Soil organic carbon in an old-growth temperate forest: Spatial pattern, determinants and bias in its quantification

Zuoqiang Yuan a, Antonio Gazol b, Fei Lin a,c, Ji Ye a,c, Shuai Shi a, Xugao Wang a, Miao Wang a, Zhanqing Hao a,⁎

a State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, PR China
b Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, Tartu 51005, Estonia
c Graduate University of Chinese Academy of Science, Beijing 100049, PR China

⁎ Corresponding author. Tel.: +86 24 8397 0209; fax: +86 24 8397 0300. E-mail address: hzq@iae.ac.cn (Z. Hao).

1. Introduction

Soil organic carbon (SOC) is one of the largest carbon reservoirs of the earth’s surface and plays an important role in the global carbon cycle (Guo and Gifford, 2002; Lal, 2004; Piao et al., 2009). In terrestrial ecosystems, SOC stock is nearly three times the vegetation biomass carbon pool, and about twice the amount of carbon stored in the atmosphere (Schlesinger, 1990). Importantly, 40% of the belowground and 80% of the aboveground carbons are held by forests (Dixon et al., 1994). Old growth forests in particular act as century-scale global carbon sinks, helping to offset increasing atmospheric CO₂ concentrations (Luyssaert et al., 2008; Zhou et al., 2006). The sequestered CO₂ and bias in its quantification

Recent efforts have been carried out at different scales (global, national and local) for SOC stock estimation in forest ecosystems (Grimm et al., 2008; Luyssaert et al., 2008; Zhang et al., 2006, 2008; Zhou et al., 2006). However, quantifying the SOC patterns is complicated since different factors can influence its spatial variation (Conen et al., 2003; Muukkonen et al., 2009). Furthermore, the spatial distribution of plant-available mineral nutrients in forest soils is often highly heterogeneous over time and space (Liski, 1995; Wilding et al., 2000). Many studies have reported that SOC patterns are influenced by topographic heterogeneity (Venteris et al., 2004; Yoo et al., 2006), soil bedrock composition (Jobbágy and Jackson, 2000), climatic conditions (Davidson and Janssens, 2006; Leirós et al., 1999) and tree species composition (Li and Han, 2008; Liski, 1995; Resh et al., 2002). However, tree species composition is interrelated with soil heterogeneity and topography (Augusto et al., 2003), as well as with litter quality and decomposition rates (Polyakova and Billor, 2007; Sariyildiz, 2008; Yang et al., 2005) but few studies have tried to discover the relative influence of these different factors on SOC variation (e.g. Jobbágy and Jackson, 2000).

Characterizing local SOC patterns requires the collection of sufficient soil samples to properly represent the small-scale spatial variation in SOC (Muukkonen et al., 2009; Yan and Cai, 2008). Unfortunately, although several studies have tried to examine how sampling density...
influences the reliability of SOC estimation (Mäkipää et al., 2008; Muukkonen et al., 2009; Yan and Cai, 2008), a consensus does not exists about the minimum number of samples needed to accurately represent SOC patterns. Soil variation displays a strong small-scale heterogeneity (Gallardo, 2003), and thus extensive and intensive sampling designs can ensure a proper representation (Grunwald and Reddy, 2008; Heim et al., 2009; Kerry and Oliver, 2007; Wilding et al., 2000). Unbiased estimates of local variation of SOC are of crucial importance because these values can be potentially used for large-scale studies (e.g. Conen et al., 2003; Luysma et al., 2008; Zhou et al., 2006) and predicting the global climate change (Davidson and Janssens, 2006). Therefore, investigating how sampling design and intensity affects SOC pattern estimation in a given region is of great importance from a global perspective.

The objective of this study was to determine the spatial pattern, determinants of the pattern and potential bias in SOC quantification in a 25 ha plot in an old-growth temperate forest of China. Our three specific objectives are (1) to accurately estimate the spatial variation of SOC density at the surface layer (0–10 cm) in this area; (2) to partition the spatial variation of SOC, determining the relative contribution of soil properties, topography and canopy composition and spatial structure; and (3) to evaluate the information loss associated with underlying spatial autocorrelation and spatial patterns along trajectories of sparser sampling density.

2. Materials and methods

2.1. Study site description

The study site is located in Changbai Mountain Natural Reserve (42°23′N, 128°05′E) in northeastern China. This region is the largest protected temperate forest in the world; it was established in 1960 and joined the World Biosphere Reserve Network under the Man and the Biosphere Project in 1980 (Stone, 2006). A survey site of 500 m × 500 m was established in the broad-leaved and conifer mixed forest (42°23′N, 128°05′E) in the summer of 2004 (Fig. 1). The soil is classified as dark brown forest (mollisol according to U.S. Soil Taxonomy Series, 1999), which formed in granite and basalt. Canopy composition is dominated by species such as Pinus koraiensis, Tilia amurensis, Quercus mongolica, Fraxinus mandshurica, Ulmus japonica, Ulmus laciniata, and Populus ussuriensis. For a detailed description of the plot see Hao et al. (2007).

2.2. Data collection

We quantified the topographic heterogeneity, tree species composition and soil variability in the survey site. To measure the different topographic variables, the study site was divided into a grid of 625 20 × 20 m quadrats and we measured elevation (with values from 793.1 m to 809.4 m), convexity (with values from −1.5 m to 1.3 m), and slope (with values from 0.3° to 16.1°). The elevation of each 20 × 20 m quadrat was obtained from the mean elevation at the four corners of a quadrat using Electronic Distance Measuring Device (EDMD) (Condit, 1998). This method is an intensive procedure that requires four people and measures elevation accurately. Basically, the method involves the use of a metric tape, a compass and sighting telescope and estimates elevation by triangulation of horizontal and linear distance. Following Harms et al. (2001), convexity of a quadrat was computed as the elevation of the focal quadrat minus the mean elevation of the eight surrounding quadrats. For the edge quadrats, convexity was taken as the elevation of the center points minus the mean elevation of the four corners. Each quadrat was divided into four triangular planes, each formed by joining three corners of the quadrat. Slope was the mean angular deviation from horizontal of each of the four triangular planes formed by connecting three of its corners.

All trees with diameter at breast height (DBH) ≥ 1 cm were tagged, identified to species level, measured, and their geographic coordinates were recorded. Yuan et al. (2010) showed that leaf litter production in this forest has a strong relationship with tree basal area and that six species (P. koraiensis, T. amurensis, Q. mongolica, F. mandshurica, U. japonica, U. laciniata and P. ussuriensis) accounted for more than 80% of litter production. Therefore, only these species were considered in the analyses. We calculated the basal area (m²/ha) of each species in each plot. After that, the basal area of the five deciduous species and that of P. koraiensis in each plot was divided by the number of individuals per plot to get a relative measure. Therefore, the relative basal area of dominate (five species) deciduous trees and P. koraiensis as two measurements were used in the analyses.

During 1–4 October 2007, we sampled soils by using a regular grid of points every 30 m (John et al., 2007). In order to capture variations in SOC at finer scales, two additional sample points (2, 5, or 15 m) were selected in a randomly assigned cardinal direction (N, NE, E, SE, etc.) from the base point (Fig. 1) (John et al., 2007; Yavitt et al., 2009). At each sample location, large debris was first removed and three subsamples (0.2 m around the sample location) were obtained to a depth of 10 cm using a 5-cm diameter cylinder. Then, these three sub samples were mixed thoroughly to ensure that the sample was representative of the surrounding area. Five sample points could not be sampled because of large roots, tree trunk, or stumps, we thus obtained a total of 967 soil samples in the 25 ha plot (Fig. 1). Finally, the volumetric soil water content (%) was measured at each sample location at a depth of 20 cm using a Time Domain Reflectometers (TDR) probe (Yuan et al., 2011).

For each soil sample, soil pH in water (1:1 soil: solution ratio) was determined using a Beckman glass electrode. Soil organic carbon content was determined by a titrimetric method using a strong oxidizing agent (K₂C₂O₇ in the presence of H₂SO₄ (Nelson and Sommers, 1975). Soil bulk density was determined by a core method. At each

![Fig. 1. The location, contour map and the soil sample distribution pattern of the 25-ha (500 × 500 m) Changbai temperate forest plot.](Image)
soil sample location, a 5.4 cm diameter cutter was inserted into the soil and the surrounding soil was removed using a trowel, after which the intact soil ped was extracted. The length (and hence volume) of the ped was determined, and the soil was transferred to an aluminum tray and dried to constant weight at 105 °C.

In this study, there is only one horizon (0–10 cm) for each soil sample location, so the calculation of SOC density \( (C_d) \) (kg m\(^{-2}\)) is based on the following formula:

\[
C_d = (1-\theta_i) \times \rho_i \times 0.58 \times c_i
\]  

(1)

where \( \theta_i \) is gravel (>2 mm) content at sample location \( i \) (%), \( \rho_i \) is soil bulk density in the surface layer (g cm\(^{-3}\)), and \( c_i \) is organic matter content at sample location \( i \) (g kg\(^{-1}\)).

2.3. Data analysis

Different methods were used to answer the questions stated in the introduction. For question 1, geostatistical analysis was used to analyze spatial variation of SOC density. Since SOC densities had a skewed distribution (Appendix A), the Box–Cox transformation (Gallardo and Paramá, 2007) was applied to obtain a normal distribution of the variable. After that, a semivariogram was calculated for SOC density to determine the degree of spatial dependence among sample locations, and the appropriate model function was fitted to the semivariogram (Govaerts, 1997).

Envelopes for empirical semivariograms were computed by permutation (99 simulations) of the data values on the spatial location. For each of the 99 simulations, data values were randomly allocated to each simulation using the same lags as for the empirical semivariogram. Semivariogram estimates above or below envelope boundaries indicate the presence of a significant spatial pattern at the spatial scale (Gallardo and Paramá, 2007). The data were converted back to original units prior to kriging (Hengl et al., 2004). Predicted maps of SOC density were obtained for 20×20 m blocks (i.e., coincident with the topographic and canopy composition data) using ordinary (block) kriging. The same procedure was applied to create maps of the spatial pattern of soil moisture (Mean: 40.1%, Range: 28.7–57.6%) and soil pH (Mean: 5.45, Range: 5.13–5.87) (Yuan et al., 2011).

For question 2, in order to assess the relative contribution of environmental and spatial variables to the total observed variation in SOC density, we used variation partitioning with multiple regression (Borcard et al., 1992). Specifically, we estimated the fraction of variation in SOC explained by the topography (elevation, slope and convexity), canopy composition (relative basal area of five deciduous tree species and that of P. koraensis) and spatial variables. The basic idea of variation partitioning consists in applying several multiple and partial linear regressions in order to discover the shared and pure fraction of each group of explanatory factors (Borcard et al., 1992). Due to the inherent spatial autocorrelation of SOC patterns and the potential interrelation between topography and canopy composition, variation partitioning is a perfectly suited method to separate the shared and pure explained fractions of each group of factors.

Principal Coordinates of Neighbor Matrices (PCNM) were used to introduce space as an explanatory variable to assess the spatial variation within 20×20 m quadrats. This approach is dependent on the calculation of a principal coordinate analysis (PCoA), which generates a set of orthogonal sine waves constructed from a truncated matrix of Euclidean distances among sampling units using the x and y coordinates from the center of each quadrant (Borcard and Legendre, 2002). These variables can be used as multi-scale predictors in canonical or regression analyses, since they represent the spatial structure of the study site at different spatial scales.

The analytical approach was composed of variation partitioning analyses in two steps. Firstly, we analyzed SOC density against the topographic, canopy composition and spatial variables. After that, SOC density was regressed against soil moisture and pH to remove the influence of these soil variables, and the residual variation of this regression was obtained. Finally, a variation partitioning of the residual variation in SOC density was performed using as predictors the topographic, canopy composition and spatial variables. We applied this approach due the potential interlinked effect of topography and canopy composition on soil conditions such as moisture and pH (Yuan et al., 2012). Therefore, we are separating the direct effect that topography and canopy composition can have on SOC from the indirect one (via modifying the soil conditions).

A variable selection was applied prior to the variation partitioning analysis to use only those variables that significantly \( (P \leq 0.05 \text{ after } 999 \text{ random permutations}) \) influence SOC density variations (Blanchet et al., 2008). Before performing PCNM spatial variable selection, data were detrended to account for the influence of topographic gradients. Similarly, to avoid the possible inflation of the model performance, and thus the increase of Type I errors, the selection was also constrained by the adjusted R\(^2\) value obtained the PCNM as explanatory factors and SOC as response in a multiple regression analysis Blanchet et al. (2008).

For question 3, to evaluate the effect of using a reduced data set to show the underlying spatial variation and distribution of SOC density, we followed the two criteria proposed by Grunwald and Reddy (2008): (i) random selection of soil samples might be biased toward specific geographic regions, resulting in clumped and clustered subsets; and (ii) a one-time selection of soil samples from the total data set ignores the impact of sampling fluctuations on prediction performances. Thus, subsets of 870, 720, 570, 420, 270, and 120 sampling points were randomly selected from the total data set \( (n=967) \) using the bootstrap re-sampling method; the six subset sampling densities were 348, 288, 228, 168, 108 and 48 samples ha\(^{-1}\) respectively.

The random selection procedure to identify each subset was repeated 999 times to account for sampling fluctuations (Grunwald and Reddy, 2008). For each selected subset, the semivariograms of SOC density were computed and kriging maps were obtained in the same way as for the dense observation model \( (n=967) \) using ordinary kriging. The standard deviations of the semivariogram parameters \( (nugget, sill \text{ and range}) \) were calculated to represent the sampling fluctuations. A set of 97 sample points was randomly selected as an independent validation data set to evaluate model performance for the different subsets using 999 iterations. Model performance was evaluated using mean prediction error \( (\text{MPE}) \) and the root mean square error (RMSE) (Zar, 1999). The MPE was used for assessing the degree of bias in the estimates and it is calculated with Eq. (2):

\[
\text{MPE} = \frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)
\]

(2)

where \( n \) is the number of values in the valid data set, \( y_i \) is the original data, and \( \hat{y}_i \) are the predicted values.

The RMSE provides a measure of the error size that it is sensitive to outliers. RMSE is calculated with Eq. (3):

\[
\text{RMSE} = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2}
\]

(3)

Statistical and geostatistical analyses were performed in “geoR” package of R statistical language (R Development Core Team, 2010). To create the PCNM variables and perform the forward selection we used the “PCNM” (Blanchet et al., 2008) and “packfor” (Dray, 2005) packages respectively; Variation partitioning analyses were performed using the “vegan” package (Oksanen et al., 2010).
3. Results

SOC density ranged widely from 2.31 to 37.7 kg m$^{-2}$ with a mean of 14.76 kg m$^{-2}$ in this old-growth temperate forest in the Changbai plot. After removing the second-order trend in SOC density, the empirical semivariogram was fitted with a spherical model. SOC density was strongly spatially autocorrelated (nugget/sill=0.238) at the studied spatial scales, since the first two points of the semivariogram lie below the lower boundary of the envelope (P<0.01) (Fig. 2a). The result of the kriging map is given in Fig. 2b. The predicted value of SOC density ranged from 5.71 to 26.8 kg m$^{-2}$ among the 625 20×20 m quadrats, but most of quadrats were within 15–20 kg m$^{-2}$. High SOC density values were found in the western part of the plot where quadrats had low elevation and slope (Fig. 2b).

The results of the variation partitioning of SOC density showed that 73% of the variation was explained by the canopy, topographic and spatial variables selected (Fig. 3). Specifically, 18% of the variation was explained by the canopy composition variables selected (relative basal area of the five deciduous trees and that of P. koraiensis), 17% by topography (elevation and slope) and 73% by the spatial variable (the x- and y-UTM coordinates and 12 PCNM’s). The results also showed that the total amount of fraction explained was completely spatially structured, i.e. the pure effect of the canopy composition and topographic variables was zero (Fig. 3). There also existed a negative correlation between basal area, five deciduous species and P. koraiensis, and SOC density (unstandardized partial regression coefficients (b) = –41.31; −0.26, respectively). Conversely, elevation (b=0.29) and slope (b=3.95) were positively related with SOC variation. The regression of SOC variations against soil moisture and pH showed that they explained 51% of the variation in SOC, and the two variables were positively related with SOC (b=0.23; 42.17, respectively). The analysis of the residual variation of SOC, after removing the influence of the soil variation, indicated that the canopy and topographic variables had significant influences on SOC variation (Fig. 3). However, only the relative basal area of the five deciduous species (b = –12.09) and slope (b=0.12) were significantly related with SOC. These variables in combination with the spatial variables explained 27% of the residual variation in SOC.

The estimated spatial variation of SOC density became weaker with reduced sampling densities (n=870–120), as indicated by increasing nugget/sill ratio (Table 1). The spatial ranges for SOC density for different sampling densities changed dramatically from 33 to 60 m (Table 1). Although there is no significant difference in the mean SOC density

![Fig. 2. Semivariograms (a) of Box–Cox transformed SOC density, and the kriged map of SOC density (b) using the 967 soil samples. Dashed lines in panel (a) show the maximum and minimum values found in 99 permutations.](image)

![Fig. 3. Variation partitioning results of Soil Organic Carbon (SOC) against the topographic, canopy and spatial selected variables, before (a) and after (b) removing the influence of the soil moisture and soil pH. Two canopy (relative basal area of the five deciduous trees and that of P. koraiensis), two topographic (elevation and slope) and 16 spatial variables (x-UTM, y-UTM and PCNM’s 5, 9, 16, 31, 47, 77, 80, 140, 148, 172, 187, 204, 233 and 254) were selected and used in the analyses. Values out of the path diagram indicate the fraction of variance explained by each set of variables (i.e. canopy, topography and space) without removing their joint effect with the other variables. Values inside of the path diagram indicate the fraction of variance explained by each set of variables when removing their joint effect with the other sets of variables. Values lower than 0 or negative variances explained are not shown. Significant values (P<0.05) after 9999 unrestricted permutations are indicated with *](image)
value of the six subsets (Table 1), the standard error of the SOC density among the 625 20 × 20 m quadrats changed from 2.42 to 0.67 depending on sampling intensity (Fig. 4). The maps of the spatial distribution patterns of SOC density tended to be more homogeneous with reduced sampling densities (Fig. 4). As expected, the validation exhibited larger MPE and RMSE values with decreasing sampling intensity \((n = 870–120)\) for SOC density (Fig. 5); The RMSE of SOC density increased by 51.7% for the reduced sample sets \((n = 120)\) when compared with the dense set \((n = 870)\).

4. Discussion

The results indicate that SOC density displays a strong spatial variation in the old-growth temperate forest under study. Moreover, we demonstrated that the spatial variation of SOC density is influenced by the soil conditions (Liski, 1995), the topographic heterogeneity (Venteris et al., 2004) and canopy composition and structure (Luyssaer et al., 2008). As has been suggested by other authors, topography, soil properties and vegetation composition are interrelated and have different implications on SOC variations (Jobbágy and Jackson, 2000). Finally, our results show that the accurate quantification of the spatial variation of SOC requires a large number of samples, as has been reported for other soil properties (Gallardo, 2003). Quantification, estimation and explanation of SOC density in old-growth forests have important implications for future climate change scenarios (Zhou et al., 2006), but a sampling with low intensity or without considering spatial variation can lead to biased results and thus to incorrect conclusions.

SOC density in the Changbai old-growth temperate forest is relatively high among global temperate forests. For example, Jobbágy and Jackson (2000) estimated SOC density around 7.4 kg m\(^{-2}\) for global temperate forests, which is half the value found here. These differences between local and regional estimations reinforce our theory that unbiased local estimates of SOC can be obtained only with local studies. Because the highly cited study of Jobbágy and Jackson (2000) was based on soil

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Sample model ((n))</th>
<th>Nugget ((C_0))</th>
<th>Sill ((C))</th>
<th>Nugget/sill ((C_0/C))</th>
<th>Range ((m))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC density ((kg \ m^{-2}))</td>
<td>967</td>
<td>0.101</td>
<td>0.422</td>
<td>0.238</td>
<td>18.5</td>
</tr>
<tr>
<td>870</td>
<td>0.109 (0.044)</td>
<td>0.424 (0.007)</td>
<td>0.257</td>
<td>33.6 (2.19)</td>
<td></td>
</tr>
<tr>
<td>720</td>
<td>0.124 (0.072)</td>
<td>0.421 (0.014)</td>
<td>0.294</td>
<td>35.5 (5.72)</td>
<td></td>
</tr>
<tr>
<td>570</td>
<td>0.142 (0.116)</td>
<td>0.422 (0.019)</td>
<td>0.337</td>
<td>38.1 (9.53)</td>
<td></td>
</tr>
<tr>
<td>420</td>
<td>0.181 (0.130)</td>
<td>0.418 (0.024)</td>
<td>0.432</td>
<td>49.1 (31.2)</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>0.175 (0.151)</td>
<td>0.436 (0.041)</td>
<td>0.402</td>
<td>114.9 (63.6)</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.209 (0.161)</td>
<td>0.424 (0.060)</td>
<td>0.492</td>
<td>59.3 (65.3)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Mean and the standard error of SOC density computed for subsets of 870, 720, 570, 420, 270 and 120 samples with 999 iterations.
profiles stored in global databases, this issue of bias also important implications from a global perspective. However, there exists strong variation in SOC density estimations even within the Changbai Mountain forest. For example, Yang et al. (2005) estimated a mean SOC density of 12.5 kg m$^{-2}$ for this ecosystem, but Zhang et al. (2006) estimated mean SOC density at 6 times that amount (73.0 kg m$^{-2}$). Variations will arise for the different methods used to estimate SOC density, but they indicate that a proper estimation of SOC density is not an easy issue. Due to the intensive and extensive samplings used in this study, we think that our results are more reliable than those reported previously, and that an unbiased estimation of SOC at given scale cannot be achieved without considering its strong spatial variation (Muukkonen et al., 2009; Yan and Cai, 2008). However, the sampling intensity reported in this study is prohibitive for global and large-scale studies. For those situations, and in light of our results, sampling schemes should be designed to include as much topographic and vegetation composition heterogeneity since those factors strongly determine SOC patterns.

Forest trees sequester atmospheric CO$_2$, which becomes fixed in living tissues or stored as organic matter in the forest floor (Luysaert et al., 2008; Zhou et al., 2006). The dynamics of organic matter can be affected by local environmental conditions in the sampling sites (Park et al., 2001; Resh et al., 2002; Venteris et al., 2004). Although different studies have addressed the influence of different factors on SOC variation (e.g. Luizão et al., 2004) the present study is, to our knowledge, the first to use variation partitioning with spatial variables to assess the variability in SOC spatial pattern. In our study we suggest that soil moisture and pH are the main determinants of SOC patterns, probably because they determine organic matter decomposition rates (Davidson and Janssens, 2006; Leirós et al., 1999) and thus SOC accumulation in soils. The two-step variation partitioning also demonstrates that the fractions explained by the topographic and soil variations were partially shared, indicating that soil variability is dependent on elevation gradients and slope variations (Yoo et al., 2006). It can be argued that the decision of which factors, i.e., topography and soil conditions, have direct and indirect influences on SOC was arbitrary. However, it was based on previous knowledge of the system indicating the potential influence of topography and canopy composition on soil conditions (Yuan et al., 2012) and on sound ecological knowledge (Yoo et al., 2006).

Our study also shows that the canopy composition and spatial structure influences the spatial pattern of SOC density. Although old-growth forests can act as carbon sinks (Luysaert et al., 2008; Zhou et al., 2006), it is assumed that their ability to accumulate carbon in soils decrease over time. Our results indicate that in those places where the relative basal area of trees is higher (i.e. trees are larger) SOC density decreases. SOC accumulation is higher in areas with low tree density, and low elevation and slope, probably because those factors favor organic matter decomposition (Li and Han, 2008; Muukkonen et al., 2009). There were several mechanisms implicated in the creation of this pattern. It has been argued that the litterfall and its spatial distribution is one of the key factors influencing small-scale spatial variation in soil organic carbon (Liski, 1995). Several studies have reported that litter accumulation and decomposition is dependent on tree species composition, litter quality (Li and Han, 2008; Polyakova and Billor, 2007) and light and temperature conditions (Sariyildiz, 2008). Therefore, the different canopy composition and structure can influence C cycles and its accumulation on the forest floor (Resh et al., 2002). Although we have not found differences between places dominated by different tree species, in contrast to other studies (Li and Han, 2008), our results show that the structure of the trees that compose the canopy influences SOC patterns. Moreover, it can be shown that the topographic heterogeneity, soil variability and canopy composition have shared effects on SOC variation. Augusto et al. (2003) showed that tree species composition can alter soil conditions, that are also important for litter decomposition (Sariyildiz, 2008), and thus SOC stock. Therefore, it can be interpreted that SOC accumulation is dependent on different factors (Jobbágy and Jackson, 2000) and to properly assess the relative contribution of these factors are help to better interpretation of the underlying processes creating those patterns.

Previous studies have suggested that sampling design (e.g., regular, random, nested) and sampling density have an impact on the estimated spatial variation and the mapped distribution pattern of soil variability (Grunwald and Reddy, 2008; Heim et al., 2009; Kerry and Oliver, 2007). Here we demonstrated that an unaligned grid design method with a high number of samples provide an unbiased estimation of SOC density and a proper representation of its spatial pattern. The reduction of the sampling intensity clearly influences the spatial pattern since most of the small-scale soil spatial heterogeneity is lost (Gallardo, 2003). Although the results show that a sparse dataset ($n=120$ samples) provide a reliable estimation of the mean SOC density, this estimation is strongly dependent on the location of the samples (Wilding et al., 2000). For example, the maps of SOC variation at different sampling densities clearly shows that due to the existence of strong linear gradients, different results can be obtained sampling only the eastern or western part of the plot. Therefore, an exhaustive sampling design is important not only to understand the local pattern, but also to provide an unbiased estimation of SOC.

5. Conclusions

Quantification, estimation and explanation of SOC density in old-growth forests have important implications for future climate
change scenarios (Grimm et al., 2008; Piao et al., 2009). Recent studies have demonstrated that old-growth forests are important SOC reservoirs in a world-wide perspective (Luysaert et al., 2008; Zhou et al., 2006) claiming to its study and conservancy. However, to understand fully the importance of SOC in old-growth forests an unbiased estimation of SOC and the factors that influence it in local-scale studies is of major importance. The results provided in this study clearly demonstrate that also within the same ecosystem strong variation exists in SOC estimation, and thus a spatially extensive and intensive design is needed to properly represent SOC variation. The small-scale spatial heterogeneity of soil conditions such as water, pH and temperature, the inherent topographic variation and the heterogeneous composition of the canopy are interrelated and together influence SOC patterns. Variation partitioning with the use of spatial variables is a powerful tool to understand SOC variations and address the relative influence of these contrasting factors. A better understanding of SOC variation at a world scale is needed and thus global studies (e.g. Jobbágy and Jackson, 2000; Luysaert et al., 2008; Zhou et al., 2006) are of great importance. However, since the sampling density and the location of the samples inside a study region, can influence SOC estimation, so local studies are also needed to support unbiased estimations of SOC stock and thus provide the basis for large-scale studies.

Acknowledgments

This study is sponsored by the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-EW-401, KSCX2-EW-Z-5), National Natural Science Foundation of China (31011120470 and 31061160188). Antonio Gazol is sponsored by ERMOS programme grant 14 (co-funded by Marie Curie Actions). We thank Dr. Fangliang He, Benjamin Turner, Tom McRae, Ryan Chisholm, Pierre Legendre, Edith Bai, and Wangming Zhou and two anonymous reviewers for their valuable suggestions to improve the manuscript. We also thank Buhang Li, Xuejiao Bai, Yuqiang Zhao, Xingding Xio, Zhaochen Zhang, and Baizhong Song for their assistance with the data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2012.11.008.

References