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Title: Tropical forest restoration: fast resilience of plant biomass contrasts with slow recovery of stable soil C stocks.

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Summary:

1. Due to intensifying human disturbance, over half of the world's tropical forests are reforested or afforested secondary forests or plantations. Understanding the resilience of carbon (C) stocks in these forests, and estimating the extent to which they can provide equivalent carbon (C) sequestration and stabilization to the old growth forest they replace, is critical for the global C balance.

2. In this study, we combined estimates of biomass C stocks with a detailed assessment of soil C pools in bare land, Eucalyptus plantation, secondary forest, and natural old-growth forest after over 50 years of forest restoration in a degraded tropical region of South China. We used isotope studies, density fractionation and physical fractionation to determine the age and stability of soil C pools at different soil depths.

3. After 52 years, the secondary forests had equivalent biomass C stocks to natural forest, whereas soil C stocks were still much higher in natural forest (97.42 t ha^{-1}) than in secondary forest (58.75 t ha^{-1}) or Eucalyptus plantation (38.99 t ha^{-1}) and lowest in bare land (19.9 t ha^{-1}). Analysis of δ^{13}C values revealed that most of the C in the soil surface horizons in the secondary forest was new C, with a limited increase of more recalcitrant old C, and limited accumulation of C in deeper soil horizons. However, occlusion of C in microaggregates in the surface soil layer was similar across forested sites, which suggests that there is great potential for additional soil C sequestration and stabilization in the secondary forest and Eucalyptus plantation.

4. Collectively, our results demonstrate that reforestation on degraded tropical land can restore biomass C and surface soil C stocks within a few decades, but much longer recovery times are needed to restore recalcitrant C pools and C stocks at depth. Repeated harvesting and disturbance in rotation plantations had a substantial negative impact on the recovery of soil C stocks. We suggest that current calculations of soil C in secondary tropical forests (e.g. IPCC Guidelines for National Greenhouse Gas Inventories) could overestimate soil C sequestration and stabilization levels in secondary forests and plantations.

Introduction

Land use change is the second most important source of anthropogenic greenhouse gas emissions, with 30% of carbon dioxide (CO_2) emission (185 ±65 Pg C) around the world generated from land use or land use change (Le Quéré *et al.* 2014). The rate of deforestation worldwide was 13 million ha yr^{-1} for the period 2000–2010 (FAO 2010), resulting in emissions of up to 2.8 ±0.5 Pg C yr^{-1} (Pan *et al.* 2011), of which 70% were from the tropics. The loss of tropical forests has been one of the most dramatic global ecological changes caused by human activities (van der Werf *et al.* 2009). Over half the tropical forest cover worldwide has been cleared (Poorter *et al.* 2016) and much of the remaining forest is affected by fragmentation and selective logging. Forest restoration has thus been proposed as a cost-effective way to mitigate anthropogenic greenhouse-gas emissions, especially in tropics (Canadell & Raupach 2008).

Following afforestation and/or reforestation, plant biomass C can increase rapidly owing to the establishment and growth of trees (Farley, Kelly & Hofstede 2004; Nave *et al.* 2013) and forest regrowth could partially offset increasing anthropogenic CO_2 emissions (Canadell & Schulze 2014). From 2000 to 2007, the worldwide estimated C sink from tropical forest regrowth after abandonment of agricultural land was 1.7 ±0.5 Pg C yr^{−1} (Pan *et al.* 2011). Recent work in neotropical secondary forests suggested that aboveground biomass recovery after 20 years was on average 122 Mg ha^{−1}, corresponding to a net carbon uptake of 3.05 Mg C ha^{−1} yr^{−1}, which was eleven times the uptake rate of old-growth forests (Poorter *et al.* 2016). As a result, aboveground biomass stocks in these secondary forests recovered up to 90% of old-growth values within a median of 66 years (Poorter *et al.* 2016). In China, secondary forests became large C sinks (176.7 ±44.8 Tg C yr^{−1}) during 1988–2001, owing to large-scale plantation establishment and forest regrowth in previously disturbed or degraded areas (Wang *et al.* 2007).

In tropical forests, soil organic carbon (SOC) represents 30-60% of the total C stored in the ecosystem (Jobbágy & Jackson 2000; Don, Schumacher & Freibauer 2011), which is comparable to the atmospheric C pool (Ciais *et al.* 2013). Although increases in aboveground biomass during tropical forest restoration have been well studied, the effects of land-use change on belowground soil C stocks have received less attention and estimates of the size, distribution and development of soil organic C stocks are highly uncertain (Martin, Newton & Bullock 2013; Wang *et al.* 2013; Mobley *et al.* 2015; Wang, Zhu & Chen 2016). Generally, afforestation of previously degraded or deforested land should increase soil C stocks (Deng *et al.* 2016). For example, a synthesis study found that afforestation of

agricultural land in the tropics could increase SOC by 29% in surface soils (Don, Schumacher & Freibauer 2011). However, the rate varied greatly among different types of land use change (Don, Schumacher & Freibauer 2011; Deng *et al.* 2016), and data was usually only available for surface soils, stressing the need for more data from deeper soil profiles (Deng *et al.* 2016). Other studies have found no clear patterns in soil carbon stocks during secondary forest succession (Martin, Newton & Bullock 2013).

We know even less about the mechanisms underlying soil C sequestration during forest restoration. Soil contains a mixture of heterogeneous C pools and bulk soils can be partitioned by physical and/or chemical methods into different C fractions of varying resistance to mineralization (Wang, Zhu & Chen 2016). The turnover time of these fractions can range from days (active pool), decades (slow pool), to centuries (recalcitrant pool) due to their specific chemical or physical stabilities. Different land use types vary in the proportions of these soil C pools (Guo & Gifford 2002), and both active and slow pools are considered to be sensitive to land use change (Schwendenmann & Pendall 2008). Conversion of natural forest to plantations decreased the physically, chemically and biochemically protected slow C pools in surface and deep soils in one study in subtropical forest (Lyu *et al.* 2016). Although identifying land-use induced changes in total soil C stocks is important, it is also imperative that we are able to identify the mechanisms of change in different soil C pools to determine long-term sequestration potential (Diochon & Kellman 2009; Chapman, Palanivel & Langley 2012).

Differences in the natural abundances of ^{12}C and ^{13}C isotopes (δ^{13}C) in organic material and soil C are a useful method to estimate C flows under steady state (Gunina & Kuzyakov 2014). During the decomposition of SOC, kinetic fractionation occurs because the reaction rates of microbial enzymes are slower for heavy isotopes (^{13}C) than for lighter isotopes (^{12}C), enriching the remaining SOC and the microbial biomass with ^{13}C (Guillaume, Damris & Kuzyakov 2015). Although the mechanisms involved in isotope fractionation during microbial metabolism remain uncertain, a methodological review of isotope partitioning studies showed an average ^{13}C enrichment of 0.5‰ in microbial biomass compared to respired CO_2 (Werth & Kuzyakov 2010). The increase in δ^{13}C during C stabilization is roughly proportional to the number of cycles in which C is utilized by microorganisms before being stabilized in SOM (Gunina & Kuzyakov 2014). In general, the δ^{13}C also increases with soil depth, which is mainly due to a higher proportion of older and more decomposed SOC in deeper horizons (Werth & Kuzyakov 2010).

In tropical China, land degradation is most prominent in the hilly red soil region, which covers over 20 million hectares (Wei *et al.* 2013). Historically, these lands were covered by evergreen forests but during the last 150 years, intensive anthropogenic disturbances, especially harvesting, slash-and-burn agriculture, and litter raking, accompanied by excessive livestock grazing, have destroyed the original forest in the region and turned it into ''bare land'' without living trees or shrubs.

As these degraded lands generally have very few or no living plants there are no new organic C inputs, which provides a unique opportunity to study changes in soil C following afforestation of these bare lands. Due to long-term microbial decomposition, the bioavailable C in active and slow pools should have been largely depleted, and the remaining C is likely to be older recalcitrant C, which is enriched in ^{13}C. Most of these "bare lands" were recently afforested with Eucalyptus plantations or secondary forests. This shift in land-use allows the use of C isotope studies to examine changes in soil C pools.

Identifying the mechanisms underlying changes in C pools during the afforestation of degraded tropical land would help us to ascertain the role of afforestation in mitigating increasing concentrations of atmospheric CO_2, and to improve models of forest growth and nutrient cycling. Here, we present new information on the changes of aboveground and belowground C pools during 50 years of afforestation of degraded land under different land use types in tropical China. In this study, we carried out a combined assessment of C stocks above- and belowground and analyzed the latter in terms of different pools and controlling factors. We hypothesized that 1) the biomass C pool would recover at a faster rate than soil C pools, and that recovery of soil C pools would be more rapid in secondary forest compared to managed plantations; 2) the recovery of soil C pools would depend strongly on soil depth and surface soil C would recover faster than deeper soil C pools; 3) stabilization of C within soil aggregates would represent an important mechanism to store soil C.

Methods

Site description

Our restoration study was initiated in 1959 at the Xiaoliang Tropical Coastal Ecosystem Research Station in Guangdong Province of China (110°54′18″E, 21°27′49″N). The climate of this region is strongly influenced by tropical monsoons, with a mean annual temperature of 23℃, and annual precipitation of 1400—1700 mm. The area was covered by evergreen broad-leaved seasonal rainforest until the 1850s, after which, clear-cut logging, firewood harvesting, and soil erosion denuded about 40000 ha of tropical seasonal forests by the early 1950s, with only small xeric shrubs, grasses, and vines sparsely found in ditches (Ren *et al.* 2007). The soil at this region was laterite developed from granite (Yao, Zheng & Peng 1984). As the topsoil was completely lost, the bare surface was covered by coarse sands and mineral aggregates rich in iron oxides and manganese (Yu & Pi 1985).

In 1959, researchers started a long-term restoration experiment by establishing one bare land reference and two restoration treatments in three geographically similar catchments (Ren *et al.* 2007). For the reference catchment (3.7 ha), the land was left bare without human interference (Fig 1). The two treatments included a eucalyptus (*Eucalyptus exserta*) plantation (3.9 ha), and broad-leaved

mixed plantation (3.8 ha). All the three catchments had a similar slope (<6°) and initial soil properties (Table S1; (Yao, Zheng & Peng 1984; Li, Weng & Yu 1996). The two restored catchments were initially both restored with *Eucalyptus exserta* seedlings in the early 1960s. One catchment remains as a eucalyptus rotation plantation, which was harvested every 5 to 8 years, the other was clear-cut in 1974 and reforested with 312 native tree species, which has since developed into a secondary forest. All tree plantings followed the same procedure: seedlings of the chosen species were planted in *c.* 1-m^3 pits in a 2.5-m × 2.5-m grid, with addition of compost and water (Ren *et al.* 2007). For the Eucalyptus plantation, the understory plants were usually removed and fertilizers (96 kg N ha^{-1} and 72 kg P ha^{-1}) were applied at the beginning of each rotation and when the plantations were two years old.

Historical soil C data

The surface soil C stocks of the four land use types have been recorded once or twice every 10 years since 1959. Until 2000, one composite soil sample, made of nine individual soil cores, was analysed for soil C in the bare land catchment and Eucalyptus plantation. However, since this was a long-term record and all the sites were sampled regularly over five decades, the historical soil C data provides valuable information on the recovery of soil C stocks. Most of the historical data were sampled at 0-20 cm depth but for some sampling points (i.e., in 1979), data were only available for 0-15 cm depth. For all sampling points and years except the most recent data in 2011, soil organic C was measured using the Walkley-Black method (Liu *et al.* 1996). The soil C content of samples collected in 2011 was measured on an Isoprime 100 IRMS (IsoPrime Ltd., Cheadle Hulme, UK).

Vegetation measurements and soil sampling

In 2011, four plots were randomly established in each of the three land use types and a nearby natural old-growth forest (henceforth 'natural forest', which has been well preserved by local residents for at least 200 years). All plots were separated by a distance of at least 40 m. Due to the heterogeneity of plants in different land use types, the plot size was 10-m × 10-m in bare land and Eucalyptus plantation, and 30-m × 30-m in the secondary forest and natural forest. Species density, tree height (H) and tree diameter at breast height (DBH) were recorded for each plot. We used a set of allometric equations (Table S2), which were developed for nearby forests (Wen *et al.* 1999), to calculate leaf, trunk, branch, and root biomass in Eucalyptus plantation, secondary forest, and natural forest. For each component, we calculated biomass C from dry weight multiplied by a conversion factor of 0.47 (IPCC 2006). The root to shoot ratio was calculated as the total belowground root biomass C divided by shoot biomass C, which was the sum of leaf, trunk and branch biomass C. At the time of the study, the trees in the Eucalyptus plantation were two years old and the secondary forest was *c*. 37 years old.

In each plot, the litter on the soil surface was collected from three 0.5-m × 0.5-m quadrats, mixed, dried at 60ºC, and weighed. Soil samples were collected from the same quadrats in four depth

increments (0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm), the samples from each quadrat were bulked to give one sample per plot and depth. For each depth increment, three additional samples were collected using a bulk density cup to calculate soil bulk density. Soil samples were air-dried after large roots and stones had been removed by hand.

We performed aggregate separation and density fractionations of the light and heavy soil fractions using methods adapted from Six *et al.* (1998). Four aggregate sizes were separated using wet sieving through a series of sieves (2000, 250, and 53 µm). Briefly, a 100-g air-dried sample was placed in a 2000-µm sieve and submerged for 5 min in de-ionized water. Aggregate separation was achieved by manually moving the sieve up and down by 3 cm with 50 repetitions during a period of 2 mins. After which, the stable >2000 µm aggregates were gently back-washed off the sieve into an aluminum pan. Floating organic material (>2000 µm) was discarded, as this is by definition not considered as SOM (Six *et al.,* 1998). Water and soil that passed through the sieve were poured into the next two sieves (one at a time) and the sieving was repeated in a similar fashion, but floating material was retained. Thus, four size fractions were obtained (>2000 µm, 250–2000 µm, 53–250 µm and <53 µm). The aggregates were oven-dried at (65ºC), weighed, and stored in glass jars at room temperature until analysis.

Density fractionation was carried out using a solution of 1.7 g cm^{-3} sodium iodine (NaI). A 5-g subsample of oven-dried (65ºC) soil was suspended in 35 ml of NaI solution and slowly shaken by hand. The material remaining on the cap and sides of the centrifuge tube was washed into suspension with 10 ml of NaI solution. The samples were centrifuged (1250 g) at 20ºC for 5 min. The floating material (light fraction; LF) was aspirated onto a 20-µm mesh nylon filter, washed multiple times with deionized water to remove NaI, and dried at 65ºC. The heavy fraction (HF) was rinsed three times with 50 ml deionized water and was dried and weighed.

To determine C and N, samples of litter and soil were dried to constant weight at 65ºC and then ground to pass through 0.84-mm mesh sieves. Total C and N concentrations and $\delta^{13}C$ were measured on an Isoprime 100 IRMS (IsoPrime Ltd., Cheadle Hulme, UK). To save costs, we only analysed the $\delta^{13}C$ of the litter, the light and heavy density fractions, <53 µm aggregate size fractions, and bulk soil C at 0-10 cm and 10-20 cm depths.

Based on the assumption that there have been no plant C inputs to the bare land in the past 50 years, we used the $\delta^{13}C$ values of the SOC to calculate the proportion of new C fraction (*fnew*, i.e., C derived from recent plant residues) and old C fraction (*fold*, i.e., the organic C from 50 years ago) with the mass balance equation (Del Galdo *et al.* 2003; Cheng *et al.* 2013):

$$f_{new} = \frac{\delta_{new} - \delta_{old}}{\delta_{veg} - \delta_{old}} \times 100\%$$

where δnew is the $\delta^{13}C$ value of a given soil sample, δold is that of soil from the bare land, and δveg is the $\delta^{13}C$ values of the corresponding litter from each plot.

Data analysis

All data analyses were performed in R version 3.3.2 (R Core Team 2016); were necessary, variables were log-transformed to meet modelling assumptions. In this study, four land-use types each with four replicate plots, and soil samples were taken at four depths (0-10 cm, 10-20 cm, 20-40 cm, and 40-60 cm). To assess the effect of land-use type on aboveground biomass C, litter C, and soil C stocks, we used linear mixed models in the *lme4* package (Bates *et al.* 2015), with land-use type as the fixed effect and plot as the random effect. For soil C pools in different fractions, $\delta^{13}C$, and fraction of new C in soils, we used linear mixed effects models with land-use type, soil depth, and their interaction as fixed effects, and plot as the random effect. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and *p*-values to check for model improvement (Pinheiro & Bates 2000) and the fit of the final model was inspected using diagnostic plots. Statistics are given for the comparison between the best-fit model and the corresponding null model. For the final model, *p*-values for each fixed effect were generated in the *lmerTest* package (Kuznetsova, Brockhoff & Christensen 2015), using Satterthwaite's approximation for degrees of freedom. Results are reported as significant at *p* <0.05.

Results

Historical soil C variation

The bare land, Eucalyptus plantation and secondary forest had similar land use history until 1959. After Eucalyptus was planted between 1959 and 1974, there was a steady increase of surface soil C but a consistent decline of soil C in the bare land. In 1974, the reforested areas were clear-cut, and replaced by native species (secondary forest site) or continued as Eucalyptus plantations. Accordingly, we observed a five-year decline in soil C after clear-cutting (Fig. 2) until 1980, since when soil C has increased in the secondary forest, but not in Eucalyptus plantation, which was harvested every 5 to 8 yrs. The soil C concentration in natural forest has not varied greatly since 1974 and has been consistently the highest of the four land-use types.

Biomass and soil C stocks

The natural forest and secondary forest plots had comparably high aboveground biomass C stocks (73.3 $\pm$11.3 Mg C ha^{-1} and 63.5 $\pm$10.1 Mg C ha^{-1}), which were much higher than the two-year old Eucalyptus plantation (2.9 $\pm$0.9 Mg C ha^{-1}), and the bare land had no aboveground vegetation. Estimated root biomass in secondary forest (25.1$\pm$0.52 Mg C ha^{-1}) was similar as in natural forest (23.0$\pm$2.63 Mg C ha^{-1}) and much higher than in the Eucalyptus plantation (1.17$\pm$0.27

Mg C ha^{-1}; Fig. 3), accordingly, the ratios of root to shoot biomass were 0.31, 0.39 and 0.40 for natural forest, secondary forest and Eucalyptus plantation, respectively. There were no differences in forest floor C stocks among the three forest types. Similar to aboveground C stocks, soil C stocks at 0-60 cm depth were highest in the natural forest plots (97 $\pm$3.2 Mg C ha^{-1}), followed by secondary forest (59 $\pm$1.6 Mg C ha^{-1}) and Eucalyptus plantation (39 $\pm$4.6 Mg C ha^{-1}). The bare land had the lowest C stocks (20 $\pm$2.4 Mg C ha^{-1}) of the four land-use types.

We calculated total ecosystem C stocks as the sum of plant biomass C and soil C. The natural forest had the highest ecosystem C stock (195 $\pm$9 Mg C ha^{-1}), which was significantly higher than the secondary forest (149 $\pm$18 Mg C ha^{-1}), but the difference was mostly due to soil C stocks, as the plant biomass C stocks in the natural forest were only 6 Mg C ha^{-1} higher than in the secondary forest. The Eucalyptus plantation had a total ecosystem C stock of 44.8 Mg C ha^{-1}, which was over twice the value in the bare land (20 Mg C ha^{-1}), as the latter did not have any plant biomass C (Fig. 3).

Vertical soil C distribution

Soil C and N concentrations decreased greatly with increasing soil depth in natural forest and secondary forest sites but the decline with depth was much less pronounced in the Eucalyptus plantation and bare land (land-use $\times$ depth: χ^2 = 116.29; p < 0.001 and χ^2 = 172.8; p = 0.001, respectively). The natural forest had the highest soil C concentrations at all four soil depths from 0-60 cm (Fig 4). At 0-10 cm depth, the soil C concentration in secondary forest was 25% lower than in the natural forest. However, in the deeper soil layers, soil C concentrations were 50% lower in secondary forest sites compared to natural forest (Fig 4a). Soil N concentrations showed a similar pattern among vegetation types and soil depths (Fig 4b). Consequently, the soil C/N ratio also varied significantly among land-use types and soil depths (land-use $\times$ depth: χ^2 = 33.4; p < 0.001; Fig 4c), whereby the bare land had the lowest C/N ratio at all soil depths, whereas the natural forest had the highest C/N ratio in all soil layers except at 0-10 cm depth. Bulk density was the highest in bare land and lowest in natural forest (Fig 4d).

Soil C fractions

Soil density fractionation revealed that there was no light C fraction in the bare land site (Fig 5a) and the light fraction was greatest in the surface soil layer (0-10 cm) and declined similarly with depth (Fig. 5a) among the remaining three land-use types (depth effect: χ^2 = 24.1; p < 0.001). The heavy soil C fraction varied greatly with land-use type but declined similarly with soil depth in all land-use types (land-use + depth: χ^2 = 120.7; p < 0.001; Fig. 5b). However, the heavy fraction was greater in in the natural forest compared to the other sites at all depths, followed by secondary forest and Eucalyptus plantations.

Physical fractionation of soil C according to aggregate sizes revealed similar differences among land-use types. Coarse macroaggregate fraction (>2000 µm) C decreased substantially with soil depth and varied greatly among land-use types (land-use × depth: $\chi^2 = 115.7$; $p < 0.001$; Fig. 6); natural forest plots had the highest soil C in coarse macroaggregates at all depths, with values almost twice as high as in secondary forest plots (Fig. 6a). The coarse macroaggregate fraction C was much lower in the Eucalyptus plantation compared to secondary forest and lowest in the bare land at all four soil depths (Fig. 6a). The fine macroaggregate fraction (250-2000 µm) C showed a similar pattern (land-use × depth: $\chi^2 = 78.7$; $p < 0.001$). Although the fine macroaggregate fraction C in the surface soil layer (0-10 cm) was similar in natural forest and secondary forest plots (Fig. 6b), it was two-fold greater in natural forest than secondary forest at all other depths. The microaggregate fraction (53–250 µm) C showed the same pattern of a substantial decline with depth across land-use types (land-use + depth: $\chi^2 = 26.6$, $p < 0.001$; Fig. 6c). The bare land had the lowest microaggregate C at all soil depths and there was little variation among depths. There were no significant differences in the silt and clay fraction among the three forest types (Fig. 6d), but the bare land tended to have the lowest values of silt and clay-associated C at all soil depths.

^{13}C isotopes of litter and soil

Litter δ^{13}C ranged from -30.74‰ to -31.29‰ in all three forests, and there was no difference among forest types (Table 1). Light fraction δ^{13}C also varied little among the three forests but there was a significant enrichment of δ^{13}C with increasing soil depth (depth effect: $\chi^2 = 11.0$, $p < 0.001$). The δ^{13}C values for the heavy fraction were higher than the light fraction and differed among sites: δ^{13}C in the heavy fraction was the most depleted in secondary forest and natural forest (-27.35 to -26.38‰) and more enriched in bare land (-21.95‰) at 0-10 cm depth, whereas differences in δ^{13}C depletion among land-use types were much less pronounced at 10-20 cm depth (land-use × depth: $\chi^2 = 33.3$, $p < 0.001$).

Old and new soil C stocks

We calculated the fraction of new soil C in each forest and soil layer from the difference in δ^{13}C between plant-derived new C and older soil C (Table 1 and Fig 7). The secondary forest had the highest proportion of new plant-derived C (59.8%) in the surface soil layer (0-10 cm), followed by the natural forest with 50.5%. In the Eucalyptus plantation, only 30% of the soil C at 0-10 cm depth was new plant-derived C. At 10-20 cm depth, the secondary forest still had the highest proportion of new soil C (40.8%), followed by the natural forest (38.8%), and Eucalyptus plantation still had the lowest proportion of new C (18.5%).

New C stocks at 0-10 cm depth were similar in natural forest and secondary forest, which were four times higher than in the Eucalyptus plantation (Fig 7). However, old C stocks in the natural forest (12.68 $\pm$1.06 Mg C ha^{-1}) were 44% higher than in secondary forest (8.74 $\pm$0.51 Mg C ha^{-1}); the

Eucalyptus plantation and bare land had lower old soil C stocks than either forest type (7.04 $\pm$1.12 Mg C ha^{-1} and 4.07 $\pm$0.65 Mg C ha^{-1}, respectively). Hence, almost all (96.7%) of the difference in soil C stocks between natural forest and secondary forest can be attributed to differences in old C. We further analyzed old C stocks in different density and physical fractions. There were no differences in old C among land-use types in the light fraction (Fig. 7), but in the heavy fraction, the old C stock was highest in natural forest (11.6 $\pm$0.99 Mg C ha^{-1}), followed by secondary forest (7.73 $\pm$0.48 Mg C ha^{-1}) and Eucalyptus plantation (6.11 $\pm$1.02 Mg C ha^{-1}). There was no light fraction in bare land and all the soil C in bare land was old. The old soil C stock in macro- and micro-aggregates was higher in natural forest (12.32 $\pm$1.11 Mg C ha^{-1}) than in secondary forest (8.23 $\pm$0.48 Mg C ha^{-1}), Eucalyptus plantation (5.85 $\pm$1.22 Mg C ha^{-1}), and bare land (3.39 $\pm$1.55 Mg C ha^{-1}). The stock of old C in the silt and clay fraction was very low and did not differ among vegetation types.

There was a similar pattern in old and new C stocks at 10-20 cm depth. The natural forest had the largest new C stock (6.47 $\pm$1.10 Mg C ha^{-1}), followed by secondary forest (4.16 $\pm$0.98 Mg C ha^{-1}) and Eucalyptus plantation (1.23 $\pm$0.98 Mg C ha^{-1}). The natural forest also had the largest old C stock (10.15 $\pm$1.14 Mg C ha^{-1}), which was 56% higher than in secondary forest (6.02 $\pm$0.74 Mg C ha^{-1}) and 58% higher than in the Eucalyptus plantation (5.93 $\pm$1.06 Mg C ha^{-1}). The bare land had the smallest old C stock at 10-20 cm depth (4.04 $\pm$0.58 Mg C ha^{-1}), which was similar to the surface layer. Most of old C was distributed in the heavy fraction and within macro- and micro-aggregates. The natural forest generally had the largest new C stocks in both density fractions, followed by secondary forest and Eucalyptus plantation, which did not differ (Fig. 7).

Discussion

Biomass C stocks

Due to intensifying human disturbance, over half of the world's tropical forests are now afforested secondary forests or plantations (FAO 2010). Understanding the resilience of these forests, and estimating the extent to which they can provide equivalent levels of ecosystem services (such as C sequestration) to the old growth forest they have replaced is critical for the global C cycle (Poorter *et al.* 2016). In this study, after over 50 years of restoration, aboveground biomass recovery in the secondary forest had already reached 86% of the nearby natural forest reference site, which is remarkably similar to the recovery to 90% of old-growth values after 66 years reported for neotropical secondary forests (Poorter *et al.* (2016). The corresponding net carbon uptake of 1.26 Mg C ha^{-1}yr^{-1} in our secondary forest site is much higher than in old-growth forest in the Amazon (Brienen *et al.* 2015) or in African tropical forests (Lewis *et al.* 2009), but similar to results from logged and well-managed Amazonian forest (Rutishauser *et al.* 2015). Root biomass generally represents 22-33% of the aboveground biomass in tropical forests (Mokany, Raison & Prokushkin 2006; Preece *et al.* 2015) and

the root to shoot ratio tends to decrease with plant age. Consequently, young plantations usually have a higher root to shoot biomass ratio than old forests (Cairns *et al.* 1997; Raich *et al.* 2014). Our results are consistent with this pattern, indicating rapid recovery of both above- and belowground biomass after disturbance.

Soil C stocks

Compared to biomass, changes in soil C in secondary forests are less well studied (Martin, Newton & Bullock 2013; Poorter *et al.* 2016). Several global and tropical syntheses of soil C dynamics have reported increased soil C pools with afforestation (Post & Kwon 2000; Don, Schumacher & Freibauer 2011), but our knowledge of C sequestration mechanisms during afforestation is incomplete (Wang, Zhu & Chen 2016). By combining soil fractionation and ^{13}C isotope methods, we were able to examine the variation in different soil C stabilization mechanisms (surface *vs.* deeper soils, silt and clay *vs.* larger soil aggregates, light *vs.* heavy C fractions) under different vegetation types.

We showed that, after 50 years of afforestation, the soil C content in secondary forest at 0-60 cm depth was still substantially lower than in the natural forest, which is consistent with the general pattern for tropical land-use change (Don, Schumacher & Freibauer 2011). Contrasting patterns in soil C stocks following afforestation are likely to arise, in part, from the land-use history and the level of disturbance. For example, studies that detected no clear increase in soil C stocks in secondary forests considered afforestation of abandoned agricultural land, which had comparably high C content (Martin, Newton & Bullock 2013). Furthermore, many previous studies only report changes in surface soil stocks (0-10 cm or 0-20 cm depth), which recover more rapidly, and there are much larger differences in soil C across the whole soil profile (Don, Schumacher & Freibauer 2011). Our study investigated long-term changes in soil C stocks after afforestation of bare land, in which soil organic C had been severely depleted. This allowed us to investigate the recovery of soil C stocks in plantations and secondary forest regrowth at different soil depths.

The historic soil C data showed that the soil C content of the bare land declined from 1959 to 1985, as there were no new organic C inputs from vegetation. We propose two mechanisms responsible for the decline in soil C: 1) erosion of surface soils, which have a high C content; and 2) the decomposition of bioavailable SOC in the absence of new C inputs. Previous studies have shown that soil erosion increases substantially when forest is converted to other land uses, because the physical protection of the soil surface (vegetation and litter) has been removed (Guillaume, Damris & Kuzyakov 2015). Previous work at our study sites, showed that rates of soil erosion are high in the bare land but low in the Eucalyptus plantation and negligible in the secondary forest (Ren *et al.* 2007; Li 2009). Consequently, erosion is likely to contribute to the continued loss of soil C from the bare land.

Nonetheless, our isotope study indicates substantial microbial transformation of the soil C in the past: the soil C in the bare land had the highest $\delta^{13}C$ values, whereas litter had the most depleted ^{13}C, followed by the light and heavy C fractions. This corroborates the pattern of continuous ^{13}C enrichment of the density fractions observed in many previous studies (Dorodnikov *et al.* 2011; Gunina & Kuzyakov 2014). The enrichment of $\delta^{13}C$ occurs via kinetic fractionation during microbial transformation of soil C; microbial decomposers preferentially break down ^{12}C, releasing it as CO_2, leaving ^{13}C-enriched microbial products and organic residues (Werth & Kuzyakov 2010). Thus, increasing $\delta^{13}C$ corresponds to the degree of microbial transformation, and represents the level of soil C stabilization (Dorodnikov *et al.* 2011; Gunina & Kuzyakov 2014). As erosion involves the mechanical transportation of soil particles, it does not directly affect the $\delta^{13}C$ of soil organic matter. We thus conclude that the ^{13}C-enrichment of the soil C in the bare land is largely due to the microbial transformation of C in the absence of fresh plant C inputs. The soil C/N ratio in the bare land was also the lowest of all sites, which provides further evidence of the microbial depletion of soil C as the C/N ratio of organic matter generally decreases during microbial decomposition. We estimated the C turnover time of soil C in the bare land at nearly 400 years, indicating high recalcitrance of the remaining C.

The historic data also showed a substantial decline in soil C during the conversion of Eucalyptus plantation to secondary forest in 1974, which persisted over a period of five years. Many previous studies have observed similar reductions in soil C stocks at the beginning of afforestation (Deckmyn *et al.* 2004; Vesterdal *et al.* 2008) due to increased C decomposition after disturbance and low C input from new plants (Wang, Zhu & Chen 2016). Unsurprisingly, surface soil C stocks recovered more rapidly than soil C pools in deeper soil horizons, which suggests that there is still high C sequestration potential in the deeper soils of secondary forests and plantations after 50 years of forest regeneration, and that it takes much longer for stabilized deep soil C pools to recover after severe disturbance. The accumulation of surface soil C was much greater in secondary forest compared to Eucalyptus plantations, which is likely due to the repeated disturbance of the Eucalyptus plantation site by rotational harvesting and the young age of the trees at the time of the study.

The difference in total soil C stocks between natural and secondary forest plots was largely due to the larger stock of old C in the natural forest. Although the soil C stocks in the surface soil layer were similar in the secondary forest and the natural forest, most of the soil C in the secondary forest was new C. Moreover, although the secondary forest had a greater surface soil C stock compared to the Eucalyptus plantation, the proportion of old C was similar between the two reforested sites and much lower than the natural forest. This was also consistent with the patterns of soil C stocks in depth: the natural forest had much larger soil C stocks at depth than the secondary forest. Since deep soil C is also regarded as older C with much longer turnover times than surface soil C (Chaopricha & Marin-Spiotta 2014), these results suggest that, after 50 years, the secondary forest has greatly

increased the stocks of new C in the surface soil, but has had a limited effect on the accumulation of old C and soil C stocks at depth. The litter C pools did not differ among forest sites and it is therefore unsurprising that the light fraction C pools were also remarkably similar because soil C in the light density fraction generally represents unprotected particulate organic matter derived from plant residues (Six *et al.* 2002). By contrast, the distribution of heavy fraction C was consistent with the occlusion of C within microaggregates.

Soil aggregates play an important role in protecting soil C in forests (Six *et al.* 1998) and afforestation increased the C content in microaggregates in the surface soil layer in our study. Indeed, in comparison with the natural forest, both the secondary forest and the Eucalyptus plantations had similar soil C pools in microaggregates and in the silt and clay fraction in the surface soil layer, which suggests that much of the new C in the soil is being stabilized. In the deeper soil layers, total soil C and the C associated with microaggregates was lower in secondary forest and plantations compared to the natural forest, which is consistent with previous work demonstrating that reforested sites had lower aggregate-C in comparison with natural forests (Del Galdo *et al.* 2003). However, we would expect C pools in surface soils to respond more rapidly than deeper soil horizons and our results suggest that there is still great C sequestration potential in these afforestation sites if they remain undisturbed.

The net effect of afforestation on total soil carbon depends not only on new C gained, but also on old C lost (Bashkin & Binkley 1998). Under IPCC guidelines for greenhouse gas (GHG) inventory (IPCC 2006), it is assumed that the carbon stock in the mineral soil will remain constant as long as the land remains forested, regardless of changes in forest management, forest types, and disturbance regime. This is, however, in contrast to many data syntheses and empirical studies (Don, Schumacher & Freibauer 2011; van Straaten *et al.* 2015; Wang, Zhu & Chen 2016). Our study suggests that this assumption only holds true for old C under similar land-use history (i.e. secondary forest and Eucalyptus plantation). However, the total C stocks and the pool of old soil C in the natural forest were still significantly higher than in secondary forest after 50 years of recovery. Thus, despite substantial soil C accumulation in secondary forest, the stock of stabilized C has not recovered to natural forest levels. If the patterns we observe at our study site are more widely relevant, this suggests that the current assumption of C sequestration by afforestation (IPCC 2006) could overestimate soil C stabilization in secondary forests.

Conclusions

After over 50 years of recovery of severely degraded land, we found that tropical secondary forests performed well in sequestering C in plant biomass, with comparable biomass C stocks to natural forest. However, although the secondary forest had similar surface soil C stocks to natural forest, the C stocks in deeper soils were still much smaller, and our isotope study revealed that the majority of

the soil C in secondary forests was new C, which has not yet been stabilized. Our results indicate that there is still high C sequestration potential in these secondary forests and plantations. However, this process is slow in comparison with aboveground biomass C recovery, and much longer recovery time will be needed to replenish depleted stocks of recalcitrant C, especially in deeper soil horizons.

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Author's Contributions

FW and ZL conceived the ideas and designed methodology; FW, QL, BZ, YL and YD collected the data; FW, EJS and YD analysed the data; FW, EJS, XL, JT, ZL and WZ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

All data used in this manuscript are present in the manuscript and its supporting information. Data available from the Dryad: doi:10.5061/dryad.k53b7.

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Table 1. δ13C values for bulk soil and soil fractions and the proportion of new C fraction(*f*new) at two soil depths in four land-use types after the restoration of degraded land in tropical South China; NF is natural forest, SF is secondary forest, EP is Eucalyptus plantation, BL is bare land, LF is the light density fraction, and HF is the heavy density fraction; values are means±SE for *n*=4; statistical data was generated from the final model (see statistical analysis).

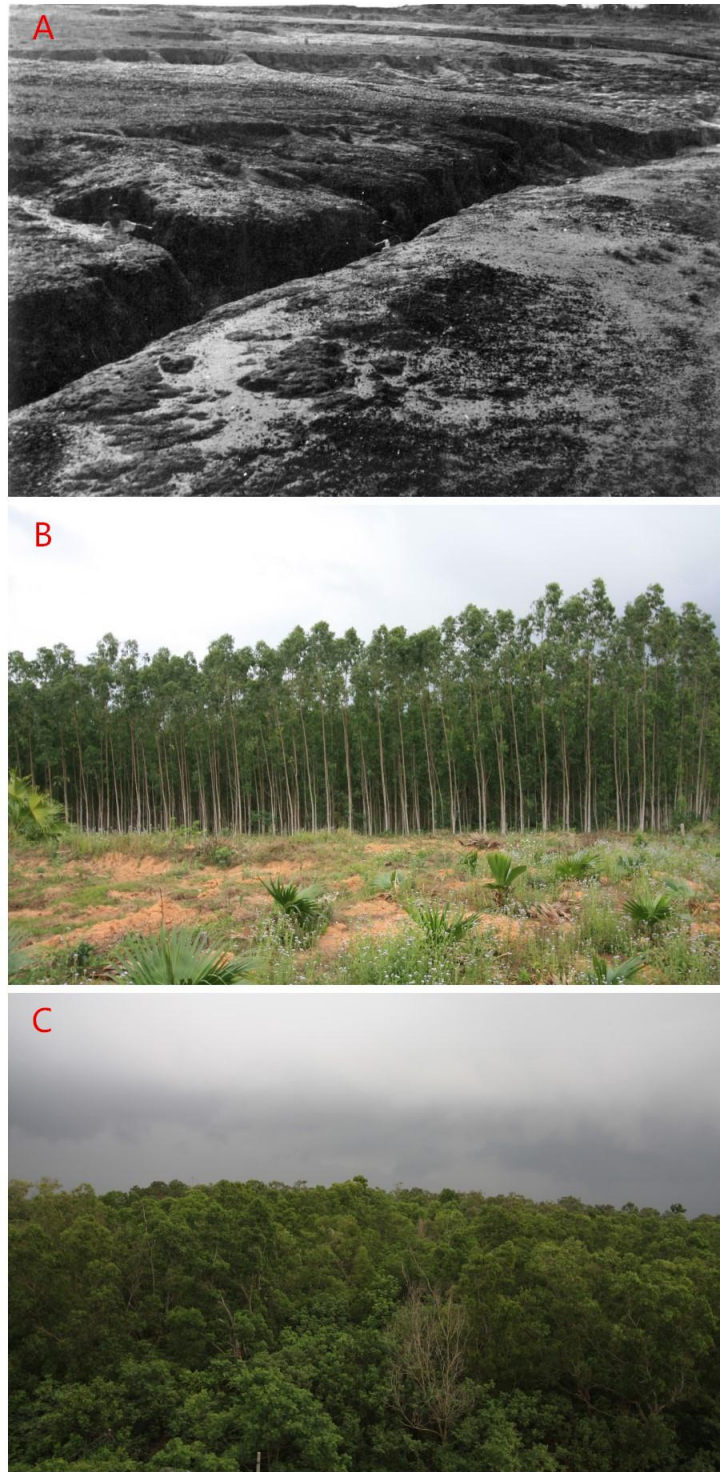| Type	Depth	$\delta^{13}C$(‰)				*f*new(%)
		Litter-C	LF-C	HF-C	Soil C	
NF	0-10	-30.74±0.13	-27.50±0.45	-26.38±0.46	-26.50±0.44	50.5±5.3
	10-20		-25.98±1.12	-25.49±0.62	-25.51±0.64	38.8±7.3
SF	0-10	-31.22±0.16	-28.79±0.27	-27.35±0.29	-27.57±0.26	59.8±2.9
	10-20		-27.06±0.89	-25.77±0.47	-25.88±0.44	40.8±4.9
EP	0-10	-31.29±0.16	-27.59±0.45	-24.11±0.28	-24.92±0.40	30.0±4.4
	10-20		-25.14±1.27	-23.68±0.47	-23.88±0.44	18.5±6.0
BL	0-10	-	-	-21.95±0.78	-22.19±0.83	-
	10-20	-	-	-22.87±0.41	-22.85±0.41	-
Source of variation						
Type			-	*p<0.001*	*p=0.001*	*p<0.001*
Depth			*p<0.001*	*ns*	*p=0.033*	*p<0.001*
Interaction			-	*ns*	*ns*	*ns*

Figure 1. The bare land before restoration (A), Eucalyptus plantation (B) and restored secondary forest (C) at the Xiaoliang Station. The bare land photo was taken in 1959 by Zuoyue Yu and the remaining two photos were taken in 2009 by Faming Wang.
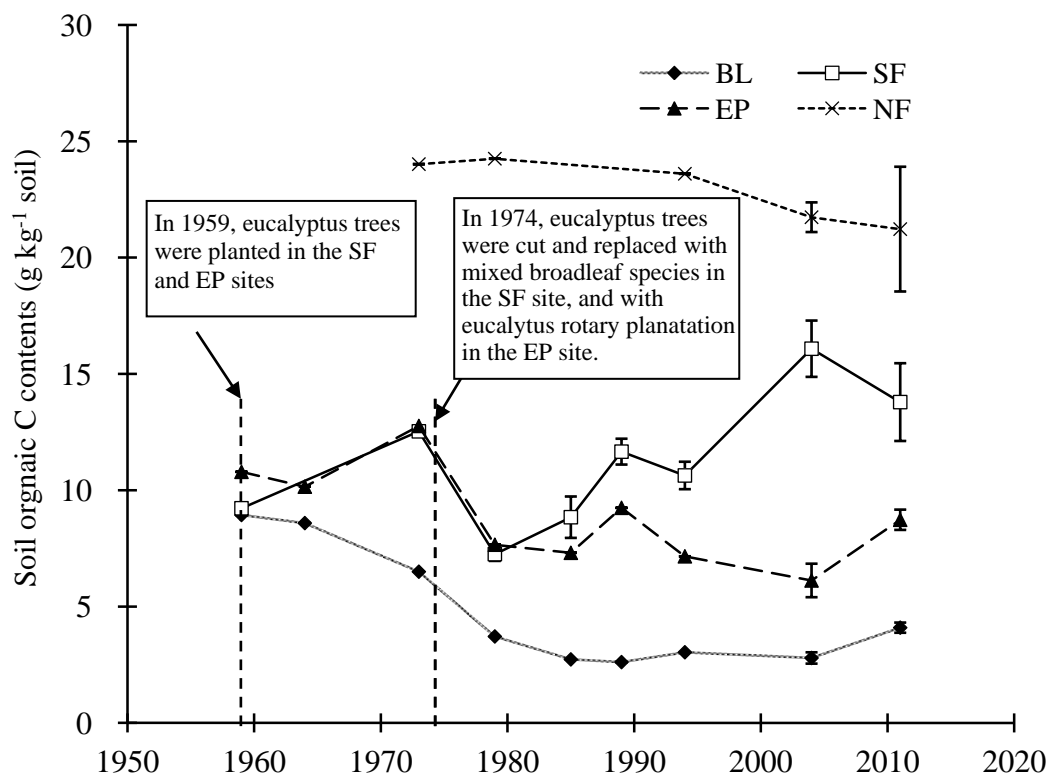
Figure 2. Historical variation in surface soil C (0-20 cm) in four land-use types after the restoration of degraded land in tropical South China, where BL is bare land, EP is Eucalyptus plantation, SF is secondary forest, and NF is natural old-growth forest. The data for 1979 were collected at 0-15 cm depth, data in 2004 are from Gu et al., 2007; and Ren et al., 2007 has reported the BL data from 1959 to 2004; for details see Table S3. Data with error bars shows means ± S.E., data with no error bar indicated only one data available.

Figure 3. Total carbon stocks and carbon pools in four land-use types after the restoration of degraded land in tropical South China, where BL is bare land, EP is Eucalyptus plantation, SF is secondary forest, and NF is natural old-growth forest; error bars indicate standard errors for $n = 4$ per carbon pool.
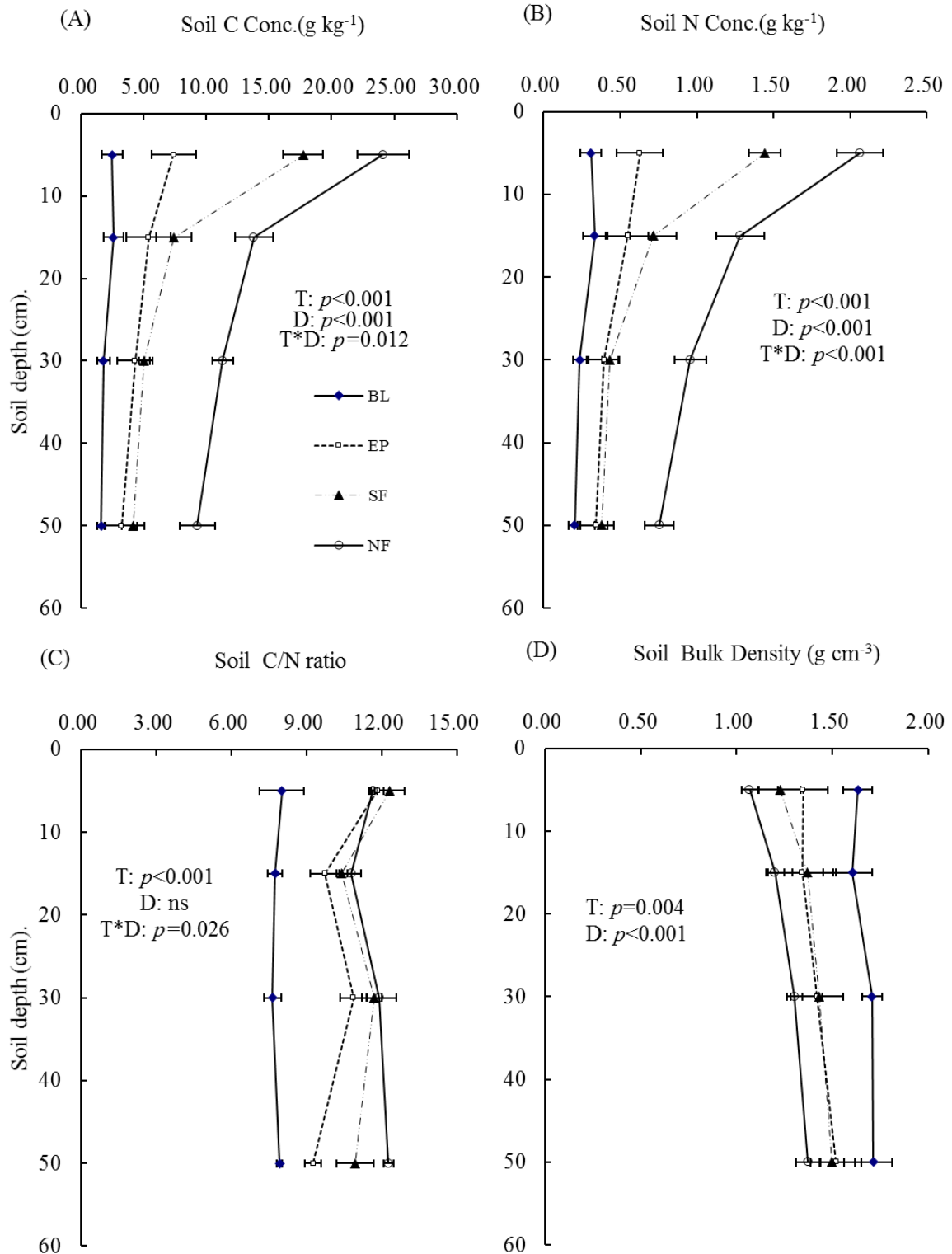
Fig 4. Depth distribution of A) soil C, B) soil N, C) soil C/N ratio and D) bulk density in four land-use types after the restoration of degraded land in tropical South China, where BL is bare land, EP is Eucalyptus plantation, SF is secondary forest, and NF is natural old-growth forest; significance values are given for main effects from a linear mixed model (see statistical analysis), where T is the effect of land-use type and D is the effect of soil depth; error bars indicate standard errors for $n = 4$.
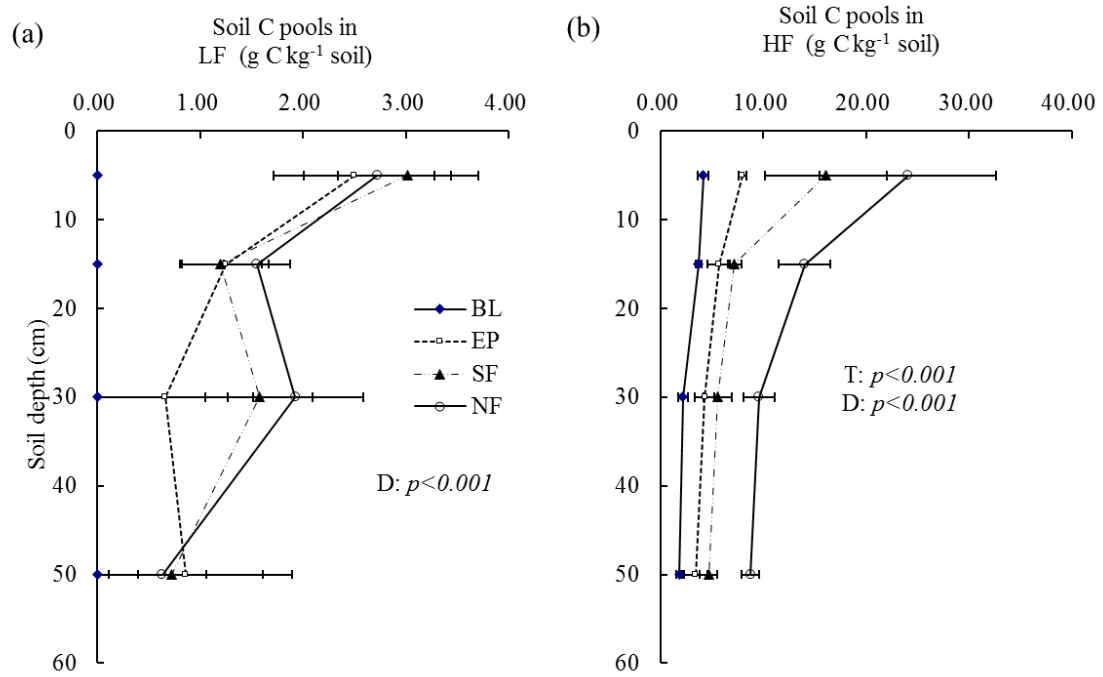
Figure 5. Soil carbon pools in the light density fraction (LF) and heavy density fraction (HF) at different soil depths in four land-use types after the restoration of degraded land in tropical South China, where BL is bare land, EP is Eucalyptus plantation, SF is secondary forest, and NF is natural old-growth forest; significance values are given for main effects from a linear mixed model, where T is the effect of land-use type and D is the effect of soil depth; error bars indicate standard errors for $n = 4$.
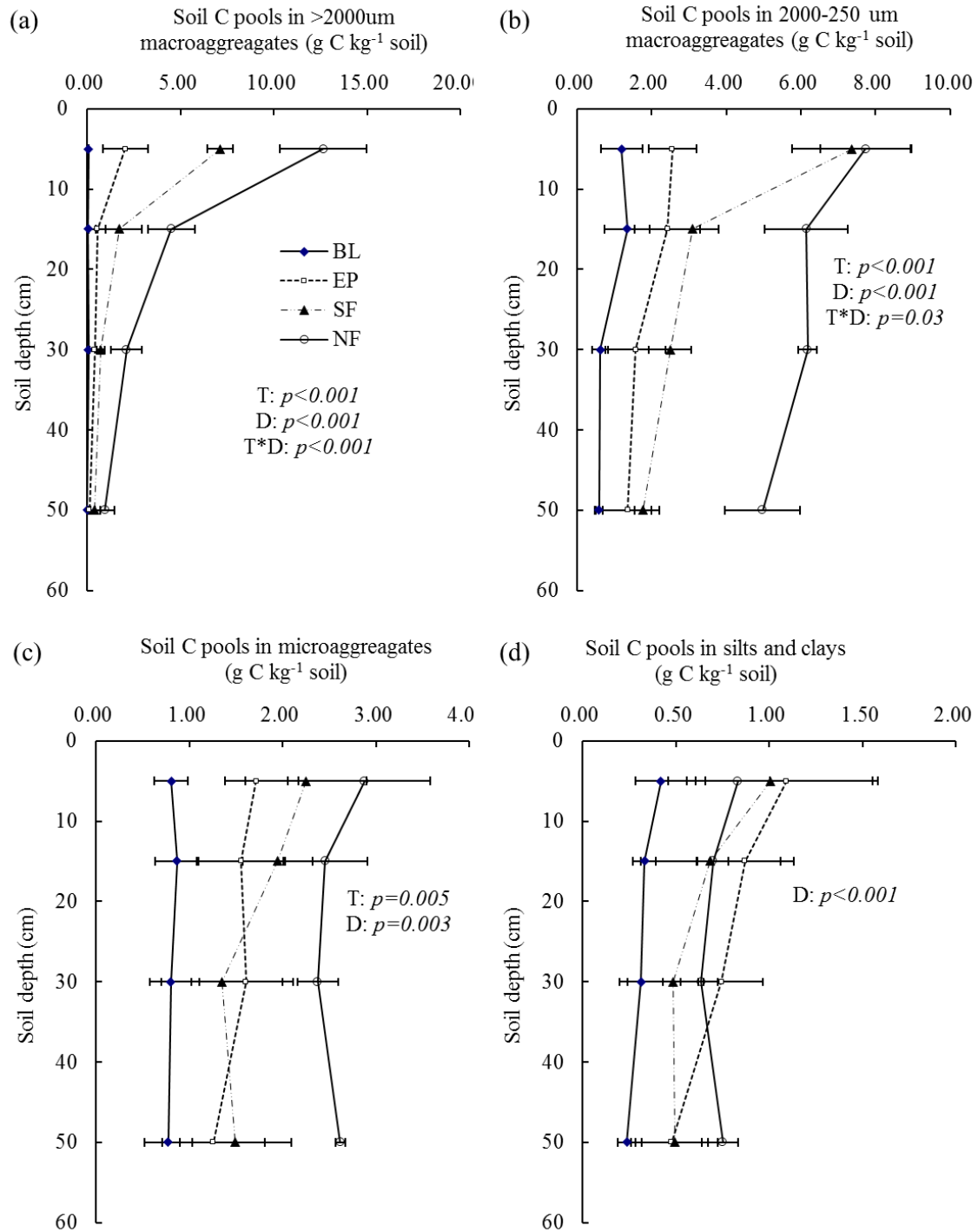
Fig 6. Soil aggregate size fractions at different soil depths in four land-use types after the restoration of degraded land in tropical South China, where BL is bare land, EP is Eucalyptus plantation, SF is secondary forest, and NF is natural old-growth forest; significance values are given for main effects from a linear mixed model, where T is the effect of land-use type and D is the effect of soil depth; error bars indicate standard errors for $n = 4$.
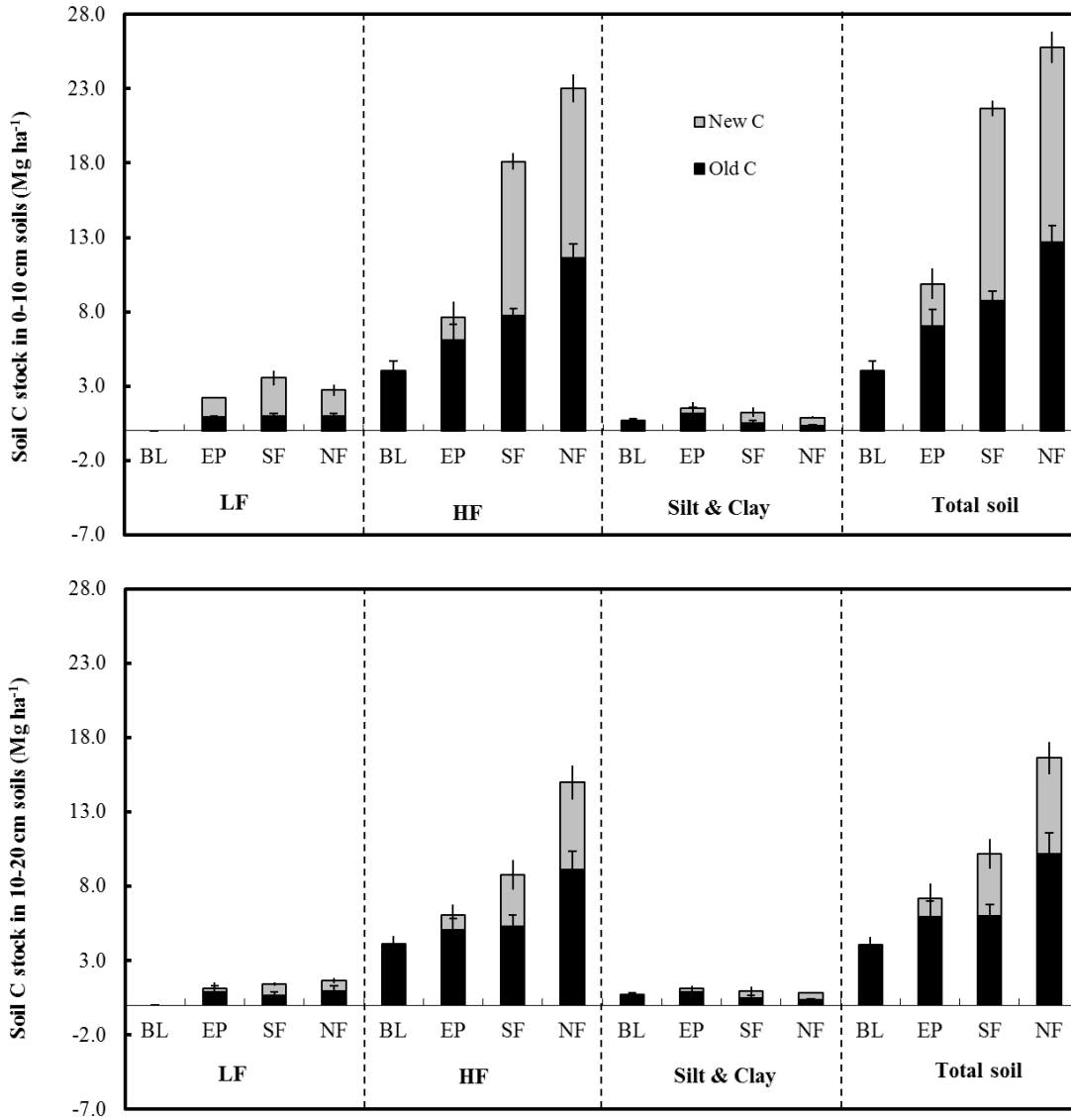
Fig 7. Stocks of new and old carbon in different soil density fractions, soil aggregates and the bulk soil at different soil depths in four land-use types after the restoration of degraded land in tropical South China, where BL is bare land, EP is Eucalyptus plantation, SF is secondary forest, and NF is natural old-growth forest, LF is the light density fraction, HF is the heavy fraction, error bars indicate standard errors for $n = 4$.