Spatial variability of soil organic carbon in a tropical montane landscape: Associations between soil organic carbon and land use, soil properties, vegetation and topography vary across plot to landscape scales.

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Abstract. Presently, the lack of data on soil organic carbon (SOC) stocks in relation to land-use types and biophysical characteristics prevents reliable estimates of ecosystem carbon stocks in montane landscapes of mainland SE Asia. Our study, conducted in a 10,000-ha landscape in Xishuangbanna, SW China, aimed at assessing the spatial variability in SOC concentrations and stocks, and the relationships of SOC with land-use types, soil properties, vegetation characteristics and topographical attributes at three spatial scales: (1) land-use types within a landscape (10,000 ha) (2) sampling plots (one ha) nested within land-use types (plot distances ranging between 0.5 - 12 km) and (3) subplots (10-m radius) nested within sampling plots. We sampled 27 one-ha plots including 10 plots in mature forests, 11 plots in regenerating or highly disturbed forests, and six plots in open land including tea plantations and grasslands. We used a sampling design with a hierarchical structure. The landscape was first classified according to land-use types. Within each land-use type, sampling plots were randomly selected, and within each plot we sampled within nine subplots. SOC concentrations and stocks did not differ significantly across the four land-use types. However, within the open-land category, SOC concentrations and stocks in grasslands were higher than in tea plantations ($P<0.01$ for 0-0.15-m, $P=0.05$ for 0.15-0.30-m, $P=0.06$ for 0-0.9-m depth). The SOC stocks to a depth of 0.9 m were 177.6 ± 19.6 (SE) Mg C ha^{-1} in tea plantations, 199.5 ± 14.8 Mg C ha^{-1} in regenerating or highly disturbed forests, 228.6 ± 19.7 Mg C ha^{-1} in mature forests, and 236.2 ± 13.7 Mg C ha^{-1} in grasslands. In this montane landscape, variability within plots accounted for more than 50% of the overall variance in SOC stocks to a depth of 0.9 m, and the topsoil SOC concentrations. The relationships of SOC concentrations and stocks with land-use types, soil properties, vegetation characteristics and topographical attributes varied across spatial scales. Variability in SOC within plots was determined by litter layer carbon stocks ($P<0.01$ for 0-0.15-m and $P=0.03$ for 0.15-0.30-m and 0-0.9-m depth) and slope ($P\leq0.01$ for 0-0.15-m, 0.15-0.30-m and 0-0.9-m depth) in open land, and by litter layer carbon stocks ($P<0.001$ for 0-0.15-m, 0.15-0.30-m and 0-0.9-m depth) and tree basal area ($P<0.001$ for 0-0.15-m and $P=0.01$ for 0-0.9-m depth) in forests. Variability in SOC among plots in open land was related to the differences in SOC concentrations and stocks between grasslands and tea plantations. In forests, the variability in SOC among plots was associated with elevation ($P<0.01$ for 0-0.15-m and $P=0.09$ for 0-0.9-m depth). The scale-dependent relationships between SOC and its controlling factors demonstrate that studies that aim to investigate the land-use effects on SOC need an appropriate sampling design reflecting the controlling factors of SOC so that land-use effects will not be masked by the variability between and within sampling plots.

1. Introduction

Soils are the largest pool of terrestrial organic carbon, storing more carbon than the combined total of carbon stocks in the atmosphere and vegetation (Schlesinger, 1997). The carbon pools in soil and atmosphere are tightly linked to the photosynthetic activity of plants and decomposition of soil organic matter by soil fauna. The flux from the soil organic carbon (SOC) pool to atmospheric CO_2 is one of the largest in the global carbon cycle and is sensitive to changes in land use (e.g., Powers et al., 2011) and climate (Amundson, 2001). Apart from the important role of the SOC pool in the global carbon cycle, SOC is a dominant controlling factor of important soil functions such as soil fertility, soil structure, and soil water-holding capacity. SOC stocks typically display considerable spatial variability across landscapes. Understanding the drivers of this variability is essential for the development of management strategies that aim at enhancing soil functions, and for SOC accounting purposes with a relevance for policy makers. Examples of such SOC accounting purposes are the Clean Development Mechanism (CDM) and Reducing Emissions from Deforestation and Degradation (REDD+) initiatives that aim to generate financial compensation for local communities if they protect and enhance ecosystem carbon stocks (UNFCCC, 2009).

Spatial variability in SOC is the result of soil-forming factors acting and interacting across various spatio-temporal scales (Trangmar et al., 1986). Soil-forming factors affecting SOC are soil parent material, topographical attributes, biota, human activity (which includes land-use type and land management), time, and climate (Jenny, 1941). The importance of these controlling factors differs with spatial scale and environmental setting (Chaplot et al., 2010; Liu et al., 2013; Powers and Schlesinger, 2002). At the landscape scale, parent material (which often affects soil group, and clay minerology and content) is an important driver of SOC (e.g. de Koning et al., 2003; Schimel et al., 1994; Six et al., 2002). Within the same soil group, SOC is mainly influenced by land-use type and management (e.g. de Blécourt et al., 2013; de Koning et al., 2003; Mekuria et al., 2009; Post and Kwon, 2000), and geomorphological characteristics such as slope and slope position (Chaplot et al., 2005; Corre et al., 2015; Pennock and Corre, 2001). Spatial patterns of SOC are also greatly influenced by small-scale variability in biophysical factors that influence plant productivity and decomposition of soil organic matter (Hook et al., 1991; Stoyan et al., 2000). A comprehensive understanding of the sources of spatial variability of SOC and its key drivers at multiple scales is an important prerequisite for upscaling SOC data to larger areas.

In this study, we used a hierarchical sampling design to examine spatial variability in SOC concentrations (SOCc) and stocks (SOCs) and its relationships with land-use types, soil properties, vegetation characteristics, and topographical attributes at multiple spatial scales in a tropical montane landscape in Xishuangbanna, SW China. For centuries the area's land use has been characterized by swidden agriculture (also called slash-and-burn agriculture, or shifting cultivation) (Xu, 2006). The long history of swidden agriculture has resulted in a mosaic of secondary forests, agricultural fields, paddy rice, tea plantations, and rough grasslands (i.e. grasslands invaded with shrubs). Similar multi-use landscapes extend throughout SW China and the northern areas of Laos, Myanmar, Thailand and Vietnam (Garrity, 1993). In recent decades, large areas, formerly under swidden agriculture, have been transformed into landscapes with a more uniform land-use cover dominated by commercial crops and monoculture tree plantations (Rerkasem et al., 2009). The impact of the demise of swidden agriculture on ecosystem carbon stocks remains hard to predict, which is caused, among other factors, by limited SOC data (Fox et al., 2014). There were only three studies so far that evaluated the

impact of land use and various biophysical factors on the spatial variation in SOCc and SOCs at a landscape or larger scale in montane mainland southeast Asia; these were conducted in northern Thailand (Aumtong et al., 2009; Pibumrung et al., 2008) and Laos (Phachomphon et al., 2010).

Our specific objectives were (i) to quantify the SOCs of the four dominant land-use types: tea plantation, rough grasslands, regenerating or highly disturbed forests, and mature forests, (ii) to determine the proportions of the overall variance of SOCc and SOCs as well as soil, vegetation and topographical properties that were accounted for by land-use types within the landscape (10,000 ha), by sampling plots (one ha) nested within land-use types (plot distances ranging between 0.5 - 12 km), and by subplots (10-m radius) nested within sampling plots, and (iii) to assess the relationships between SOCc and SOCs with land-use types, soil properties, vegetation characteristics and topographical attributes. Our data provide important information on SOCs for an understudied region, give insights into factors that drive SOCs at multiple spatial scales, and will help to design better sampling strategies for SOCs.

2. Material and Methods

2.1 Study area

The studied landscape covered an area of about 10,000 ha and was located in Mengsong township, Xishuangbanna prefecture, Yunnan province, China (21˚29'25.62"N, 100˚30'19.85"E) (Figure 1a), bordering with Myanmar. The topography is mountainous with elevations of 1100-1900 m above sea level (asl). The climate is tropical monsoon and has a mean annual temperature (MAT) of 18 ˚C (at 1600 m asl). Mean annual precipitation (MAP) ranges from 1600-1800 mm, of which 80 % falls in the wet season lasting from May to October (Xu et al., 2009).

Land-use types in the area cover a disturbance gradient ranging from intensively managed tea plantation, rough grasslands, regenerating or highly disturbed forests, to mature forests, with minimal human influence. Forests in the area are classified as seasonal tropical montane rainforest in valleys, with transitions to seasonal evergreen broadleaf forest on hill slopes and ridges (Zhu et al., 2005). Our sampling plots ranged in elevation from 1147 to 1867 m asl, with slopes up to 49% (Table 1). The soils at the sampling plots varied from Haplic and Ferralic Cambisols in narrow valleys, to Cambic and Ferralic Umbrisols and Umbric and Haplic Acrisols and Ferralsols at both midslope and upslope positions (IUSS Working Group WRB., 2006). Soil texture ranged from sandy clay loam to clay, soil pH (H_2O) from 3.2-6.2, and the effective cation exchange capacity (ECEC) in the subsurface soil ranged from 4.8-45.8 $cmol_c$ kg^{-1} clay (Table 2).

2.2 Sampling design

We selected 27 one-ha sampling plots of which 10 plots were in mature forests, 11 plots in regenerating or highly disturbed forests, and six plots were categorized as open land used as tea plantations or rough grasslands (Figure 1b). In each sampling plot, we established nine circular subplots with a 10-m radius on a square grid with 50-m spacing (Figure 1c). Plots were selected using double sampling for stratification, also known as two-phase sampling (Fleischer, 1990). In phase 1, we classified the land-use types of the 10,000-ha landscape based on grid points (400 points with 500-m spacing) that were placed on satellite images (SPOT5 acquired in 2009

and RapidEye acquired in 2010) of the study area. Each point was identified as mature forest, regenerating or highly disturbed forest, open land, or other. In phase 2, the study area was divided in 16 equal-area units. From these 16 units, 12 were randomly selected, and within these 12 units we randomly selected the sampling plots from the classified grid points. Minimum distance between the sampling plots was 500 m. The land-use classification of the selected sampling plots was verified through field validations and interviews with local informants. Of the selected sampling plots, three sampling plots included a maximum of four out of the nine subplots, which did not belong to the original land-use classifications. To reduce noise in the dataset we removed these subplots from the dataset. The fieldwork, which included soil, litter and vegetation sampling, was done in 2010 and 2011.

We defined mature forests as forest sites dominated by trees with stem diameters more than 30 cm that did not show signs of recent disturbances due to timber extraction or fire. Regenerating or highly disturbed forests included both younger forest sites dominated by smaller trees, and older forest sites that had been strongly disturbed due to timber extraction or recent burning. Dominant tree families in the forest are Lauraceae, Fagaceae, Pentaphylaceae, Euphorbiaceae, and Rubiacea (Paudel et al., 2015). The selected open land plots included three plots in tea plantations and three plots in rough grasslands. Sampled tea plantations consisted of tea bushes planted in rows parallel to the slopes with few or no trees. One of the sampled tea plantations was terraced. Management practices applied in the tea plantations involved weeding and the use of chemical fertilizers and pesticides. Weeded plants were typically left between or under the tea bushes. Rough grasslands were dominated by *Imperata cylindrica* (L.) Raeusch grass, some small shrubs and a few trees. These grasslands are typically used for extensive cattle grazing and are maintained by regular burning. We observed that some of our grassland plots burnt at least two times between 2010 and 2013. According to local informants, sampling plots in each land-use type had been burnt in the past, as is inherent to the areas with a long history of swidden agriculture. Evidence of fire in the past was also observed by pieces of charcoal in the collected soil samples down to the deepest sampling depth of 0.9-1.2 m.

2.3 Soil and litter sampling

Soils were sampled down to 1.2 m at five depth intervals: 0-0.15 m, 0.15-0.3 m, 0.3-0.6 m, 0.6-0.9 m and 0.9-1.2 m. At each of the nine subplots per plot, we collected samples for the top three depths from four systematically (2 m east, 2 m north, 2 m west and 2 m south of the subplot center) positioned points using a Edelman auger (4-cm diameter). Soil samples collected from each subplot were mixed thoroughly in the field to form one composite sample per sampling depth per subplot. Soil samples at 0.6-0.9-m and 0.9-1.2-m depth were taken in soil pits at four subplots and one subplot per sampling plot, respectively. These pits were also used to measure soil bulk density for each sampling depth using the core method (Blake and Hartge, 1986). The bulk density measurements were corrected for gravel content (pebbles > 2 mm). The litter layer (including leaves, seeds, and twigs with a length < 0.2 m) was collected at each subplot with a 0.04-m^2 quadrant sampling frame. Samples of the litter layer were collected between May-August 2010. This one-time sampling of the litter layer coincided with the start of the rainy season and does not reflect seasonal or annual fluctuations in litterfall (Paudel et al., 2015). The litter layer mainly consisted of fresh and partly decomposed plant material.

2.4 Tree inventory and topographical attributes

At all nine subplots (10-m radius) per plot we measured the diameter at breast height (DBH), at 1.3 m above the soil surface, of all trees with a DBH ≥ 10 cm. Within a 5-m radius of the subplot center we also measured the DBH of all trees with a DBH ≥ 2 cm. Tree basal area at each subplot was calculated as the sum of the basal area of all measured trees. Topographical data obtained for each subplot included slope, elevation, and compound topographic index (CTI). We measured the slope from the center of each subplot to a target point situated 5-m downslope of the subplot center using a clinometer. Elevation was derived from a SRTM digital elevation model with a 90-m resolution resampled to 30-m resolution. The CTI, also known as steady state wetness index, quantifies landscape positions based on slope and upstream contributing area orthogonal to flow direction (Gessler et al., 1995; Moore et al., 1993). High CTI values refer to valleys with large catchments and low CTI values denotes to ridges or steep slopes. We calculated the CTI from the 30-m SRTM digital elevation model using ArcGIS.

2.5 Laboratory analyses and calculations

We analyzed the soil samples for total organic carbon and nitrogen concentrations, soil pH, soil texture and ECEC. Litter layer samples were analyzed for total organic carbon and nitrogen concentrations. Prior to analyses, the soil samples were air dried (5 days) and sieved (< 2 mm). Litter layer samples were oven dried at 60 °C for 48 hours and weighed. Total organic carbon and nitrogen concentrations were analyzed by dry combustion for ground subsamples of each soil and litter sample using a CNS Elemental analyzer (Elementar Vario EL, Hanau, Germany). Since soil pH (H_2O) was below 6.2, we did not expect carbonates in these soils and carbonate removal was not necessary. Soil pH (H_2O), pH (KCl) and soil texture were measured on each sample from the 0-0.15-m, 0.15-0.3-m, and 0.9-1.2-m depth intervals, and on a pooled sample per sampling plot for the 0.6-0.9 m depth interval. Soil pH (H_2O) and pH (KCl) were measured in a 1:2.5 soil-to-solution ratio. Soil texture was determined using the pipette method distinguishing the fractions clay (<0.002 mm), silt (0.002-0.063 mm), and sand (0.063-2 mm). ECEC was measured on soil samples of the 0-0.15 m depth interval and on a pooled sample from each sampling plot for the 0.6-0.9 m depth interval. The soil samples were percolated with unbuffered 1 M NH_4Cl and the percolates were analyzed for exchangeable cations using ICP-EAS (Spectroflame, Spectro Analytical Instruments, Kleve, Germany).

We calculated the litter layer organic carbon stocks using the carbon concentration, the mass of the litter layer and the sample frame area. SOCs of each sampling depth was calculated using:

$$SOCs(\text{Mg C ha}^{-1}) = \frac{\%C}{100} \times BD \ (Mg \ m^{-3}) \times \Delta D \ (m) \times 10,000 \ m^2 ha^{-1},$$

where BD is the soil bulk density (corrected for gravel content, pebbles > 2mm) and ΔD is the thickness of the sampling depth. Since the soil depth of some sampling plots did not reach down to 1.2 m, we reported both the total SOCs down to 0.9 m and the total SOCs down to 1.2 m. The total SOCs of each subplot was calculated as sum of SOCs of the constituent soil depths per subplot, the mean SOCs of the 0.6-0.9-m depth of the respective sampling plot, and the SOCs of the 0.9-1.2-m depth obtained at the plot level.

2.6 Statistical analyses

Statistical analyses were carried out using the statistical software R version 3.2.3 (R Core Team, 2015). Statistical tests were conducted for each sampling depth separately. Prior to analyses, we tested the data for normality (Shapiro-Wilk test) and equality of variances (Levene's test). Significant differences were accepted at $P \leq 0.05$, and differences at $P \leq 0.1$ were considered as marginally significant.

Data at the subplot level (SOCc and SOCs, soil C:N ratio, other soil characteristics (sand, silt plus clay, bulk density, pH (H_2O), pH (KCl), ECEC, Al saturation and base saturation) down to 0.3 m, tree basal area, litter layer characteristics and topographical attributes) were analyzed using linear mixed effects models (LME) with sampling plot included as random intercept, using the package nlme (Pinheiro et al., 2012). We tested if land-use types (fixed effect term) differed in SOCc and SOCs, tree basal area, soil, litter and topographical attributes (response variables). Multiple comparisons of the means of each land-use type were done using Tukey's test in the package multcomp (Hothorn et al., 2008). We conducted multiple regression analyses, using LMEs with sampling plot as random intercept, to test the relationships between SOCc or SOCs (response variables) with the following potential explanatory variables (fixed effect terms): land-use type, silt-plus-clay percentage, ECEC of the subsurface soil (0.6-0.9-m depth), litter layer carbon stock, litter layer C:N ratio, tree basal area, slope, relative elevation (change in elevation relative to the lowest situated sampling plot), and CTI. We conducted regression analyses separately for forests (mature forest and regenerating or highly disturbed forest combined) and open land (tea plantation and grassland combined). Correlation tests showed that the explanatory variables included in the LMEs were not strongly correlated with each other (Spearmans Rho < 0.44). Minimum adequate LMEs were selected using a stepwise model selection based on the Akaikes Information Criterion with the function stepAIC in the package MASS (Venables and Ripley, 2002). Residuals of the selected LMEs were examined for normality and equality of variances. In cases where we detected unequal variances, we included variance functions and if the assumption of normality was violated we used a logarithmic transformation of the response variable. The proportion of the variance explained by the fixed effect terms (marginal R^2) of each LME was calculated according to Nakagawa and Schielzeth (2013). We used a variance component analysis to partition the overall variance of each response variable into the variability among land-use types, among sampling plots within land-use types, and among subplots within plots. For the variance partitioning, we refitted the LME with sampling plot nested within land-use type as random intercept. Subsequently, we tested if both random factors were required in the LMEs by leaving out the random effect for land-use type, and comparing the two LMEs using a likelihood ratio test (Crawley, 2007).

For data that were only available at plot level (soil characteristics below 0.3-m depth other than SOCc and SOCs, and SOCc of the 0.9-1.2-m depth), we tested the effect of land-use type using either one-way analysis of variance (ANOVA) (parametric test) followed by Tukey's HSD test, or Kruskal-Wallis ANOVA (non-parametric test) followed by a pairwise Wilcoxon test with Holm's correction for multiple comparisons.

3. Results

3.1 Soil properties, vegetation characteristics and topographical attributes

240   Comparison of soil characteristics across land-use types revealed significant differences in soil pH and ECEC (Table 2). The soil pH (H_2O) down to 0.3 m was lowest in mature forest (data 0.15-0.3 m not shown), and the pH (KCl) down to 0.15 m was lower in mature forests than in the tea plantations. Compared to grasslands, the ECEC of the top 0.15 m was lower in tea plantations. Tree basal area and litter layer carbon stocks were higher in regenerating or highly disturbed forests and mature forests than in tea plantations and grasslands (Table 1).

245   Litter layer C:N ratios were narrower in mature forest compared to grassland. Comparison of topographical attributes showed that the land-use types were located on similar altitudes and topographical positions (reflected by CTI). However, the tea plantations had more gentle slopes compared to the other land-use types (Table 1).

3.2 Soil organic carbon concentrations and stocks

We did not detect differences in SOCc and SOCs across the four land-use types for any of the sampling depths
250   nor for the total SOCs down to 0.9 m and 1.2 m (Figure 2, Table 3). In forests, SOCc and total SOCs were positively associated with litter layer carbon stock, tree basal area, and elevation (marginal $R^2 = 0.51$ for 0-0.15-m, marginal $R^2 = 0.25$ for 0.15-0.3-m, marginal $R^2 = 0.18$ for 0-0.9-m depth, marginal R^2 refers to the variance explained by the fixed effect terms of each LME) (Table 4). However, the effect of elevation on total SOCs was only marginally significant, and for the 0.15-0.3-m depth litter layer carbon stock was the only controlling factor
255   of SOCc that was statistically significant. The effect of silt-plus-clay percentage on SOCc was included in the regression LME for the 0.15-0.3-m depth but was not statistically significant (Table 4).

        In open land, the most important controls of SOCc and total SOCs were land-use type, vegetation characteristics (litter layer carbon stocks, litter layer C:N ratio and tree basal area) and slope (marginal $R^2 = 0.57$ for 0-0.15-m, marginal $R^2 = 0.54$ for 0.15-0.3-m and marginal $R^2 = 0.60$ for 0-0.9-m depth) (Table 4). SOCc and
260   total SOCs increased with increasing litter layer carbon stocks and decreased with increasing slope. Furthermore, SOCc and total SOCs in grasslands were higher than tea plantations when controlling for the variability related to other explanatory variables (Table 4). Litter C:N ratio was included as explanatory variable for SOCc at 0.15-0.3-m depth; however, this effect was marginally significant (Table 4). Tree basal area was included as explanatory factor for SOCc in open land at 0.15-0.3 m and for total SOCs, but its effects on SOCc
265   was marginally significant at 0.15-0.3 m and not significant on total SOCs (Table 4).

3.3 Variance partitioning of soil properties, vegetation characteristics and topographical attributes

Variance partitioning showed that in the top 0.3 m of the soil, with the exception of soil pH H_2O, land-use type did not contribute significantly to any of the variation in soil properties (Figure 3a; for 0.15-0.3 m, data not shown). Instead, the variability among plots (nested within land-use type) and among subplots (nested within
270   plots) contributed relatively equally to the variances in SOCc, total SOCs down to 0.9 m and all other soil properties (except for soil texture) (Figure 3a). For soil texture, the variability among plots was the most important component of the overall variance. Most of the overall variance in the litter layer carbon stocks and litter layer C:N ratio was accounted for by the variability among subplots within plots (Figure 3b). For tree basal area, the variability among land-use types was the most important component of the overall variance followed

275 by the variability within plots. The main proportion of the overall variance in slopes was covered by the variability among subplots within plots, and the overall variance in elevation was almost completely due to the variability among plots (Figure 3c).

4. Discussion

4.1 Effects of land-use type on soil organic carbon concentrations and stocks

280 Our values of SOCs in mature forest, regenerating or highly disturbed forests, tea plantations and grasslands (Table 3) were at the high end of the range of SOCs reported for these land-use types in other studies from montane areas of mainland SE Asia (Table 5, our comparisons are based on equivalent sampling depths). SOCs to a depth of 0.3 m in mature forest and regenerating or highly disturbed forest were comparable to national estimates of SOCs in forests in Laos (Chaplot et al., 2010). However, our total SOCs within 0-0.9-m and 0-1.2-

285 m depth were higher than the regional estimates of SOCs within 1-m depth in subtropical forests in China (Yu et al., 2011), and than the SOCs within the same depths in other tropical forests in Xishuangbanna, SW China, (de Blécourt et al., 2013; Lü et al., 2010) and northern Thailand (Aumtong et al., 2009; Pibumrung et al., 2008). Data on SOCs in tea plantations and grasslands in the montane regions of SE Asia are scarce. Our observed SOCs in tea plantations within 0-0.6 m were in the range of the regional estimates of SOCs in tea plantations

290 reported for SW China (Li et al., 2011). However, our values of SOCs within 0-1.2-m depth in grasslands were higher than the amounts reported for fallow fields with a vegetation consisting of grasses and shrubs in northern Thailand (Aumtong et al., 2009). Compared to the cited studies, our study site was located at a higher elevation (1100-1900 m asl), had a relatively low MAT (18 °C) and a relatively high MAP (1600-1800 mm) (Table 5). Elevation and MAP have commonly been observed to positively affect SOCs (Amundson, 2001; Chaplot et al.,

295 2010; Dieleman et al., 2013) while MAT is known for being negatively associated with SOCs (Amundson, 2001; Powers et al., 2011). These factors may have contributed to the large total SOCs we observed.

Although land-use type is often considered an important controlling factor of SOC, we did not observe differences in SOCc and SOCs among land-use types (Table 3, Figure 2). Possible explanations for the high total SOCs in grasslands, which were similar to SOCs in mature forest, are higher belowground net primary

300 production and charcoal inputs compared to forests (van der Kamp et al., 2009; Yonekura et al., 2010). Higher belowground NPP in *Imperata* grasslands compared to forests may result in greater inputs of organic matter to the soil. To our knowledge, no comparable data (i.e. from sites with similar biophysical characteristics) exists on belowground NPP in these land-use types. Belowground NPP of regularly burnt *Imperata* grasslands in northeast India ranges from 973.8 to 1326.7 g m^{-2} y^{-1} (Astapati and Das, 2010) and is far greater than the

305 reported 111 and 379 g m^{-2} y^{-1} for tropical forests on Ultisols and Oxisols, respectively (Vogt et al., 1996). Charcoal input in grasslands is probably relatively high due to the high fire frequencies. However, results from field measurements on impacts of fire and charcoal additions on SOC quantities are contradicting, ranging from SOC losses (Bird et al., 2000; Fynn et al., 2003) to no change or increases in the SOC pool (Eckmeier et al., 2007; Ojima et al., 1994). Studies conducted in Kalimantan, Indonesia (van der Kamp et al., 2009; Yonekura et

310 al., 2010) reported even higher SOCs in *Imperata* grasslands compared to primary forests. Similarly, a meta-study of tropical land-use conversions (Powers et al., 2011) reported an increase in SOCs of 26% following forest-to-grassland conversions on soils with low activity clays and annual precipitation of 1501-2500 mm,

which are similar to the biophysical conditions in our study area. However, this meta-study also included managed grasslands as opposed to the semi-managed (mainly by regular burning) grasslands in our study.

The large proportion of variability within and among plots from the overall variance in SOCc and SOCs (Figure 3a) reflects our probability sampling technique (double sampling for stratification) for selecting plot locations. Studies with sampling designs based on prior knowledge of factors controlling SOC at a specific spatial scale of investigation (e.g. using space-for-time substitution, chronosequences, or stratification based on soil groups) generally result in smaller variability among plots nested within land-use types, as opposed to probability sampling designs. Results of the variance component analysis showed that large variability in SOCc and SOCs, other soil properties, vegetation characteristics, and topographical attributes within and among sampling plots (Figure 3) masked possible land-use effects on SOC in our study area.

4.2 Effects of soil properties, vegetation characteristics and topographical attributes on soil organic carbon concentrations and stocks

Our findings that the majority of the overall variance in SOCc and total SOCs down to 0.9 m was accounted by the variability within sampling plots and a smaller proportion was accounted by the variability among plots (Figure 3a), is similar to the findings of a study in subtropical northern New South Wales, Australia with plots of 30 m x 30 m (Paul et al., 2013). A large small-scale variability was also observed on a hill slope in Laos, where 85% of the variance in SOCc and SOCs occurred at a 20-m scale (Chaplot et al., 2009). In contrast, in lowland landscapes of Sumatra, Indonesia, where plots of 50 m x 50 m had slopes ranging from 3-16 %, only a small proportion of the overall variance in SOCs was accounted by the variability within plots (Allen et al., 2016). Paul et al. (2013) related the high within plot variability in SOCc to the heterogeneous nature of vegetation and microclimate in their plots. Chaplot et al. (2009) attributed the large small-scale variability in SOCs and SOCc to land use, clay content and hill-slope surface morphology. The study of Allen et al. (2016) was on well-drained areas of the landscape with gentle slopes and stratified by soil group, which may have resulted in the small within-plot variability they observed. Our study was in a montane landscape, wherein large within-plot variability in SOCc and SOCs may have been due to a large heterogeneity in slope and vegetation characteristics especially tree basal area and litter layer carbon stocks, within the one-ha plots (and therein the possible microclimate variability) (Table 4 and Figure 3). We base this on the associations of SOC with tree basal area and litter layer carbon stocks in forest, and with litter layer carbon stocks and slope in open land (Table 4), in combination with the high proportion of within-plot variability of these parameters from the overall variances (Figure 3). We attributed the variability in SOCc and SOCs among plots in open land to land-use effects (tea plantations versus grasslands) whereas in forests, elevation was the most important factor controlling the variability in SOCc and SOCs among plots (Table 4, Figure 3). The low marginal R^2 of our SOC-models in forests, for 0.15-0.3-m and 0-0.9-m depths, indicated a large amount of unexplained variance and suggests that other controlling factors may have contributed which we did not include in our measurements. These factors could include vegetation composition and land-use history, which we tried to document but which proved difficult to categorize meaningfully.

The observed increase in SOCc and SOCs in forests and open land with increasing tree basal area and litter layer carbon stocks (Table 4) was in accordance with findings from previous studies (de Blécourt et al., 2013; Powers and Schlesinger, 2002; Woollen et al., 2012) and is attributed to biomass productivity. Enhanced biomass productivity may increase SOC input through increases in litterfall and root residues. The use of tree

basal area and litter layer carbon stocks as a proxy for biomass productivity is supported by positive associations between yearly litterfall and increases in tree basal area and litter layer carbon stocks, observed in a subset of

355 our forest plots (Table A1, Paudel et al., 2015). The decrease in SOCc and SOCs in open land with increasing slope (Table 4) was most likely related to surface erosion, which is common in montane landscapes (e.g., Arrouays et al., 1995; Corre et al., 2015). The importance of erosion and sedimentation processes on the redistribution of SOC was shown in studies conducted in Laos (Chaplot et al., 2005) and Ecuador (Corre et al., 2015); soil erosion was highest at the upper slopes and most of the eroded soil and SOC was deposited within a

360 short distance at the lower slopes. The observed increase in SOCc and SOCs in forests with increase in elevation is consistent with other studies (Chaplot et al., 2010; Dieleman et al., 2013; Powers and Schlesinger, 2002). Elevation effects on SOC are often related to changes in precipitation, temperature, soil characteristics, and biomass productivity. However, despite the large elevation gradient of the forest plots in our study (1147-1867 m asl) we did not observe any elevation effects on silt-plus-clay percentage, ECEC of the subsurface soil

365 (reflecting clay mineralogy), soil pH H_2O, soil C:N ratio or tree basal area (data not shown). Although microclimatic data for our plots were not available, the commonly occurring reduction in temperatures with increase in elevation may influence SOC decomposition rates, which could possibly explain the positive trend between elevation and SOC in our forest plots. Soil texture within a similar soil group is regarded as an important control for plant productivity, decomposition of soil organic matter, and SOC stability (Silver et al.,

370 2000). In our study area, silt-plus-clay percentage did not influence SOCc and SOCs (Table 4). Possibly the influence of soil texture on SOC was masked by the large variability in soil groups (and thus clay mineralogy) in our study area.

4.3 Implications for sampling soil organic carbon stocks

Probability sampling techniques as applied in our study are appropriate for assessing spatial variability of SOC

375 and its driving factors across scales (subplot to plot and landscape scale) but fall short in detecting land-use effects on SOC. In montane landscapes, large variability in SOC due to variability in vegetation characteristics, slope and elevation within and among plots (Figure 3) may conceal the land-use effects on SOC, unless sample sizes are very large. An often used approach that has proven to be effective in detecting land-use effects on SOC is space-for-time substitution (e.g., de Blécourt et al., 2013; de Koning et al., 2003; van Straaten et al., 2015;

380 Veldkamp, 1994). This approach aims to select plots that mainly differ in land-use type, with soil group and thus clay minerology and content, and topographical and climatic characteristics being comparable. However, in contrast to our probability sampling technique, plot selection using the space-for-time substitution approach is non random in order to meet the criteria for comparison, and thus SOC levels measured in those studies can only be extrapolated to larger scales under similar soil type and biophysical characteristics.

385 **5. Conclusions**

In this tropical montane landscape in SW China, the spatial variability in SOCc and SOCs was largest at the plot scale. This high within-plot variability in SOC reflected the variability in litter layer carbon stocks and slope in open land, and the variability in litter layer carbon stocks and tree basal area in forests. Therefore, to achieve a reliable estimate of SOC stocks within plots, it is important to have a plot size that encompasses the inherent

390 slope and vegetation variability. Furthermore, since the variability in SOCc and SOCs among plots was related to elevation in forests, and to land-use type in open land, stratification of similarly montane landscapes, should be based on elevation and land-use types as the principal drivers of SOC at the landscape scale. These scale-dependent relationships between SOCc and SOCs with controlling factors demonstrate that sampling designs must consider the controlling factors at the scale of interest in order to elucidate their effects on SOC against the

395 variability within and between plots.

Data availability

Data associated with this paper are available in Dryad: doi:10.5061/dryad.f4m6k.

Author contributions

Conceived and designed the study: Marleen de Blécourt, Rhett Harrison and Rainer Brumme. Performed the
400 study: Marleen de Blécourt and Ekananda Paudel. Analyzed the data: Marleen de Blécourt, Marife D. Corre and Edzo Veldkamp. Wrote the paper: Marleen de Blécourt. All co-authors read and contributed to revisions of the manuscript.

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560 **Table 1: Means (± SE)[a] of vegetation characteristics and topographical attributes of four different land-use types in a tropical montane landscape in SW China.**

Characteristics	Mature forest (n=10)	Regenerating or highly disturbed forest (n=11)	Grassland (n=3)	Tea plantation (n=3)	P value
Litter layer C concentration (%)	40.0 (1.1)	40.1 (1.1)	42.8 (0.2)	39.7 (2.3)	0.38
Litter layer C:N ratio	29.7 (1.5) b	36.4 (2.1) ab	43.2 (6) a	35 (3.4) ab	0.02
Litter layer carbon stock (Mg C ha^{-1})	5.6 (0.6) a	4.2 (0.5) a	1.7 (0.2) b	1.5 (0.2) b	<0.01
Tree basal area (m^2 ha^{-1})	29 (2.5) a	18.2 (1.9) b	3 (0.7) c	0.8 (0.2) c	<0.01
Slope (%)	29.7 (1.6) a	26.7 (1.1) ab	31 (3.8) a	12.9 (1.3) b	0.05
Elevation (m)	1664 (66)	1559 (67)	1719 (59)	1573 (119)	0.54
Compound topographic index[b]	9.9 (0.4)	8.9 (0.2)	8.4 (0.2)	9.8 (0.8)	0.29

[a]Within a row, means followed by different letters indicate significant differences among land-use types, and means without letters indicate no significant difference among land-use types (linear mixed effects model, one-way ANOVA or Kruskal-Wallis ANOVA at $P \le 0.05$).

565 [b]Compound topographic index (Gessler et al., 1995; Moore et al., 1993) quantifies landscape positions based on slope and upstream contributing area orthogonal to flow direction. High CTI values refer to valleys with large catchments and low CTI values denotes to ridges or steep slopes.

Table 2: Means (± SE)[a] of soil properties of four different land-use types in a tropical montane landscape in SW China.

Characteristic	Depth (m)	Mature forest (n=10)	Regenerating or highly disturbed forest (n=11)	Grassland (n=3)	Tea plantation (n=3)	P value
Sand (%)	0-0.15	39.8 (3.9)	36.3 (3.1)	47.4 (3.7)	37.6 (10.6)	0.55
	0.6-0.9	40.9 (4.4)	31.5 (4.3)	47.5 (3.9)	33.6 (9.3)	0.24
Silt plus clay (%)	0-0.15	60.2 (3.9)	63.7 (3.1)	52.6 (3.7)	62.4 (10.7)	0.54
	0.6-0.9	59.1 (4.4)	68.5 (4.3)	52.5 (3.9)	66.4 (9.3)	0.24
Bulk density (g cm^{-3})	0-0.15	0.8 (0.05)	0.8 (0.02)	0.8 (0.03)	0.7 (0.1)	0.59
	0.6-0.9	1.1 (0.05)	1.1 (0.03)	1.0 (0.03)	1.1 (0.0)	0.5
Soil C:N ratio	0-0.15	15.1 (0.6)	14.3 (0.4)	16.3 (1.1)	14.2 (0.8)	0.21
	0.6-0.9	10.7 (0.5)	10.4 (0.3)	12.5 (0.9)	10.4 (0.7)	0.18
pH (H$_2$O)	0-0.15	4.5 (0.1) b	4.8 (0.1) a	5.0 (0.2) a	5.0 (0.1) a	<0.01
	0.6-0.9	5.0 (0.1)	5.0 (0.1)	5.0 (0.2)	4.9 (0.3)	0.82
pH (KCl)	0-0.15	3.6 (0.1) b	3.8 (0.1) ab	3.9 (0.1) ab	4.1 (0.1) a	0.02
	0.6-0.9	3.8 (0.1)	3.9 (0.1)	3.9 (0.2)	4.1 (0.1)	0.30
ECEC[b] (cmol$_c$ kg^{-1} clay)	0-0.15	47.3 (7.6) ab	32.5 (3.6) ab	53.6 (4.4) a	24.1 (3.3) b	0.04
	0.6-0.9	23.6 (4.3)	16.2 (3.2)	17.5 (1.6)	7.7 (1.5)	0.10
Al saturation (%)	0-0.15	72.4 (3.1)	64.2 (6.1)	60.5 (12.2)	49.3 (12.4)	0.27
	0.6-0.9	86.3 (1.4)	80.5 (6.1)	87.8 (1.4)	62.8 (14.8)	0.22
Base saturation (%)	0-0.15	20.5 (3.1)	29.3 (6.0)	35.6 (11.8)	43.5 (11.3)	0.12
	0.6-0.9	8.5 (1.5)	12.0 (5.3)	7.9 (1.5)	29.1 (13.3)	0.11

570   [a]Within a row, means followed by different letters indicate significant differences among land-use types, and means without letters indicate no significant difference among land-use types (linear mixed effects model, one-way ANOVA or Kruskal-Wallis ANOVA at P ≤0.05).

[b]ECEC, effective cation exchange capacity.

Table 3: Means (± SE)[a] of soil organic carbon stocks (Mg C ha^{-1}) of four different land-use types in a tropical montane landscape in SW China.

Depth (m)	Mature forest (n = 10)	Regenerating or highly disturbed forest (n = 11)	Tea plantation (n = 3)	Grassland (n = 3)	P value
0-0.15	65.5 (6.8)	58.4 (4)	44.3 (7)	66 (2.6)	0.15
0.15-0.3	51.7 (4.5)	47.5 (3.7)	40.3 (3.6)	55.1 (5)	0.32
0.3-0.6	73.4 (8)	58.9 (4.8)	59.1 (7.6)	67.7 (2.6)	0.37
0.6-0.9	38 (3.2)	34.6 (3.5)	34 (5.3)	47.4 (4)	0.40
0.9-1.2[b]	18 (3.1)	23.2 (6)	20.8 (7.7)	38.5 (14.9)	0.35
Sum 0-0.9	228.6 (19.7)	199.5 (14.8)	177.6 (19.6)	236.2 (13.7)	0.34
Sum 0-1.2[b]	252.1 (25.4)	230.5 (24.6)	216.2 (32.1)	274.6 (28.2)	0.71

[a]Within a row, means followed by different letters indicate significant differences among land-use types, and means without letters indicate no significant difference among land-use types (linear mixed effects model and one-way ANOVA at $P \leq 0.05$).

[b]The number of replicates per land-use type deviates from the original number of replicate plots because the soil depth of some sampling plots did not reach down to 1.2 m. For the 0.9-1.2-m depth and total SOC stocks to 1.2 m, the number of replication is as follows: mature forest (n = 8), regenerating or highly disturbed forest (n=8), tea plantation (n=3), grassland (n=3).

Table 4: Coefficient estimates[a] (± SE) of effects of soil texture, effective cation exchange capacity (ECEC), vegetation characteristics and topographical attributes on soil organic carbon (SOC) concentrations and total SOC stocks in forests (regenerating or highly disturbed forest and mature forest combined) and open land (tea plantation and grassland combined) in a tropical montane landscape in SW China.

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Response	Effect	Forest (n = 21)		Open land (n = 6)	
		Estimate	P value	Estimate	P value
SOC concentration (%) at 0-0.15 m	Intercept	2.22 (0.66)	<0.001	6.47 (0.48)	<0.001
	Land-use type[b]		ns	-2.01 (0.30)	<0.01
	Silt-plus-clay percentage (%)		ns		ns
	ECEC[c] at 0.6-0.9 m (cmol$_c$ kg^{-1} clay)		ns		ns
	Litter layer carbon stock (Mg C ha^{-1})	0.16 (0.04)	<0.001	0.29 (0.1)	<0.01
	Litter layer C:N ratio		ns		ns
	Tree basal area (m^2 ha^{-1})	0.03 (0.01)	<0.001		ns
	Slope (%)		ns	-0.04 (0.01)	<0.01
	Relative elevation[d] (m)	0.01 (0.001)	<0.01		ns
	Compound Topographic Index		ns		ns
SOC concentration (%) at 0.15-0.30 m	Intercept	0.94 (0.86)	0.28	4.79 (0.54)	<0.001
	Land-use type[b]		ns	-1.64	0.05
	Silt-plus-clay percentage (%)	0.02 (0.01)	0.16		ns
	ECEC[c] at 0.6-0.9 m (cmol$_c$ kg^{-1} clay)		ns		ns
	Litter layer carbon stock (Mg C ha^{-1})	0.17 (0.03)	<0.001	0.34 (0.14)	0.03
	Litter layer C:N ratio		ns	-0.02 (0.01)	0.10
	Tree basal area (m^2 ha^{-1})	0.01 (0.006)	0.13	-0.17 (0.08)	0.06
	Slope (%)		ns		ns
	Relative elevation[d] (m)	0.01 (0.001)	0.13		ns
	Compound Topographic Index		ns		ns
Total SOC stock (Mg C ha^{-1}) at 0-0.9 m	Intercept	109.8 (24.1)	<0.001	247.4 (27.1)	<0.001
	Land-use type[b]		ns	-63.22 (16.3)	0.06
	Silt-plus-clay percentage at 0.15-0.3 m (%)		ns		ns
	ECEC[c] at 0.6-0.9 m (cmol$_c$ kg^{-1} clay)		ns		ns
	Litter layer carbon stock (Mg C ha^{-1})	5.3 (1.53)	<0.001	14.27 (6.05)	0.03
	Litter layer C:N ratio		ns		ns
	Tree basal area (m^2 ha^{-1})	0.89 (0.35)	0.01	-3.53 (3.71)	0.36
	Slope (%)		ns	-2.54 (0.87)	0.01
	Relative elevation[d] (m)	0.08 (0.05)	0.09		ns
	Compound Topographic Index		ns		ns

[a]Linear mixed effects models with sampling plot as random intercept. All effects were included in the full model, and model simplification resulted in the minimum adequate model. ns - not significant (i.e., the effects excluded by model simplifications)

590 [b]The land-use effect in open land is calculated as SOC in tea plantation minus SOC in grassland.

[c]ECEC, Effective Cation Exchange Capacity.

[d]Relative elevation is the change in elevation compared to the lowest situated sampling plot.

Table 5: Overview of published soil organic carbon (SOC) stocks in four different land-use types from montane areas of mainland SE Asia.

Land use	Country, site	Soil type	Elevation (m)	Climate MAP (mm)	MAT (˚C)	Depth (m)	SOC stock (Mg C ha⁻¹)	Reference
Forest	Laos, total country	-	-	-	-	0-0.3	112	Chaplot et al. (2010)
	China, Xishuangbanna	Haplic Acrisol	600	1539	21.7	0-1	84-102	Lü et al. (2010)
	China, Menglong, Xishuangbanna	Ferralsols and (hyper) ferralic Cambisols	700-830	1377	22.7	0-0.9	170	de Blécourt et al. (2013)
	Thailand, Nam Hean watershed	Red Yellow Podzolic soils and Reddish Brown Lateritic soils	215-1674	1405	16.9 (DS[a])-32.5 (WS[a])	0-1	196.24	Pibumrung et al. (2008)
	Thailand, Khun Samun Watershed	Hyperalic Alisols (Humic) and Endogleyic Luvisol (Chromic)	300-800	1400	22-29	0-1.2	~170	Aumtong et al. (2009)
	China, Subtropical zone	-	-	-	-	0-1	104.4-111.2	Yu et al. (2011)
Tea	China, Southwest	Haplic Acrisol	-	1000-1700	15-19	0-0.6	132.3-158.7	Li et al. (2011)
Fallow	Thailand, Khun Samun Watershed	Hyperalic Alisols (Humic) and Endogleyic Luvisol (Chromic)	300-800	1400	22-29	0-1.2	~210	Aumtong et al.(2009)

595   ~ an approximate value, deciphered from a figure.

[a] DS - dry season, WS - wet season.

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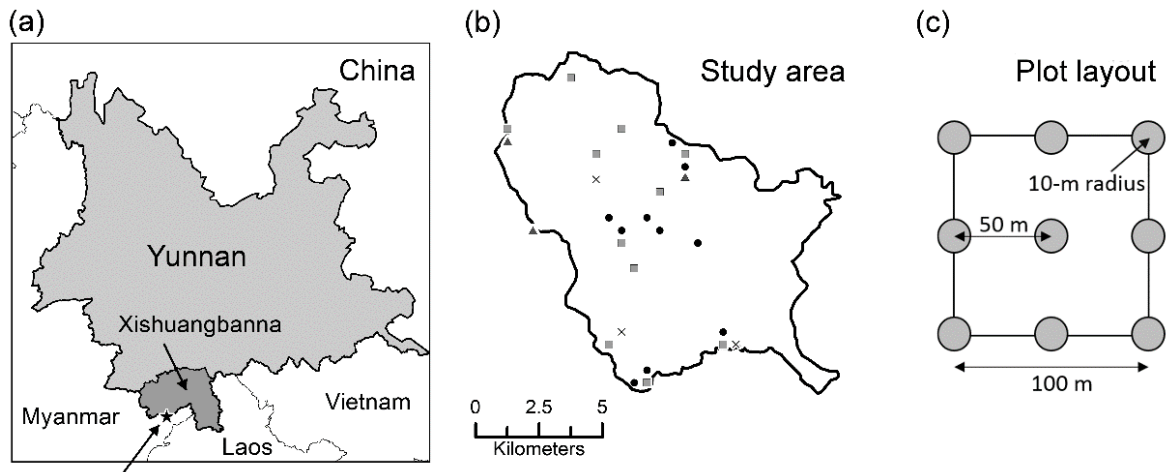
Figure 1: Sampling design: (a) The location of the study area in Xishuangbanna prefecture, Yunnan province, China, is depicted by the black star. (b) Location of the 27 sampling plots in the study area (10,000 ha), black circles were classified as mature forests (n=10), grey squares as regenerating or highly disturbed forests (n=11), black stars as rough grasslands (n=3), and black crosses as tea plantations (n=3). (c) Sampling plots of 100 m x 100 m with nine 10-

605   **m radius subplots (grey cycles) arranged on a square grid with 50-m spacing.**
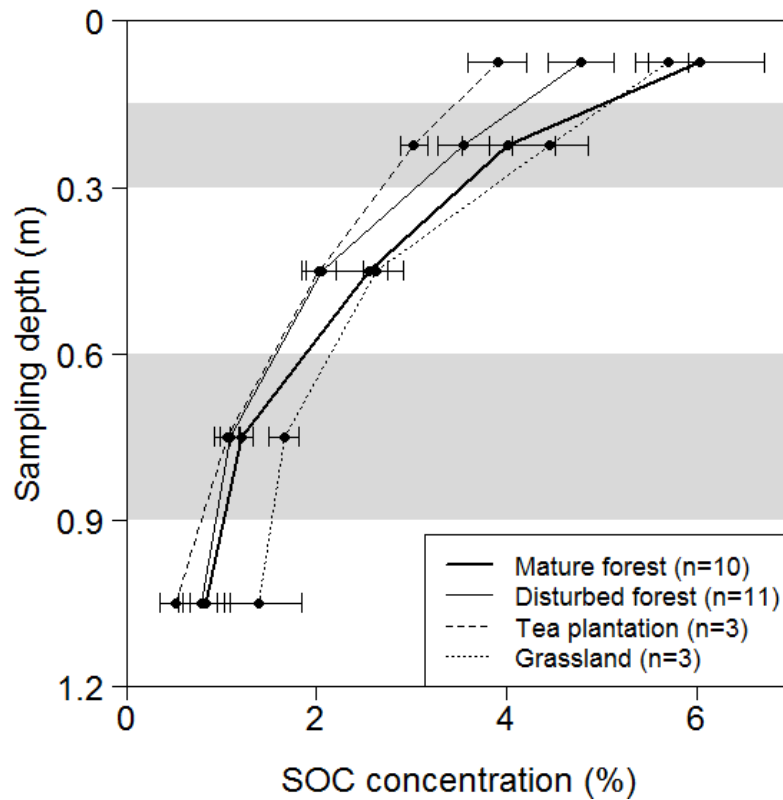
Figure 2: Soil organic carbon (SOC) concentrations in relation to sampling depth for four different land-use types in a tropical montane landscape in SW China. Alternating white and grey bands show the sampling depths. For each depth, means (SE bars) did not differ among land-use types (linear mixed effects model with P = 0.22-0.49 at sampling depths < 0.9 m, and one-way ANOVA with P = 0.37 at 0.9-1.2 m).

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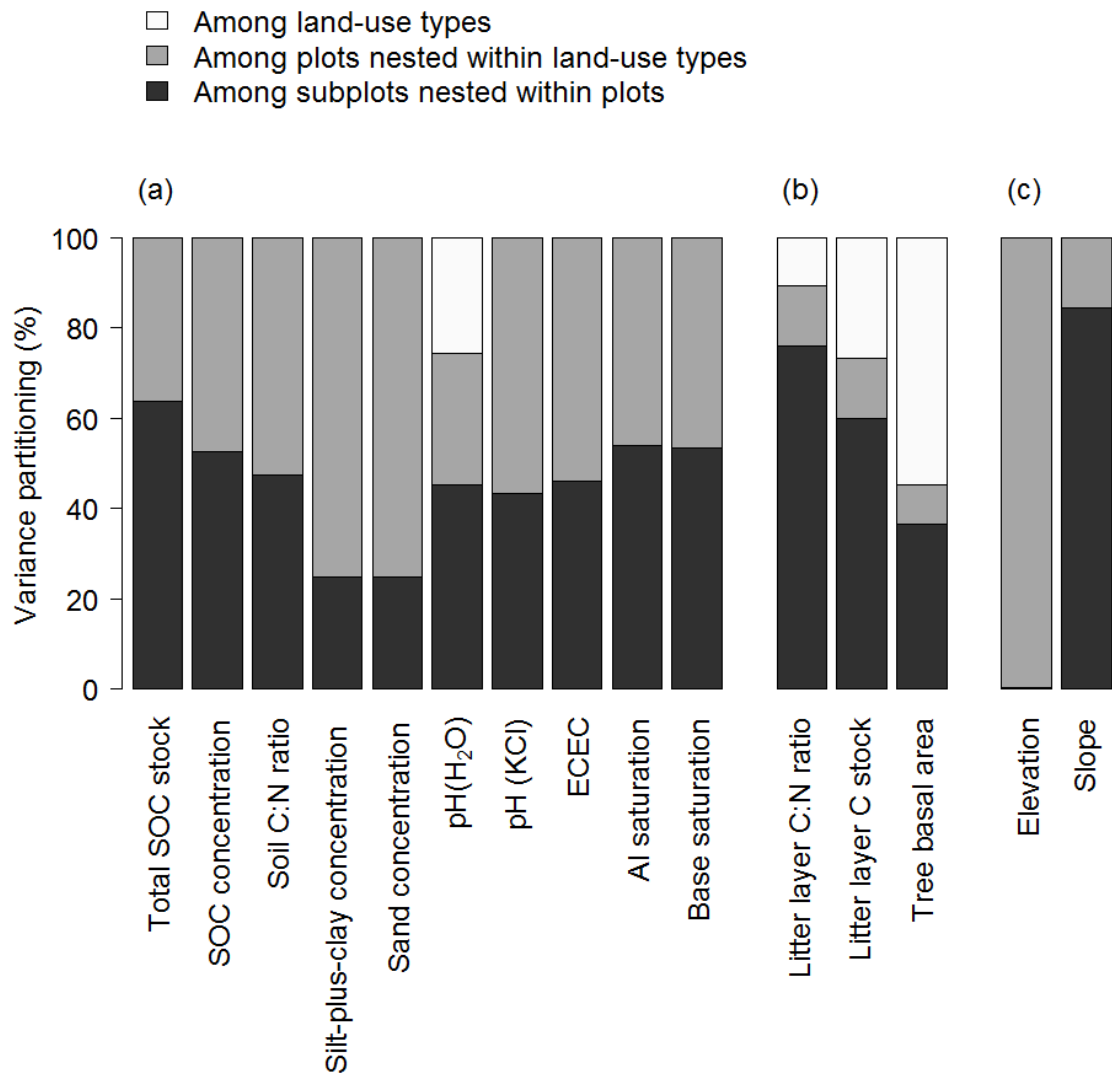
Figure 3: Partitioning of the overall variance in (a) total soil organic carbon (SOC) stocks to 0.9-m depth, and SOC concentrations and soil properties at 0-0.15-m depth, in (b) vegetation characteristics, and in (c) topographical attributes, which can be attributed to the variability among land-use types, sampling plots nested within land-use types, and subplots nested within sampling plots (Linear mixed effects model with likelihood ratio test at P ≤ 0.05, the variability in litter C:N ratio among land-use types is marginally significant with P = 0.06).

Table A1. Direction of effects[a] of soil properties, vegetation characteristics and topographical attributes on litter layer carbon stock and yearly litterfall[b] in forest in a tropical montane landscape in SW China.

Response	Effect	Direction of effect	P value	R^2
Litter layer carbon stock	Elevation	+	0.05	0.19
	Soil pH H_2O (0-0.15-m depth)	-	0.07	
	Yearly litterfall	+	0.03	
Yearly litterfall	Tree basal area	+	<0.01	0.19
	ECEC subsoil	+	0.14	

620 [a]Linear mixed effects model with sampling plot as random intercept. Fixed effects included in the full models were elevation, slope gradient, compound topographic index, silt-plus-clay percentage, soil pH H_2O, tree basal area, litter C:N ratio, yearly litterfall.

[b]Litterfall was collected every month from 9 of the 21 forest plots. Details on the materials and methods are described by Paudel et al. (2015).

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