

Soil aggregate fraction-based ^{14}C analysis and its application in the study of soil organic carbon turnover under forests of different ages

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Received August 6, 2012; accepted December 17, 2012; published online January 16, 2013

There still exist uncertainties in the trend, magnitude and efficiency of carbon sequestration with regard to the changes in soil organic carbon (SOC) pools after afforestation. In this study, SOC turnover times of the meadow steppe and planted forests at Saihanba Forest Station of Hebei Province, China are estimated by means of the radiocarbon (^{14}C) method. Our results show that the SOC turnover times can be as long as from 70 to 250 years. After planting the *Pinus sylvestri* var. *mongolica* in the *Leymus chinensis* meadow steppe, the turnover times of organic carbon in both bulk samples and soil aggregate fractions of the topsoils are decreased with an increase of the stand age. Such a lowering of the turnover time would cause an increase in soil CO_2 flux, implying that afforestation of grassland may reduce the capacity of topsoil to sequester organic carbon. Combined stable isotope and ^{14}C analyses on soil aggregate fractions suggest that there are different responses to afforestation of grassland between young and old carbon pools in topsoils. In the young and middle-age planted forests, the proportion of CO_2 emission from the older soil carbon pool shows an increasing trend. But in the mature planted forest, its proportion tends to decline, indicating that the stand age may influence the soil carbon sequestration mechanism. The CO_2 emission from the topsoils estimated using the ^{14}C method is relatively low compared to those by other methods and may be caused by the partial isolation of the young carbon component from the soil aggregates. For more accurate estimation of CO_2 flux, future studies should therefore employ improved methodology for more effective separation of different soil carbon components before isotope analyses.

^{14}C , soil organic carbon turnover, aggregate fractions, Saihanba, afforestation of grassland, stand age

Citation: Tan W B, Zhou L P, Liu K X. Soil aggregate fraction-based ^{14}C analysis and its application in the study of soil organic carbon turnover under forests of different ages. *Chin Sci Bull*, 2013, 58: 1936–1947, doi: 10.1007/s11434-012-5660-7

Land-use change, especially the change from natural ecosystems to agro-ecosystems, is an important cause for soil organic carbon (SOC) pool change [1–5]. Land-use change controls the fate of carbon in the terrestrial ecosystem mainly through its impact on SOC input and decomposition rate [6,7]. Statistics show that, from 1850 to 1998, changes in land use pattern and tillage practice have led to a net emission of about 136 Pg CO_2 from the global soil carbon pool [8]. Therefore, rational land use to improve the capacity of soil to store carbon is an important measure for mitigating climate change as well as for the world food security [9–11]. Afforestation is a type of land-use change associat-

ed with human activities, and any small changes in SOC pool caused by afforestation can alter the function of the soil as a carbon source or sink [12–16]. Most previous studies concerning mechanisms of the SOC sequestration and emission in planted forest have focused on the SOC content and storage change [12,15], and there is no consensus on the changes in SOC pools following afforestation. Studies have reported accumulation [17–19], no net change [20–22], or loss [12,16,23] of SOC storage after afforestation. The disparity may be due to a number of factors, including sampling depth, regional climate, previous land use, tree species, and stand ages [23–25]. SOC turnover time, as a parameter for estimating soil carbon flux, is important for understanding the dynamics of SOC [26–28], but little has been done

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to evaluate the soil carbon pool change based on the SOC turnover times for the soils under planted forests. Therefore, systematic investigation on the turnover time of SOC in planted forests will be highly valuable for unraveling the mechanisms involved in the changes of the SOC pools.

Atmospheric weapons testing in the late 1950s and early 1960s injected a large quantity of bomb- ^{14}C into the atmosphere, leading to a rapid rise in ^{14}C abundance of atmospheric CO_2 with a peak value in 1963–1965. Subsequently, such ^{14}C entered the terrestrial carbon pool through plant photosynthesis, and eventually enriched in the soil organic matter [29–31]. Therefore, the ^{14}C peak can provide an extremely useful tool to quantify the turnover of the soil organic matter at timescales of a few years to a few hundred years through models [32]. For regions lacking long-term observations, the ^{14}C tracer method can provide a powerful means for estimating the SOC turnover at the meso- and long-term scale. Such an approach has a potential advantage compared with that through direct observations for SOC storage or soil respiration. The SOC is not a homogeneous pool but composed of a complex mixture of organic components with a variety of turnover times ranging from days to centuries or even millennia [33]. The responses of different SOC fractions to climate and environmental change also show significant difference. Different particle-size aggregates protect organic carbon in different ways, which will lead to differences in the turnover times of organic carbon [26]. Therefore, soil aggregate fractions separation method can help us understand the soil carbon cycle from the perspective of the heterogeneity of soil organic matter, and when combined with the ^{14}C tracer technique, it can help us better understand the SOC turnover mechanism and predict its response to climate change [34–38].

Saihanba, with the largest cover of planted forests and as a typical forest-steppe ecotone in North China, is extremely

sensitive to climate change. It can serve as an ideal site for studying the responses of planted forests ecosystem to climate change in the future. In this study we collected bulk topsoils from *Leymus chinensis* meadow steppe and *Pinus sylvestri* var. *mongolica* planted forests with different stand ages (young, middle-age and mature) in the Saihanba region, and fractionated them into different particle-size aggregate fractions, and analysed the ^{14}C contents in bulk soils and soil aggregate fractions to obtain their SOC turnover times. We then combined these data with soil property, vegetation type, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements in order to evaluate the impact of afforestation of grassland on SOC turnover.

1 Materials and methods

1.1 Site description

The study was carried out at the Saihanba Forestry Center, located at $42^\circ 02' - 42^\circ 36' \text{N}$, $116^\circ 51' - 117^\circ 39' \text{E}$, 1500–1940 m a.s.l., in the north of Weichang Manchu and Mongol Autonomous County, Hebei Province, China (Figure 1). The Saihanba Forestry Center, being 240 km and 460 km away from Chengde and Beijing respectively, is in the south of Hexigten Banner, east of Duolun Country, north of Yudaokou Pasture, and west of Weichang Manchu and Mongolian Autonomous County.

The study site has continental monsoon climate in the transition from the warm temperate zone to the temperate zone, with annual mean temperature of -1.5°C , extreme maximum temperature of 33.4°C , extreme minimum temperature of -43.2°C , $\geq 5^\circ\text{C}$ annual accumulated temperature of 1957.5°C , $\geq 10^\circ\text{C}$ annual accumulated temperature of 1645.2°C , $\geq 15^\circ\text{C}$ annual accumulated temperature of 866.4°C . Annual mean precipitation is 530 mm with a trend of high in the southeast and low in the northwest, and annual

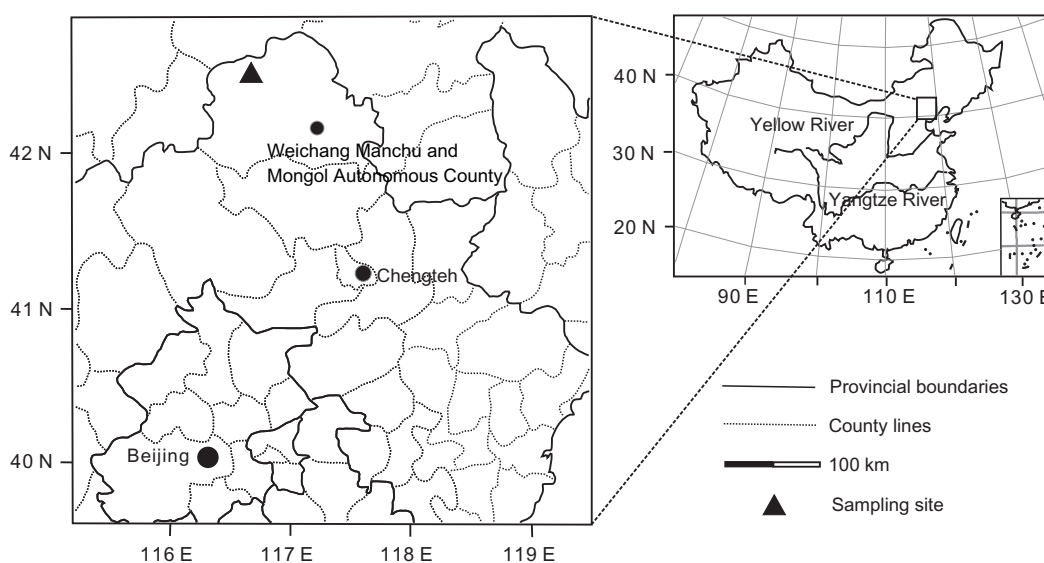


Figure 1 Location of the study site.

evaporation is 1388 mm that is high in the spring and summer but low in the autumn and winter.

The area was once a desert, and climate change is the main driving force for its vegetation degradation [39]. Since the Forestry Center was built in the 1960s, the ecological environment at the site has gradually been restored, now a unique vegetation landscape with forest, shrub, shrub-grassland, grassland, meadow and swamp [40]. The soils in the region are dominated by aeolian sandy soil, together with meadow and swamp soils.

1.2 Soil sampling

Soil sampling was conducted in July 2009, including a site in the plot of *Leymus chinensis* meadow steppe, a site in the plot of young *Pinus sylvestri* var. *mongolica* planted forest, two sites in the plot of middle-age *Pinus sylvestri* var. *mongolica* planted forest, and a site in the plot of mature *Pinus sylvestri* var. *mongolica* planted forest. At each sampling site, we collected two parallel surface soil (0–5 cm) samples with foil sampler for soil density determination, and then collected about 1 kg soil at the same depth for the determinations of other soil properties. The plot description and the soil properties are given in Tables 1 and 2, respectively.

1.3 Soil aggregates fractionation

About 100 g frozen and dried bulk soils were dry sieved at 2 mm. For the soil materials > 2 mm, after removing the residues of plant roots, we weighed the remaining soil skeleton particles to get the percentage of > 2 mm gravels and the

data was then used for calculating the soil CO₂ flux. For the fraction < 2 mm, after collecting the residues of plant roots with the water flotation method, we combined them into the > 2 mm plant roots residues. The remaining materials are treated as bulk soils. For soil aggregates fractionation, the > 250 μm and 63–250 μm fractions were isolated by wet sieving [41], and the 2–63 μm and < 2 μm fractions were separated by settling from a suspension [42], the different particle-size aggregate fractions were then freeze-dried and weighed for calculating their relative percentages. Finally the bulk soils and soil aggregate fractions obtained with the above processes were prepared for analysis of the organic carbon (TOC) and total nitrogen (TN) contents, $\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}$, and ^{14}C .

1.4 TOC, TN, $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$

All bulk soils and different particle-size aggregate fractions were pretreated with excess 1 N hydrochloric acid to remove carbonates at room temperature, then rinsed and freeze-dried for 24 h or more and ground into fine powder over 100 meshes. Plant root residues were washed with distilled water, then freeze-dried and ground into fine powder as the above treatments for soils.

TOC and TN contents were determined by elemental analyzer (VARIO EL cube) in Analytical Centre of Peking University, and $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ values were measured using Finnigan MAT253 mass spectrometer coupled with a COSTECH elemental analyzer in the Godwin Laboratory, University of Cambridge. The standard deviations are less than 0.05% for TOC and TN and 0.15‰ for $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$, respectively.

Table 1 Plot descriptions at the sampling site

Plot type	Stand age (a)	Soil type	Soil texture	Species composition
Meadow steppe	–	Aeolian sandy soil	Sandy loam	Dominant species: <i>Leymus chinensis</i> ; accompanying species: <i>Potentilla tanacetifolia</i> , <i>Sanguisorba officinalis</i> , <i>Galium verum</i> , <i>Heteropappus hispidus</i> (Thunb.) Less., <i>Trollius chinensis</i> Bunge, etc.
Young forest	12	Aeolian sandy soil	Sandy loam	Dominant species: <i>Pinus sylvestris</i> var. <i>mongolica</i> ; the herbaceous layer under forest mainly consists of <i>Carex</i> sp., <i>Saussurea</i> sp., <i>thalictrum</i> sp.
Middle-age forest	25	Aeolian sandy soil	Sandy loam	Dominant species: <i>Pinus sylvestris</i> var. <i>mongolica</i> ; the herbaceous layer under forest is scarce.
Mature forest	39	Aeolian sandy soil	Sandy loam	Dominant species: <i>Pinus sylvestris</i> var. <i>mongolica</i> ; the herbaceous layer under forest is scarce.

Table 2 Soil properties in different plots

Plot type	Sample identifier	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	Soil density (g cm ⁻³)	Soil water content (%)	Soil pH	The percentage of soil aggregate fractions (%)			
							> 250 μm	63–250 μm	2–63 μm	< 2 μm
Meadow steppe	S1	26.5	1.8	1.20	7.2	6.2	53.4	33.8	10.3	2.5
Young forest	S2	27.6	2.5	1.19	8.8	6.8	44.2	29.1	23.6	3.1
Middle-age forest	S3	27.7	2.6	0.98	9.3	7.1	47.1	25.8	23.8	3.3
	S4	27.8	2.4	1.02	9.0	7.0	48.8	24.8	23.1	3.3
Mature forest	S5	22.3	1.5	1.18	9.7	7.3	50.7	30.7	15.8	2.8

The $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ values are expressed in the following formulas:

$$\delta^{13}\text{C}_{\text{org}} (\text{‰}) = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} \times 1000, \quad (1)$$

$$\delta^{15}\text{N} (\text{‰}) = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \times 1000. \quad (2)$$

The standard samples for $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ are PDB (Pee Dee Belemnite) and atmospheric N_2 , respectively.

1.5 ^{14}C analysis

Sample preparation for ^{14}C analysis was undertaken using the method of Xu et al. [43] in the Laboratory of Quaternary Global Change Group of Peking University. Soil materials containing about 1–1.5 mg carbon were placed into quartz tubes with clean CuO and desulphurization powder. After the tubes were vacuumed and sealed, subsamples were combusted at 900°C for 2–3 h. The quartz tubes with CO_2 were cracked open in the tube cracker, then purified by a dry ice and ethanol slurry on the first trap and liquid nitrogen on second trap. The purified CO_2 was transferred to a known-volume reservoir and quantified by measuring the CO_2 pressure. Approximately 1 mg carbon as CO_2 was frozen into the reduction tube containing Zn, TiH_2 , and Fe powder, then sealed by torch. After reduction at 550°C for 7–8 h, graphite is formed at the surface of the Fe powder. For the reagents mentioned above, TiH_2 provided the source of H_2 , Fe powder acted as catalyst, and Zn was not only for reducing CO_2 to CO, but most importantly, for recycling the H_2O back to H_2 for promoting graphitization [43].

Measurements of ^{14}C were made with the NEC 500 kV compact Accelerator Mass Spectrometry (AMS) at the Institute of Heavy Ion Physics, Peking University. The precision for AMS is better than 0.4‰, and the instrumental background is less than 0.03 pMC [44].

^{14}C data are reported as pMC [45], which is defined as

$$\text{pMC} (\%) = \frac{A_{\text{SN}}}{A_{\text{ON}} \times e^{\lambda(y-1950)}} \times 100, \quad (3)$$

where A_{SN} is the $^{14}\text{C}/^{12}\text{C}$ ratio of the sample corrected to a $\delta^{13}\text{C}$ value of -25‰ to account for the assumption that plants discriminate twice as much against ^{14}C as they do against ^{13}C , A_{ON} is the $^{14}\text{C}/^{12}\text{C}$ ratio of the oxalic acid activity normalized to $\delta^{13}\text{C}$ value of -19‰ , $\lambda = 1/8267$ is based on the 5730 a half-life, and (y) is the year of Oxalic measurement.

1.6 SOC turnover times and soil CO_2 flux

The SOC turnover times were estimated using a time-dependent steady-state box model [46–48]. This model assumes that the decomposition of soil organic carbon follows

a first-order kinetic law. And, therefore, for each year (y), the variation in ^{14}C in a soil with time can be described by the following mass balance equation:

$$C_y \times ^{14}\text{C}_y = C_{y-1} \times ^{14}\text{C}_{y-1} \times (1 - k - \lambda) + I \times ^{14}\text{C}_{\text{atm}, y-lag}, \quad (4)$$

where C is the organic carbon inventory of a soil sample (g C m^{-2}), ^{14}C is the pMC of a soil sample (‰), k is the first-order decomposition constant for homogeneous C pools (a^{-1}), λ is the ^{14}C decay constant ($1/8267$), I is the annual carbon input ($\text{g C m}^{-2} \text{ a}^{-1}$), $^{14}\text{C}_{\text{atm}}$ is the pMC of the atmosphere CO_2 (‰), lag is the average number of years that atmospheric CO_2 is retained in plant tissue before becoming part of the soil organic matter pool.

At steady state, $C_y = C_{y-1}$ and $I = kC$, eq. (4) can be transformed into

$$^{14}\text{C}_y = ^{14}\text{C}_{\text{atm}, y-lag} \times k + ^{14}\text{C}_{y-1} \times (1 - k - \lambda). \quad (5)$$

The decomposition constant, k , or its inverse, τ (a), is obtained by matching the modeled and measured pMC for the year in which the soil was sampled.

The soil CO_2 flux at 0–5 cm depth was calculated as

$$F (\text{g C m}^{-2} \text{ a}^{-1}) = \sum_i \frac{\text{SOC}(i) \times \gamma \times \text{AP}(i) \times H \times (1 - \delta_{2\text{mm}}/100)/10}{\tau(i)}, \quad (6)$$

where $\text{SOC}(i)$ is the SOC contents of different particle-size aggregate fractions (g kg^{-1}), γ is the bulk soil density (g cm^{-3}), $\text{AP}(i)$ is the percentages of different particle-size aggregate fractions (‰), H is soil depth (cm), $\delta_{2\text{mm}}$ is the percentages of >2 mm gravels in bulk soil (‰), and $\tau(i)$ is the SOC turnover times of different particle-size aggregate fractions (a).

2 Results and discussion

2.1 Impact of lag values on SOC turnover time estimation

As the time needed to transform the standing plants into the plant litter as soil organics inputs will vary with plants, the lag values used in the estimation of the SOC turnover time, will directly affect the ^{14}C -derived dynamics of soil carbon pool. In the plot of *Leymus chinensis* meadow steppe, the ^{14}C content of plant litter annually entering soil is equivalent to the atmospheric ^{14}C level in the same year, thus we can assume $lag = 1$. For plots of *Pinus sylvestri* var. *mongolica* planted forests, the life of evergreen coniferous pine needles is usually 3–5 years [49], so we can choose the intermediate 4 as the lag value. Considering that the soil organic matter inputs consist of not only the pine needles litter, but also understory herbaceous plants and their roots, we need to evaluate the effects of different lag values assumed on the SOC turnover time estimation for the plots of *Pinus sylves-*

tri var. *mongolica* planted forests. The results from a comparative analysis show that the turnover times estimated when the *lag* value is set to 4 are in good agreement with those when the *lag* values are set to 1, 2, 3 and 5 (Figure 2(a)), and the differences of turnover time ($\Delta\tau$) between them are all within ± 3 years (Figure 2(b)), suggesting that the *lag* values ranging from 1 to 5 have no significant effect on the SOC turnover times estimation. Therefore, the *lag* values in our model are set to 1 in the plot of *Leymus chinensis* meadow steppe and 4 in plots of *Pinus sylvestri* var. *mongolica* planted forests, respectively. With these *lag* values, the pMC of bulk soils and different particle-size aggregate fractions (Table 3) are modeled to estimate the

SOC turnover times, the results are listed in Table 4.

2.2 SOC turnover times for different particle-size aggregates

The SOC turnover times in soil aggregate fractions of all samples increase gradually with decreasing particle-size (Table 4), indicating that soil organic matter in smaller particle-size aggregates have higher stability. Chemical protection of soil organic matter arises from the interaction of different types of organic carbon with metal oxides and clay minerals, and thus, the decomposition rate of SOC is generally slower in smaller particle-size aggregate [36,50–53].

Table 3 pMC (%) of bulk soils and different particle-size aggregate fractions

Plot type	Sample identifier	Bulk soil	Different particle-size aggregate fractions			
			> 250 μm	63–250 μm	2–63 μm	< 2 μm
Meadow steppe	S1	98.7	101.7	99.1	96.6	93.7
Young forest	S2	102.0	103.3	102.9	98.8	97.2
Middle-age forest	S3	104.3	106.0	105.9	101.2	98.9
	S4	105.8	106.5	105.6	102.0	98.9
Mature forest	S5	112.6	113.8	112.6	109.8	107.6

Table 4 SOC turnover times (a) of bulk soils and different particle-size aggregate fractions

Plot type	Sample identifier	Bulk soil	Different particle-size aggregate fractions			
			> 250 μm	63–250 μm	2–63 μm	< 2 μm
Meadow steppe	S1	398	263	377	524	735
Young forest	S2	251	210	222	390	483
Middle-age forest	S3	184	148	151	281	386
	S4	152	140	156	251	386
Mature forest	S5	70	61	70	95	122

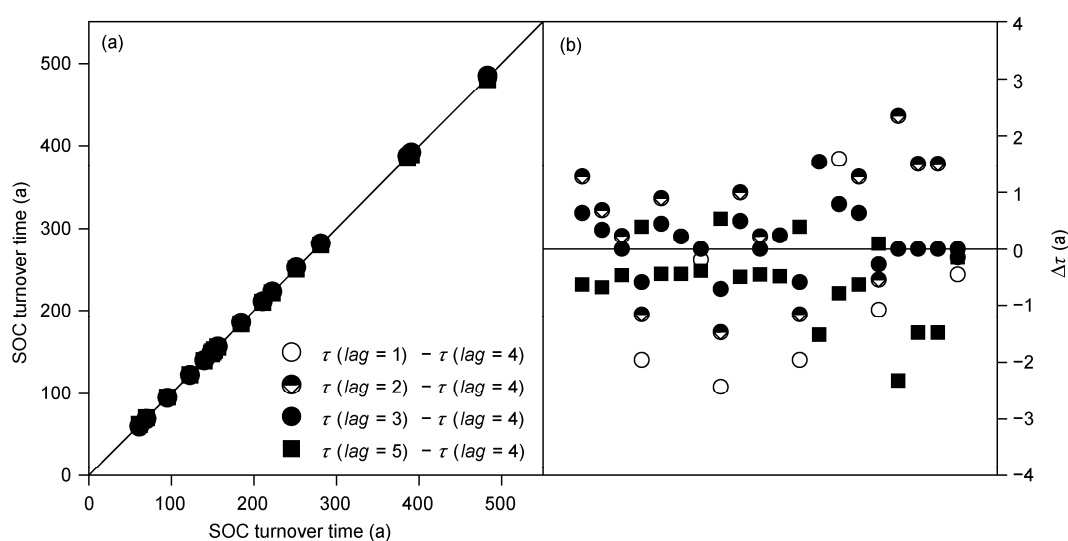


Figure 2 Correlations (a) and differences (b) of SOC turnover times between different *lag* values assumed in plots of *Pinus sylvestri* var. *mongolica* planted forests.

By contrast, occlusion is the dominant form for stabilizing soil organic matter in larger particle-size aggregates, so the decomposition rate of SOC is relatively faster [51–53]. There is a sharp increase of porosity for smaller particle-size aggregate, within which the organic matter is decomposed by virtue of diffusion of extracellular enzymes secreted by microbes. This is an extremely large energy-consuming process for microbes, hence leading to a slower decomposition rate of SOC [54]. With the increase in particle-size of soil aggregate fraction, the condensation and molecular complexity of humic acid will decrease, which is accompanied by a rise in its activation grade [55]. This may be another important reason for the faster turnover of SOC in the larger particle-size aggregate. Previous studies have shown that greater enrichment of ^{13}C and ^{15}N across soil carbon fractions reflects microbial processing during plant litter decomposition and soil organic matter formation [56–58]. In addition, decreasing C/N ratios in soil carbon fractions have been shown to be coupled with increasing decomposition of soil organic matter [59]. With the decrease of soil aggregate particle-size, the C/N ratios show a decreasing trend (Figure 3(a)), and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in soil aggregate fractions of all samples increase (Figure 3(b) and (c)). This indicates that the degree of soil organic matter decomposition in small particle-size aggregate is higher than that of large particle-size aggregate. An increase in the SOC decomposition would reduce SOC availability, thus preventing their decomposition [60]. In

this case, the differences in the degree of SOC decomposition may also be one of the reasons for different particle-size aggregates having different SOC turnover times.

The difference in the degree of soil organic matter decomposition among different particle-size aggregates provides us an opportunity for understanding the formation mechanism of soil aggregates. Currently, various models have been employed to explain the formation of aggregates [61–63]. Our results seem to suggest that the formation of larger particle-size aggregates in soil is prior to that of smaller particle-size aggregates. After the organic matter in the larger particle-size aggregate decomposes to a certain degree, its residual organic matter can form smaller particle-size aggregates through interaction with cements to improve the stability of the SOC. The microbial activities will then stop due to the available organic matter being exhausted, leading to the breakdown of the larger particle-size aggregates and the release of the smaller particle-size aggregates. The above scenario supports the embryonic development model proposed by Six et al. [64] as the dominant mechanism for the formation of soil aggregates, that is, the redistribution of SOC in soil is from the larger particle-size aggregates to the smaller particle-size aggregates, which results in the higher degree of SOC decomposition in the latter.

2.3 SOC turnover times

The SOC turnover times for both bulk soils and soil aggregate fractions are significantly greater in the plot of meadow steppe than in the plots of planted forests (Table 4). This suggests that afforestation of grassland may accelerate the decomposition of soil organic matter in topsoil. For planted forests, the SOC turnover times decrease gradually with increasing stand ages (Table 4). While the vegetation type is considered to be a primary factor for different plots having different SOC turnover times, the sources for the soil organic matter must also play an important role. Compared with above-ground litterfall, root debris input forms a greater contribution to the formation of stable soil carbon pool [65–69]. Therefore, the root-to-shoot ratio of plant debris incorporated into the soil is an essential controlling factor for the formation and stabilization of soil organic matter [70]. The root-to-shoot ratios are related to vegetation types. Generally, grassland and steppe soils receive a higher proportion of total carbon input as root litter in comparison to forest ecosystems under similar climatic conditions [71,72]. This may explain why the planted forests have faster decomposition rates of soil organic matter than meadow steppe in our study. Understory vegetations abundance has been shown to increase gradually with increasing stand ages [73–75]. This could promote the activity of soil microbes [76], thus resulting in the decrease of the SOC turnover times.

Water-soluble organic carbon (WSOC), as a part of ac-

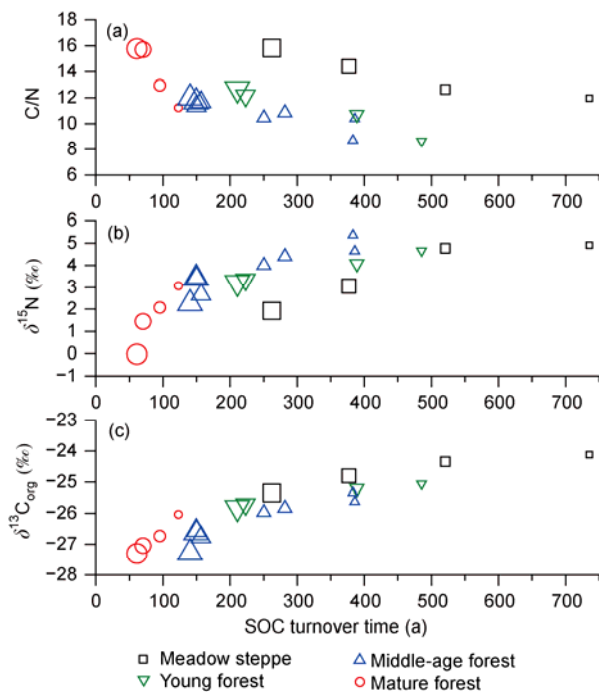


Figure 3 Relationships of $\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}$, and C/N ratios with SOC turnover times in soil aggregate fractions. Symbol sizes from large to small are in order of $>250\ \mu\text{m}$, $63\text{--}250\ \mu\text{m}$, $2\text{--}63\ \mu\text{m}$, and $<2\ \mu\text{m}$ soil aggregate fractions.

tive carbon pool, is widely known to play a dominant role in improving microbial activity for being easily assimilated by microbes [77,78]. Higher content of WSOC was observed in coniferous forest soil than in grassland soil [79,80]. This may be one of the reasons that the decomposition rate of SOC in *Pinus sylvestri* var. *mongolica* planted forests is greater than in *Leymus chinensis* meadow steppe. Plant litter is one of the major sources of WSOC [81]. Its production would significantly increase with increasing stand ages [82–84], thus supplying more WSOC to the soil. In addition, the increase in the production of plant litter could accelerate the decomposition of the native organic matter in soil by priming effect [85,86]. Hence, the turnover times of SOC are observed to decrease with the increase of stand age.

During the decomposition process of plant litter, decomposing organisms would prefer ^{13}C -depleted molecules for respiration, leaving the ^{13}C -enriched molecules incorporated into the soil organic matter pool. As a result, the $\Delta\delta^{13}\text{C}_{\text{org}}$ and $\Delta\delta^{15}\text{N}$ values, defined as the degree of difference in stable isotope values between soil organic matter and plant litter, is generally proportional to the degree of soil organic matter decomposition [57]. Figure 4 shows that the soil in the plots of planted forest exhibiting lower values of $\Delta\delta^{13}\text{C}_{\text{org}}$ and $\Delta\delta^{15}\text{N}$ than in the plot of meadow steppe, and a trend of decreasing $\Delta\delta^{13}\text{C}_{\text{org}}$ and $\Delta\delta^{15}\text{N}$ values with increasing stand ages. This suggests that the decreasing trend in SOC turnover times after afforestation is likely related to the difference in the degree of SOC decomposition.

The C/N ratio of organic matter in soils is a well-known

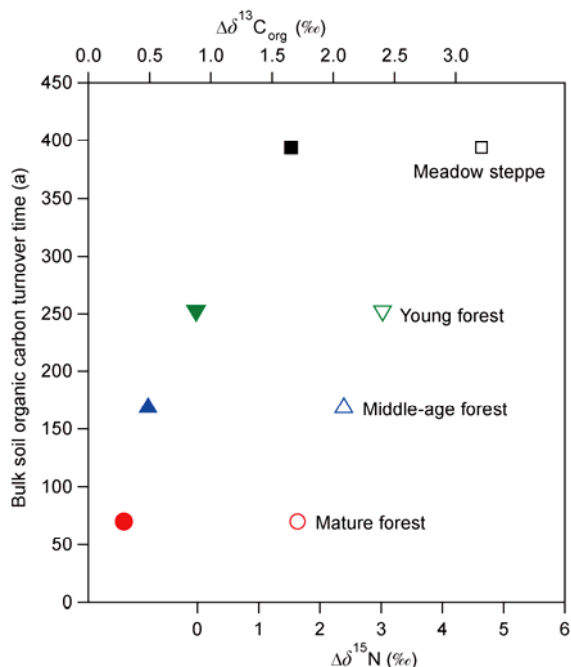


Figure 4 Relationship of SOC turnover times with enrichment degrees of stable isotope in soil organic matter. $\Delta\delta^{13}\text{C}_{\text{org}} = \delta^{13}\text{C}_{\text{soil}} - \delta^{13}\text{C}_{\text{root}}$, $\Delta\delta^{15}\text{N} = \delta^{15}\text{N}_{\text{soil}} - \delta^{15}\text{N}_{\text{root}}$. Solid and open symbols represent $\Delta\delta^{13}\text{C}_{\text{org}}$ and $\Delta\delta^{15}\text{N}$, respectively.

parameter that could affect C and N release during decomposition; too high C/N ratio will prevent soil organic matter decomposition due to the lack of nitrogen, while appropriately reducing C/N ratio can accelerate the soil organic matter decomposition [87]. Figure 5 shows that the C/N ratios in both bulk soils and soil aggregate fractions under meadow steppe are greater than under young planted forest, suggesting that the decrease of the SOC turnover time at the initial stage after afforestation is at least partly attributable to the decline of the soil C/N ratios. It is interesting to note that soil C/N ratios do not continue to decrease with increasing stand ages, but increase from middle-age to mature planted forests (Figure 5). This implies that the impact of soil C/N ratio on SOC decomposition may gradually decrease with increasing stand ages.

The physical and chemical properties of soils are also critical in controlling the organic matter decomposition. Previous studies show that the soil porosity increase in the early stage after afforestation [88], which can provide a good ventilation environment for microbial growth, and therefore may also be a factor to accelerate the SOC decomposition [89].

By comparing the topsoil organic carbon turnover times across different ecosystems in China (Figure 6), we find that the SOC in our study area has significantly greater turnover times than in other ecosystems. This may be attributed to the unique environmental and climatic conditions in the Saihanba area, including low soil temperature, limited rainfall, poor soil property and special vegetation composition. But the most important reason may lie in the differences among the approaches used for estimating SOC turnover times.

The first-order kinetic equation fitting method can be used to estimate soil organic matter decomposition rates by employing dynamic model to match the data from incubation studies [90–95]. The natural abundance ^{13}C labeling tracer approach is based on the physiological differences during the photosynthetic fixation of CO_2 between C_3 and C_4 plants, which lead to plants with distinct $\delta^{13}\text{C}$ values; where a vegetation change from C_3 to C_4 plants (or from C_4 to C_3) has occurred, the rate of change of $\delta^{13}\text{C}$ values in soil organic matter will give us a turnover time of soil organic

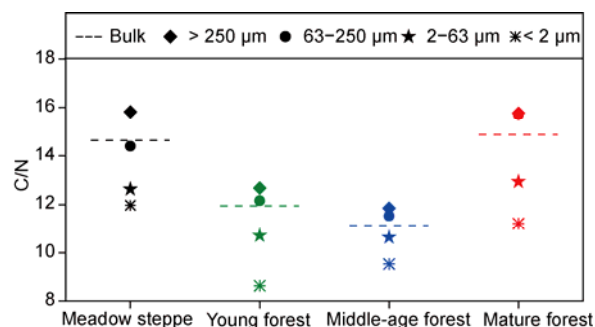


Figure 5 C/N ratios of bulk soil and soil aggregate fractions in meadow steppe and planted forests with different stand ages.

matter [96]. The experimental periods involved in the above two methods are usually within a year or a few years. Therefore, they do provide suitable ways to study decomposition of readily biodegradable organic matter in soils, and to quantify the dynamics of soil carbon pool at short-term scale. However, they are not suitable for evaluating the dynamics of the dominant soil carbon pools at long-term scale [32,105]. Hence, it would result in an overestimation of the SOC decomposition rate. Laboratory incubation is generally difficult to exclude the interference of human factors on SOC decomposition [27], thus not suitable for extrapolating the decomposition rates from the laboratory to ecological settings. The natural abundance ^{13}C labeling tracer approach also needs to consider the impact of carbon isotope fractionation on $\delta^{13}\text{C}$ values during the decomposition process of soil organic matter [106]. The vegetation shifts may also change the amount and quality of carbon input to soils, affecting the decomposition process and potentially confounding measurements of the soil organic matter decomposition rate.

Although soil respiration measurement approach is widely applied in estimating soil CO_2 flux [107], it is difficult to distinguish the relative contributions of root autotrophic respiration and of microbial heterotrophic respiration to soil [108]. The matrix for heterotrophic respiration of soil microbe is mainly from labile organic matter which only accounts for a smaller proportion in soils. The increase or decrease in soil respiration does not necessarily mean a fast or slow of decomposition rate of soil organic matter, hence the soil respiration measurement approach could give rise to large uncertainty in SOC turnover time estimation.

The ^{14}C tracer technique is mainly used to quantify soil turnover times across decade-to-century-scale based on models [27,32]. Therefore, the soil turnover times estimated by ^{14}C analysis are generally greater than by other approaches (Figure 6), but of more significance to evaluate the dynamics of SOC in ecological settings.

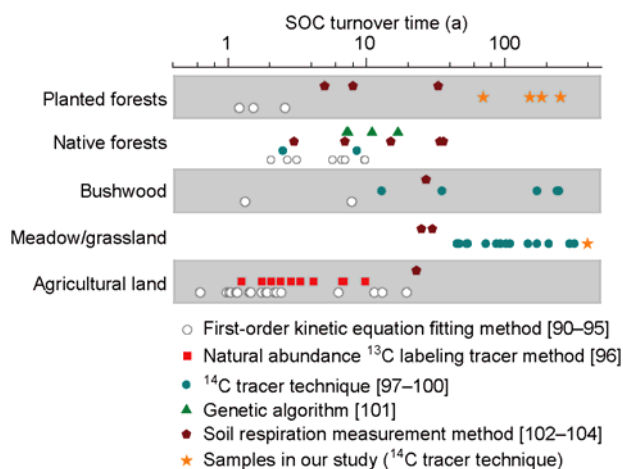


Figure 6 Turnover times of topsoil organic carbon under different ecosystems in China.

2.4 Soil CO_2 flux

We have calculated the CO_2 fluxes of topsoil in different plots using the SOC contents (Table 5) and percentages (Table 2) of different particle-size aggregate fractions. The values are $4.3 \text{ g C m}^{-2} \text{ a}^{-1}$ for meadow steppe, $5.9 \text{ g C m}^{-2} \text{ a}^{-1}$ for young planted forest, $6.8 \text{ g C m}^{-2} \text{ a}^{-1}$ for middle-aged planted forest and $17.5 \text{ g C m}^{-2} \text{ a}^{-1}$ for mature planted forest, respectively. Figure 7 shows that the topsoil CO_2 flux in planted forests is significantly higher than in meadow steppe. Within the planted forests, the topsoil CO_2 flux gradually increases with increasing stand ages. The trend of increasing topsoil CO_2 flux after afforestation may be mainly caused by the decrease in SOC turnover times, suggesting that carbon sequestration in soil is limited by rapid decomposition after the afforestation. However, compared with the grassland, planted forests can accumulate more plant litter on the surface soil to compensate the SOC loss caused by the afforestation [109,110]. Moreover, planted forests can fix more atmospheric CO_2 through plant photosynthesis with the greater aboveground biomass [2], which would guarantee the capacity of planted forest as a carbon sink in ecosystem [20,21].

From meadow steppe to young planted forest and then to middle-aged planted forest, the proportion of topsoil CO_2 flux contributed from $<2 \mu\text{m}$ soil aggregate fraction remains at a relatively low value, while that from $2\text{--}63 \mu\text{m}$ soil aggregate fraction is gradually increased, and that from $>250 \mu\text{m}$ and $63\text{--}250 \mu\text{m}$ soil aggregate fractions gradually decrease except for that from the $>250 \mu\text{m}$ soil aggregate fraction during the period from young to middle-age after afforestation (Figure 8). As discussed earlier, the degree of organic carbon decomposition in the smaller particle-size aggregate fraction is higher than in the larger particle-size aggregate fraction, that is, the apparent age of organic carbon in former is older than in the latter. Overall, there is an increase in the proportion of topsoil CO_2 emission from older organic carbon pool ($2\text{--}63 \mu\text{m}$ soil aggregate fraction), but a decreasing trend from younger organic carbon pools ($>250 \mu\text{m}$ and $63\text{--}250 \mu\text{m}$ soil aggregate fractions) during the young and middle-age stage after afforestation of grassland. This is because the increase in fresh carbon from plant

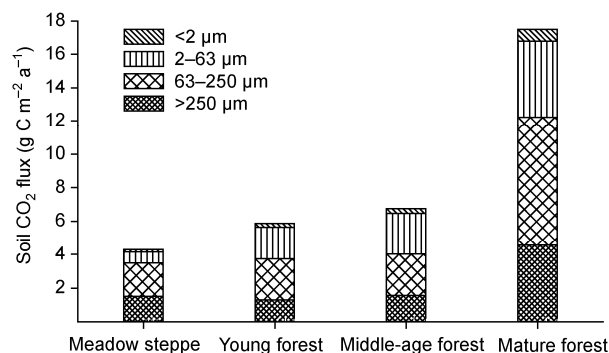
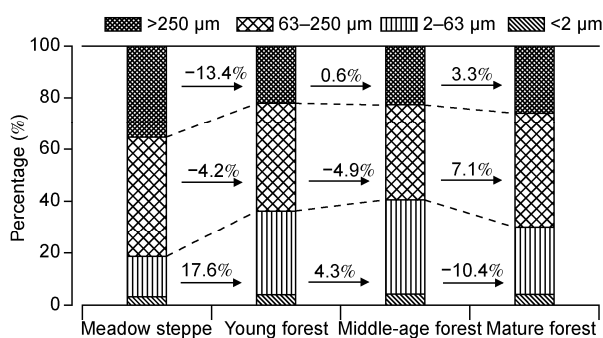


Figure 7 Topsoil CO_2 flux in meadow steppe and planted forests with different stand ages.

Table 5 SOC contents (g kg^{-1}) in different particle-size aggregate fractions

Plot type	Sample identifier	Different particle-size aggregate fractions			
		$> 250 \mu\text{m}$	$63\text{--}250 \mu\text{m}$	$2\text{--}63 \mu\text{m}$	$< 2 \mu\text{m}$
Meadow steppe	S1	11.7	34.4	51.8	64.6
Young forest	S2	10.4	31.6	52.5	61.3
Middle-age forest	S3	10.6	27.3	55.3	68.7
	S4	10.8	32.7	54.3	62.2
Mature forest	S5	9.3	29.8	46.6	52.7

**Figure 8** Change of relative percentages of soil CO₂ flux in different particle-size aggregate fractions under meadow steppe and planted forests with different stand ages.

litter input to soils may have stimulated the microbial activity by priming effect, accelerating mineralization of native old carbon in soils [85]. However, the proportion of topsoil CO₂ emission from older organic carbon pool ($2\text{--}63 \mu\text{m}$ soil aggregate fraction) begins to decrease while that from younger organic carbon pools ($>250 \mu\text{m}$ and $63\text{--}250 \mu\text{m}$ soil aggregate fractions) begins to rebound. This may be attributed to the re-adaptation of microbes to soil environment.

It has been shown that soil CO₂ flux gradually decreases with increasing depth along a soil profile, and the surface soil (0–10 cm) accounts for about 40%–80% of the whole soil profile [98]. Therefore, it can be inferred that the soil CO₂ in topsoil (0–5 cm) can contribute at least 20%–40% to whole soil profile. In this study, we calculated the soil profile CO₂ flux in different plots assuming a 20%–40% contribution of topsoil CO₂ flux to the whole soil profile, the values are $10.7\text{--}21.5 \text{ g C m}^{-2} \text{ a}^{-1}$ for meadow steppe, $14.7\text{--}29.3 \text{ g C m}^{-2} \text{ a}^{-1}$ for young planted forest, $16.9\text{--}33.8 \text{ g C m}^{-2} \text{ a}^{-1}$ for middle-aged planted forest and $43.8\text{--}87.5 \text{ g C m}^{-2} \text{ a}^{-1}$ for mature planted forest, respectively. However, the above estimated soil CO₂ flux only includes that produced by oxidative decomposition of soil organic matter, and we need to consider the contributions from root respiration [111]. Although it is difficult to assess the relative contributions of root autotrophic respiration and microbial heterotrophic respiration to soil CO₂ flux, approximately 30%–70% of soil CO₂ flux are produced by root respiration [103,112–114]. We assumed the intermediate value 50% as

the average contribution of soil respiration and calculated the soil CO₂ flux in different plots. The CO₂ flux estimates became $21.5\text{--}42.9 \text{ g C m}^{-2} \text{ a}^{-1}$ for meadow steppe, $29.3\text{--}58.7 \text{ g C m}^{-2} \text{ a}^{-1}$ for young planted forest, $33.8\text{--}67.6 \text{ g C m}^{-2} \text{ a}^{-1}$ for middle-aged planted forest and $87.5\text{--}175.1 \text{ g C m}^{-2} \text{ a}^{-1}$ for mature planted forest, respectively. These values are significantly lower than the soil CO₂ fluxes calculated on the basis of the exponential equation or Arrhenius equation [115]. Torn et al. [27] showed that the soil CO₂ flux will be underestimated by using the ¹⁴C tracer method if treating all the carbon in bulk soil as homogeneous with respect to turnover times. The soil CO₂ flux in alpine meadow estimated by Tao et al. [98] also exhibited relatively low values. This may be partly caused by not dividing soil organic matter into fractions with different intrinsic turnover times. The soil aggregate fractionation method used in our study has successfully distinguished the differences in SOC turnover times among different particle-size aggregate fractions (Table 4), but failed to yield the active soil organic matter pool with turnover time at the inter-annual scale, thus resulting in an underestimation of the soil CO₂ flux. Therefore, it is necessary to combine the physical fractionation approach with the chemical fractionation or the biological fractionation methods for more effectively separating organic matter into pools that cycle with different intrinsic turnover timescales [35,41,42,116,117]. Such an approach would significantly improve the accuracy of soil CO₂ flux estimates in future studies.

3 Conclusions

We have investigated the topsoil organic carbon ¹⁴C under *Leymus chinensis* meadow steppe and *Pinus sylvestri* var. *mongolica* planted forests with different stand ages at Saihanba, and have reached the following conclusions:

- (1) The SOC turnover times under *Pinus sylvestri* var. *mongolica* planted forests vary from 70 to 250 years, which are significantly shorter than the SOC turnover time (400 years) under *Leymus chinensis* meadow steppe.
- (2) The SOC turnover times in soil aggregate fractions gradually increase with decreasing particle-size, which is attributed to the different mechanisms of soil aggregate carbon stabilization and different degrees of organic carbon

decomposition within the soil aggregates.

(3) The SOC turnover times are found to decrease after afforestation of grassland, and show a decreasing trend with increasing stand ages. This may be caused by a number of factors, including the increase in plant litter, water soluble organic carbon, understory vegetation abundance and soil porosity, and the decrease in the degree of soil organic matter decomposition. The soil C/N ratio is shown to decrease at the initial stage after afforestation of grassland, which has contributed partly to the enhanced SOC decomposition. However, there is no obvious drop in soil C/N ratio with further increase of stand ages.

(4) The soil CO₂ flux is significantly increased after afforestation, which can be attributed to the lowering of the SOC turnover times. The proportion of soil CO₂ emission from older soil carbon pool is increased during the young and middle-age stages of the afforestation. But no such trend is observed in the mature stage, suggesting that the stand age may influence the soil carbon sequestration mechanism.

(5) The ¹⁴C tracer method has great potential for studying the SOC turnover at the meso- and long-term scale, and for estimating the soil CO₂ flux. It is especially useful for evaluating the relative contributions from different soil carbon pools with different turnover times. The CO₂ emission from the topsoils estimated using the ¹⁴C analysis approach combined with soil aggregate fractionation is relatively low, which may be due to the partial isolation of the young carbon components from the soil aggregates. Therefore, it is necessary to develop new methodology for more effective separation of different soil carbon components prior to isotope analyses, and thus to improve the accuracy of soil CO₂ flux estimates at meso- and long-term scales.

We thank Piao Shilong and Zheng Chengyang for help and encouragement during the fieldwork, Ding Xingfang for assistance in the AMS measurements.

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