design efficient point sampling strategies to minimize sampling intensity while achieving a desired accuracy for broader-scale estimates. Sampling designs can be evaluated either by comparing results generated from

reduced data sets with those based on a full data set (Atkinson *et al.*, 1994), or by comparing their spatial properties (Chang *et al.*, 1998).

The purpose of this study was to determine how spatial uncertainty in SOC pools might affect estimates of SOC storage in a subtropical savanna landscape where woody plants have increased in abundance during the past century. Specific objectives were to: (1) quantify the spatial pattern and uncertainty of SOC; (2) evaluate the performance of different sampling designs in estimating SOC storage; and (3) develop efficient and effective sampling designs for estimating soil attributes in landscapes with complex vegetation patterns.

Methods

Study site

The study was conducted at the Texas AgriLife La Copita Research Area (LCRA, 27°40'N, 98°12'W), 65 km west of Corpus Christi, Texas. Elevation ranges from 75 to 90 m above sea level. The climate is subtropical with warm, moist winters and hot, dry summers. Mean annual temperature is 22.4 °C with an average growing season of 289 days. Mean annual precipitation is 680 mm with bimodal peaks in May and September. Uplands are characterized by sandy loam soils (Typic and Pachic Argiustolls), whereas lowlands and playas have clay loam or clay soils (Pachic Argiustolls). The site has been continuously grazed by cattle since the late 1800s until its designation as a research area in early 1980s, at which time progressive livestock grazing management practices were implemented.

The landscape grades with gentle slopes (<3%) from uplands to drainage lowlands and bottom playas. Uplands are savanna parklands with discrete woody patches scattered in a continuous C_4 grassland matrix (Fig. 1a). Woody patches include smaller shrub clusters (generally <100 m²) and larger groves (generally >100 m²). Shrub clusters typically consist of a central honey mesquite (*Prosopis glandulosa*) tree with a diverse assemblage of understory shrub species beneath its canopy. Discrete shrub clusters expand laterally and coalesce to form groves with overlapping mesquite canopies (Archer, 1995; Bai *et al.*, 2009). Shrub clusters and grasslands occur where the argillic horizon is well-expressed, whereas groves occur on soils lacking an argillic horizon; and plants in groves are larger than those in clusters. Understory shrub species composition is similar in both clusters and groves. Common species include *Zanthoxylum fagara*, *Celtis pallida*, *Condalia hookeri*, *Diospyros texana*, *Schaefferia cunefolia*, *Ziziphus obtusifolia* and *Berberis trifoliolata*. The herbaceous grassland matrix is dominated by C_4 grasses including *Paspalum setaceum*, *Setaria geniculata*, *Bouteloua rigidiseta*, and *Chloris cucullata*, but also has a significant C_3 forb component.

Field sampling

Sequential temporal sampling of SOC subsequent to the establishment of shrubs would be the most direct way to evaluate
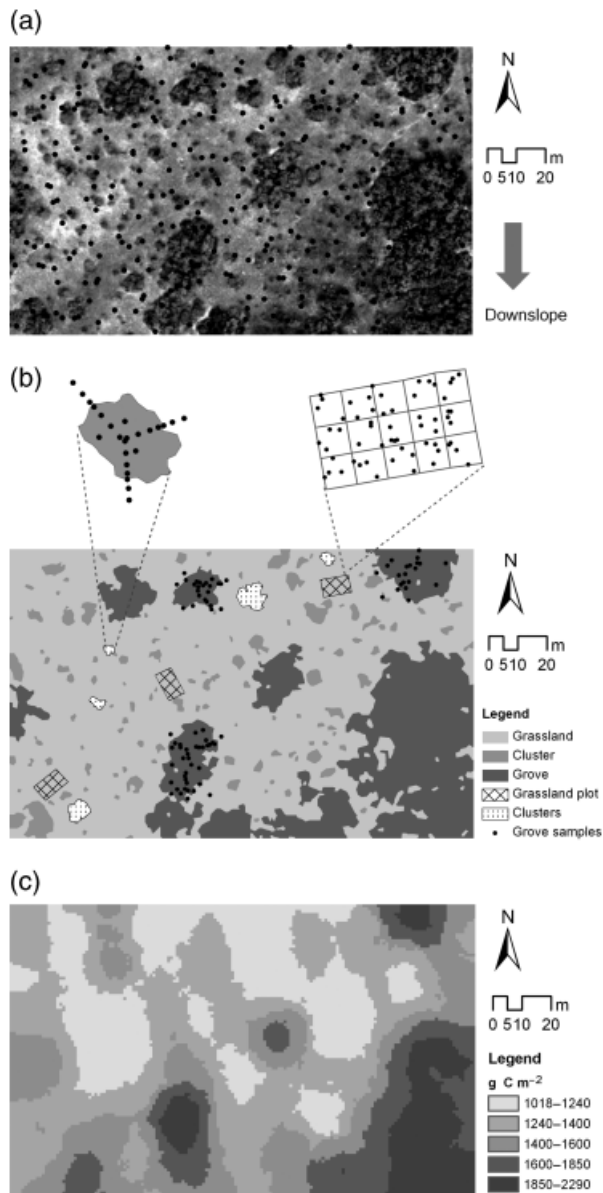
Fig. 1 (a) Aerial photograph of the $160\,m \times 100\,m$ plot within a sandy loam upland savanna parkland landscape (1%–3% slope) and locations of 320 random soil samples (dark points). Dark patches are shrub clusters and groves and light grey color indicates open grassland. (b) Classified vegetation map of the plot showing locations of intensively sampled shrub clusters, groves, and grassland subplots. (c) Kriged map of soil organic carbon (SOC, $g\,C\,m^{-2}$) of the plot based on 320 random points.

effects of vegetation change on soil properties. However, SOC changes induced by shrubs take place over the course of decades to centuries (Hibbard *et al.*, 2003; Boutton *et al.*, 2009) and long-term plots with such documentation do not exist. The formation of shrub communities in grasslands at our field site is initiated with the establishment of mesquite (Archer, 1995). Hence, the age of woody patches corresponds to the age of the largest mesquite in that patch; and SOC

increases with mesquite age (Boutton *et al.*, 2009). We capitalized on this knowledge of shrub community development to quantify spatial uncertainty of SOC in different woody elements using a space-for-time substitution approach, assuming spatial variation along mesquite bole (point of longest shrub influence) to canopy dripline (point of most recent shrub influence) transects represent time; and that processes determining shrub influences on spatial patterns are consistent over time.

A $160\,m \times 100\,m$ plot divided into a grid of $10\,m \times 10\,m$ cells was established on the upland portion of a landscape in January 2002. The corners of each cell were georeferenced using a GPS unit (Trimble Pathfinder Pro XRS, Trimble Navigation Limited, Sunnyvale, CA, USA), given a unique identification number (X, Y coordinate) and marked with a PVC pole fastened to rebar. Two random points within each cell were selected for soil sampling (320 total, Fig. 1a). Distances from each soil sampling point to two uniquely identified cell corners were measured. Vegetation cover at each sample point was recorded as grassland, shrub cluster or grove.

Color infrared aerial photography was acquired in April 2003 for the plot. Negative photography was scanned (0.25 m nominal resolution), georeferenced using ERDAS Imagine (ERDAS, 1999) and subjected to unsupervised classification with the Iterative Self-Organization Data Analysis (ISODATA) method (ERDAS, 1999). This classification initially grouped pixels into 30 classes based on reflectance value similarities; and these were subsequently collapsed into one of two classes: woody or nonwoody. Woody patches $<1\,m^2$ were treated as nonwoody because data showed little difference in SOC between grasslands and clusters $<1\,m^2$ canopy area (S. R. Archer *et al.*, in preparation). Woody patches $>1\,m^2$ were subsequently classified as either clusters ($1–100\,m^2$) or groves ($>100\,m^2$) based on canopy area. The classified vegetation map is shown in Fig. 1b.

In addition to the random soil samples mentioned above, three groves, five clusters, and three grassland subplots were designated via a directed random process for intensive sampling (Fig. 1b). The three groves selected were large, medium, and small in size (areal extent); and each was divided into a grid of $5\,m \times 5\,m$ cells. PVC poles anchored to rebar were used to mark the corners of each cell and their coordinates were determined using the GPS unit mentioned above. Two random points were located within each cell for soil sampling. Soils were also collected along tree-to-tree transects ($n = 3$ per grove) radiating from near the grove center and into adjoining grasslands. Soil samples were collected at the following locations along these transects: (a) the bole of each tree, (b) midway between the bole of each tree and its canopy dripline, and (c) canopy dripline. Coordinates of soil sample points in grid cells and along transects were determined by measuring distances to two uniquely identified cell corners. A total of 63, 37, and 24 soil samples were collected from three groves, respectively.

Five shrub clusters were selected via a directed random process for intensive sampling. For each shrub cluster, transects ($n = 3$) radiated from the cluster center into the adjacent grasslands with orientations ca. 120° from each other. Each transect consisted of seven soil sample points, determined by

their relative locations along the transect: (a) the center of each shrub cluster (near mesquite bole), (b) one-third the distance between the cluster center and its canopy dripline, (c) two-third the distance from the center to the canopy dripline, (d) 15 cm inside canopy dripline, (e) 15 cm beyond the canopy dripline, (f) beyond the canopy dripline at a distance equal to one-third the distance between the shrub cluster center and its canopy dripline, and (g) beyond the canopy dripline at a distance equal to two-third of the distance from the cluster center to its canopy dripline (Fig. 1b). Additional soil samples were collected at three or four random locations within canopy of each shrub cluster. A total of about 25 soil samples were thus collected for each cluster. Steel rebars ($n = 4$ or 5) were installed in each cluster as reference markers and their coordinates determined using a high-resolution GPS. Coordinates of soil sample points were determined by measuring distances to two uniquely identified steel rebars.

Three grassland subplots (6 m × 10 m) were also established. Each grassland plot was divided into a grid of 2 m × 2 m cells whose corners were marked with rebar. Corners were georeferenced using a high-resolution GPS and assigned a unique identification number (X, Y coordinate). Four random points were selected within each cell for soil sampling (Fig. 1b). Coordinates of each soil sample point were determined by measuring the distances to the two nearest cell corners. A total of 60 soil samples were collected in each grassland plot.

All soil samples were collected to a depth of 15 cm using a 2.24 cm diameter corer. Soil samples were collected during 2 multiple-day periods in December 2002 and January 2003 respectively. Surface litter, if present, was brushed away before coring. Two adjacent soil cores were collected at each sampling point. SOC was determined from one core; and soil bulk density from the other. Coordinates of these soil-sampling points were calculated and imported into ArcGIS (ESRI, Redlands, CA, USA). Groves, shrub clusters, and grassland plots were surveyed for visible animal disturbance (e.g., burrows, excavations, wallows, nests, etc.). Animal disturbances were delineated in relation to known georeferenced points (e.g., cell corners).

Laboratory analysis

Soil cores for SOC were dried at 60 °C for at least 48 h, passed through a 2 mm screen to remove gravel and coarse organic fragments, and then pulverized to a fine powder in a centrifugal mill (Angstrom Inc., Belleville, MI, USA). Samples were weighed into silver capsules (5 × 7 mm) using a microbalance, treated with HCl vapor in a desiccator to remove carbonates (Harris *et al.*, 2001), and dried. SOC concentration (%) was determined by combustion/gas chromatography using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ, USA) elemental analyzer. Soil bulk density was determined by the core method. SOC concentration was converted into density (g C m^{-2} to a depth of 15 cm) by multiplying soil bulk density.

Data analysis

ANOVA (SPSS for Windows version 12.0, SPSS Inc., Chicago, IL, USA) using the 320 random samples were conducted to compare SOC density in grassland, shrub cluster, and grove vegetation types. A sample variogram fitted with a variogram model (VARIOWIN version 2.2; Pannatier, 1996) was then constructed to quantify SOC spatial pattern using the same 320 random samples; and Kriging (GSLIB; Deutsch & Journel, 1998) was used to predict SOC at unsampled locations.

SIS, a type of conditional stochastic simulation (Isaaks & Srivastava, 1989), was used to quantify spatial uncertainty of SOC. We used SIS (SISIM program of GSLIB; Deutsch & Journel, 1998) to generate multiple alternative estimates, each of which honored the global statistics and the spatial structure of the 320 random samples. We ran 500 simulations of SOC for 1 × 1 m cells using the 320 randomly collected soil samples. The standard deviation (SD) of SOC estimates in a given cell is an indication of the degree of uncertainty in the estimate of SOC at that location (Isaaks & Srivastava, 1989; Rossi *et al.*, 1993; Goovaerts, 2001). The cutoffs used to transform sample SOC into an indicator variable included nine deciles plus the SOC densities at the 0.05 and 0.95 cumulative distribution fractions. VARIOWIN (Pannatier, 1996) was used to construct sample variograms and to fit variogram models at each cutoff.

Means and SDs of SOC from the 500 SISs were overlaid with the classified vegetation map in ArcGIS to examine the spatial pattern and uncertainty of SOC in each vegetation type. This enabled us to generate a complete set of SOC means and SDs within each vegetation type (grassland, shrub cluster and grove). Frequency distributions of SOC means and SDs were then developed for each vegetation type.

Sampling designs

Complete random sampling in different vegetation types. Kriging of SOC density was first conducted using all data in the 160 × 100 m plot [random samples from 10 × 10 m cells, along with transect (clusters, groves), grid cell (grassland), and random data from the three vegetation types]. The sampling intensity in these sampled plant communities was very high: ca. 800 ha^{-1} for groves, ca. 500 ha^{-1} for shrub clusters, and 1000 ha^{-1} for grassland subplots. Kriged maps of SOC within these communities using all the data were used as baseline reference maps to which sample methods were compared. Various intensities of complete random sampling were then conducted on these vegetation-specific baseline reference maps to evaluate their accuracy in estimating SOC storage. Estimation errors were calculated as the absolute difference between SOC density obtained from random samplings and the SOC density from the reference maps.

Effectiveness of alternative sampling designs. Proceeding on the assumption that the kriged maps of intensively sampled areas of grassland, shrub cluster and grove communities were representative of the actual SOC density, we extrapolated these data in a spatially explicit fashion across the 160 × 100 m entire plot using actual percentages of the fractional cover of grassland, shrub cluster and grove communities derived from the classified imagery. This extrapolated map was considered as a complete baseline dataset with reasonably well-known SOC values.

Based on the spatial uncertainty of SOC in the different vegetation types suggested by SIS simulation results, three alternative sampling designs were applied to the baseline data set. For each sampling design, total SOC storage for a given vegetation type was estimated as the product of the mean SOC density and the area occupied by that vegetation type. Landscape-scale storage was then determined by summing the values for the three vegetation types.

The first design was completely random on the whole plot. The second design was a stratified random sampling of grassland, shrub clusters, and groves with equal sampling intensity. The third design was a stratified random sampling of vegetation types with unequal sampling intensities. Preliminary results, as shown by SIS simulations, indicated a higher spatial uncertainty of SOC in groves and shrub clusters compared with grasslands. We therefore set the sampling intensity for shrub clusters and groves as twice that of the grassland in the third design. For each alternative sampling design, a range of overall sampling intensities (10–200 samples ha^{-1}) were used. This enabled us to compare the accuracy of alternative sampling designs as a function of sampling intensity.

Estimation errors and woody plant cover. Do SOC estimation errors for grasslands undergoing woody plant encroachment vary as a function of the type and extent of woody plant cover? To address this question, estimation errors were calculated for simulated landscapes with varying percentages of grassland, shrub cluster, and grove vegetation. In these scenarios, the ratio of shrub cluster area to grove area was kept constant for simplicity.

How important are within-community gradients?. Previous studies at our site have shown that shrub clusters have a higher mean SOC content than grassland (Archer *et al.*, 2001; Boutton *et al.*, 2009). In addition, both the magnitude and variability of SOC decrease exponentially from the centers to the canopy edges of these communities (S. R. Archer *et al.*, in preparation). To determine how within-community spatial pattern of SOC might affect estimation accuracy, we compared results from random and transect-based sampling designs against baseline reference maps obtained from intensively sampled shrub clusters.

Results

Spatial pattern of SOC

The random sampling of 320 points indicated significant differences ($P < 0.001$) among vegetation types ($P < 0.001$). The average SOC density for the upper 15 cm of the soil profile decreased from groves ($\bar{x} = 1832\,\mathrm{g\,C\,m}^{-2}$; $n = 62$) to clusters ($\bar{x} = 1500\,\mathrm{g\,C\,m}^{-2}$; $n = 29$) and grasslands ($\bar{x} = 1282\,\mathrm{g\,C\,m}^{-2}$; $n = 204$). SOC variability was highest in groves [coefficient of variation (CV) = 0.23] and lowest in grasslands (CV = 0.17).

Fossorial rodents [e.g., pack rats (*Neotoma micropus*)] and ants (e.g., *Atta texana*) occur on this site and

Fig. 2 Top down view of a typical pack rat (*Neotoma micropus*) disturbance in a *Prosopis* grove developing on grasslands at the La Copita site in the Southern Great Plains, TX, USA. Burrow entrances are covered with twigs and coarse woody debris. (Photo by Feng Liu).

influence soils. No surface animal disturbances were observed in the three 6 × 10 m grassland subplots. Only one of the five shrub clusters sampled had a rodent burrow disturbance of ca. 2 m^2. However, animal disturbances, primarily pack rat nests and burrows (Fig. 2), along with other small rodent burrows, armadillo (*Dasypus novemcinctus*) diggings and collared peccary (*Tayassu tajacu*) wallows, were observed in each of the three groves. The total area impacted by animal disturbances in groves was 5%–9% (data not shown).
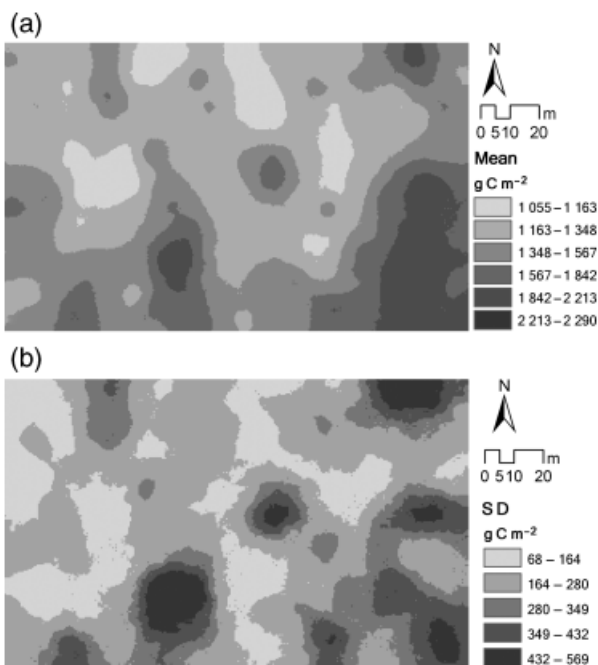
SOC was spatially autocorrelated. The spherical model fitted to the sample variogram had a range of 36.8 m, which indicates the extent of spatial autocorrelation. The sill and nugget of the variogram model were 10.92×10^4 and 5.07×10^4, respectively. The structure variance to sill ratio, which represents the proportion of the total variance explained by the spatial structure, was 0.54. The predicted map of SOC generated by ordinary kriging using 320 random samples displayed a spatial pattern similar to that of vegetation cover, especially the distribution of woody patches (Fig. 1a and c). The kriged map indicated SOC densities were the highest in the core regions of woody patches (shrub clusters and groves), decreasing towards the perimeter of woody patches and reaching lowest values within the grassland matrix. The extensive areas of high SOC density in the southeast corner of the plot were associated with a large grove. Grasslands covered 82% of the area with low SOC densities ($<1500\,\mathrm{g\,C\,m}^{-2}$). Since this kriging was based on 320 random points (2 per 10 m × 10 m grid cell), not all woody patches had soil samples; and hence their impacts on SOC are not fully represented in Fig. 1c.
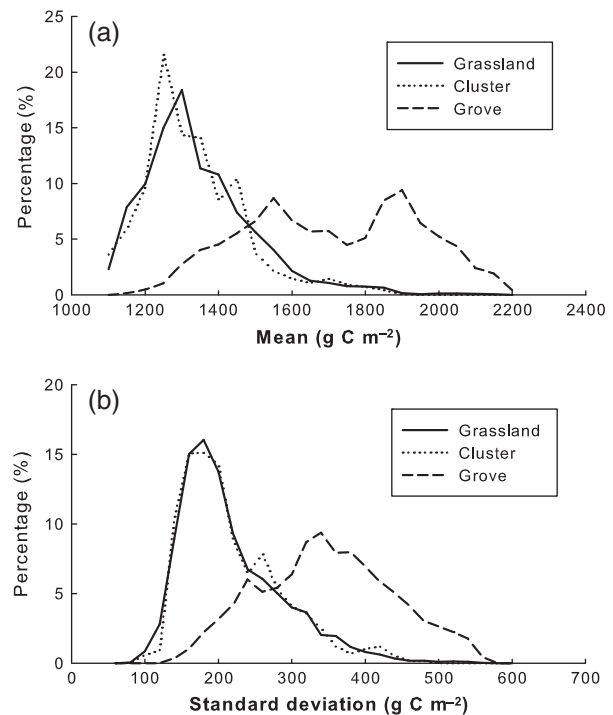
Spatial uncertainty of SOC

Spatial patterns of SOC in the SIS simulations were similar to those in the kriged map of SOC, with the former exhibiting greater variation than the latter (Figs 1c and 3a). Consistent with the kriged map of SOC, the map of the means of the 500 simulations showed that SOC was closely related to the spatial distribution of woody vegetation (Figs 1a and 3a), with core regions of shrub clusters and groves having high SOC densities that attenuated towards canopy edges.

SDs of SOC based on the 500 SIS simulations quantify the amount of uncertainty associated with estimates of SOC at a given location (Fig. 3b). Higher SDs indicate higher spatial uncertainty. Uncertainty for SOC was typically greatest within portions of the landscape dominated by woody plants and lowest in grassland areas.

Frequency distributions of mean simulated SOC densities demonstrated that the grassland areas typically had lower SOC densities with a relatively narrow spread, indicative of low spatial variability (Fig. 4a). Shrub clusters had a frequency distribution similar to that of grassland, but with higher frequencies at ca. 1250 and $1450\,g\,C\,m^{-2}$. In contrast to grasslands and shrub clusters, groves had a notably higher proportion of high mean SOC values. The range of SOC densities in groves was also substantially wider than that in shrub clusters
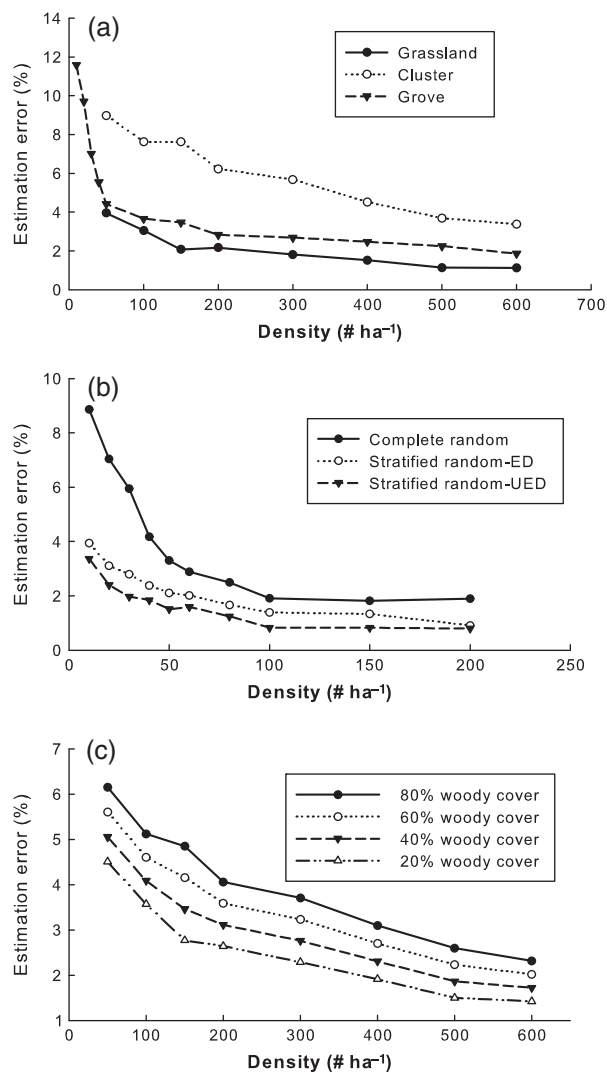

Fig. 4 Frequency distribution of soil organic carbon (SOC) means (a) and standard deviations (b) within intensively sampled grassland subplots, shrub clusters, and groves based on results from 500 sequential indicator simulations.

and grassland, another indication of greater SOC variability in groves compared with clusters and grassland.

Grassland and shrub clusters also had similar frequency distributions of SDs in simulated SOC densities, with the exception of higher frequencies around $260\,g\,C\,m^{-2}$ in shrub clusters (Fig. 4b). The SDs of simulated SOC densities in groves were much higher than those in shrub clusters and grassland, indicating substantially greater uncertainty in SOC estimates in this vegetation type.

Sampling designs

Estimation errors associated with complete random sampling decreased with increasing sampling intensity for all vegetation types (Fig. 5a). At any given sampling intensity, shrub clusters consistently had the highest estimation errors and grasslands the lowest, suggesting SOC in shrub clusters was more variable than that in grassland. Estimation errors in groves decreased dramatically as sampling intensity increased to approximately 50 samples ha^{-1}, suggesting a threshold in sampling intensity for estimating SOC via complete random sampling in this vegetation type. Shrub clusters did not exhibit a clear threshold sampling intensity, but error appeared to be minimized at ca. 500 samples ha^{-1},

Fig. 3 Mean (a) and standard deviation (b) of soil organic carbon (SOC, $g\,C\,m^{-2}$) derived from 500 sequential indicator simulations based on the 320 random sample points in Fig. 1a.

Fig. 5 Error (%) of soil organic carbon (SOC) estimates as a function of sampling intensity (samples ha^{-1}) on kriged maps of intensively sampled grasslands, shrub clusters, and groves. (a) Complete random sampling on intensively sampled plots; (b) Complete random, stratified random with equal density (ED) in grassland and woody patches, and stratified random with unequal density (UED) in grassland and woody patches and (b) complete random sampling on simulated landscapes with different percentages of woody cover, assuming a constant ratio of shrub cluster and grove area.

Table 1 Estimation error of SOC for different sampling designs in shrub clusters

Number of samples	Sampling design	Estimation error (%)
1	Random	25.54
	(b or c)	18.68
2	Random	20.61
	(b or c) for two clusters	13.85
	(b, c)	11.15
3	Random	17.58
	(b or c) for three clusters	12.66
	(b, c, d)	9.25
4	Random	15.66
	(b or c) for four clusters	9.71
	(b, c) for two clusters	7.95
	(a, b, c, d)	9.31
5	Random	11.21
	(b or c) for five clusters	10.53
	(b, c), (b, c, d)	6.98
6	Random	10.84
	(b or c) for six clusters	9.19
	(b, c) for three clusters	6.62
	(b,c, d) for two clusters	7.5

Letters indicate soil samples inside clusters along transects: (a) near the center of the cluster; (b) one-third the distance between the cluster center to canopy dripline; (c) two-third the distance between the cluster center to the canopy dripline; (d) 15 cm inside cluster canopy dripline.
SOC, soil organic carbon.

an order of magnitude greater than that in groves and 3.3 times that of grasslands.

Consistent differences in estimation error occurred among the three sampling regimes (complete random sampling, stratified random sampling with equal density, and stratified random sampling with unequal density) when tested using the extrapolated data set (Fig. 5b). Stratified random sampling with unequal intensities (higher sampling densities in shrub clusters

and groves) generated the lowest estimation error and complete random sampling the highest, regardless of sampling intensity. At low sampling intensities (<50 ha^{-1}), estimation error in the complete random sampling decreased more quickly than the other two designs as sampling density increased. When sampling intensity reached ca. 100 ha^{-1}, estimation errors of all three designs leveled off, suggesting further increases in sampling density would not substantively improve estimation accuracy.

Results of the complete random sampling experiments on simulated landscapes indicate that errors in estimates of SOC storage would steadily increase with increasing shrub cover, regardless of sampling intensity (Fig. 5c).

Sampling along center-to-edge transects consistently yielded better estimation of SOC density than random sampling in shrub clusters known from previous work to have strong gradients of SOC (Table 1). This was generally true even when the number of random samples was relatively high. Using sample points from the middle section of the center-edge transect yielded less estimation error than using samples obtained near the center or obtained near the canopy edge. A sampling

regime consisting of two or three points on one transect within one cluster yielded better results than sampling a single point in multiple clusters, regardless of whether that single point was selected at random or from a targeted location.

Discussion

Land cover change in drylands often involves the displacement of herbaceous life forms with woody life forms. This shift in vegetation is often in response to extensive livestock grazing which has the concomitant effect of reducing fire, herbaceous primary production, and ground cover (Archer, 2009). These grazing-induced changes may substantially alter SOC pools, albeit in ways that are difficult to generalize (Milchunas & Lauenroth, 1993; Derner *et al.*, 2006), and thus influence patterns of SOC and its spatial variability before and during the course of shrub encroachment. Once established, shrubs alter soils in and around their canopies in a variety of ways, both direct and indirect (Virginia, 1986). The resulting 'islands of fertility' wherein SOC and soil nutrients accumulate as a result of shrub stem flow, above- and belowground shrub litter inputs or translocation of nutrients from intercanopy areas to zones beneath shrub canopies via extensive lateral root, aeolian, fluvial, or animal transport processes, can thus transform grasslands with a relatively homogenous distribution of SOC to shrublands with a very heterogeneous distribution of SOC (e.g., Schlesinger *et al.*, 1990; Hibbard *et al.*, 2001; Ravi *et al.*, 2007; Okin *et al.*, 2009). The contrast in SOC between shrub islands and the herbaceous zones that separate them increases with time of shrub occupation (Wheeler *et al.*, 2007; Throop & Archer, 2008; Boutton *et al.*, 2009) and diminishes when shrubs die (McClaran *et al.*, 2008). Characterizing landscape-scale changes in SOC pools within the context of woody plant encroachment thus presents numerous challenges. A key first step in addressing this challenge and improving our estimates of land cover change effects on SOC pools is to understand their spatial uncertainties.

Spatial uncertainty of SOC and sampling designs

Spatial uncertainty of ecosystem properties is related to their underlying spatial patterns, and its elucidation is influenced by the number of samples, sampling designs, and the spatial distribution of samples (e.g., Buscaglia & Varco, 2003; Conant *et al.*, 2003). SIS has been effective in quantifying spatial uncertainty of ecological variables (Rossi *et al.*, 1993; Goovaerts, 2001; Juang *et al.*, 2004; Zhao *et al.*, 2005); and in this case illustrated how the spatial uncertainty of SOC estimates

increase when woody plant communities develop on grassland. It should be noted that the results from this work reflect shrub communities that are relatively young (<100-year-old) from a SOC perspective (Archer *et al.*, 2004; Bai *et al.*, 2009); and dynamic simulations suggest it may take another 100 or more years to reach a carbon steady state (Hibbard *et al.*, 2003). During that time SOC heterogeneity may peak (if it has not already done so) and subsequently become more uniform.

Although shrub cluster landscape elements had a comparable or lower degree of spatial uncertainty in SOC estimates compared with grasslands and groves (Fig. 4b), SOC estimation errors in the complete random sampling design were considerably and consistently higher for shrub clusters (Fig. 5a). There are two probable explanations for this discrepancy. Firstly, our random sampling design and intensity may not have been sufficient to adequately capture the spatial uncertainty of SOC in shrub clusters. Shrub clusters are relatively small (ca. 10–65 m^2 on the sampled landscape) and only two random samples were collected per 100 m^2 cell. Simulations based on these relatively few random samples may therefore have failed to capture the spatial variability of SOC in shrub clusters as few or no random sample fell within them (Fig. 1a). Secondly, SOC in shrub clusters declines steadily along a gradient extending from the center of clusters (where shrubs have been present and influencing soils for the longest time) to their canopy edge (where shrub influences on SOC would have been relatively recent) (S. R. Archer *et al.*, in preparation). Similar gradients of SOC have also been described for other shrub-invaded grasslands (Throop & Archer, 2008). Complete random sampling performed poorly in this setting compare to transect sampling designs taking the spatial pattern into consideration (Table 1).

Our analyses also revealed that sampling one shrub cluster with two or three samples was more effective at reducing estimation error than taking single samples from multiple clusters. Furthermore, sampling more clusters with sufficient sample intensity and spatial arrangement yielded better results than random sampling at higher densities in fewer clusters (Table 1). More widespread and smaller sampling blocks would also generate more power in testing significance when spatial autocorrelation is present (Legendre *et al.*, 2004). These results illustrate the need to carefully consider spatial patterns when designing sampling regimes to estimate SOC and other soil attributes in landscapes characterized by mixtures of contrasting plant communities.

The high spatial uncertainty of SOC in groves (Fig. 4b) potentially reflects several interacting factors. First, SOC is known to be strongly influenced by

root and surface litter biomass (Boutton *et al.*, 1998, 2009; Hibbard *et al.*, 2001; Rasse *et al.*, 2005); and mean ($\pm$ SE) litter and root biomass were much higher in groves than in grassland (litter: groves $542 \pm 59\,\mathrm{g\,m^{-2}}$, grasslands $30 \pm 3\,\mathrm{g\,m^{-2}}$; roots, 0–15 cm depth: groves $696 \pm 68\,\mathrm{g\,m^{-2}}$, grasslands $195 \pm 17\,\mathrm{g\,m^{-2}}$) (Liu *et al.*, 2009). In turn, SOC density was higher and more variable in groves (CV = 0.35) than in grassland (CV = 0.15), which makes estimation of SOC less accurate (more uncertain) in these landscape elements. Second, faunal disturbances, well-known to locally impact soils (e.g., Greene & Reynard, 1932; Titus *et al.*, 2002; Whitford, 2002), were common in groves. However, there are virtually no such disturbances in grassland and little in clusters. Third, groves have more complex and variable vegetation structure than that in clusters and grassland (Archer, 1995), which may also affect the spatial distribution of root and litter mass.

Sampling strategies for complex landscapes

Given the widespread occurrence of shrub encroachment into grasslands around the world, there is considerable interest in quantifying the potential impacts of woody expansion on global carbon pools (Houghton & Hackler, 2000; CCSP, 2007; Asner & Archer, 2009). However, detection of change in SOC pools resulting from vegetation cover change is challenging due to complications caused by spatial variability, land use legacies, erosion, and small signal to noise ratios (Conant *et al.*, 2003). Results from this study are consistent with other studies showing that woody plant invasion into grassland ecosystems increases the spatial heterogeneity of soil properties (Archer, 1995; Schlesinger *et al.*, 1996; Bekele & Hudnall, 2006). This elevates the challenge of accurately quantifying changes in SOC pools. Studies of woody plant encroachment effects on SOC pools to date are typically quite limited in their sample density and distribution and this may partially account for the wide divergence of perspectives (ranging from increases to decreases to no change) as to how this vegetation change has affected SOC (Wessman *et al.*, 2004). Spatial autocorrelation in ecological variables affects classic tests of statistical significance (Legendre *et al.*, 2002) and should be considered in the design of experiments and field surveys (Dutilleul, 1993; Cole *et al.*, 2001; Hewitt *et al.*, 2007). Results of this study illustrate the need to design efficient point sampling strategies for quantifying SOC storage in complex landscapes where spatial variability must be considered and quantified. Our sampling experiments provide a basis and some guidelines in developing such sampling strategies.

Nested (Bellehumeur & Legendre, 1998) or systematic grid point or grid cell sampling (Buscaglia & Varco, 2003) was suitable for quantifying spatial structures of soil properties in our grassland system undergoing shrub encroachment. Our results indicated that random sampling, stratified within a systematic grid, worked well in capturing the overall spatial pattern of SOC on a landscape comprised of multiple vegetation elements. However, results of the SIS simulations revealed marked differences in spatial variability and uncertainty among vegetation elements. As a result, accurate estimates of SOC density can be achieved by systematic or nested sampling in some settings, whereas sampling stratified by vegetation elements with differential sampling intensities is required on others. Sampling designs demonstrated the importance of structured sampling within vegetation elements characterized by strong within-unit spatial patterns (shrub clusters) where SOC density can not be reliably estimated with a random design. Thus, when multiscale spatial patterns occur, incorporation of spatial structures at different scales into experimental and sampling designs is essential (e. g., Noda, 2004). Our results suggested an approach combining stratified random sampling with unequal point densities and transect sampling of landscape elements exhibiting strong internal gradients yield best estimate of SOC density while capturing the spatial pattern of SOC at both broad and fine scales. Decisions on how to allocate sampling effort among landscape elements could be informed by preliminary surveys.

The sample distribution and intensity needed to account for spatial pattern and uncertainty (Hewitt *et al.*, 2007) must be balanced against the cost of sample collection, preparation, and analyses. Several general recommendations can be offered to achieve this balance in arid and semi-arid landscapes where complex spatial pattern exist for a variable(s) of interest: (1) conduct a preliminary survey to explore spatial structures within specific vegetation types and estimate the appropriate sampling intensity for each, (2) use (1) to develop a stratified random sampling protocol with unequal densities in different landscape elements according to their variability or uncertainty, (3) use structured sampling (e.g. transects) within landscape elements with strong spatial patterns (shrub clusters in our case).

Woody plant invasion of grassland ecosystems is a geographically extensive phenomenon that has the potential to significantly alter global biogeochemical cycles (Schlesinger *et al.*, 1990; Houghton *et al.*, 1999; Asner *et al.*, 2004; Knapp *et al.*, 2008). Evaluation of the effects of this land cover change on regional or global carbon cycles requires the extrapolation of fine scale, local inventories of SOC storage to broad scales. Spatial

heterogeneity of soil properties is amplified as woody plant abundance increases in grasslands, thus making it more difficult to accurately estimate the impact of this vegetation change on SOC (Fig. 5c). This study demonstrates how spatial pattern and uncertainty of SOC influence the accuracy of its estimation in heterogeneous landscapes characterized by cooccurring plant life forms. The guidelines suggested herein will improve the efficacy of sampling aimed at quantifying edaphic responses to changes in land cover and land use.

Acknowledgements

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