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Supporting Online Material for

Old-Growth Forests Can Accumulate Carbon in Soils

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Materials and Methods References

1 Sites and Methods

2 Monsoon Evergreen Broadleaved Forests. This long-term research was 3 conducted at the Dinghushan Man and Biosphere (MAB) Reserve (23°09'21"N-4 23°11'30"N, 112°30'39"E–112°33'41"E) in Guangdong Province, China. The Reserve 5 covers an area of 1,155 ha and has a subtropical monsoon climate. The Reserve was 6 established in 1950 to protect a remnant of undisturbed natural monsoon evergreen 7 broadleaf forests in the subtropics, and was accepted as the first MAB reserve in China 8 in 1978. It has 2,054 recorded native species of higher plants. Annual mean 9 precipitation is 1,678 mm, concentrated from April to September. Annual mean relative 10 humidity is 78 percent and annual mean temperature is 22.3° C. Elevation in the reserve 11 ranges from about 14 m to 1,000 m above sea level. The bedrock is typically sandstone 12 and shale. Soils, with serious natural acidification and pH 4.0–4.9, are classified as 13 hydration laterites.

14 A 200 m by 400 m plot, representative of the monsoon evergreen broadleaf forests 15 in the region, was established in 1979 at an altitude of about 300 m above sea level on a 16 south-facing slope. The forest at this site has not been disturbed for more than 400 years, according to ${}^{14}C$ dating (S1). The above ground community can be divided into 17 18 five layers: three arbor layers, one shrub layer, and one grass layer. In addition, the 19 aboveground community has many kinds of interlayer plants (liana and epiphytes). The 20 upper canopy is dominated by species with high importance values but few individuals. 21 Evergreen plants are predominant and most often natives of the tropics and subtropics, 22 such as Castanopsis chinensis, Canarium pimela, Schima superba, and Engelhardtia 23 roxburghiana. The sub-canopy layer is mainly composed of Cryptocarya concinna and *Machilus chinensis*. The aboveground biomass of this community is about 380 Mg ha⁻¹. 24

1 Soil Organic Carbon (SOC) and Bulk Density Measurements. The data set 2 used for this study was compiled from four major field campaigns spanning 24 years. 3 Field measurements were consistently collected during the study period to a depth that 4 included the entire top 20 cm soil layer, which did not reflect the change in soil 5 thickness presumably caused by bulk density change. Substances of the organic layers 6 (O horizons) were excluded. The first field campaign was conducted in 1979 with 96 7 composite soil samples collected using an auger 30 mm in diameter. During this 8 campaign, the plot was divided into 800 subplots of 10 m by 10 m. A composite sample 9 of five soil cores was taken from each of the 96 randomly selected subplots. The second 10 campaign started in 1983 and ended in 1986. During this campaign, the plot was divided 11 into 200 subplots of 20 m by 20 m. Thirty-three subplots were sampled in the same way 12 as in 1979, except 10 soil cores were taken for each composite sample. The purpose of 13 the third field campaign was to investigate the vertical distribution of SOC. During this 14 campaign, one composite sample of 20 soil cores was collected from the plot. During 15 the last campaign, an ongoing effort to monitor the long-term dynamics of SOC since 16 1998, 20 composite samples (10 soil cores for each composite) from 20 randomly 17 located subplots were collected each year.

Sampling was carried out primarily in September, at the end of the rainy season and the beginning of the dry season. The soil samples were bulked, mixed, and airdried. Plant residues, including roots, were discarded. The soils were then milled to pass a sieve of 93,000 openings m⁻². Soil organic carbon was determined using the wet oxidation method (*S2*).

Roughly in parallel, soil bulk density was determined by samples taken from 12,
8, and 6 subplots in 1979, 1983, and 1986, respectively, and from 20 subplots between

1999 and 2003. Undisturbed soil cores were taken from five randomly selected
locations within each subplot using a stainless steel corer (5.65 cm in diameter, 4 cm in
depth, and 100 cm³ in volume). All of the sample cores within each subplot were pooled
together and oven-dried at 105° C to constant weights. Soil bulk density was calculated
as the ratio of total dry weight to total soil volume.

6 Calculation of Soil Carbon Stock Change. SOC stock is the product of SOC 7 concentration, layer thickness, and bulk density (S3, S4). The determination of the 8 temporal change of SOC stock becomes challenging when these three factors change 9 over time. In this study, two extreme values, representing the upper and lower limits of 10 the possible SOC change, were used to define the uncertainty introduced by the lack of 11 observations on soil thickness dynamics. The upper limit of SOC change was defined 12 based on mass conservation. A decrease in bulk density will be compensated by an 13 increase in soil thickness in the calculation of SOC stock change under mass 14 conservation. Nevertheless, decreased bulk density, as shown by our field 15 measurements, would result in an expansion of the original top 20 cm layer under mass 16 conservation. Consequently, the SOC concentration measured using samples collected 17 from the top 20 cm layer would be higher than that of the expanded soil column, 18 because the relatively low carbon-bearing portion of the expanded column at the bottom 19 was not included in the samples. The change in SOC concentration measured by this 20 sampling strategy would represent the maximum change possible, because the SOC 21 change rate usually decreases with soil depth and the relatively slow changing part of 22 the expanded column was not included in the samples. The upper limit of the change of 23 SOC stock can be calculated as follows:

24

1
$$SOCu = 0.2*D_{1979}*(SOC_{2003}-SOC_{1979})$$
 (1)

where 0.2 and D₁₉₇₉ are soil sample thickness (m) and bulk density (Mg mass m⁻³) in
1979, respectively, SOCu is the upper limit of SOC stock change (Mg C ha⁻¹) from
1979 to 2003, and SOC₁₉₇₉ and SOC₂₀₀₃ are SOC concentration in the top 20 cm layer
(Mg C Mg⁻¹ mass) in 1979 and 2003, respectively.

6 The lower limit of SOC stock change is defined using the assumption of constant
7 thickness and calculated as follows:

8
$$SOC_1 = 0.2^* (D_{2003} * SOC_{2003} - D_{1979} * SOC_{1979})$$
 (2)

9 where D₂₀₀₃ is soil bulk density (Mg mass m⁻³) in 2003, and SOC₁ is the lower limit of
10 SOC stock change in the top 20 cm layer (Mg C ha⁻¹) from 1979 to 2003. In essence, it
11 is assumed in equation 2 that the expanded part of the original top 20 cm soil layer,
12 resulting from decreased bulk density, did not contain SOC. Therefore, equation (2)
13 gives the lower limit of SOC stock change.



15 References

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