

Changes in soil carbon sequestration in *Pinus massoniana* forests along an urban-to-rural gradient of southern China

H. Chen^{1,3}, W. Zhang¹, F. Gilliam², L. Liu¹, J. Huang¹, T. Zhang¹, W. Wang¹, J. Mo¹

¹ Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

² Department of Biological Science, Marshall University, Huntington, WV 25755-2510, U.S.A.

³ University of Chinese Academy of Sciences, Beijing 100039, China

Abstract

Urbanization is accelerating globally, causing a variety of environmental changes such as increases in air temperature, precipitation, atmospheric CO₂, and nitrogen (N) deposition. However, effects of these changes on forest soil carbon (C) sequestration remain largely unclear. Here, we used urban-to-rural environmental gradients in Guangdong Province, southern China, to address the potential effects of these environmental changes on soil C sequestration in *Pinus massoniana* forests. In contrast with our expectations and earlier observations, soil C content in urban sites was significantly lower than those in suburban and rural sites. Lower soil C pools in urban sites were correlated with a significant decrease in fine root biomass and a potential increase in soil organic C decomposition. Variation of soil C pools was also a function of change in soil C fractions. Heavy fraction C content in urban sites was significantly lower than those in suburban and rural sites. By contrast, light fraction C content did not vary significantly along the urban-to-rural gradient. Our results suggest that urbanization-induced environmental changes may have negative effect on forest soil C in the studied region.

1 Introduction

Urbanization is accelerating globally, with 50% of the world's population currently living in cities, with projected increases to 70% by 2050 (UNFPA, 2007). Rapid urban development has the potential to alter regional C budgets through urbanization-induced environmental changes (Trusilova and Churkina, 2008; Pouyat et al., 2002). Urbanization-induced environmental changes includes a variety of environmental changing factors caused by accelerating urbanization, such as increases in air temperature, precipitation, atmospheric CO₂, and nitrogen (N) deposition (Shen et al., 2008). Numerous studies have shown air temperature (Jones et al., 1990), precipitation (Botkin and Beveridge, 1997; Gilbert et al., 1989), atmosphere CO₂ (Idso et al., 2002; Pataki et al., 2003), and N deposition (Lovett et al., 2000; Fenn et al., 2003) to be higher in urban areas than in rural surroundings. This environmental gradient may even be a useful tool for investigating how global environmental change influences forest ecosystem structure and function, since such changes in cities are also known to be major drivers of global change (Carreiro and Tripler, 2005; Shen et al., 2008;).

The current scientific evidence supports that urbanization-induced environmental changes should increase soil C sequestration of urban forests. Results from long-term N addition experiments in the United States and Europe have shown that N deposition can increase forest soil C sequestration of 0.51 to 0.69 Mg C ha⁻¹ yr⁻¹ (Hyvonen et al., 2008; Pregitzer, et al., 2008). Using a meta-analysis of experiments carried out over >2 yr periods, Jastrow et al. (2005) reported that elevated CO₂ concentration would increase soil C sequestration of 0.19 Mg C ha⁻¹ yr⁻¹. If combined with N addition, this positive effect of elevated CO₂ on soil C storage would be more pronounced (van Groenigen et al., 2006; Hungate et al., 2009). This belief was also supported by recent direct field measurements along an urban-to-rural gradient in New York red oak (*Quercus rubra* L.) forests (Pouyat et al., 2002) and in a semi-arid tropical desert ecosystem in Phoenix, Arizona (Koerner et al., 2010). However, besides the above mentioned two direct measurements, this belief has not been tested in other cities, forests and (or) climate zone (Pouyat et al., 2003; Yesilonis and Pouyat 2012). Soil warming induced by elevated urban air temperate may reduce soil C storage in the short-term by increasing decomposition, this may be offset by increasing C input and SOM stabilization in the

long-term (Conant et al., 2008; Giardina et al., 2000). As a result, diversity in the responses of forest soil C to urbanization-induced environmental changes may also be existent.

China has undergone rapid urbanization, largely resulting from economic reform and the “open door policy” initiated in late 1978 (Chen et al., 2006). The population of Guangdong Province, southern China, increased nearly two-fold from 1982 to 2010 (i.e., 53.6 million to 104.3 million persons) (SBGP, 2011). Despite this notable increase, no data are available that relate the response of forest soil C to these urbanization-induced changes.

To address this, we established urban-to-rural gradients in Guangdong Province, beginning with the Pearl River Delta (PRD) economic region at the center of development; the PRD covers nearly 25% of the provincial area and supports ~54% of the population (SBGP, 2011). The purpose of this study was to assess the potential effects of urbanization changes on forest soil C in southern China utilizing this urban-to-rural gradient. Masson pine (*Pinus massoniana* L.) plantations were chosen because of their wide distribution in southern China, accounting for 45% of total plantation area in Guangdong Province (Kuang et al., 2008). In addition, Masson pine forests have relatively structural and spatial homogeneity, eliminating the confounding of other factors. We hypothesized that urbanization-induced environmental changes would increase soil C sequestration in these pine forests.

2 Materials and methods

2.1 Study region

This study comprised sites located throughout Guangdong Province, southern China (Fig. 1). The PRD economic region is the fastest developing area in the Province. The following environmental gradients have been related to patterns of urbanization extending from the core of PRD to its surrounding areas: (1) air temperature is approximately 0.5-2.0 °C higher in the core of PRD than in its surroundings due to the effect of “urban heat island” (Mai et al., 2011; Dou et al., 2011); (2) CO₂ emissions are relatively elevated in PRD, accounting for 70% of total emissions in Guangdong Province (Liu, 2009); (3) rates of N deposition vary from approximately 46 kg ha⁻¹y⁻¹ toward the

core of PRD to $< 20 \text{ kg ha}^{-1}\text{y}^{-1}$ in the most distant rural areas (Huang et al., 2012; Kuang et al., 2011); and (4) annual average precipitation is also higher in urban area than in surrounding areas (Li et al., 2009).

Because the pattern of urbanization of this region is not always linear, we combine both distance from center and land-use status to determine our gradients. We initially use distance to define four urbanization classes in this study region: (1) urban, 0-65 km from urban core; (2) urban/suburban, 65-130 km from urban core; (3) suburban/rural, 130-195 km from urban core; (4) rural, 195-260 km from urban core (Fig. 1). We further divided each class into 10 subzones of equal area. In each class we randomly chose 3 or 4 subzones to locate our sampled forests based on a land-use map. In total, 14 forests were selected in this study — three in the urban class (Huolushan, Maofengshan, and Shunfengshan, abbreviated to HLS, MFS, and SFS, respectively), four in the urban/suburban class (Heshan (HS), Dinghushan (DHS), Guanyinshan (GYS), and Xiangtoushan (XTS)), four in the suburban/rural class (Heishiding (HSD), Shimentai (SMT), Yunjishan (YJS), and Dachouding (DCD)), and three in the rural class (Huaiji (HJ), Dadongshan (DDS), and Wuzhishan (WZS) (Fig.1). Longitude of these forests ranges from $\text{E}111^{\circ}54'19.78''$ to $\text{E}114^{\circ}25'37.54''$, and latitude ranges from $\text{N}22^{\circ}40'13.31''$ to $\text{N}24^{\circ}46'40.25''$ (Table S1). Annual precipitation ranges from 1566 to 2133 mm, and mean annual air temperature ranges from 19.45 to 22.2 °C in the study region (Table S1).

All pine plantations used in this study had remained unmanaged following planting. Several criteria were used in site selection to ensure comparability among forests: (1) no disturbance after planting, including fire, insect infestations, logging, and fertilization; (2) stand age between 40 and 60 years; (3) stand density between 600 and 800 trees ha^{-1} (Table S1); (4) soils of lateritic red earth (Ultisols in USDA soil taxonomy or Acrisols in the FAO soil classification). In addition, sampling was carried out in the center of the selected site to avoid edge effects.

2.2 Soil sampling

Soil sampling was conducted during January to May of 2011. In each forest site, three random subplots (5m×5m) were selected to sample soil from three soil layers (0-10 and 10-20 and 20-40 cm

122 depths) using a 10-cm inside diameter (ID) corer. Soil samples passed through a 2 mm sieve, and
123 roots and plant residues were removed. Soil organic carbon (SOC) was determined by dichromate
124 oxidation and titration with ferrous ammonium sulfate (Walkley and Black, 1934). Soil total
125 nitrogen (TN) was measured using the micro-Kjeldahl method (Jackson, 1964). For bulk density
126 determination, soil was collected in a $0.25 \text{ m}^2 \times 0.5 \text{ m}$ deep pit in each subplot using a 5-cm ID
127 corer. Bulk density measures were used to calculate SOC content.

128

129 Soil microbial biomass carbon (MBC) was estimated by chloroform fumigation extraction
130 technique (Vance et al., 1987). Soluble C was extracted using a 0.5 M K_2SO_4 solution from 10-g soil
131 samples before and after fumigation. Extracts were analyzed for total dissolved C using a total C
132 analyzer (Shimadzu model TOC-500, Kyoto, Japan). Soil MBC was calculated as the difference in
133 extractable C between fumigated and non-fumigated soil, divided by 0.45. Soil extractable
134 dissolved organic carbon (DOC) was measured on the same samples used for the analysis of MBC,
135 and calculated as the K_2SO_4 -extractable C concentration.

136

137 **2.3 Soil density fractions**

138 Soil C was separated into two fractions using a density fraction method: (1) light fraction (LF),
139 which tends to have younger soil C pools and includes undecomposed or partly decomposed
140 organic residues and micro-biomass (Christensen et al., 2001); (2) heavy fraction (HF), which
141 generally contains older soil C pools and includes C associated with mineral surfaces or concealed
142 within micro-aggregates (Trumbore, 1993). Methodology for soil C fractionation followed
143 McLauchlan and Hobbie (2004) with alterations as noted. Approximately 15 g of air-dried soil was
144 weighed into a 100 ml centrifuge tube with 50 mL NaI (a density of 1.7 g cm^{-3}). Tubes were
145 centrifuged at 1000 rpm for 10 min. The materials floating on the surface of tubes (LF) were
146 decanted into a vacuum filter unit with 0.45 μm nylon filter paper. This process was repeated until
147 no floating material remained. The materials remaining at the bottom (HF) of the centrifuge tube
148 were also rinsed into the vacuum filter unit. All samples on the filter paper were washed with 75 mL
149 of 0.01 mol/L CaCl_2 , followed by at least 75 mL of distilled water. The light and heavy materials
150 were dried at 60°C for 48 h and weighed. All samples passed a 60-mesh sieve and analyzed for
151 SOC and TN concentration as previously described.

152

153 **2.4 Fine root biomass**

154 Root cores were collected using a 10-cm ID corer from 0-10 cm soil layer. Fine roots (≤ 2 mm
155 diameter) were sorted from washed cores by hand into living and dead components following
156 procedures from Silver and Vogt (1993). Root samples were washed by distilled water, oven dried,
157 and measured for living and dead fine roots biomass. The SOC and TN of live fine root samples
158 were also analyzed as described.

159

160 **2.5 Statistical analysis**

161 All data analyses were carried out using SAS software (SAS Institute Inc., Cary NC, USA).
162 One-way analysis of variance (ANOVA) was performed to compare the differences among four
163 urbanization classes (urban, urban/suburban, suburban/rural, and rural) in fine root biomass, fine
164 root C and N concentration, and soil respiration. Two-way ANOVA was used to test differences
165 among urbanization classes and soil depths in the variables which were measured in multiple soil
166 layers. Correlation and regression analyses were used to examine relationships between variables
167 and distance from urban center to rural. Statistical significant differences were set at $P < 0.05$ unless
168 otherwise stated. Mean values are expressed ± 1 standard error of the mean.

169

170 **3 Results**

171

172 **3.1 SOC and TN concentrations**

173 SOC and TN concentrations both varied significantly with urbanization class, with both increasing
174 from urban to rural condition (Table 1). Significant and positive correlation existed between SOC
175 concentrations, soil TN concentrations and distance from urban to rural in all soil depths ($0.52 \leq R^2$
176 ≤ 0.66 , all $P < 0.001$). Distance explained approximately 24 - 31% and 21- 36% of changing for
177 SOC and soil TN among sites, respectively. Two-way ANOVA showed that urbanization-induced
178 environmental changes significantly reduced SOC and TN concentrations in urban compared with
179 those in suburban and rural sites in all soil depths (Table 1, all $P < 0.05$). As a result, no significant
180 difference among gradient classes was shown for the soil C: N ratio in any soil layer (Table 1, all P
181 > 0.05).

182

183 **3.2 SOC content**

184 When SOC was calculated as content (i.e., as Mg ha^{-1}) it increased significantly from urban to rural
 185 conditions, exhibiting a positive linear relationship with distance across all soil depths (Fig 2 A, R^2
 186 $= 0.717$, $P < 0.001$). Two-way ANOVA showed that SOC content significantly increased from urban
 187 to rural at 0-10 cm depth (Fig 2B, $P < 0.001$), but not at 10-20 and 20-20 cm depths (Fig 2B, $P =$
 188 0.5060 and 0.0821 , respectively). When calculating SOC content to 40 cm depths, the mean SOC
 189 content were 64.87 ± 4.17 , 79.12 ± 11.7 , 93.83 ± 8.71 , and $96.43 \pm 6.60 \text{ Mg ha}^{-1}$ in urban,
 190 urban/suburban, suburban/rural and rural sites, respectively.

191

192 **3.3 Soil density fractions**

193 LF and HF showed different patterns along the urban-to-rural gradient. HF comprised $> 94\%$ of
 194 total soil mass and contained the majority of soil C content (approximately 70 - 85%) for all sites
 195 combined (Table 2). Mass proportion of LF and HF, LF organic carbon (LF-OC) concentrations,
 196 and the LF-OC content did not vary significantly along the gradient (Table 2). In contrast, heavy
 197 fraction organic carbon (HF-OC) concentration increased from urban to rural conditions in 0-10 and
 198 10-20 cm soil layer (Table 2, both $P < 0.0001$). N concentrations in LF showed no significant
 199 difference among four urbanization classes, but significantly increased in HF from urban to rural in
 200 both 0-10 and 10-20 cm soil layer (Table 2, $P = 0.0001$ and 0.0244 , respectively). No significant
 201 change was observed for the C: N ratio of LF and HF in two soil layers (Table 2, both $P > 0.05$).

202

203 **3.4 Fine root, microbial biomass C, and extractable DOC**

204 Live and dead fine root biomass exhibited similar patterns along the urban-to-rural gradient. Live
 205 fine root biomass was significantly higher than dead root biomass ($P < 0.001$, $n = 14$), and
 206 comprised approximately 70% of total fine root biomass (live plus dead). Live, dead and total fine
 207 root biomass was all significantly lower in urban sites than in other urbanization classes (Fig 4A).
 208 Live fine root C concentration exhibited no significant difference among four gradient classes, but
 209 N concentrations of live fine root increased significantly from urban to rural (Fig 5, $P < 0.0001$).
 210 C:N ratios declined from 44 ± 4 in urban sites to 40 ± 3 , 33 ± 2 and 28 ± 4 in urban/suburban,
 211 suburban/rural, and rural sites, respectively ($P < 0.0001$).

212

213 Microbial biomass C decreased significantly from urban to rural sites in 0-10 cm soil layer (Fig 4bB,
214 $P < 0.05$), but not significantly in 10-20 and 20-40 cm (Fig 4B, both $P > 0.05$). Conversely, the
215 extractable DOC was not significantly different among urbanization classes in any soil layer (Fig
216 4C, $P > 0.05$ for each layer).

217

218 **4 Discussion**

219

220 SOC content ranged along the urban-to-rural gradient from 64.87 to 96.43 Mg ha⁻¹ in top 40 cm soil,
221 well within the range (41.74 to 102.17 Mg ha⁻¹) reported for pine forests in Guangdong province
222 and other subtropical regions of China (Fang and Mo 2002; Kang et al., 2006; Zheng et al., 2008;
223 Jiang et al., 2011). Our results suggest that urbanization-induced environmental change has
224 significantly decreased soil C content (Fig. 2B), rejecting our initial hypothesis and contradicting
225 results from other studies. Pouyat et al. (2002) analyzed soil in New York red oak (*Quercus rubra* L.)
226 forests and showed that soil C content significantly increased in urban sites compared to those in
227 rural sites. In a semi-arid tropical desert ecosystem, similar results were also found by Koerner et al.
228 (2010) along an urban-to-rural gradient in Phoenix, Arizona.

229

230 Although the reasons for our observed pattern are not clear, we suggest two possible explanations.
231 First, C input may be decreased in urban sites due to the reduction of belowground root input to the
232 soil. We found that fine root biomass was significantly lower in urban sites than those in suburban
233 and rural sites (Fig. 4A). Indeed, C input via fine roots can equal C input from above-ground
234 production (Nadelhoffer and Raich 1992). Furthermore, because annual productivity of fine roots
235 typically decreases with excess N availability (Nadelhoffer, 2000), it is likely that decreased fine
236 root production arose from higher N deposition in more urbanized areas (Gilliam, 2006, 2007).

237

238 Second, soil C loss from urban sites may be enhanced by increasing SOM decomposition.
239 Decomposition of SOM can be influenced by a variety of factors, including organic matter quality,
240 microbial activity, and microclimate (Chapin et al., 2002). In our study, organic matter quality did
241 not appear to change with degree of urbanization, since there were no significant differences in soil

242 C:N ratio along the urban-to-rural gradient (Table 1). There was, however, a significant increase in
243 microbial biomass in urban sites (Fig. 4B), indicating a potential increase in microbial activity.
244 Meanwhile, the elevated air temperatures associated with urban sites would also increase SOM
245 decomposition. Pouyat et al. (2002) suggested that the elevated temperature in urban areas
246 increased litter decay rate, and that the magnitude even can offset increased litter input to the soil.

247
248 Although there were no significant differences in DOC among four gradient classes (Fig. 4C), some
249 studies have reported that land-use change and land management can increase DOC fluxes in urban
250 areas (Aitkenhead-Peterson et al., 2009; Williams et al., 2005). Compared to such anthropogenic
251 influences, our results suggest that the effects of urbanization on soil DOC flux may be negligible.

252
253 Decreases in soil C storage in urban areas appears largely driven by the change in HF-OC pool
254 (often considered passive C), rather than in LF-OC pool (labile C) (Fig. 3). Contrary to our results,
255 other work has found that higher total passive C and lower labile C in soil from urban forests
256 compared to soil from rural forests (Groffman et al., 1995), which was attributed to decreasing
257 SOM recalcitrance, which was strongly linked with the reduction of air pollution and earthworm
258 activity.

259
260 It has been suggested that the recalcitrance of SOM would increase with the formation of stable
261 organo-mineral complexes via adsorption reactions (Sollins et al., 1996). We found that N
262 concentration of HF was higher in rural sites than in suburban and urban sites (Table 2), suggesting
263 that increasing amounts of N-rich material was adsorbed into mineral soil, possibly forming stable
264 organo-mineral complexes in rural areas. N-rich proteinaceous compounds are important in the
265 formation of organo-mineral complexes (Kleber et al., 2007). We suggest that these N-rich materials
266 may arise from dead roots, considering that both dead fine root biomass and root N concentrations
267 increased toward rural sites (Fig. 5). In addition, the enzyme-kinetic hypothesis predicts that
268 degradation of low-quality substrate (recalcitrant molecular structure) has a higher temperature
269 sensitivity compared to labile substrate because the former requires higher total activation energy to
270 fully mineralize substrate (Bosatta and Agren 1999). Therefore, higher temperature in urban areas is
271 likely cause accelerated decomposition of HF-C and may be another reason for the lower HF-C

272 content in urban sites.

273

274 In conclusion, we measured the forest SOC content along an urban-to-rural gradient in Guangdong
275 province, southern China. We found SOC content was significantly lower in urban areas than those
276 in suburban and rural areas. It was suggested that decreased fine root biomass and a potential
277 increased SOC decomposition were the possible reasons for this lower soil C pool in urban forests.
278 We further found that HF-OC content also increased from the urban to the rural, which was the
279 main driver of the change of total soil C pool. By contrast, LF- OC had not significant change in
280 this study. These results are contrary to the general belief and the earlier studies, suggesting that
281 urbanization-induced environmental changes may decrease soil C sequestration in the studied
282 forests. Our findings would be typical for tropical plantation forests, however, the results and
283 corresponding control mechanism should be further validated in various ecosystems and regions in
284 the future.

285

286 **Acknowledgements**

287

288 This study was supported by the National Natural Science Foundation of China (nos. 41273143 and
289 31000236), and National Key Basic Research 973 Program (2010CB833502). The authors wish to
290 acknowledge Chuan Ma and Feifei Zhu for their skilful assistance in field work, Xiaomei Chen for
291 her assistance in laboratory work, and Dr. Per Gundersen and Dr. Weixing Zhu, Dr. Yunting Fang,
292 and Dr. Xiankai Lu for their invaluable suggestions in earlier version of the manuscript.

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454 **Table 1.** Comparison of SOC (%), TN (%), soil C/N ratio and soil bulk density (g cm⁻²) (in
 455 0-10,10-20, and 20-40 cm soil layers) among four urbanization gradient classes.

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Soil depth (cm)	Urbanization classes	SOC (%)		TN (%)		C/N ratio	Soil bulk density (g cm ⁻³)	
0-10 cm	Urban	2.10 (0.13)	a	0.19 (0.02)	a	10.92 (1.05)	1.25 (0.17)	a
	Urban/Suburban	2.63 (0.47)	a	0.23 (0.03)	ab	12.03 (2.09)	1.22 (0.14)	a
	Suburban/Rural	3.75 (0.40)	b	0.28 (0.04)	bc	13.47 (2.91)	1.04 (0.13)	b
	Rural	3.99 (0.63)	b	0.31 (0.03)	c	12.91 (2.52)	1.03 (0.05)	b
10-20 cm	Urban	1.33 (0.16)	a	0.10 (0.01)	a	14.28 (2.55)	1.41 (0.10)	a
	Urban/Suburban	1.59 (0.48)	ab	0.11 (0.02)	a	14.98 (3.12)	1.34 (0.12)	ab
	Suburban/Rural	2.04 (0.40)	ab	0.15 (0.03)	ab	14.18 (2.92)	1.15 (0.08)	ab
	Rural	2.19 (0.06)	b	0.15 (0.01)	b	15.46 (1.07)	1.19 (0.03)	b
20-40 cm	Urban	0.81 (0.09)	a	0.05 (0.02)	a	18.05 (1.23)	1.48 (0.10)	a
	Urban/Suburban	0.93 (0.20)	a	0.05 (0.02)	a	18.23 (1.02)	1.41 (0.06)	ab
	Suburban/Rural	1.47 (0.20)	b	0.08 (0.01)	ab	18.28 (1.03)	1.21 (0.13)	ab
	Rural	1.51 (0.12)	b	0.08 (0.02)	b	18.34 (0.94)	1.26 (0.01)	b

457 Notes: The different letters indicate significant differences at $P < 0.05$ level, and no letters indicate
 458 no significant differences among different urbanization gradient classes, respectively (SNK test).
 459 Values are means with S.E. in parentheses (N = 3 for urban and rural, N = 4 for urban/suburban and
 460 suburban/rural).

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469 **Table 2.** Characteristics of two soil fractions.

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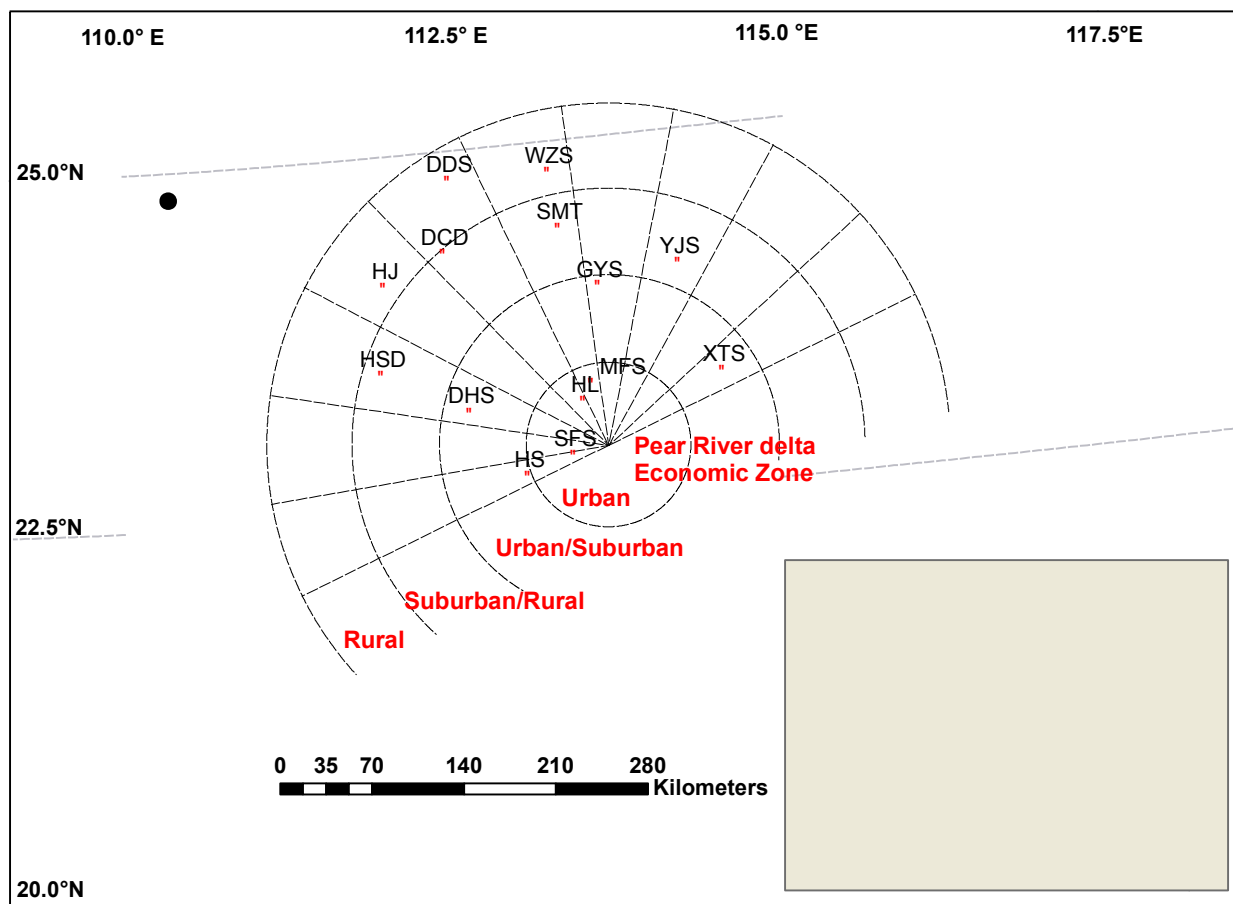
Soil fraction	Depth (cm)	Urban classes	C (%)	N (%)	C/N ratio	Percent of		Percent of	
						bulk mass (%)	soil	bulk soil (%)	C
LF	0-10	Urban	25.96 (3.66)	0.93 (0.11)	28.04 (0.91)	3.62 (0.53)		28.80 (4.02)	
		Urban/Suburban	21.50 (3.84)	0.87 (0.13)	25.29 (4.01)	3.54 (0.99)		28.25 (5.34)	
		Suburban/Rural	26.72 (5.89)	0.91 (0.09)	29.48 (4.31)	4.10 (1.34)		27.22 (5.47)	
		Rural	21.68 (2.92)	0.81 (0.05)	26.46 (2.46)	5.87 (1.33)		26.40 (4.04)	
	10-20	Urban	25.29 (3.97)	0.64 (0.03)	40.67 (7.68)	1.06 (0.06)		19.81 (1.48)	
		Urban/Suburban	21.72 (2.50)	0.57 (0.02)	38.09 (5.52)	1.35 (0.21)		20.14 (1.40)	
		Suburban/Rural	27.23 (5.30)	0.66 (0.11)	41.27 (5.43)	1.19 (0.24)		17.91 (1.62)	
		Rural	25.55 (7.24)	0.69 (0.12)	36.74 (7.03)	1.55 (0.56)		15.06 (2.59)	
HF	0-10	Urban	1.66 (0.10) a	0.12 (0.02) a	14.30 (2.99)	96.37 (0.48)		71.20 (4.02)	
		Urban/Suburban	1.99 (0.40) a	0.15 (0.03) ab	14.21 (2.12)	96.45 (0.99)		71.75 (5.34)	
		Suburban/Rural	2.93 (0.54) b	0.19 (0.04) bc	14.97 (1.91)	95.90 (1.34)		72.78 (3.42)	
		Rural	3.16 (0.44) b	0.25 (0.07) c	16.67 (3.10)	94.12 (1.33)		73.95 (4.49)	
	10-20	Urban	1.15 (0.18) a	0.09 (0.01) a	13.77 (2.32)	98.94 (0.06)		80.28 (1.48)	
		Urban/Suburban	1.21(0.25) ab	0.09 (0.02) a	13.46 (2.93)	98.64 (0.21)		79.83 (1.40)	
		Suburban/Rural	1.52(0.36) bc	0.13 (0.03) ab	11.71 (2.06)	98.80 (0.24)		82.54 (1.62)	
		Rural	1.75 (0.22) c	0.17 (0.09) b	15.45 (4.14)	98.44 (0.56)		84.94 (1.15)	

471 Notes: The different letters indicate significant differences at $P < 0.05$ level, and no letters indicate
472 no significant differences among different urbanization gradient classes, respectively (SNK test).
473 Values are means with S.E. in parentheses (N = 3 for urban and rural, N = 4 for urban/suburban and
474 suburban/rural).

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479 **Fig 1.** Location of our study sites in Guangdong province of southern China. A total of fourteen
 480 Masson Pine forests were selected along the transect. The detailed information for each forest is
 481 listed in Table S1

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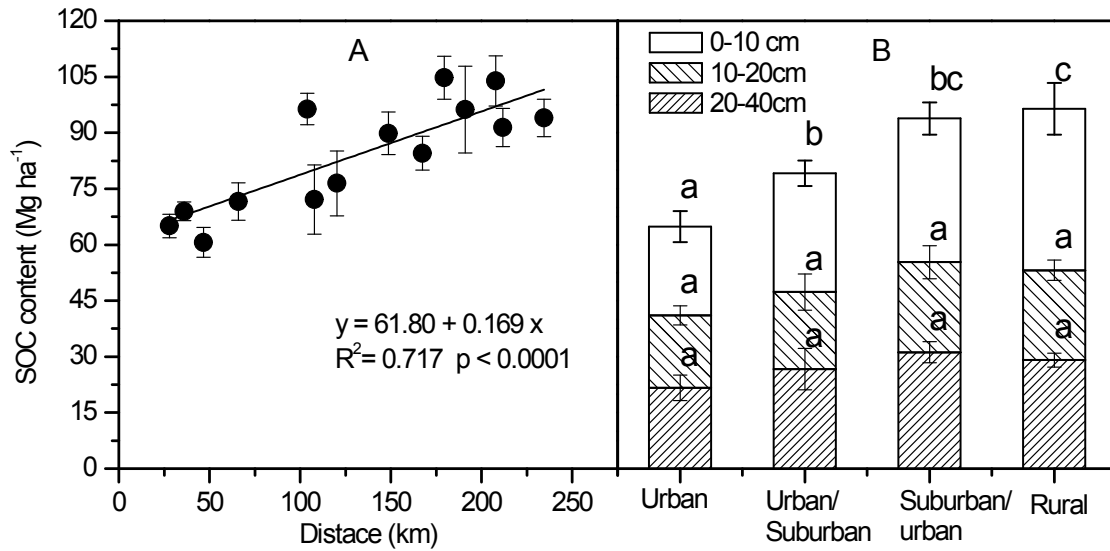
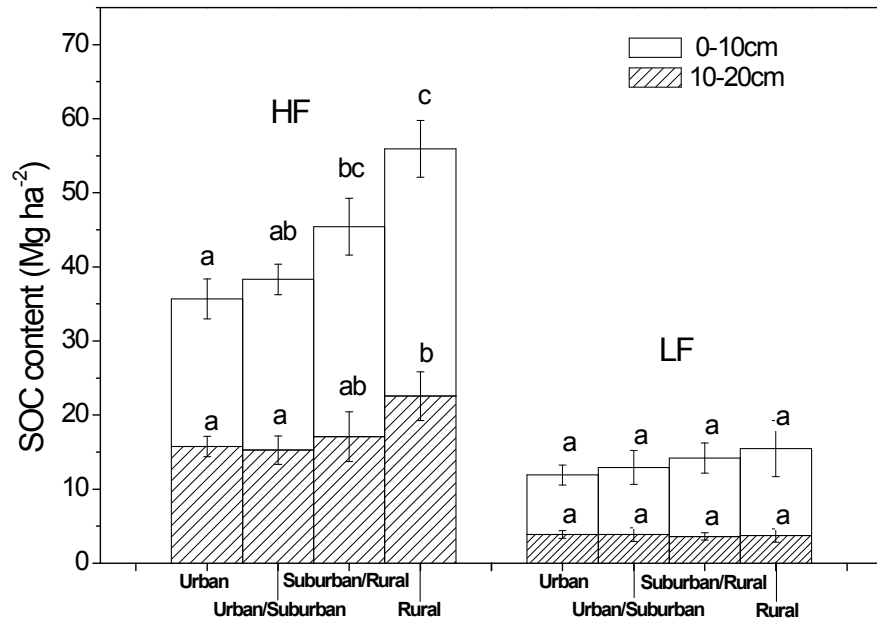


Fig 2. Change of SOC content in the top 40 cm soil. (A) correlation analysis of bulk SOC content (in 0-10 cm, 10-20 cm, and 20-40 cm soil layer) and the distance from urban to rural; (B) comparisons of SOC content among four urbanization gradient classes. Error bars indicate ± 1 S.E. (N = 3 for urban and rural, N=4 for urban/suburban and suburban/rural). Different letters denote significant difference ($P \leq 0.05$) between gradient classes (SNK test).



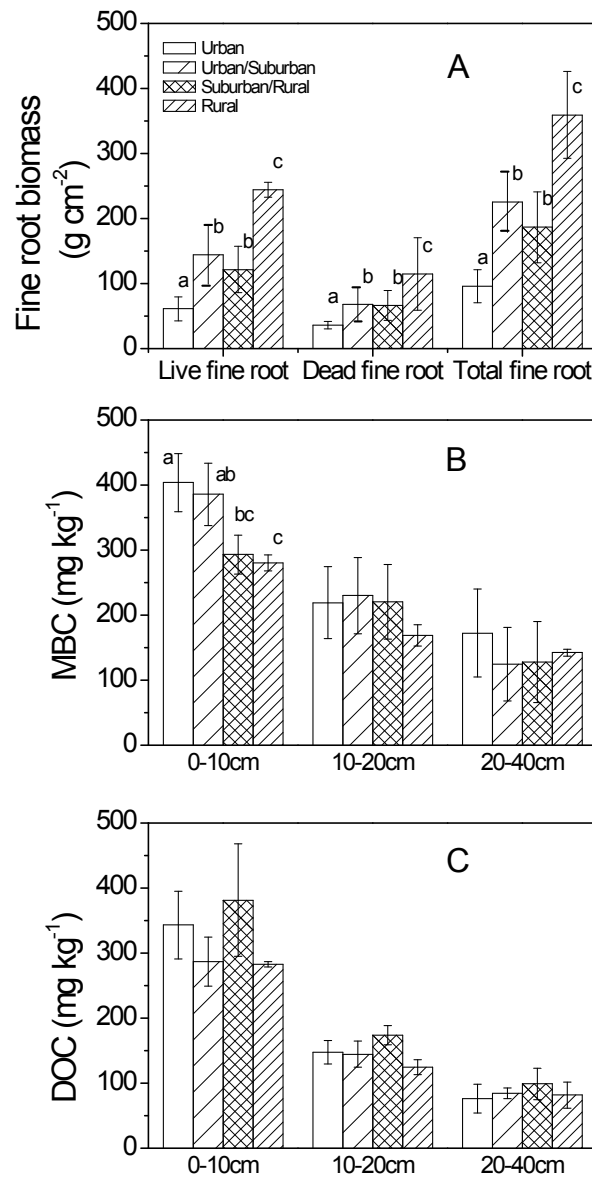
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509 **Fig 3.** Comparisons of HF-OC and LF-OC content (in 0-10 and 10-20 cm soil layer) among four
 510 urbanization gradient classes. Error bars indicate ± 1 S.E. (N = 3 for urban and rural, N=4 for
 511 urban/suburban and suburban/rural). Different letters denote significant difference ($P \leq 0.05$)
 512 between gradient classes (SNK test).

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 517 **Fig 4.** Comparisons of fine root biomass (A), MBC (B), DOC (C) among different urbanization
 518 gradient classes. Error bars indicate ± 1 SE (N = 3 for urban and rural, N=4 for urban/suburban and
 519 suburban/rural). Different letters indicates significant difference ($P \leq 0.05$) between gradient classes,
 520 and no letters indicate no significant differences ($P > 0.05$) among different urbanization gradient
 521 classes, respectively (SNK test).

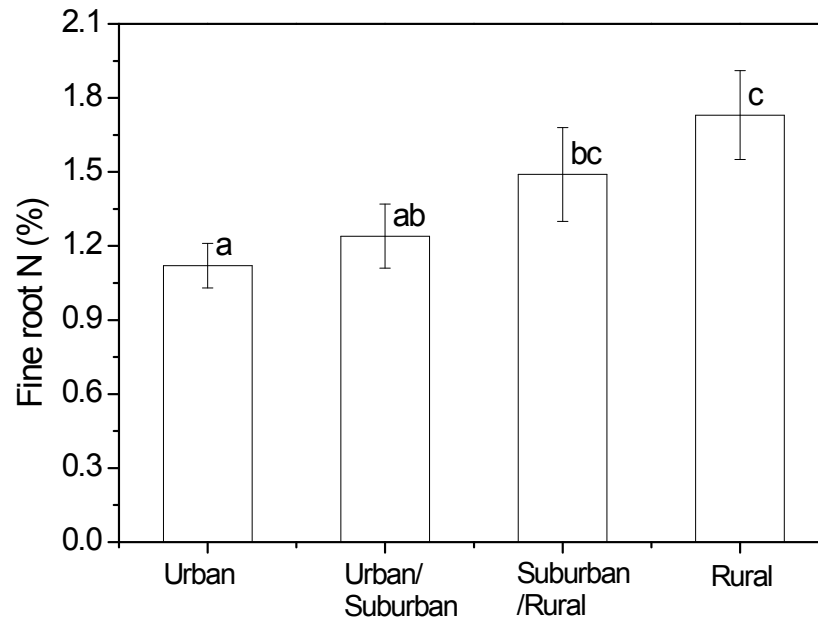


Fig 5. Comparisons of N concentration in live fine root (0-10 cm soil layer) among four urbanization gradient classes. Error bars indicate ± 1 S.E. (N = 3 for urban and rural, N=4 for urban/suburban and suburban/rural). Different letters denote significant difference ($P \leq 0.05$) between gradient classes (SNK test).

Supplementary material

Table S1. Site characteristics

Site (code)	Latitude (N)	Longitude (E)	Distance from urban core (km)	Elevation (m)	MAP (mm)	MAT (°C)	Stand density (trees ha ⁻¹)	Tree age (year)
HLS	23°10'53.30"	113°23'2.00"	36.1	45	1742 (351)	22.09 (0.52)	700	40
MFS	23°18'5.87"	113°27'0.57"	46.7	50	1742 (351)	22.09 (0.52)	700	40
SFS	22°49'7.65"	113°16'38.99"	28.0	48	1742 (351)	22.09 (0.52)	700	50
HS	22°40'13.31"	112°54'14.01"	66.0	60	1701 (283)	21.15 (0.43)	700	40
DHS	23°8'57.27"	112°31'3.07"	107.8	283	1625 (275)	22.22 (0.47)	800	60
GYS	23°58'9.34"	113°33'49.22"	120.3	385	2133 (383)	20.95 (0.41)	700	50
XTS	23°18'26.87"	114°25'37.54"	103.8	366	1730 (340)	22.01 (0.49)	700	40
HSD	23°27'42.85"	111°54'19.78"	179.3	400	1690 (265)	20.99 (0.47)	700	50
SMT	24°23'7.47"	113°18'8.49"	167.5	56	1675 (243)	19.45 (0.43)	700	40
YJS	24°4'55.65"	114°10'18.33"	148.6	462	1758 (314)	19.93 (0.50)	700	40
DCD	24°16'58.67"	112°25'25.81"	191.0	891	1597 (328)	19.65 (0.45)	700	40
HJ	24°4'7.45"	111°57'50.40"	207.8	432	1597 (328)	19.65 (0.45)	700	40
WZS	24°46'40.25"	113°15'28.59"	211.7	500	1566 (281)	20.38 (0.39)	750	60
DDS	24°46'17.29"	112°30'3.17"	234.5	815	1597 (328)	19.65 (0.45)	700	60

Notes: MAP = mean annual precipitation, for the years 1978 - 2011; MAT = mean annual temperature, for the years 1978 - 2011; Temperature and precipitation in each sites interpolated from the nearest meteorological station data. Latitude, longitude and elevation are from GPS readings taken on site.

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