

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, but the extent depended on

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18 ~~both sampling depths and calculation methods selected (i.e., FD or ESM).~~ On average, the shifting from  
19 native forest to cropland led to SOC losses by 19.1 %, 25.1 %, 30.6 %, 36.8 % and 37.9 % for the soil  
20 depths 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 ~~layers~~. 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.

删除的内容: The results showed that converting forest to cropland and other types significantly led to SOC and TN losses, although the effect magnitude partly depended on both sampling depths and soil mass considered

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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;

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

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

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

61 mass (ESM) approach is required to correct the calculation (Ellert and Bettany, 1995; Lee et al., 2009).

62 The ESM method, which can account for the differences in soil masses among treatments, is being  
63 increasingly employed (Don et al., 2011; Wiesmeier et al., 2015). As land-use conversions are often  
64 associated with the changes in soil BD, depth-based method would result in a considerable difference in  
65 soil masses for the quantification of SOC stocks.

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66 Karst is a distinctive topography, and the rocky desertification has become a serious environmental  
67 issue in this landscape of Guangxi Province, Southwestern China (Wang et al., 2004; Zhao et al., 2014).

68 The typical karst areas are characterized by high fragility own to their natural settings and  
69 anthropogenic impacts (Xu and Zhang, 2014). This, in turn, has not only damaged eco-environment  
70 including soil erosion, lower biodiversity and decreased soil productivity, but also exacerbated the  
71 poverty level in the rural areas of the region (Hu et al., 2014; Liu et al., 2005; Wang et al., 2004). Thus,  
72 karst area is considered as small environment capacity, weak anti-disturbance, low stability and  
73 powerless self-adjustment.

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

80 important to evaluate the changes in SOM status, which are accompanied by changes in ecosystem  
81 services, processes, and functions (Carter, 2002; Smith et al., 2015). We hypothesized that the  
82 ecological resettlement program may deplete SOM stocks, and thus cause soil degradation. Recently,  
83 some researchers have paid more attention about the effect of land use change on the soil  
84 physicochemical properties and soil quality in this karst region (Fu et al., 2015; Hu et al., 2014; Xie et  
85 al., 2014; Xu et al., 2008). However, studies on soil C dynamics were mainly focused on the surface  
86 layers (e.g.,  $\leq 30$  cm) and the FD method are utilized to assess SOC stocks (Chen et al., 2012; Fu et al.,  
87 2015; Liu et al., 2015b; Zhang et al., 2012). Very little effort has been devoted to the effect of land  
88 conversion on SOC stocks in the deeper soils. Thus, the present study was aimed to (1) quantify the  
89 changes in SOC and TN stocks down to 100 cm after conversion from native forest to croplands and  
90 other managed systems; (2) to examine whether the calculation methodology (i.e., ESM vs. FD) may  
91 change the interpretation of the results.

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## 92 2 Materials and methods

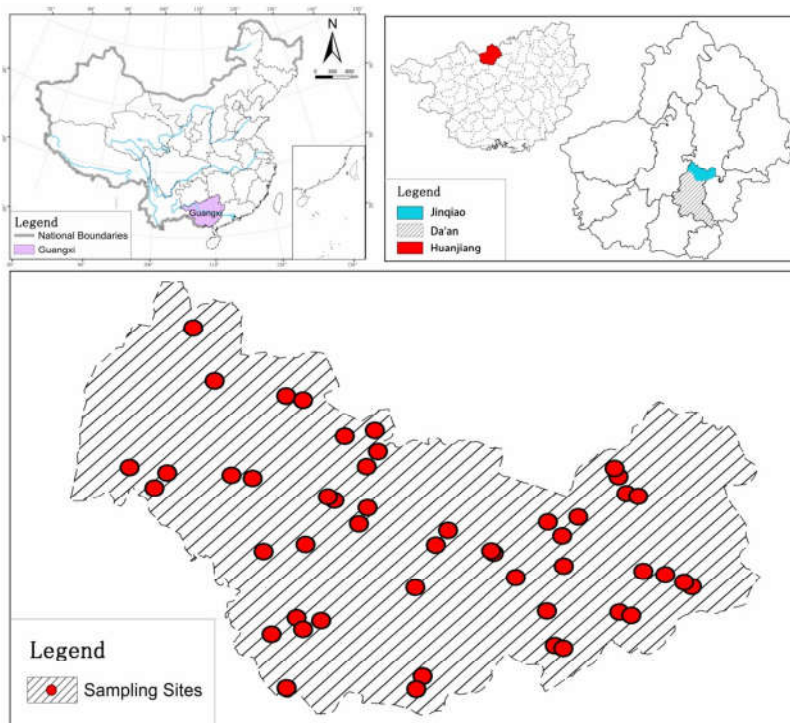
### 93 2.1 Study area

94 This study was conducted at the Huanjiang County (107°51'–108°43' E, 24°44'–25°33' N), Guangxi  
95 Province, Southwestern China (Fig. 1). This county is surrounded by mountains, and has a total land  
96 area of 4 572 km<sup>2</sup> with elevation ranging from 149 to 1 693 m. The natural vegetation mainly consisted  
97 of shrubs, herbs and lianas. The region is characterized by a subtropical monsoon climate with a distinct

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

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105 **Figure 1.** Location of the study area and the distribution of soil sampling sites in northwest Guangxi, Southwestern

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

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119 **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>Caesalpiniaadecapetala</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 the1990s

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

## 122 2.2 Soil samples collection and analysis

123 All samples were taken from July to August 2012. Before soil sampling, each plot was divided into five  
124 subplots and samples were collected using a auger (4.1 cm diameter) down to 100 cm (0–10, 10–20,  
125 20–40, 40–60, 60–80, and 80–100 cm) after removing the litter layer if available. The five samples were  
126 taken randomly in an “S” form at each sampling subplot, and then were composited together to gain a  
127 representative sample at each depth. Soil bulk density (BD) was measured in all six layers by the core  
128 method using metal cylinders (5 cm in height and 5 cm in diameter). The core samples were oven-dried  
129 at 105 °C for 24 h and then weighed. A total of 150 subplots were investigated, and 180 soil samples  
130 were collected. All samples were sieved through a 2-mm screen, and roots and other coarse debris  
131 fraction were removed. A portion of the samples were air-dried and stored at room temperature for soil  
132 physicochemical analysis. Another portion of samples for microbial biomass mass analyses was stored  
133 at 4 °C for no longer than one week before analyzing.

134 Soil organic C was analyzed with the Walkley and Black's dichromate oxidation method and total soil  
135 N was determined via the semi-micro Kjeldahl digestion procedure. Soil available phosphorus (AP) was  
136 extracted with 0.5 M NaHCO<sub>3</sub> at a pH of 8.5 and was analyzed with a colorimetric method. The above

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138 mentioned soil C, N and P analysis was measured according to (Bao, 2000). Soil texture analysis was  
139 determined by a pipette method and the soil particle was divided into three fractions according to USDA  
140 classification system: sand 2–0.05 mm, silt 0.05–0.002 mm and clay < 0.002 mm. The soil microbial  
141 biomass carbon (SMC) was measured by chloroform fumigation–K<sub>2</sub>SO<sub>4</sub> extraction carbon automatic  
142 analysis (Wu et al., 2006).

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### 143 2.3 Soil organic C and total N calculations

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

$$147 \quad M_i = BD_i \times Z_i \times 100 \quad (1)$$

148 where  $M_i$  is dry soil mass (Mg ha<sup>-1</sup>),  $BD_i$  is soil bulk density (g cm<sup>-3</sup>),  $Z_i$  is the thickness of the  $i$ th soil  
149 layer (cm), and 100 is a unit conversion factor. The SOC stock in the fixed depth was calculated as:

$$150 \quad SOC_{i, \text{fixed}} = Con_i \times M_i \times 0.1 \quad (2)$$

151 where  $SOC_{i, \text{fixed}}$  is the SOC mass to a fixed layer (Mg ha<sup>-1</sup>) and  $Con_i$  is the SOC concentration (g kg<sup>-1</sup>),  
152 0.1 is a unit conversion factor. Hence, the equivalent C mass (Mg ha<sup>-1</sup>) in a soil layer is calculated as:

$$153 \quad M_{i, \text{ex}} = M_i - M_{i, \text{equiv}} \quad (3)$$

156 
$$SOC_{i,equiv} = SOC_{i,fixed} - (Con_{bottom} \times M_{i,ex})/1000 \quad (4)$$

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

## 162 2.4 Statistical analysis

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

## 168 3 Results

### 169 3.1 Selected soil physicochemical properties

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

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175 **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 <sup>-1</sup> )	15.76a	13.95b	12.40c	11.47cd	11.28d	10.99d	25.02**
TN (g kg <sup>-1</sup> )	1.53a	1.25b	1.17b	1.02c	1.02c	0.98c	22.35**
AP (mg g <sup>-1</sup> )	0.129	0.109a	0.091a	0.088a	0.104a	0.097a	2.46ns
SMC (mg kg <sup>-1</sup> )	257a	134b	143b	111b	106b	119c	24.64*
BD (g cm <sup>-3</sup> )	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	–

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

181 Similarly, the highest TN concentration was found under NF, and followed by UG and SS, and the  
 182 lowest was EF, SF and CF. The SMC concentration was also different among the six vegetation types ( $P$   
 183 < 0.05), and were significantly greater in the native forest than in the other land-use types. However, the  
 184 averaged soil BD and available phosphorus (AP) under different ecosystems did not exhibit  
 185 significantly differences (Table 2). The clay and silt contents ranged from 21.3 % to 30.4 %, and from

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187 18.9 % to 27.0%, respectively among the six land-use types. Except for the two agricultural soils (SF  
188 and CF) that belonged to the clay loam soil, the other four land-use types all were sandy clay loam soils.

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### 189 3.2 Soil BD, SOC and TN concentrations across soil profile

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

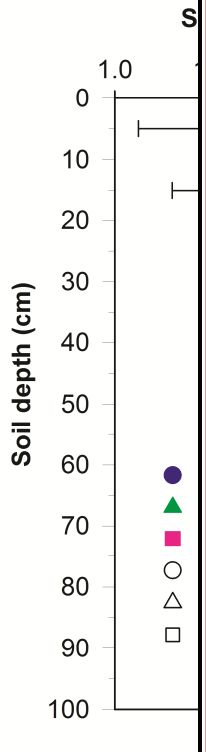
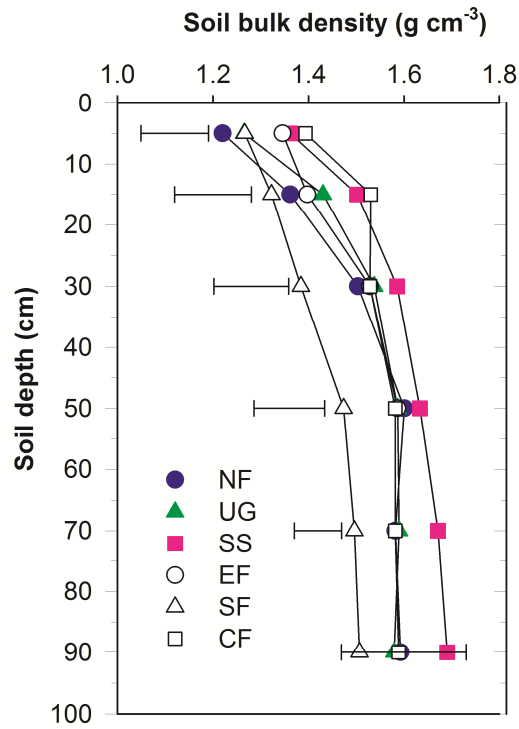
193 **Table 3.** Results ( $P$ -values) of two-way ANOVAs on the effects of land use types, soil depth and their interaction on  
194 soil bulk density (BD), soil organic C (SOC) and total nitrogen (TN) concentrations and stocks.

	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

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

202 interpretation of SOC stocks calculated on the FD basis.

203



204

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

207

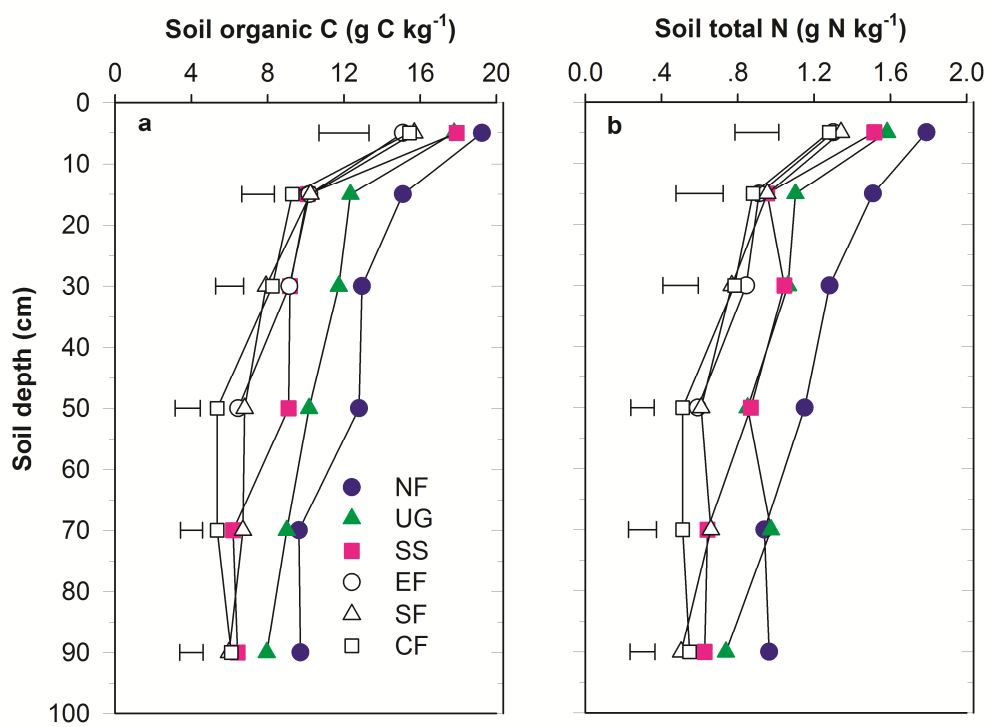
< 0.05) for comparison among treatments at the same depth.

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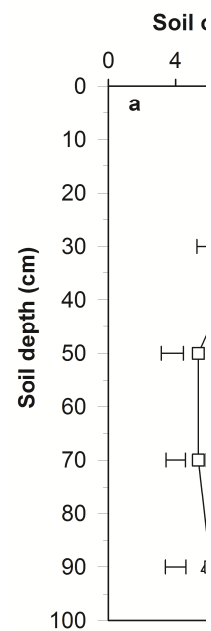
210 There was a significant interaction between land use types and depths on SOC and TN concentrations

211 ( $P < 0.001$ ; Table 3). Overall, SOC and TN concentrations generally decreased with depths across soil

212 profile (Fig. 3).



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214 **Figure 3.** Vertical distribution of soil organic C (a) and total N (b) concentrations as influenced by changes in land use

216 types (NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF,  
217 corn field). Error bars represent LSD ( $P < 0.05$ ) for comparison among treatments at the same depth.

218 The highest value was observed in NF soil, while the difference among NF, UG and SS soils was not  
219 significant ( $P > 0.05$ ) in the 0–10 cm layer. Afterwards, a sharp and significant decline happened in the  
220 10–20 cm layer (Fig. 3). In the 20–40 cm layer, SOC concentration under NF and UG were greater than  
221 those in other ecosystems, whereas no changes in SOC and N concentrations existed between SS, EF,  
222 SF and CF soils. Similarly, the TN concentration followed the order of  $NF > UG = SS > EF = SF = CF$ .  
223 At the deeper layers (i.e., 40–60, 60–80, and 80–100 cm), both SOC and TN concentrations greatly  
224 decreased from native forest to other managed ecosystems (Fig. 3).

### 225 3.3 Soil organic C and TN stocks calculated on FD and ESM basis

226 The SOC and TN stocks calculated on FD and ESM basis are presented in Table 4. On the FD basis, no  
227 differences in SOC stock between land use types were found in the 0–10 cm layer ( $P > 0.05$ ; Table 4),  
228 except for the SS, which had higher SOC stock than SF. In the 0–20 cm layer, NF had higher SOC stock  
229 by 31.8 % and 23.0 % than that of SF and CF treatments, respectively. Taken together the 0–40 cm  
230 depth, the SOC stock was in the order of  $NF = UG > SS = EF$ , and  $SS > SF = CF$ , and NF increased  
231 SOC by 50.2 % and 36.4 % than SF and CF respectively. Instead, for the 0–60 cm profile, SOC stock  
232 showed a trend of  $NF > UG > SS > EF = SF = CF$ , and the SOC under NF was higher by 14.0 %, 25.5 %,   
233

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235 49.6 %, 64.4 % and 59.1 % than that of UG, SS, EF, SF and CF, respectively. Similar trend of SOC  
 236 stock induced by land use conversion was found in the 0–100 cm profile. These data indicated that the  
 237 SOC stocks changes heavily depended on the soil sampling depth considered. The TN at different soil  
 238 depths shares similar [a](#) pattern with the SOC in these ecosystems.

239 **Table 4.** Soil organic C (SOC) and total N (TN) stocks calculated using fixed-depth (FD) and equivalent soil mass  
 240 (ESM) methods in different soil depths under land-uses types (NF, native forest; UG, unused grassland; SS, secondary  
 241 shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field).

Land use types	Soil depth cm	FD-based stock Mg ha <sup>-1</sup>			ESM-based stock Mg ha <sup>-1</sup>	
		SOC	TN	Soil mass	SOC	TN
NF†	0–10	23.48ab	2.18a	<b>1220b</b>	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	<b>2582b</b>	43.97a	4.25a
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	7.79a
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	<b>5357b</b>	55.28d	5.09c



CF		60.84c	5.52c	5982a	55.69d	5.03c
NF	0–60	123.69a	11.76a	8791ab	117.47a	11.20a
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	<b>14310b</b>	113.30d	10.37c
CF		113.94d	10.48c	15486a	106.78d	9.84c

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

244 On the ESM basis, the highest SOC stock stored in the NF soil of the 0–10 cm layer ( $\approx 1220 \text{ Mg ha}^{-1}$ )  
 245 <sup>1</sup>). Compared with NF, the SOC stock in this layer was reduced by 7.3 % under UG, 6.9 % under SS  
 246 and 19.6 % under CF, though the differences between NF, UG and SS were not significant ( $P > 0.05$ )  
 247 (Table 4). For the 0–20 cm depth ( $\approx 2582 \text{ Mg ha}^{-1}$ ), the SOC stock showed an order of  $\text{NF} > \text{UG} = \text{SS}$ ,  
 248 and  $\text{UG} > \text{EF} = \text{SF} = \text{CF}$ . Specifically, SOC stock was lower by 11.7 %, 16.7 % and 20.6 % following  
 249 the shift from NF to UG, SS and CF respectively. Under the 0–40 cm profile selected ( $\approx 5357 \text{ Mg ha}^{-1}$ ),  
 250 the averaged SOC stock under NF reduced by 10.8 % under UG, 22.1 % under SS and 30.4 % under CF  
 251 soil. When deeper profile was considered ( $\geq 60 \text{ cm}$ ), for example, the SOC stored in the NF of 0–60 cm  
 252 soil was reduced by 24.1 % under SS and 37.7 % under CF. For the whole 0–100 cm profile, the

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255 conversion from NF to SS and CF decreased SOC stocks by 26.8 % and 39.8 %, respectively. TN

256 stocks followed similar patterns ~~with~~ SOC stocks. These data indicated that sampling depth considered

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257 may impact the interpretation of SOC losses upon the land use conversion.

## 258 **4 Discussion**

### 259 **4.1 Sampling depth for assessing SOC and TN stocks**

260 According to our study, the SOC and TN stocks markedly decreased following native forest conversion

261 to managed ecosystems by human activities (Table 4; Fig. 3). These results are in line with findings that

262 soil C and N stored in natural ecosystems are more than that in the converted lands (de Moraes Sá et al.,

263 2015; Don et al., 2011; Fialho and Zinn, 2014; Guo and Gifford, 2002). In karst region of Southwestern

264 China, similar results were also reported (Chen et al., 2012; Liu et al., 2015a). Consequently, the

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265 depletion of SOM may be directly related to the loss of ecosystem services and land productivity.

266 It is worth noting that the effect magnitude of SOC and TN stocks losses by land-use change might

267 partly depend on sampling depth considered. For example, the conversion from NF to croplands

268 (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

269 cm layers, respectively (Table 4). Moreover, the decreased SOC stock loss under NF relative to CF was

270 36.8 % in the 0–60 cm depth and 37.9 % in the 0–100 cm whole profile. The data clearly suggest that

271 the SOC loss due to land use changes generally increased with depth. We recommend that shallow

274 sampling may bias the interpretation of soil C data, and should be paid more attention in further study.

275 Our results are supported by other studies (Baker et al., 2007; Olson and Al-Kaisi, 2015) . Otherwise, if

276 the shallow layers of soil profile ~~only are~~ collected, the SOC loss following land use changes might be

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277 underestimated to some extent.

278 In the karst area, most early reports have focused on the top soil layer (e.g.,  $\leq 30$  cm) to assess the

279 SOC stocks changes (Chen et al., 2012; Fu et al., 2015; Liu et al., 2015b; Zhang et al., 2012). These

280 above mentioned works and other superficial studies on how land use change may alter SOM dynamics

281 (Post and Kwon, 2000; West and Post, 2002), may lead to erroneous results. As a result, the whole soil

282 profile, rather than the tilled layer, should be sampled to capture the possible differences in SOC stocks

283 upon land use change. Lal (2009) considered that it is important to measure the land use induced

284 changes in SOC to at least 1 m, preferably 2 m depth in forest systems where deep tap roots may

285 transfer biomass C to deeper layers.

#### 286 **4.2 Changes in SOC and TN stocks calculated by FD and ESM approaches**

287 In our study, the ESM method has been proposed in which soil C and N data for a fixed depth can be

288 adjusted to normalize to a particular soil mass within specific layer (Ellert and Bettany, 1995). Overall,

289 the patterns of SOC and TN stocks were better clarified by the ESM approach, especially in upper

290 layers (e.g.,  $\leq 40$  cm; Table 4). Using the FD method, the SOC stored in the NF soil was reduced by

292 11.8 %, 21.4 %, 30.0 %, 38.2 %, and 38.7 % in the 0–10, 0–20, 0–40, 0–60, and 0–100 cm layers,  
293 respectively, as compared with the croplands (including SS and CF). By contrast, these corresponding  
294 values were 19.1 %, 25.1 %, 30.6 %, 36.8 %, and 37.9 %, when calculation on the ESM basis. These  
295 data indicate that FD method underestimated SOC loss in the 0–40 cm layer, but overestimated in the 0–  
296 60 and 0–100 cm profiles, although the two groups were not statistically different (Paired T-test,  $P =$   
297 0.305). The contrasting results are probably associated with the variations in soil BD across soil profile  
298 (Fig. 2). Thus, in studies which use an FD approach for the comparison of SOC stocks may misinterpret  
299 the SOC changes, which is supported by other studies (Don et al., 2011; Lee et al., 2009). Instead, ESM  
300 calculation appears to be more effective for detecting temporal trends in SOC and other nutrients stocks.

删除的内容: Specifically, using the FD method, we found that the SOC stock under 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 0–100 cm layer, respectively, compared with the croplands.

301 It should be noted that the calculation methods also influence SOC interpretation even in deeper  
302 profiles (e.g., 0–60 and 0–100 cm; Table 4). This case indicates that even if deeper soil profile (e.g.,  $\geq$   
303 60 cm) is collected, it is still necessary for ESM method to accurately assess SOC stock. Otherwise, the  
304 variations in soil BD, which usually occurs at surface soils, may obscure changes in profile SOC stock.  
305 In future study, we should reassess the archived soil C data using ESM instead of FD approach, which  
306 will help to improve our ability to detect the relatively small changes in SOM pool in response to  
307 land-use change.

#### 308 **4.3 Implications for future land-use management**

316 Our study had shown that converting native forest towards agricultural ecosystems caused the highest  
317 SOM losses (as high as 39.8 % for SOC and 42.4 % for TN) down to 100 cm profile (Table 4; Fig. 2).  
318 Historically, any land-use changes that disrupt the prior long-standing balance of input and decay  
319 processes will induce a shift in SOC stocks (Batjes, 2014; Janzen, 2015). Almost invariably, for  
320 example, converting forest to grassland or arable cropland resulted in loss of soil C and N (Don et al.,  
321 2011; Foley et al., 2005; Guo and Gifford, 2002; Wei et al., 2014), not only because disturbance  
322 stimulates SOM decay (Post and Kwon, 2000; Six et al., 2002), but also because inputs from  
323 aboveground and belowground are reduced (Guo and Gifford, 2002). Consequently, the declined SOM  
324 might drive land degradation that directly impairs on human society through loss of ecosystem goods  
325 and services (Carter, 2002; Jaiarree et al., 2014; Lal, 2010; Smith et al., 2015). Regretfully, we did not  
326 measure the aboveground biomass and crop yields in the present study and the knowledge should be  
327 determined for productivity analysis in future investigation.

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328 In the karst regions of Southwestern China, the declined SOM had caused soil degradation, leading to  
329 the increased soil erosion and reduced land productivity (Hu et al., 2014; Liu et al., 2005; Xu et al.,  
330 2008). Xie et al. (2014) highlighted that the degradation trend of soil fertility was almost parallel to the  
331 aggravation of karst rocky desertification. Thus, how to conserve and restore the degraded karst areas is  
332 promoted by Chinese central and local governments (Hu et al., 2008; Zhao et al., 2014). Our results

334 showed that the conversion of native forest to croplands had exported SOC from soils as higher as 39.8 %  
335 (Table 4). Instead, for the unused grassland (UG) and secondary shrub (SS) ecosystems, the SOC stock  
336 was reduced by 12.8 % and 26.8 %, respectively, compared with the native forest (NF). Based on these  
337 results, we suggest that it is necessary to conduct some engineering programs (e.g., afforestation) to  
338 accelerate SOC recovery in the karst region. A recent study by Liu et al. (2015a) found that the soil  
339 microbial activities, texture, and litter fall played important roles in SOC accumulation along vegetation  
340 successions, and relevant strategies such as increased N import and less soil disturbances were proposed  
341 to enhance SOC sequestration, and thus ecological restoration in this vulnerable karst landscape. Thus,  
342 further understanding of the mechanisms of SOM protection and recovery under land use change is  
343 heavily needed. This is particularly important in Southwestern China, because of the large area of native  
344 forests being increasingly converted to agricultural land and other human managed ecosystems.

## 345 **5 Conclusion**

346 Our study demonstrates that the conversion of native forest into cropland and other managed systems  
347 led to SOC and TN losses. However, the effect magnitude of soil C and N stocks losses were dependent  
348 on sampling depths (i.e., surface vs. subsoil) and calculation approaches. The averaged SOC stocks on  
349 ESM basis in the depths of 0–10, 0–20, 0–40, 0–60 and 0–100 cm of croplands (including SF and CF)  
350 were 19.1 %, 25.1 %, 30.6 %, 36.8 % and 37.9 % lower, respectively, than those of NF soil. Generally,  
351 the FD based soil C and N stocks differed with ESM based ones, particularly in the surface soil where

352 soil bulk density varies largely, suggesting it is crucial for soil mass correction for estimating SOC and  
353 TN stocks with land-use changes. Further study is needed to elucidate the mechanisms behind SOM  
354 losses after the land-use conversions in the typical karst landscapes.

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