



1 **Combined deep sampling and mass-based approaches to assess soil carbon and nitrogen losses**
2 **due to land-use changes in karst area of Southwestern China**

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10 **Abstract.** The conversion of natural vegetation to managed ecosystems may negatively influence soil
11 organic carbon (SOC) and total nitrogen (TN) stocks, particularly in the fragile ecosystems. The
12 objective of present study was to assess SOC and TN stocks losses by combining deep sampling with
13 mass-based calculations upon land-use changes in a typical karst area of Southwestern China. We
14 quantified the changes from native forest to grassland, secondary shrub, eucalyptus plantation,
15 sugarcane and corn fields (both defined as croplands), on the SOC and TN stocks down to 100 cm depth
16 using fixed-depth (FD) and equivalent soil mass (ESM) approaches. The results showed that converting
17 forest to cropland and other types significantly led to SOC and TN losses, although the effect magnitude



18 partly depended on both sampling depths and soil mass considered. On average, the shifting from native
19 forest to cropland led to SOC losses by 19.1 %, 25.1 %, 30.6 %, 36.8 % and 37.9 % for the soil depths
20 of 0–10, 0–20, 0–40, 0–60 and 0–100 cm, respectively, which highlighted that shallow sampling
21 underestimated SOC losses. Moreover, the FD method underestimated SOC and TN losses for the upper
22 40 cm layer, but overestimated the losses in the deeper profiles. We suggest that the ESM together with
23 deep sampling should be encouraged to detect the differences in SOC stocks. In conclusion, the
24 conversion of forest to managed systems, in particular croplands significantly decreased in SOC and TN
25 stocks, although the effect magnitude to some extent depended on sampling depth and calculation
26 approach selected.

27 **Keywords:** Land-use change, karst area, soil carbon and nitrogen stocks, sampling depth, equivalent
28 soil mass

29 **1 Introduction**

30 Land-use change, like deforestation has become a significant concern in terms of environmental
31 degradation and global climate change (Harris et al., 2012; Mukhopadhyay et al., 2016; Wiesmeier et al.,
32 2015). Globally, the large-scale conversions of natural ecosystems to croplands and other managed
33 ecosystems have already resulted in historically large emissions of C into the atmosphere (as higher as
34 320 Pg C), since the dawn of settled agriculture (Lal, 2010). In turn, land degradation due to soil organic
35 C (SOC) loss may damage ecosystem services and functions (Brevik et al., 2015; Costantini et al., 2016;



36 Foley et al., 2005), directly affecting the hydrological and biogeochemical cycles in the earth system
37 (Brevik et al., 2015; García-Díaz et al., 2016; Sonneveld et al., 2016). Thus, the changes in quality and
38 quantity of SOC may inevitably influence the soil degradation, agricultural productivity and food
39 security (Carter, 2002; Janzen, 2015; Srinivasarao et al., 2014).

40 Many studies have shown serious decreases of soil organic matter (SOM) pools owing to human
41 activities, such as cultivation of soils under forest or natural vegetation (Lal, 2009; Post and Kwon,
42 2000). However, most soil C studies have focused only on the surface layers (i.e., ≤ 30 cm) to clarify
43 the SOC in response to land-use changes (Baker et al., 2007; Post and Kwon, 2000; Wei et al., 2014;
44 West and Post, 2002). Because more than one third of roots and more than one half of soil C are stored
45 below 20 cm depth (Jobbágy and Jackson, 2000), the conversion of land use change may well influence
46 soil C in the subsoils. Therefore, the soil C stored in the deeper layers may be heavily neglected, but
47 little is known about the quantity of SOC at depth and how it responds to land management (Guo and
48 Gifford, 2002; Lozano-García and Parras-Alcántara, 2014; Wei et al., 2014). A more complete and
49 accurate evaluation of management effects on SOC stocks should involve collecting deeper depths to
50 identify the real changes (Baker et al., 2007; Olson and Al-Kaisi, 2015; Parras-Alcántara et al., 2015).

51 The SOC stock is widely quantified to a fixed-depth (FD) as the product of soil bulk density (BD),
52 depth and concentration. This FD method, however, has been considered to introduce substantial errors
53 when soil BD differs between treatments (VandenBygaart and Angers, 2006). Instead, equivalent soil



54 mass (ESM) approach is required to correct the calculation (Ellert and Bettany, 1995; Lee et al., 2009).
55 The ESM method, which can account for the differences in soil masses among treatments, is being
56 increasingly employed (Don et al., 2011; Wiesmeier et al., 2015). As land-use conversions are often
57 associated with the changes in soil BD, depth-based method would result in a considerable differences
58 in soil masses for the quantification of SOC stocks.

59 Karst is a distinctive topography, and the rocky desertification has become a serious environmental
60 issue in this landscape of Guangxi Province, Southwestern China (Wang et al., 2004; Zhao et al., 2014).
61 The typical karst areas are characterized by high fragility own to their natural settings and
62 anthropogenic impacts (Xu and Zhang, 2014). This, in turn, has not only damaged eco-environment
63 including soil erosion, lower biodiversity and decreased soil productivity, but also exacerbated the
64 poverty level in the rural areas of the region (Hu et al., 2014; Liu et al., 2005; Wang et al., 2004). Thus,
65 karst area is considered as small environment capacity, weak anti-disturbance, low stability and
66 powerless self-adjustment.

67 Since the late 1990s, China initiated the Grain for Green project, and the karst region of Southwest
68 China is one of the main regions involved in this project. Accordingly, the Guangxi governments had
69 implemented an environmental resettlement program that transferred about 4 million people from this
70 rocky region to the “in-migration” areas (Hu et al., 2008). The natural and land ecosystems in the
71 in-migration areas exerted by ecological migrants might be negatively influenced. It is critically



72 important to evaluate the changes in SOM status, which are accompanied by changes in ecosystem
73 services, processes, and functions (Carter, 2002; Smith et al., 2015). We hypothesized that the
74 ecological resettlement program may deplete SOM stocks, and thus soil degradation. Recently, some
75 researchers have paid more attention about the effect of land use change on the soil physicochemical
76 properties and soil quality in this karst region (Fu et al., 2015; Hu et al., 2014; Xie et al., 2014; Xu et al.,
77 2008). However, studies on soil C dynamics were mainly focused on the surface layers (e.g., ≤ 30 cm)
78 and the FD method are utilized to assess SOC stocks (Chen et al., 2012; Fu et al., 2015; Liu et al.,
79 2015b; Zhang et al., 2012). Very little effort has been devoted to the effect of land conversion on SOC
80 stocks in the deeper soils. Thus, the present study was aimed to (1) quantify the changes in SOC and TN
81 stocks down to 100 cm after conversion from native forest to croplands and other managed systems; (2)
82 to examine whether the calculation methodology (i.e., equivalent soil mass vs. fixed depth) may change
83 the interpretation of the results.

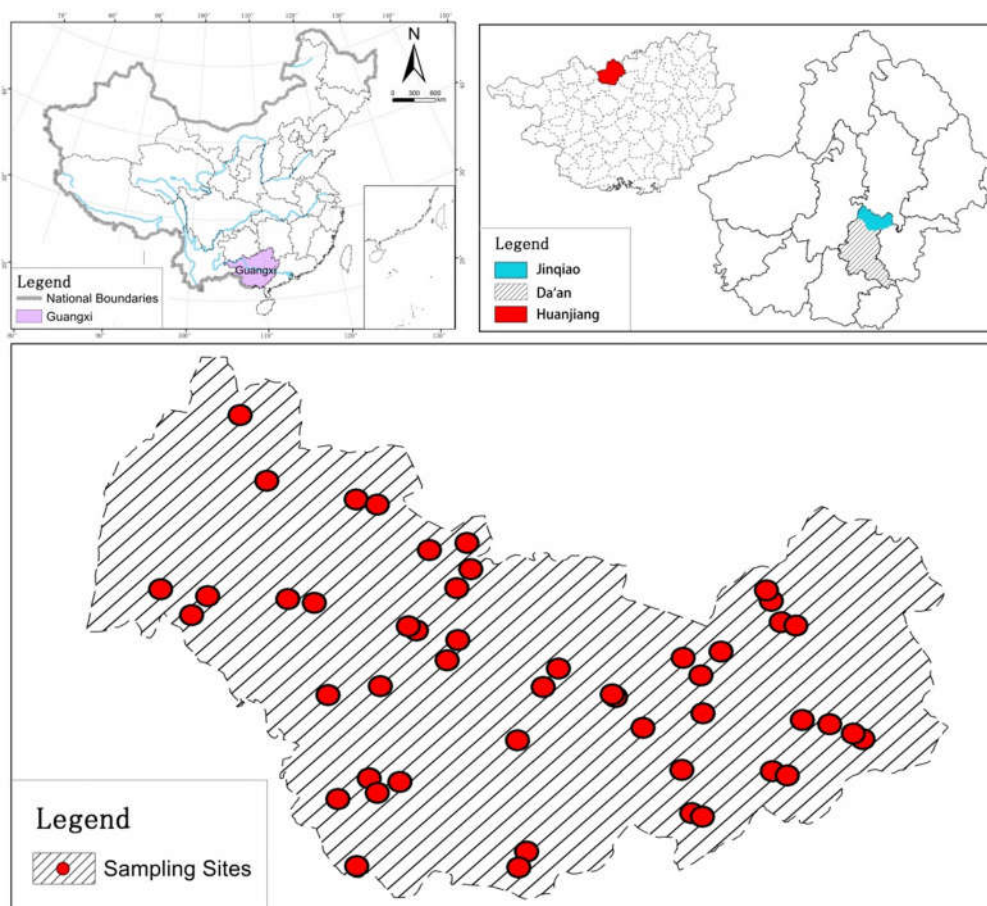
84 **2 Materials and methods**

85 **2.1 Study area**

86 This study was conducted at the Huanjiang County (107°51'–108°43' E, 24°44'–25°33' N), Guangxi
87 Province, Southwestern China (Fig. 1). This county is surrounded by mountains, and has a total land
88 area of 4 572 km² with elevation ranging from 149 to 1 693 m. The natural vegetation mainly consisted
89 of shrubs, herbs and lianas. The region is characterized by a subtropical monsoon climate with a distinct



90 wet (from Apr to Sep) and dry season (from Oct to Mar). The annual mean temperature is 19.9 °C, and
91 the annual average precipitation is 1750 mm. The rainfall was uneven distributed and concentrated
92 during rainy seasons (about 70 %) from April to September. In this area, the soil is calcareous and has
93 been mainly developed from limestone parent materials.



94
95 **Figure 1.** Location of the study area and the distribution of soil sampling sites in northwest Guangxi, Southwestern
96 China



97 Since the 1999, the Huanjiang county had received about 70,000 environment migrants, and became
98 the largest environmental resettlement region in the Southwestern karst area. In this region, we selected
99 five ecosystems, i.e., native forest (NF), unused grassland (UG), secondary shrubland (SS), eucalyptus
100 forest (EF) and croplands (including sugarcane field: SF and corn field: CF). The later four ecosystems
101 are all originated from the native forest due to deforestation or land-use change. All the five ecosystems
102 selected are adjacent to each other. Within the each land use type, five plots (about 0.067–0.10 ha) were
103 chosen. All sites were located on the similar physiographical conditions (e.g., with slope about 15–25 °,
104 and elevation) and the distance between sampling sites ranged about from 250 to 350 m. The detailed
105 information for the land use types including vegetation, dominant plant species and management history
106 are listed in Table 1.

107 **Table 1.** The general information of land-use types selected in this study

Land-use types	Dominant species	Vegetation form	Management history
Native forest (NF)	<i>Platycaryalongipes</i> ; <i>Carpinuspubescen</i> ; <i>Lithocarpus confines</i> ; <i>Iteayunnanensis</i>	Forest	Undisturbed natural mixed deciduous and evergreen broadleaved forest
Unused grassland, (UG)	<i>Themeda japonica</i> ; <i>Heteropogonontortu</i>	Grassland	Deforested and cultivated until 2005, natural recovery to grass
Secondary shrub (SS)	<i>Caesalpinia decapetala</i> ; <i>Bauhinia championi</i> ; <i>Pyracanthafloruneana</i>	Shrub land	Deforested and cultivated until 2002, then naturally recovered to secondary forest
Eucalyptus forest, (EF)	<i>Eucalyptus robusta</i> <i>Smith</i>	Eucalyptus	Deforested and converted to eucalyptus forest in the 1990s



Sugarcane field (SF)	<i>Saccharum</i>	Sugarcane	Deforested and cultivated in the 1990s
Corn field (CF)	<i>Zea mays</i>	Corn	Deforested and cultivated in the 1990s

108 2.2 Soil samples collection and analysis

109 All samples were taken from July to August 2012. Before soil sampling, each plot was divided into five
110 subplots and samples were collected using a auger (4.1 cm diameter) down to 100 cm (0–10, 10–20,
111 20–40, 40–60, 60–80, and 80–100 cm) after removing the litter layer if available. The five samples were
112 taken randomly in an “S” form at each sampling subplot, and then these five samples were composited
113 together to gain a representative sample at each depth. Soil bulk density (BD) was measured in all six
114 layers by the core method using metal cylinders (5 cm high and 5 cm in diameter). The core samples
115 were oven-dried at 105 °C for 24 h and then weighed. A total of 150 subplots were investigated, and 180
116 soil samples were collected. All samples were sieved through a 2-mm screen, and roots and other coarse
117 debris fraction were removed. A portion of the samples were air-dried and stored at room temperature
118 for soil physicochemical analysis. Another portion of samples for microbial biomass mass analyses was
119 stored at 4 °C for no longer than one week before analyzing.

120 Soil organic C was analyzed with the Walkley and Black's dichromate oxidation method and total soil
121 N was determined via the semi-micro Kjeldahl digestion procedure. Soil available phosphorus (AP) was
122 extracted with 0.5 M NaHCO₃ at a pH of 8.5 and was analyzed with a colorimetric method. The above



123 mentioned soil C, N and P analysis was measured according to (Bao, 2000). Soil texture analysis was
 124 determined by a pipette method and the soil particle was divided into three fractions according to USDA
 125 classification system: sand 2–0.05 mm, silt 0.05–0.002 mm and clay < 0.002 mm. The soil microbial
 126 biomass carbon (SMC) were conducted by chloroform fumigation–K₂SO₄ extraction carbon automatic
 127 analysis (Wu et al., 2006).

128 **2.3 Soil organic C and total N calculations**

129 We calculated SOC and TN stocks on both FD and ESM basis. The principle of ESM method is given
 130 by (Ellert and Bettany, 1995; Lee et al., 2009). When soils are sampled to the designated depth ($i = 1, \dots,$
 131 n), the soil mass is calculated as:

$$132 \quad M_i = BD_i \times Z_i \times 100 \quad (1)$$

133 where M_i is dry soil mass (Mg ha⁻¹), BD_i is soil bulk density (g cm⁻³), Z_i is the thickness of the i th soil
 134 layer (cm), and 100 is a unit conversion factor. The SOC stock in the fixed depth was calculated as:

$$135 \quad SOC_{i, \text{fixed}} = Con_i \times M_i \times 0.1 \quad (2)$$

136 where $SOC_{i, \text{fixed}}$ is the SOC mass to a fixed layer (Mg ha⁻¹) and Con_i is the SOC concentration (g kg⁻¹),
 137 0.1 is a unit conversion factor. Hence, the equivalent C mass (Mg ha⁻¹) in a soil layer is calculated as:

$$138 \quad M_{i, \text{ex}} = M_i - M_{i, \text{equiv}} \quad (3)$$



139
$$SOC_{i,equiv} = SOC_{i,fixed} - (Con_{bottom} \times M_{i,ex})/1000 \quad (4)$$

140 where $SOC_{i,equiv}$ is the equivalent SOC mass ($Mg\ ha^{-1}$), $M_{i,equiv}$ is the selected equivalent soil mass, $M_{i,ex}$
141 ex is the excess soil mass used to attain the ESM, Con_{bottom} is SOC concentration in the deepest soil core
142 segment ($g\ C\ kg^{-1}\ soil$) (core segment = n), 1000 is a unit conversion factor. The TN stock was
143 calculated using the same procedure as described above. We designated the lightest soil mass among the
144 treatments for the specific layer as equivalent soil mass according to (Lee et al., 2009).

145 2.4 Statistical analysis

146 Analysis of variance was performed using the SPSS 11.0 software (SPSS, 2001) to compare the
147 differences in SOC and TN concentrations and stocks, SMC, AP, and soil BD among different land use
148 types or soil depths. The means were compared using Fisher's protected least significant difference
149 (LSD). Unless otherwise stated, all differences discussed are significant at the $P < 0.05$ probability
150 level.

151 3 Results

152 3.1 Selected soil physicochemical properties

153 For the 0–40 cm depth, the averaged SOC concentration was in the order of $NF < UG < SS = EF$, and
154 $SS > SF = CF$, indicating deforestation significantly reduced SOC level, particularly converted into the
155 croplands (Table 2).



156 **Table 2.** Selected soil physicochemical properties for the different land-use types (0–40 cm).

Variable	Land use types						F value
	NF	UG	SS	EF	SF	CF	
SOC (g kg ⁻¹)	15.76a	13.95b	12.40c	11.47cd	11.28d	10.99d	25.02**
TN (g kg ⁻¹)	1.53a	1.25b	1.17b	1.02c	1.02c	0.98c	22.35**
AP (mg g ⁻¹)	0.129	0.109a	0.091a	0.088a	0.104a	0.097a	2.46ns
SMC (mg kg ⁻¹)	257a	134b	143b	111b	106b	119c	24.64*
BD (g cm ⁻³)	1.36a	1.41a	1.48a	1.42a	1.32a	1.48a	1.99ns
Clay (%)	21.3	21.9	24.8	25.5	31.0	30.4	–
Silt (%)	20.1	18.9	25.2	27.0	24.2	24.9	–
Sand(%)	58.6	59.2	50.0	47.5	44.8	44.7	–
Soil texture	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam	Clay Loam	Clay Loam	–

157 Note: NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF,
 158 corn field; SOC, soil organic carbon content; TN, total nitrogen; AP, available phosphorus; SMC, soil microbial
 159 biomass carbon concentration. BD, soil bulk density; Clay, soil clay (< 0.002 mm); silt, soil silt (0.05–0.002 mm); sand,
 160 soil sand (2.0–0.05 mm); Different letters following values indicate significance at $P = 0.05$; ns, *, **, no significant
 161 difference at $P = 0.05$, <0.05, <0.01, respectively.

162 Similarly, the highest TN concentration was found under NF, and followed by UG and SS, and the
 163 lowest was EF, SF and CF. The SMC concentration was also different among the six vegetation types (P
 164 < 0.05), and were significantly greater in the native forest than in the other land-use types. However, the
 165 averaged soil BD and available phosphorus (AP) under different ecosystems did not exhibit
 166 significant differences (Table 2). The clay and silt contents slightly ranged from 21.3 % to 30.4 %, and



167 18.9 % to 27.0%, respectively among the six land-use types. Except for the two agricultural soils (SF
168 and CF) which belonged to the clay loam soil, other four land-use types all were sandy clay loam soils.

169 3.2 Soil BD, SOC and TN concentrations across soil profile

170 Two-way ANOVA showed that land use types and soil depths significantly impacted soil BD, SOC and
171 TN concentrations and stocks ($P < 0.001$; Table 3). We also observed their interactive effects for all the
172 measured parameters ($P < 0.001$; only for soil BD, $P = 0.03$).

173 **Table 3.** Results (P -values) of two-way ANOVAs on the effects of land use types, soil depth and their interaction on
174 soil bulk density (BD), soil organic C (SOC) and total nitrogen (TN) concentrations and stocks.

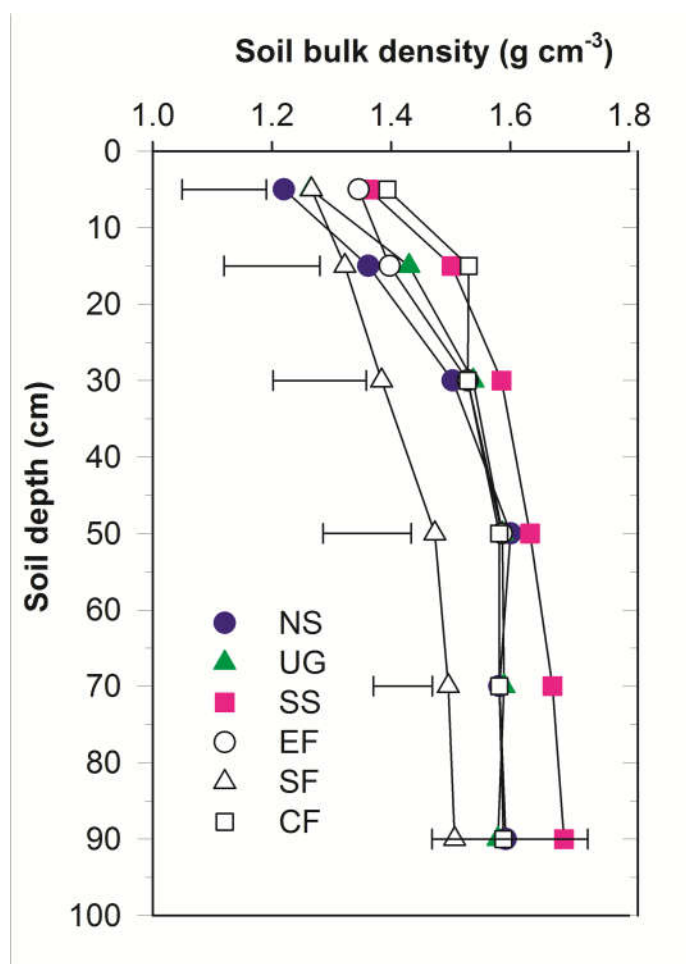
	BD	SOC concentration	TN concentration	SOC stock	TN Stock
Land use	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Soil depth	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Land use x soil depth	0.03	< 0.001	< 0.001	< 0.001	< 0.001

175 Soil BD was higher under CF and SS than that of NF in 0–10 cm layer. In the 10–20 cm depth, the
176 highest value was found in the CF soil, while the lowest occurred under SF. Below the 40 cm layer,
177 limited differences were observed among different land use types (Fig. 2). Soil BD generally ranged
178 from 1.22 to 1.69 g cm⁻³ from surface to bottom layers, showing increasing tendency across soil profile
179 irrespective of land use types (Fig. 2). Overall, soil BD was lower in upper 0–40 cm layers as compared
180 with that in the deeper layers. The changes in soil BD across the soil profile might bias the



181 interpretation of SOC stocks calculated on the FD basis.

182



183

184 **Figure 2.** Changes in soil bulk density along soil profile under different land uses types (NF, native forest; UG, unused
185 grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field). Error bars represent LSD (P

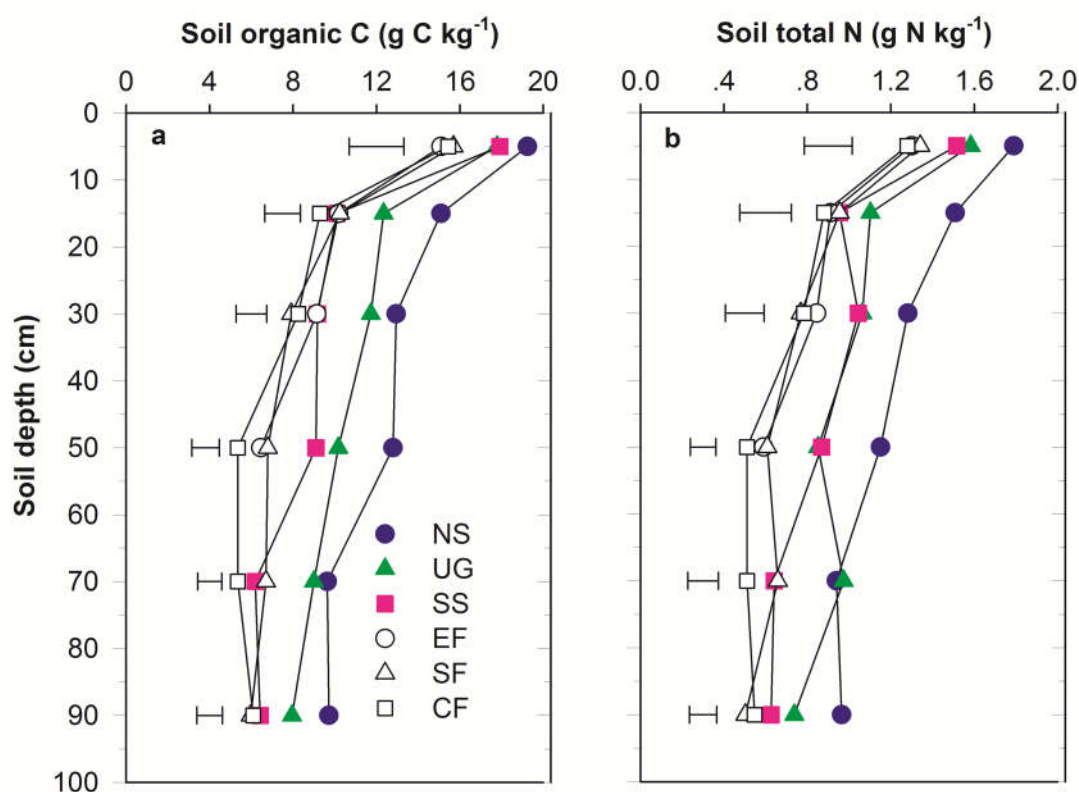
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< 0.05) for comparison among treatments at the same depth.

187



188 There was a significant interaction between land use types and depths on SOC and TN concentrations
189 ($P < 0.001$; Table 3). Overall, SOC and TN concentrations generally decreased with depths across soil
190 profile (Fig. 3).



191
192 **Figure 3.** Vertical distribution of soil organic C (a) and total N (b) concentrations as influenced by changes in land use
193 types (NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF,
194 corn field). Error bars represent LSD ($P < 0.05$) for comparison among treatments at the same depth.
195 The highest value was observed in NF soil, while the differences among NF, UG and SS soils was not



196 significant ($P > 0.05$) in the 0–10 cm layer. Afterwards, a sharp and significant decline happened in the
197 10–20 cm layer (Fig. 3). In the 20–40 cm layer, SOC concentration under NF and UG were greater than
198 those in other ecosystems, whereas no changes in SOC and N concentrations existed between SS, EF,
199 SF and CF soils. Similarly, the TN concentration followed the order of $NF > UG = SS > EF = SF = CF$.
200 At the deeper layers (i.e., 40–60, 60–80, and 80–100 cm), both SOC and TN concentrations greatly
201 decreased from native forest to other managed ecosystems (Fig. 3).

202 3.3 Soil organic C and TN stocks calculated on FD and ESM basis

203 The SOC and TN stocks calculated on FD and ESM basis are presented in Table 4. On the FD basis, no
204 differences in SOC stock between land use types were found in the 0–10 cm layer ($P > 0.05$; Table 4),
205 except for the SS, which had higher SOC stock than that of SF. In the 0–20 cm layer, NF had higher
206 SOC stock by 31.8 % and 23.0 % than that of SF and CF treatments, respectively. Taken together the 0–
207 40 cm depth, the SOC stock was in the order of $NF = UG > SS = EF$, and $SS > SF = CF$, and NF
208 increased SOC by 50.2 % and 36.4 % than SF and CF respectively. Instead, for the 0–60 cm profile,
209 SOC stock showed a trend of $NF > UG > SS > EF = SF = CF$, and the SOC under NF was higher
210 by 14.0 %, 25.5 %, 49.6 %, 64.4 % and 59.1 % than that of UG, SS, EF, SF and CF, respectively. Similar
211 trend of SOC stock induced by land use conversion was found in the 0–100 cm profile. These data
212 indicated that the SOC stocks changes heavily depended on the soil sampling depth considered. The TN



213 at different soil depths shares similar pattern with the SOC in these ecosystems.

214 **Table 4.** Soil organic C (SOC) and total N (TN) stocks calculated using fixed-depth (FD) and equivalent soil mass

215 (ESM) methods in different soil depths under land-uses types (NF, native forest; UG, unused grassland; SS, secondary

216 shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field).

Land use types	Soil depth cm	FD-based stock			ESM-based stock	
		Mg ha ⁻¹		Soil mass	Mg ha ⁻¹	
		SOC	TN		SOC	TN
NF†	0–10	23.48ab	2.18a	1220b	23.48a	2.18a
UG		22.55ab	2.00ab	1265ab	21.76ab	1.93ab
SS		24.39a	2.06ab	1362a	21.86ab	1.85abc
EF		20.27ab	1.76b	1346ab	18.38b	1.59bc
SF		19.85b	1.70b	1267ab	19.12b	1.64bc
CF		21.55ab	1.79b	1394a	18.87b	1.56c
NF		0–20	43.97a	4.25a	2582b	43.97a
UG	40.21ab		3.58b	2695ab	38.81b	3.45b
SS	39.49bc		3.49bc	2864ab	36.64bc	3.22bc
EF	34.43d		3.02cd	2744ab	32.78c	2.88c
SF	33.37d		2.96d	2589b	33.29c	2.95c
CF	35.73d		3.13bcd	2924a	32.55c	2.83c
NF	0–40		83.00a	8.09a	5589ab	79.99a
UG		76.20a	6.83b	5771ab	71.34b	6.39b
SS		68.54b	6.81b	6035a	62.33c	6.10b
EF		62.23bc	5.60c	5801ab	58.17cd	5.23c
SF		55.28c	5.09c	5357b	55.28d	5.09c
CF		60.84c	5.52c	5982a	55.69d	5.03c
NF		0–60	123.69a	11.76a	8791ab	117.47a
UG	108.46b		9.53b	8945ab	101.95b	8.99b
SS	98.29c		9.65b	9301a	89.22c	8.79b
EF	82.66d		7.47c	8974ab	78.35d	7.08c
SF	75.24d		6.88c	8304b	75.24d	6.88c



CF		77.73d	7.13c	9145a	73.23d	6.70c
NF	0–100	185.32a	17.82a	15140ab	177.24a	17.01a
UG		162.15b	14.96b	15283a	154.41b	14.24b
SS		140.68c	13.92b	16024a	129.68c	12.85b
EF		120.99d	10.40b	15225ab	115.47d	9.98c
SF		113.30d	10.37c	14310b	113.30d	10.37c
CF		113.94d	10.48c	15486a	106.78d	9.84c

217 †Values followed by a different lowercase letter in the same row among tillage treatments are significantly difference
 218 at $P < 0.05$. The bold form number for soil mass indicated the equivalent soil mass was chosen.

219 On the ESM basis, the highest SOC stock stored in the NF soil of the 0–10 cm layer ($\approx 1220 \text{ Mg ha}^{-1}$)
 220 ¹). Compared with NF, the SOC stocks in this layer was reduced by 7.3 % under UG, 6.9 % under SS
 221 and 19.6 % under CF, though the differences between NF, UG and SS were not significant ($P > 0.05$)
 222 (Table 4). For the 0–20 cm depth ($\approx 2582 \text{ Mg ha}^{-1}$), the SOC stock showed an order of $\text{NF} > \text{UG} = \text{SS}$,
 223 and $\text{UG} > \text{EF} = \text{SF} = \text{CF}$. Specifically, SOC stock was lower by 11.7 %, 16.7 % and 20.6 % following
 224 the shift from NF to UG, SS and CF respectively. Under the 0–40 cm profile selected ($\approx 5357 \text{ Mg ha}^{-1}$),
 225 the averaged SOC stock under NF reduced by 10.8 % under UG, 22.1 % under SS and 30.4 % under CF
 226 soil. When deeper profile was considered ($\geq 60 \text{ cm}$), for example, the SOC stored in the NF of 0–60 cm
 227 soil was reduced by 24.1 % under SS and 37.7 % under CF. For the whole 0–100 cm profile, the
 228 conversion from NF to SS and CF decreased SOC stocks by 26.8 % and 39.8 %, respectively. TN
 229 stocks followed similar patterns to those of SOC stocks. These data indicated that sampling depth
 230 considered may impact the interpretation of SOC losses upon the land use conversion.



231 **4 Discussion**

232 **4.1 Sampling depth for assessing SOC and TN stocks**

233 According to our study, the SOC and TN stocks markedly decreased following native forest conversion
234 to managed ecosystems by human activities (Table 4; Fig. 3). These results are in line with findings that
235 soil C and N stored in natural ecosystems are more than that in the converted lands (de Moraes Sá et al.,
236 2015; Don et al., 2011; Fialho and Zinn, 2014; Guo and Gifford, 2002). In karst region of Southwestern
237 China, similar results were also reported by other studies (Chen et al., 2012; Liu et al., 2015a).
238 Consequently, the depletion of SOM may be directly related to the loss of ecosystem services and land
239 productivity.

240 It is worth noting that the effect magnitude of SOC and TN stocks losses by land-use change might
241 partly depend on sampling depth considered. For example, the conversion from NF to croplands
242 (including EF and CF) had led to SOC loss by 19.1 %, 25.1 % and 30.6 % in the 0–10, 0–20, and 0–40
243 cm layers, respectively (Table 4). Moreover, the decreased SOC stock loss under NF relative to CF was
244 36.8 % in the 0–60 cm depth and 37.9 % in the 0–100 cm whole profile. The data clearly suggest that
245 the SOC loss due to land use changes generally increased with depth. We recommend that shallow
246 sampling may bias the interpretation of soil C data, and should be paid more attention in further study.
247 Our results are supported by other studies (Baker et al., 2007; Olson and Al-Kaisi, 2015) . Otherwise, if



248 the shallow layers of soil profile are only collected, the SOC loss following land use changes might be
249 underestimated to some extent.

250 In the karst area, most early reports have focused on the top soil layer (e.g., ≤ 30 cm) to assess the
251 SOC stocks changes (Chen et al., 2012; Fu et al., 2015; Liu et al., 2015b; Zhang et al., 2012). These
252 above mentioned works and other superficial studies on how land use change may alter SOM dynamics
253 (Post and Kwon, 2000; West and Post, 2002), may lead to erroneous results. As a result, the whole soil
254 profile, rather than the tilled layer, should be sampled to capture the possible differences in SOC stocks
255 upon land use change. Lal (2009) considered that it is important to measure the land use induced
256 changes in SOC to at least 1 m, preferably 2 m depth in forest systems where deep tap roots may
257 transfer biomass C to deeper layers.

258 **4.2 Changes in SOC and TN stocks calculated by FD and ESM approaches**

259 In our study, the ESM method has been proposed in which soil C and N data for a fixed depth can be
260 adjusted to normalize to a particular soil mass within specific layer (Ellert and Bettany, 1995). Overall,
261 the patterns of SOC and TN stocks were better clarified by the ESM approach, especially in upper
262 layers (e.g., ≤ 40 cm; Table 4). Specifically, using the FD method, we found that the SOC stock under
263 NF soil was reduced by 11.8 %, 21.4 %, 30.0 %, 38.2 %, and 38.7 % in the 0–10, 0–20, 0–40, 0–60, and
264 0–100 cm layer, respectively, compared with the croplands. By contrast, these corresponding values



265 were 19.1 %, 25.1 %, 30.6 %, 36.8 %, and 37.9 %, when calculation on the ESM basis. These data
266 indicate that FD method underestimated SOC loss in the 0–40 cm layer, but overestimated in the 0–60
267 and 0–100 cm profiles, although the two groups were not statistically different (Paired T-test, $P =$
268 0.305). The contrasting results are probably associated with the variations in soil BD across soil profile
269 (Fig. 2). Thus, in studies which use an FD approach for the comparison of SOC stocks may misinterpret
270 the SOC changes, which is supported by other studies (Don et al., 2011; Lee et al., 2009). Instead, ESM
271 calculation appears to be more effective for detecting temporal trends in SOC and other nutrients stocks.

272 It should be noted that the calculation methods also influence SOC interpretation even in deeper
273 profiles (e.g., 0–60 and 0–100 cm; Table 4). This case indicates that even if deeper soil profile (e.g., \geq
274 60 cm) is collected, it is still necessary for ESM method to accurately assess SOC stock. Otherwise, the
275 variations in soil BD, which usually occurs at surface soils, may obscure changes in profile SOC stock.
276 In future study, we should reassess the archived soil C data using ESM instead of FD approach, which
277 will help to improve our ability to detect the relatively small changes in SOM pool in response to
278 land-use change.

279 **4.3 Implications for future land-use management**

280 Our study had shown that converting native forest towards agricultural ecosystems caused the highest
281 SOM losses (as high as 39.8 % for SOC and 42.4 % for TN) down to 100 cm profile (Table 4; Fig. 2).



282 Historically, any land-use changes that disrupt the prior long-standing balance of input and decay
283 processes will induce a shift in SOC stocks (Batjes, 2014; Janzen, 2015). Almost invariably, for
284 example, converting forest to grassland or arable cropland resulted in loss of soil C and N (Don et al.,
285 2011; Foley et al., 2005; Guo and Gifford, 2002; Wei et al., 2014), not only because disturbance
286 stimulates SOM decay (Post and Kwon, 2000; Six et al., 2002), but also because inputs from
287 aboveground and belowground are reduced (Guo and Gifford, 2002). Consequently, the declined SOM
288 might drive land degradation that directly impairs on human society through loss of ecosystem goods
289 and services (Carter, 2002; Jaiarree et al., 2014; Lal, 2010; Smith et al., 2015). Regretfully, we did not
290 measure the aboveground biomass and crop yields in the present study and these knowledge should be
291 determined for productivity analysis in future investigation.

292 In the karst regions of Southwestern China, the declined SOM had caused soil degradation, leading to
293 the increased soil erosion and reduced land productivity (Hu et al., 2014; Liu et al., 2005; Xu et al.,
294 2008). Xie et al. (2014) highlighted that the degradation trend of soil fertility was almost parallel to the
295 aggravation of karst rocky desertification. Thus, how to conserve and restore the degraded karst areas is
296 promoted by Chinese central and local governments (Hu et al., 2008; Zhao et al., 2014). Our results
297 showed that the conversion of native forest to croplands had exported SOC from soils as higher as 39.8 %
298 (Table 4). Instead, for the unused grassland(UG) and secondary shrub (SS) ecosystems, the SOC stock



299 was reduced by 12.8 % and 26.8 %, respectively, compared with the native forest (NF). Based on these
300 results, we suggest that it is necessary to conduct some engineering programs (e.g., afforestation) to
301 accelerate SOC recovery in the karst region. A recent study by Liu et al. (2015a) found that the soil
302 microbial activities, texture, and litter fall played important roles in SOC accumulation along vegetation
303 successions, and relevant strategies such as increased N import and less soil disturbances were proposed
304 to enhance SOC sequestration, and thus ecological restoration in this vulnerable karst landscape. Thus,
305 further understanding the mechanisms of SOM protection and recovery under land use change is
306 heavily needed. This is particularly important in Southwestern China, because of the large area of native
307 forests being increasingly converted to agricultural land and other human managed ecosystems.

308 **5 Conclusion**

309 Our study demonstrates that the conversion of native forest into cropland and other managed systems
310 led to SOC and TN losses. However, the effect magnitude of soil C and N stocks losses were dependent
311 on sampling depths (i.e., surface vs. subsoil) and calculation approaches. The averaged SOC stocks on
312 ESM basis in the depths of 0–10, 0–20, 0–40, 0–60 and 0–100 cm of croplands (including SF and CF)
313 were 19.1 %, 25.1 %, 30.6 %, 36.8 % and 37.9 % lower, respectively, than those of NF soil. Generally,
314 the FD based soil C and N stocks differed with ESM based ones, particularly in the surface soil where
315 soil bulk density varies largely, suggesting it is crucial for soil mass correction for estimating SOC and
316 TN stocks with land-use changes. Further study is needed to elucidate the mechanisms behind SOM



317 losses after the land-use conversions in the typical karst landscapes.

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