**REGULAR ARTICLE** 



# Changes of soil C stocks and stability after 70-year afforestation in the Northeast USA

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# Abstract

*Background and aims* Forest restoration has been proposed as an effective strategy for the sequestration of atmosphere  $CO_2$ . Understanding the mechanisms of soil organic carbon (SOC) dynamics in afforestation is important to quantifying and enhancing carbon (C) sequestration.

*Methods* After 70 years afforestation with two conifer and three broadleaf tree species in monocultures in Northeast USA, we measured soil C pools as well as C fractions in these plantations and nearby pasture control. *Results* Soil C stored in forest floor was increased by afforestation, especially in conifer stands. Total C stock in mineral soils was not statistically different among species, but C fractions had been altered. Compared to pasture land, afforestation decreased coarse particle organic C (cPOC) fraction, while increased recalcitrant

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Biology Department, University of Illinois at Springfield (UIS), Springfield, IL 62703, USA mineral associated SOC (mSOC) fraction. Afforestation decreased the macro-aggregate C fraction in the 0-5 cm soil, but increased the micro-aggregate C fraction in the 5-15 cm soil.

*Conclusions* 1) Afforestation using conifers could improve the whole soil-profile carbon stock compared to broadleaves when the forest floor is included; 2) Even though the overall mineral soil C stock was not changed, afforestation could improve soil C stabilization through increasing mSOC fraction and forming more microaggregate C fraction in deeper soils.

 $\label{eq:constraint} \begin{array}{l} \textbf{Keywords} \ \ Land \ use \ change \cdot Soil \ C \ fraction \cdot Stability \cdot \\ Sequesteration \cdot Conifer \cdot Broadleaf \end{array}$ 

#### Introduction

Forests stored a substantial portion of the earth biosphere carbon (C) and were valued globally for the services they provided to the society. The current C stock in the world's forests is estimated as 861 Pg C, with 44 % in soils, and 13 % in dead woods and litters (Pan et al. 2011). Interest in the ability of forest soil to sequester and stabilize atmospheric  $CO_2$  has increased in recent decades. Because soil is the largest terrestrial C pool, more than atmospheric C and vegetation biomass C combined (Jobbágy and Jackson 2000). even small changes in site conditions such as climate or landuse change can contribute to significant C fluxes to the atmosphere.

Forest restoration has been proposed as an effective strategy for the sequestration of atmosphere  $CO_2$  (Deng et al. 2014). Following afforestation or reforestation, plant biomass C can increase rapidly owing to the establishment and growth of trees (Farley et al. 2004; Nave et al. 2013). However, the effects of forest restoration on soil C stocks and related mechanisms are not fully understood (Mobley et al. 2015). Previous studies has observed either increase (Deng et al. 2014; Lemma et al. 2006; Mao et al. 2010). decrease (Mao and Zeng 2010; Zhao et al. 2014) or negligible change (Sartori et al. 2007) of soil C stocks following restoration (Li et al. 2012). Nevertheless, some trends appear to emerge in the previous meta-synthesis studies: Firstly, prior land use has a great impact on soil C dynamics. Afforestation of previously cultivated agriculture soils generally increased soil C (Bashkin and Binkley 1998). For instance, Hernandez-Ramirez et al. (2011) reported that 35 yrs. afforestation of former cropland caused substantial (>57 %) increase in soil C, and most of the new C accrued was due to tree-derived inputs. However, plantations on grasslands or pastures do not necessarily lead to soil C accrual. Farley et al. (2004) found that afforestation of grasslands led to a decrease of soil C. Secondly, the changes in soil C during afforestation is typically a function of stand age. Generally, soil C declined relative to the initial soil C content during the first 5 years of afforestation, followed by a decrease in the rate of decline and eventually recovery to C contents found in agricultural soils after 30 years (Paul et al. 2002). However, most studies have only investigated the changes in the first 20 years of afforestation; data from older afforestation sites (> 30 yrs) are very limited (Martens et al. 2004).

Some meta-synthesis studies have suggested that broadleaf species performed better than coniferous species in soil C accrual (Laganiere et al. 2010; Li et al. 2012; Nave et al. 2013). However, this conclusion may be biased due to the lack of long term data. Coniferous species generally produce more recalcitrant plant litter and have slower decomposition rate than broadleaf species. Given enough time, soil C stock under coniferous species may be higher than or equal to those under broadleaf species (Vesterdal et al. 2008). The conflict between the above perspectives and meta-analysis observations reflected an urgent need to include more data from older (over 30 yrs) afforestation studies.

Furthermore, there are few studies investigated the mechanisms involved in the soil C dynamics during

afforestation, especially the bio-chemical-physical mechanisms of C stabilization (Schmidt et al. 2011). Soil contains a mixture of heterogeneous C pools. Detecting changes of soil C pools during afforestation is often difficult. Soil fractionation techniques offer a pathway to link soil C dynamics to the mechanisms of C sequestration and stabilization. Through physical and/or chemical methods, bulk soils can be separated into different factions that differ in chemical composition and/or location in the soil matrix. Turnover time of these fractions can range from days, decades, to centuries due to their specific chemical or physical stabilities. Soil fractionations based on particle size allow bulk soil to be fractionated into functional pools of particulate organic matters (POM) and silt and clay bound fractions and can help to identify the stability mechanisms involved (Mao and Zeng 2010; Mendham et al. 2004). Soil POM represents most of semi-decomposed plant residues at an early stage of decomposition, and thus characterizes a transitional stage in the humification process (Mao and Zeng 2010). Recent studies have shown the pivotal role of mineral-organic association (bound with silt and clay) on the long-term stability of soil organic matter (Chen et al. 2014; Del Galdo et al. 2003; Kögel-Knabner et al. 2008; Kramer et al. 2012); for example, the capacity of soil organic C accumulation during afforestation has been found positively correlated to soil clay content (Laganiere et al. 2010).

The objective of this study was to explore the changes in C stocks and various C fractions in forest floor (FF) and mineral soil layers in different conifer and broadleaf plantations that had been afforested from agriculture land for about 70 years. We hypothesized that (1) plantations, compared to pasture land, would have higher total soil C stock, POM-C, mineral bound SOC and aggregate stability, whereas soil bulk density would be lower; and (2) coniferous species would perform better than broadleaf in increasing soil C stocks after 70 yrs. afforestation as their litters are low-quality, and slow to decompose.

#### Methods

#### Site description

The study was conducted in monospecific tree plantations and a nearby pasture land at the Turkey Hill Plantations (THP) in the Tompkins County, New York (42°27'0" N, 76°25'0"W; elevation 430–460 m). All trees at the THP were planted in 0.4 ha stands between 1939 and 1941 in order to examine the long-term effects of tree species on soils. The soils at the THP are Typic Fragiudepts (Bath, Mardin Series) and Typic Dystrudepts (Lordstown Series), are coarse-loamy textured, moderately well-drained, and acidic (pH ranging from 3.8 to 5.9). The soils were under intensive cultivation for 100 years prior to the tree planting (Phillips and Fahey 2006).

## Soil sampling

In spring 2010, two replicated stands each of Norway spruce (Picea abies, NS), red pine (Pinus resinosa, RP), sugar maple (Acer saccharum, SM), black locust (Robinia pseudoacacia, BL), red oak (Quercus rubra, RO) and one nearby (< 1 km) pasture field (PA) were chosen for this study. In each forest stand and the pasture stand, a diagonal transect was laid and three sampling plots were randomly selected along the transect. At each plot, forest floor layer was carefully collected by cutting a 30  $\times$  30 cm square, and five 5 cm diameter  $\times$  15 cm depth soil cores were taken from each plot, using a corer attached to a slide hammer. The forest floor samples were dried to constant weight at 60 °C and weighted. In the laboratory, mineral soils were divided into two segments: 0-5 cm and 5-15 cm, and cores from the same plot were pooled by depth. A subsample of soil was dried at 60 °C to calculate soil water content. Soil bulk density (BD) was calculated by using the soil mass and volume. The remaining soils were sieved through an 8 mm sieve to remove stones and gravel. Fine and coarser roots were removed by hand.

# POM and mSOC

The method for separating POM and mSOC fractions was from Gregorich and Beare (2008). Briefly, 20 g of the 8-mm sieved soil was dried at 60 °C, and then dispersed for 18 h in 50 mL of 5 g/L sodium hexametaphosphate (NaPO<sub>3</sub>)<sub>6</sub> solution on a reciprocating shaker (180 rpm). The suspension was then passed a 250  $\mu$ m and a 53  $\mu$ m sieve, the remains on the 250  $\mu$ m sieve was defined as coarse POM (cPOM, >250  $\mu$ m) and on the 53 sieve as fine POM (fPOM, 53–250  $\mu$ m), the solutions passed the 53  $\mu$ m sieve was dried at 60 °C, the soils were collected as the mineral bound SOC (mSOC).

For forest floor C we measured soil organic matter (SOM) using the loss-on-ignition (2 h at 550 °C), and calculated the C concentration in forest floor with the equation: SOC = SOM/1.724 (Wang et al. 2010). Mineral soils and fractions were grounded using a mortar-pestle and analyzed for SOC content by dry combustion in a CHN analyzer (PerkinElmer, Waltham, MA, USA) at the University of Illinois Springfield lab. Total soil C stock was the sum of forest floor C and mineral soil C.

## Aggregate fractions

The aggregate separation was according to Six et al. (1998) by wet sieving soil through three sieves (2000, 250, and 53 µm) to obtain four aggregate-size classes: large macroaggregate (>2000 µm), macroaggregate (2000 µm - 250 µm), microaggregate (250 µm -53  $\mu$ m) and free mSOC (< 53  $\mu$ m). Prior to wet sieving, all field moist soils were passed through an 8 mm sieve and air-dried. A subsample of 100 g of 60 °C oven-dried soil was submersed in deionized water on top of a 2000 µm sieve for 5 min prior to wet sieving. This process leads to slaking, which is the breaking of unstable aggregates because of air pressure buildup inside the aggregates upon submersion in water. The sieving was done manually by moving the sieve up and down 3 cm, 50 times in 2 min. The fraction that remained on the 2000 µm sieve was collected in an aluminum pan and oven dried. Water plus soil that went through the 2000 µm sieve was poured onto the next sieve and sieving was repeated. All fractions were gently backwashed into aluminum pans, dried at 60 °C, and weighed. During sieving, both aggregates and sand particles of the same size class were retained on the sieves. Sand content (>53  $\mu$ m) of the aggregates was determined on a subsample of aggregates that were muffle-furnaced at 550 °C and then dispersed with  $(NaPO_3)_6$  solution (5 g/L).

## Data analysis

In this study, five species (two conifer species and three broadleaf species) each with 2 replicated stands and a pasture control were selected. Each replicated stand and pasture have 3 replicated plots. The 70-year old monotypic stands and the nearby pasture land were used to detect tree species effects (plantation vs. pasture and conifer vs. broadleaf) on soil C stock in forest restoration. In the statistical analysis, NS and RP stands were classified as conifer species (four stands each has three plots), and BL, RO and SM were broadleaf species (6 stands each has three plots). One way analysis of variance (ANOVA) was used to detect the difference of soil C stock among different species. Contrast comparison was conducted following ANOVA to detect the difference between broadleaf and conifer species, and between plantations and the pasture. Pearson correlation analysis was used to test the relationship among soil parameters. All statistical analyses were conducted on IBM SPSS Statistics V22.0 (IBM Crop. Armonk, NY, USA).

# Results

Soil bulk density and total soil C stock

Soil bulk density was the lowest in the PA at the 0-5 cm soils (0.47 g/cm<sup>3</sup>, Table 1), and there was significant differences between forested stands and PA (P = 0.007, Table 2). Soil C stocks varied greatly among stands with different species, and between the replicated stands with the same planted species, in either forest floor (FF) layer or mineral soil layers. From the FF layer to 15 cm mineral soil, the total soil C stocks ranged

from 2772 to 5275  $g/m^2$ , with the highest in the NS1 stand and the lowest in the BL2 stand (Table 1). There was no significant difference between PA and overall forested stands in terms of total soil C (P = 0.07). Conifer species on average  $(4547 \pm 183 \text{ g/m}^2)$  had significantly higher total soil C than broadleaf species  $(3472 \pm 110 \text{ g/m}^2)$ , P = 0.001). Such difference was mainly due to the higher FF layer C accumulation in the coniferous stands (Table 1), and there was no significant difference in the mineral soil C stocks (0-15 cm) (P = 0.50). In the pasture stand, the total soil C was  $3235 \pm 143$  g/m<sup>2</sup>, which was much lower than the two NS stands (Table 1). The higher C stock in the NS stands was largely due to the FF accumulation, which was absent in the BL1, BL2, SM2 and PA. In the 0-5 cm mineral soil, the highest C pool was found in the BL1 stand (1867  $\pm$  373 g/ m<sup>2</sup>). In the 5–15 cm layer, soil C stocks ranged from 1465 to 2188 g/m<sup>2</sup> with no significant difference among stands (Table 1).

#### POC and mSOC in mineral soils

Comparing to the pastureland, 70-year old plantations did not increase cPOC, fPOC, and mSOC (Table 2); in the 0-5 cm soil, PA actually had higher cPOC concentration than the planted stands

Table 1 Soil bulk density and C stock (mean  $\pm$  SE) in different stands and pastures after 70 years afforestation

Types	Stand	Bulk Density (g/cm <sup>3</sup> )		C Stock (g/m <sup>2</sup> )			
	Species	0–5 cm	5–15 cm	FF	0–5 cm	5–15 cm	Total
Conifer	NS1	$0.54\pm0.02$	$0.97\pm0.03$	$1550\pm203$	$1537\pm80$	$2188\pm92$	$5275\pm218$
	NS2	$0.62\pm0.01$	$0.90\pm0.03$	$2169 \pm 147$	$1101\pm39$	$1522 \pm 171$	$4793\pm328$
	RP1	$0.63\pm0.01$	$0.87\pm0.03$	$1110\pm94$	$1125 \pm 140$	$2146\pm326$	$4381\pm509$
	RP2	$0.64\pm0.04$	$0.89\pm0.01$	$697 \pm 164$	$1151 \pm 138$	$1891\pm228$	$3740\pm250$
	Average	$0.61 \pm 0.01$	$0.91\pm0.02$	-	$1228\pm72$	$1937 \pm 126$	$4547 \pm 183$
Broadleaf	BL1	$0.55\pm0.01$	$0.95\pm0.05$	0	$1867\pm373$	$1905\pm102$	$3771\pm337$
	BL2	$0.71\pm0.05$	$1.02\pm0.04$	0	$1179 \pm 100$	$1594 \pm 106$	$2772\pm26$
	RO1	$0.70\pm0.05$	$0.93\pm0.03$	$1159 \pm 183$	$787 \pm 102$	$1465\pm53$	$3410\pm175$
	RO2	$0.61\pm0.09$	$0.94\pm0.03$	$624\pm71$	$1137 \pm 186$	$1881 \pm 103$	$3643\pm293$
	SM1	$0.57\pm0.03$	$1.00\pm0.03$	$970\pm65$	$1028\pm36$	$1889\pm98$	$3887 \pm 182$
	SM2	$0.56\pm0.00$	$0.89\pm0.02$	0	$1364 \pm 51$	$1990\pm288$	$3354\pm246$
	Average	$0.62\pm0.02$	$0.96\pm0.02$	-	$1226 \pm 128$	$1787 \pm 67$	$3472 \pm 110$
Pasture	-	$0.47\pm0.02$	$0.92\pm0.07$	0	$1315\pm98$	$1920\pm240$	$3235\pm143$

FF Forest floor, NS Norway spruce, RP, Red Pine; SM Sugar maple, BL Black locust, RO Red oak

Table 2The statistical analysisof soil variables in different for-ested stands and the pasture after70 years afforestation

Source	Stands		Plantations vs Pasture	Conifer vs Broadleaf	
	F	Р	Р	Р	
0–5 cm					
BD	3.46	0.007	0.006	0.774	
cPOC	4.59	0.001	0.001	0.060	
fPOC	3.64	0.006	0.079	0.034	
mSOC	5.34	0.001	0.448	0.303	
> 2000 µm	1.26	0.310	N/A	N/A	
2000-250 µm	4.83	0.001	0.271	0.032	
250-53 μm	1.92	0.097	N/A	N/A	
< 53 µm	4.18	0.002	0.061	0.992	
Macro-aggregate C	14.28	< 0.001	< 0.001	< 0.001	
Micro-aggregate C	4.43	0.002	0.094	0.561	
5–15 cm					
BD	1.85	0.110	N/A	N/A	
cPOC	0.81	0.625	N/A	N/A	
fPOC	2.85	0.019	0.316	0.440	
mSOC	2.05	0.078	0.324	0.976	
> 2000 µm	1.77	0.126	N/A	N/A	
2000-250 µm	3.22	0.011	0.056	0.432	
250-53 μm	2.77	0.022	0.129	0.025	
< 53 µm	4.44	0.002	0.015	0.673	
Macro-aggregate C	1.80	0.120	N/A	N/A	
Micro-aggregate C	2.62	0.029	0.024	0.395	

*BD* bulk density, *cPOC* coarse particle organic carbon, *fPOC* fine particle organic carbon, *mSOC* mineral associated soil organic C. N/A indicates that the contrast comparison was not run as there was no significant stands effects after ANOVA

(P = 0.004, Tables 2 and 3). There was also no significant difference between conifer species and broadleaf species for cPOC, fPOC and mSOC fractions (Table 2). In the 0-5 cm soils, cPOC, fPOC, and mSOC concentrations were higher than the corresponding variables in the 5-15 cm soils. The highest cPOC was found in PA  $(15.09 \pm 1.67 \text{ g/kg soil})$ , followed by BL1  $(12.80 \pm 1.64 \text{ g/kg soil})$ , both of which were 3 times higher than the lowest value in RO1  $(4.33 \pm 0.29 \text{ g/kg soil}, \text{ Table 3})$ . Patterns of fPOC among stands were similar to those of cPOC, with the highest value in BL1  $(7.18 \pm 2.33 \text{ g/kg soil})$ , followed by PA  $(6.81 \pm 1.19 \text{ g/kg soil})$ , and the lowest in RO1  $(1.60 \pm 0.19 \text{ g/kg soil})$ . Most SOC laid in mSOC, which accounted for two thirds of the total SOC. The highest mSOC concentration was found in BL1 (36.41  $\pm$  4.8 g/kg soil), and was significantly higher than those in other stands (P < 0.05) except NS1, SM2 and PA. In the 5-15 cm soils, cPOC, fPOC, and mSOC concentrations were lower than the 0–5 cm layer and less variable. The cPOC values in the 5–15 cm soils ranged from 2.79 to 4.01 g/kg soil, which were 1/5 to 2/3 of those in the corresponding 0–5 cm layer (Table 3). Concentrations of fPOC ranged from 0.74 to 1.65 g/kg soil, and were only 1/7 to 1/2 of those in the 0–5 cm soils. The mSOC values in the 5– 15 cm layer were about half of those in the upper layer (P < 0.001, Table 2), likely reflecting the "decreasing rate" of mSOC with increasing soil depth is much slower than cPOC and fPOC. Both cPOC and fPOC contents were positively correlated to the content of mSOC (r = 0.78, P < 0.001 and r = 0.81, P < 0.001, respectively).

The proportion of cPOC and fPOC in the 0–5 cm soils ranged from 18.09 % to 30.90 % and 6.56 % to 17.22 %, respectively, and were always higher than those in the corresponding 5–15 cm soils. The mSOC proportion, in contrast, was markedly higher in the 5–15 cm soils than the 0–5 cm soils (P < 0.001, Fig. 1).

Table 3 Soil cPOC, fPOC and		G. 1			
mSOC concentrations (mean $\pm$ SE) in different forested	Vegetation Types	Stands	cPOC	fPOC	mSOC
$(\text{fiteal} \pm \text{SE})$ in different forested stands and the pasture			(g/kg soil)	(g/kg soil)	(g/kg soil)
1	0-5 cm soils				
	Conifer	NS1	$7.97\pm0.08$	$5.24\pm0.33$	$23.84\pm2.13$
		NS2	$5.35 \pm 1.45$	$2.02\pm0.50$	$15.80\pm1.05$
		RP1	$6.42 \pm 1.19$	$2.05\pm0.44$	$16.48\pm2.49$
		RP2	$4.37\pm0.41$	$1.91\pm0.71$	$18.54\pm3.34$
		Average	$6.03 \pm 0.47$	$\textbf{2.81} \pm \textbf{0.39}$	$18.67 \pm 1.14$
	Broadleaf	BL1	$12.8\pm1.64$	$7.18\pm2.33$	$36.41\pm4.8$
		BL2	$6.35\pm1.46$	$1.62\pm0.23$	$16.42\pm1.37$
		RO1	$4.33\pm0.29$	$1.60\pm0.19$	$10.68\pm1.31$
		RO2	$8.08\pm2.4$	$5.93 \pm 1.82$	$19.52\pm3.39$
		SM1	$5.49\pm0.53$	$3.71\pm0.71$	$16.27\pm1.62$
		`SM2	$8.70\pm0.67$	$5.46\pm0.85$	$23.79 \pm 1.40$
		Average	$7.63 \pm 0.63$	$4.25\pm0.53$	$20.52 \pm 1.68$
	Pasture	-	$15.09\pm1.67$	$6.81 \pm 1.19$	$27.18\pm2.53$
	5-15 cm soils				
	Conifer	NS1	$3.13\pm0.05$	$1.65\pm0.24$	$14.28\pm0.70$
		NS2	$3.18\pm0.38$	$0.89\pm0.17$	$10.38\pm0.93$
		RP1	$4.01\pm0.43$	$1.14\pm0.19$	$15.81\pm2.20$
		RP2	$3.05\pm0.62$	$1.11\pm0.23$	$12.65\pm2.31$
		Average	$\textbf{3.34} \pm \textbf{0.18}$	$1.20\pm0.10$	$13.28\pm0.77$
	Broadleaf	BL1	$3.30\pm0.40$	$1.10\pm0.14$	$14.55\pm0.89$
		BL2	$3.02\pm0.23$	$0.74\pm0.09$	$11.83\pm0.48$
		RO1	$3.49\pm0.22$	$0.85\pm0.09$	$8.48 \pm 1.03$
		RO2	$3.49\pm0.14$	$1.53\pm0.25$	$12.37\pm1.02$
		SM1	$2.79\pm0.11$	$1.02\pm0.11$	$13.23\pm2.12$
		SM2	$3.58\pm0.12$	$1.29\pm0.11$	$15.41\pm0.92$
NS: Norway spruce, RP Red Pine,		Average	$\textbf{3.28} \pm \textbf{0.08}$	$1.09\pm0.06$	$12.65\pm0.52$
<i>SM</i> Sugar maple, <i>BL</i> Black locust, <i>RO</i> red oak	Pasture	-	$3.27\pm0.26$	$0.99\pm0.36$	$14.51 \pm 1.66$

The percentage of mSOC in the 0-5 cm soils were lowest in PA (55.46 %), which was significantly lower

# Soil particle size and aggregate C

The >2000  $\mu$ m and 2000–250  $\mu$ m size classes ranged from 29.7 to 39.3 % and 28.9 to 43.2 % in the 0-5 cm soils, respectively, and were significantly higher than those in the 5–15 cm soils (Fig.2). In the 250–53  $\mu$ m size class, SM1 had the highest proportion in both soil layers, and PA had nearly the lowest proportion. In the 5-15 cm soils, conifer species generally had lower proportion of 250-53 µm size classes than broadleaf

than the average value of the forested site (65.59 %,

*P* < 0.05).

species (P = 0.045, Table 2, Fig. 2b). In the <53  $\mu$ m fraction, PA had the lowest proportion among all stands (P = 0.067, Table 3, Fig. 2b), suggesting that the  $<53 \ \mu m$  size class would increase when land-use changed from pasture to plantations. In addition, the sand content ranged from 21 to 34 % in the 0-5 cm layer, and 27 to 31 % in the 5–15 cm layer.

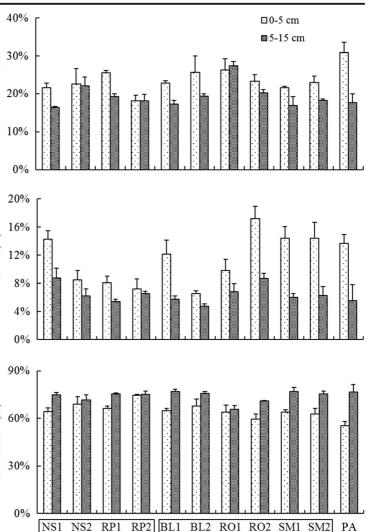
In the 0-5 cm layer, C concentration of macroaggregate (>250 µm) was lower in forested stands  $(23.84 \pm 1.45 \text{ g C/kg soil}, \text{ Table 4})$  in comparison with PA  $(33.63 \pm 1.46 \text{ g C/kg soil})$  (P = 0.044, Table 2). Moreover, the broadleaf stands ( $26.36 \pm 2.06$  g C/kg soil) had 30 % higher macro-aggregate C concentrations than the conifer stands (20.07  $\pm$  1.34 g C/kg soil, P = 0.03). Contrary to macro-aggregate, the microFig. 1 The proportions of cPOC, fPOC and mSOC in different stands and the pasture after 70 years afforestation (NS: Norway spruce; RP: Red Pine; SM: Sugar maple; BL: Black locust; RO: Red oak; PA: Pasture land; mean + 1 standard error)

cPOC/SOC (%)

fPOC/SOC (%)

mSOC/SOC (%)

Conifers



aggregate C concentration was higher in forested stands (8.20  $\pm$  0.34 g C/kg soil) than in PA (5.92  $\pm$  0.57 g C/kg soil) in the 5–15 cm layer (P = 0.046, Table 2), and there was no difference between conifer and broadleaf stands (P = 0.461, Table 2).

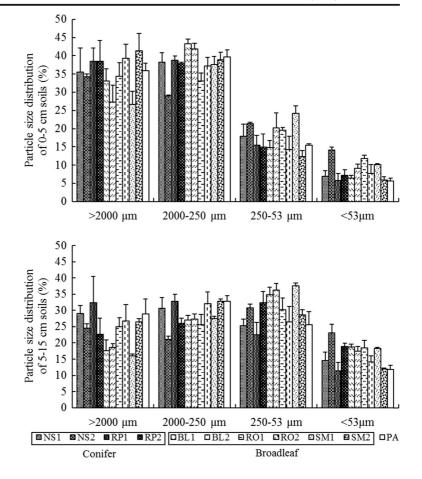
#### Discussion

Forest floor layer was observed in coniferous species stands (NS and RP) and some deciduous species stands (RO and SM), but not in the N-fixing BL stands, which had high litter quality and fast decomposition rate (De Marco et al. 2012). Generally, forests have higher soil surface C stock than pasture land due to the presence of

forest floor. However, in the mineral soil layers, there were no significant difference of soil C stock between PA and afforested stands (Table 1). This result was similar to the findings by Corre et al. (1999). who did not find difference of C contents in a comparison of mineral soils under grassland and forest in northeast American. Richter et al. (1999) found that C accumulation in forest biomass, forest floor, and the upper 7.5 cm of the mineral soil accounted for 80 %, 20 %, and <1 % of the carbon stock, respectively, in 30 yrs. old afforested loblolly pine stands. Despite higher aboveground biomass and C inputs in forested sites, C sequestration in mineral soils would be limited by slow vertical movement of organic matters between forest floor and mineral soils. Carbon stocks in forest floor (0 to 2169 g/

Broadleaf

Fig. 2 Soil particle size distribution of 0–5 cm and 5– 15 cm soils in different stands and the pasture after 70 years afforestation (NS: Norway spruce; RP: Red Pine; SM: Sugar maple; BL: Black locust; RO: Red oak; PA: Pasture land; mean + 1 standard error)



 $m^2$ ) and mineral soils (2242 to 3771 g/m<sup>2</sup> in 0–15 cm soils) measured in our site, however, were within the range reported in other 80–100 yrs. old North America sites (Garten 2011).

There was only marginally significant increase of total C stocks in forest floor and mineral soils after 70 years of afforestation (P = 0.07, Table 1). This result was similar to several previous studies which suggested that afforestation could result in increasing sequestration of C in forest floor and upper mineral soil layer, but decreasing or unchanged C contents in lower soil layers (Richter et al. 1999; Richter et al. 1994; Vesterdal et al. 2002). In a chronosequence study of red oak and Norway spruce stands planted on former arable land, Vesterdal et al. (2002) found C stock increased in forest floor and the upper 5 cm of the mineral soil could be offset by decreased C in deeper soil layers with increasing stand age. In a Germany study of poplar, aspen and willow forests that reestablished from agriculture land, Jug et al. (1999) observed a pattern of increasing C

contents in the upper 5 cm and decreasing C contents below 20 cm over only 9 years.

Previous meta-analysis studies have suggested that soil C stock tended to decrease following afforestation with coniferous species but increase following afforestation with broadleaf trees (Nave et al. 2013). However, soil C from coniferous species, generally having more recalcitrant litter derived C, have slower decomposition rate than that from broadleaf species. We thus expected that soil C stock under coniferous species should be higher than or equal to those under broadleaf species after long term restoration. After 70 years afforestation, our study indicated that total C stock from forest floor up to 15 cm deep soil was higher in coniferous stands than that in broadleaf stands (P = 0.001, Table 1). This was mainly due to the higher C sequestration in forest floor under conifer stands. If we only calculate the mineral soil, there would be no difference between conifer and broadleaf stands (Table 1). Contrary to many short term (<30 yrs) studies (Laganiere et al. 2010; Li et

Table 4 The values (mean  $\pm$  SE) of soil macro-aggregate and micro-aggregate C in different forested stands and the pasture

Types	Stands	Macro-aggregate C (g C/kg soil)	Micro-aggregate C (g C/kg soil)
0–5 cm			
Conifer	NS1	$25.63 \pm 0.67$	$10.41 \pm 1.51$
	NS2	$17.26 \pm 1.17$	$6.77\pm0.35$
	RP1	$18.37 \pm 1.31$	$5.64\pm0.41$
	RP2	$19.03 \pm 1.77$	$5.58\pm0.92$
	Average	$\textbf{20.07} \pm \textbf{1.34}$	$7.10\pm0.71$
Broadleaf	BL1	$41.00\pm2.20$	$8.22\pm0.71$
	BL2	$25.46 \pm 1.32$	$6.23 \pm 0.90$
	RO1	$14.25\pm0.55$	$5.37\pm0.53$
	RO2	$24.60 \pm 3.71$	$5.52\pm1.68$
	SM1	$23.74\pm0.67$	$10.03\pm0.87$
	SM2	$29.09\pm2.30$	$5.20\pm0.79$
	Average	$26.36 \pm 2.06$	$6.76 \pm 0.55$
Pasture	-	$33.63 \pm 1.46$	$8.59\pm0.26$
5-15 cm			
Conifer	NS1	$15.72\pm0.76$	$7.58\pm0.59$
	NS2	$12.07\pm0.69$	$7.52\pm0.39$
	RP1	$15.16 \pm 1.28$	$6.27 \pm 1.23$
	RP2	$14.50 \pm 2.71$	$10.21\pm1.57$
	Average	$14.36 \pm 0.79$	$7.89\pm0.62$
Broadleaf	BL1	$15.79\pm1.92$	$9.69\pm0.85$
	BL2	$12.97\pm0.96$	$9.14\pm0.54$
	RO1	$11.03\pm0.21$	$7.37\pm0.92$
	RO2	$15.44 \pm 1.24$	$6.94 \pm 1.01$
	SM1	$14.52\pm0.50$	$9.63\pm0.85$
	SM2	$16.26 \pm 1.14$	$7.72\pm0.64$
	Average	$14.33 \pm 0.59$	$8.41 \pm 0.39$
Pasture	-	$16.42 \pm 1.24$	$5.92\pm0.57$

NS Norway spruce, RP Red Pine, SM Sugar maple, BL Black locust, RO Red oak

al. 2012). this result indicated that long term afforestation (>70 yrs. in this study) using conifer species would have higher whole soil-profile carbon stocks compared with broadleaf species when the surface organic layer is included.

Land use changes often affect SOC quality, which can be assessed by the proportion of total C present in different soil C fractions (Eclesia et al. 2012; Martens et al. 2004). In this study, although the total C contents in mineral soils had no difference between forest stands and pasture, there were significant difference of cPOC and mSOC fractions between them. In the 0-5 cm soils, PA had higher cPOC concentration and lower mSOC proportion than forested sites. Since cPOC was mainly comprised by plant residuals, the higher cPOC concentration in pasture soils may be due to their higher root biomass distributed in surface soils. Trumbore et al. (2006) have found greater amounts of roots in the top 10 cm of soil, and also higher fine root production, in pastures than in forests. Unlike cPOC, which was regarded as active C pool, mSOC represents the mineral associated recalcitrant C pool (Garten 2011). Of all fractions, mSOC fraction contained the most C in either surface (0-5 cm) or deeper mineral soils (5-15 cm), with about 65 % of soil C in plantations was mSOC, while this proportion was 55 % in pasture land. We also found mSOC proportion increased with soil depth. Although mineral soil C stock did not increase by afforestation, this result suggested that afforestation could improve the soil C stability by increasing mineral associated soil C. Similar results has been reported by Del Galdo et al. (2003) that afforestation resulted in significant sequestration of new C and stabilization of old C in physically protected mSOC fraction (<53 um). The increased C input and less soil disturbance in the forest soils and possibly the switch in quality of the woodland litter, compared to the pasture residue, may have promoted C flows into mSOC fractions (Del Galdo et al. 2003; Six et al. 2002).

Aggregate is another important physical mechanism of soil to stabilize C. In this study, pasture land had higher macro-aggregate C than forest sites in mineral soils (Table 4), especially in the surface soils (P = 0.044). This pattern coincides with the distribution of cPOC, which was mainly derived from plant residuals (Verchot et al. 2011). Hence, our result suggested that pasture had higher cPOC and macro-aggregate C contents, both of which is more active C fractions, than forested stands. The macro-aggregate C data also showed that coniferous trees had significantly lower macro-aggregate C than broadleaf trees in the surface soils. This result may be due to the contrasting litter quality effect on decomposition: conifer species have lower litter quality and thus lower decomposition rate than broadleaf species. Although conifer had higher forest floor C stock, the vertical movement of these plant C into mineral soils was highly restricted by their lower decomposition rate and soil organism activities.

Unlike macro-aggregate, soil micro-aggregate C was higher in forested site than pasture in the 5–15 cm soils. Previous studies have demonstrated that microaggregates performed better than macro-aggregates in stabilizing and sequestering C in the long term (Six et al. 2002; Six et al. 1998; Verchot et al. 2011). Microbial processes have an important role in the formation of stable micro-aggregates (Verchot et al. 2011). The plant roots in forests distribute deeper than the roots in pasture. More C inputs from dead root in forests could improve microbial activities, and further contribute to the formation of micro-aggregates.

#### Conclusion

Understanding the mechanisms of SOC dynamics in long term afforestation is important to identifying and enhancing C sequestration to mitigate the climate change. Following 70 years afforestation of former agricultural soils with conifer and broadleaf trees, the C stored in forest floor was greatly increased, especially in conifer stands. Although mineral soil C stock did not differ among treatment stands, the soil C fractions have been altered. Comparing to pasture land, afforestation decreased active cPOC fractions, while increased the more recalcitrant mineral associated mSOC fractions. Moreover, afforestation decreased the macroaggregate C fraction in surface mineral soils, but increased the micro-aggregate C fraction in deeper mineral soils, which would contribute to more stable C sequestration. This study implies that: 1) over longer time period, afforestation by conifer species could have similar level of mineral soil C stock, and higher whole soil-profile C stocks compared to broadleaf species when the surface organic layer is included; 2) In mineral soils, afforestation could improve soil C stabilization through enhancing the formation of mineral associated organic C fraction and more micro-aggregate C fraction in deeper soils.

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