



Accumulation of soil organic C and N in planted forests fostered by tree species mixture

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Abstract: With the increasing trend of converting monocultures into mixed forests, more and more studies were carried out to investigate the admixing effects on the tree growth and aboveground carbon storage. However, few studies have considered the impact of mixed forests on the belowground carbon sequestration, and changes of soil carbon and nitrogen stock as forest grows in particular. In this study, paired pure *Pinus massoniana* plantations, *Cinnamomum camphora* plantations and mixed *Pinus massoniana* -*Cinnamomum camphora* plantations at ages of 10, 24 and 45 years were selected to test whether the mixed plantations sequester more organic carbon (OC) and nitrogen (N) in soils and whether this admixing effect becomes more pronounced with stand ages. The results showed that tree species identity and composition as well as stand age significantly affect soil OC and N stock. The soil OC and N stocks were the highest in mixed *Pinus-Cinnamomum* stands compared to those in counterpart monocultures within the same age in the whole soil profiles or specific soil depth layers (0-10 cm, 10-20 cm and 20-30 cm) for most cases, followed by *Cinnamomum* stands, and the *Pinus* stands the lowest. And these positive admixing effects were mostly non-additive. Along chronosequence, the soil OC stock capacity peaked at 24-yr-old stand and maintained relatively stable thereafter. The admixing effects were also the highest at this stage. However, at topsoil layer, the admixing effects increased with stand ages in terms of soil OC stocks. When comparing mixed *Pinus-Cinnamomum* plantations with corresponding monocultures within the same age, the soil N stock in mixed stands was 8.30%, 11.17% and 31.45% higher than the predicted mean value estimated from counterpart pure species plantations in 10-yr-old, 24-yr-old and 45-yr-old stands, respectively, suggesting these admixing effects were more pronounced along chronosequence.



1 Introduction

Soil carbon is more stable than that stored in plants, which makes soil carbon more resistive to disturbance (Cunningham et al., 2015). Organic carbon from forest soils accounts for 70%-73% global soil organic carbon (Six et al., 2002), and there is an adorable part of the global forests being plantations. Thereby, plantations play an important role on soil carbon sequestration and mitigating global carbon budget (Cunningham et al., 2015). However, considering the problems caused by pure plantations (Peng et al., 2008), nowadays planting mixed forest with different species in one stand is becoming more and more popular in worldwide plantation management (Felton et al., 2016; Oxbrough et al., 2012), for the potential positive effects (Knoke et al., 2007). Since mixed forests, compared with monocultures, were generally characterized by the sustainability to resist disturbance, potential of higher yield and better ecological services (Grime, 1998; Knoke et al., 2007). It is generally accepted that mixed forests exert favorable effects over monocultures by two main mechanisms-complementary effect and selection effect (Isbell et al., 2009). The former is explained by inducing facilitation or ease interspecific competition and enhancing resource use efficiency by niche partitioning, and the latter increased the possibilities of including highly productive species to increase the yield. Though a lot of work has been done to study positive effects of biodiversity to ecological function (Balvanera et al., 2006; Marquard et al., 2009), while the attention is rarely being attached to soil organic carbon (Vesterdal et al., 2013) and nitrogen stocks, which are closely related to global climate change. The limited researches showed that the soil OC and N stocks in mixed stands do not necessarily exceed that of corresponding pure stands (Berger et al., 2002; Forrester et al., 2012; Wiesmeier et al., 2013; Wang et al., 2013; Cremer et al., 2016), while most of them did not consider the relative portion of the component species (Berger et al., 2002; Wang et al., 2013; Cremer et al., 2016), which may result in underestimation of admixing effects. For example, admixing effect could also exist, because of the small portion of the higher-production species which leads to low expected production, even if the production of two-species mixed stand is between that of two corresponding pure stands. The studies of monocultures suggested that soil organic carbon stock of the upper soil layer fluctuated in the early stage of afforestation until it reached a new equilibrium which depends on the rates of litter input and decomposition (Paul et al., 2002; Tremblay et al., 2006; Sartori et al., 2007). And for soil nitrogen stock, it increases with increasing stand age in the upper soil layer because of the accumulative biological fixation, litterfall, cycling from deeper mineral soil via root mortality, and even atmospheric N deposition. (Hume et al., 2016). However, the changes of organic carbon and nitrogen stocks at different stand ages are poorly understood and quantified, and, to our knowledge, especially in the context of comparing pairwise mixed and pure forest stands along chronosequence. In this study we investigated the soil organic carbon and nitrogen stocks of *Pinus massoniana* and *Cinnamomum camphora* pure stands as well as their mixed stands at the 10, 24 and 45 years old. All the soil samples were taken from 0-10, 10-20 and 20-30 cm soil of above stands in Hunan province in China. We hypothesized that (1) the soil organic carbon and nitrogen stocks under mixed stands are higher than those under corresponding monocultures within the same age in whole soil profile;



(2) these positive admixing effects become more pronounced along chronosequence.

2 Materials and methods

2.1 Study site

5 Three parallel forest stands at different stand ages were selected, comprising mixed *P. massoniana* and *C. camphora* plantations, pure *P. massoniana* plantations and pure *C. camphora* at 10, 24 and 45 years old. In between some *Pinus. elliotii* were spotted and we treated them as *P. massoniana* because of the similarity of growth and biological characteristics of them. Three plots were established in 24 and 45 years old pure *Pinus*, pure *Cinnamomum* and their corresponding mixture stands in 2013 in the Botanical Garden in Hunan Province, China(28°06'N,113°02'E), and the 10 years old counterparts in Taolin forestry station (28°55'N,113°03'E) in Miluo city, Hunan Province, China, due to the lack of young stands in the Botanical Garden. Three 20 m×20 m plots were established in each of the Botanical Garden stands, and in Taolin forestry station the plots are 12m×12m constrained by the smaller patches there. These two sites are of 200km in distance and with similar climate and soil type. The regional climate is typical mid-range subtropical monsoonal with mean annual air temperature of 17.2°C, mean annual precipitation of 1422 mm in Botanical Garden and mean annual air temperature of 16.9°C, mean annual precipitation of 1353.6 mm in Taolin forestry station. The soil is well-drained clay-loam red soil developed from slate parent rock, classified as Alliti-Udic Ferrosols, according to Acrisol in the World Reference Base for Soil Resources (Institute of Soil Science, Chinese Academy of Science 2001). The depth of soil layer is deeper than 80 cm, but the content of soil humus was not rich. More detailed information about the experimental site and soil condition referred to Wen et al. (2014).

2.2 Soil sampling and laboratory analyses

20 Four samples were randomly collected in each plot using a metal corer(10 cm in diameter) from three depth: 0-10, 10-20, 20-30 cm and mixed as one sample then air-dried and sieved through 0.25mm sieve. Soil organic carbon was determined by the wet combustion method using oxidization of potassium bichromate (Walkley-Black method) and then titrated with 0.5 N ferrous ammonium sulfate solution by using diphenylamine indicator. Soil total nitrogen was measured using the Semimicro-Kjeldahl method digested with a mixture of H₂SO₄, K₂SO₄, CuSO₄ and Se (Institute of Soil Science, Chinese Academy of Science).

25 2.3 Data analysis

Soil OC stock (t ha⁻¹) and N stock (t ha⁻¹) at different soil depth in different stands were calculated with formula: stock =bulk density* depth *[OC or N concentration]. The effects of the experimental factors, species composition, stand age and soil depth, on soil OC, N concentrations and soil OC, N stocks were tested by means of three-way analysis of variance (ANOVA).



Differences in soil OC or N concentration and stock among mixed *P. massoniana* and *C. camphora* stands, pure *P. massoniana* stands and pure *Cinnamomum* stands within the same age stages and soil layers were analyzed by using one-way ANOVA, followed by Tukey's test. In order to detect whether the admixing effects were additive or non-additive, an alternative analytical method was used as suggested by Ball et al. (2008). Expected values of OC and N stock in mixed stands at different stand ages in different soil depths were calculated by adjusted values based on basal area in monocultures with formula:

$$\text{Expected value} = (\text{Ba}_{p,\text{mix}} / \text{Ba}_{p,\text{pure}}) * \text{Stock}_{p,\text{pure}} + (\text{Ba}_{c,\text{mix}} / \text{Ba}_{c,\text{pure}}) * \text{Stock}_{c,\text{pure}}$$

Where the $\text{Ba}_{p,\text{mix}}$ is the basal area of *Pinus* in the mixed stand, $\text{Ba}_{p,\text{pure}}$ is the basal area of *Pinus* in the pure *Pinus* stand, the $\text{Ba}_{c,\text{mix}}$ is the basal area of *C. camphora* in the mixed stand, $\text{Ba}_{c,\text{pure}}$ is the basal area of *Cinnamomum* in the *Cinnamomum* pure stand, $\text{Stock}_{p,\text{pure}}$ is the soil OC or N stock under pure *Pinus* stand and $\text{Stock}_{c,\text{pure}}$ is the soil OC or N stock under pure *Cinnamomum* stand. All the calculations above are conducted in the same given stand age (10-yr, 24-yr or 45-yr old). Then these expected values of OC or N stock were compared with observed values that were measured experimentally in mixed stands as: (observed-expected)/expected. For soil OC and N stock in different soil depth at different stand ages, 95% confidence intervals (CI) were also calculated. If the CIs for mixtures did not cross $y = 0$, the admixing effect was considered non-additive. Otherwise, we regard the admixing effect as additive. All the statistical analysis were conducted with statistical software R project (R 3.3.0) (R Development Core Team, 2010).

3 Results

3.1 Soil organic carbon and nitrogen stocks

The three-way ANOVA indicated the forest stand types, soil depth and their interactions exerted significant influence on the soil OC and N concentrations, while the age effect were not significant for soil OC concentration (table 2). When compared with parallel forest stand within the same ages, soil OC and N concentrations were highest in *Pinus-Cinnamomum* mixed stands for almost all the cases in the whole soil profiles or in different soil layers, but the significant differences were only detected in 24-yr old and 45-yr-old stands ($P < 0.05$) (Fig. 1).

Total soil OC stock was highest in *Pinus-Cinnamomum* mixed stands (44.86 Mg ha^{-1}) compared to corresponding monocultures at the same stand age in the whole soil profiles investigated, followed by evergreen broad-leaved *Cinnamomum* stand (36.37 Mg ha^{-1}), and conifer *Pinus* stand showed the lowest values (31.51 Mg ha^{-1}). The significant differences were detected in 24-yr old and 45-yr-old stands, but not in 10-yr old stands ($P < 0.05$). Along chronosequence, soil OC stocks increased with increasing stand age in these three forest types, with mean value of 30.50 Mg ha^{-1} , 41.96 Mg ha^{-1} and 43.85 Mg ha^{-1} in 10, 24 and 45 years old stands, respectively (Fig. 2). When we take a closer look at stratification distribution of the soil OC stocks in these three forest stands at different stand ages, the results showed the similar pattern as the total soil OC stocks as showed above, but the over-performance of OC stock in *Pinus-Cinnamomum* mixed stands



compared to the counterpart monoculture stands within the same age were mainly attributed to the top 10 cm soil depth (Fig. 3). In 0-10 cm soil layers, OC stocks under mixed stands had no significant differences compared with individual stands in the young stands, and then those under mixed stands significantly exceeded the *Pinus* individual stands in middle-age stands, and finally exceeded all two individual stands in the oldest stands; in 10-20cm soil layers, both youngest and oldest stands suggested no significant differences in soil OC stock among three stand types while in the middle-age stands mixture had remarkably higher soil OC stock than the individual ones; in the 20-30cm soil layers mixed stands showed similar soil OC stock compared with the individual stands and in the middle-age stands mixture had significant higher soil OC stock than two individuals, while this significant effect can only be detected between *Pinus* and mixed stands.

Soil depth and stand type, but not stand age exerted significant effects on soil N stock in mineral soil (Table 2). Only in 0-10cm and 10-20cm soil layers of 24-yr old stands and 20-30cm soil layer of 45-yr old did N stock significantly differ among stand types, and pure *Cinnamomum* and *Pinus-Cinnamomum* mixed stands always had similar N stocks, which were higher than *Pinus* stands. The N stock in topsoil of mixed stand increased in chronosequence, while in *Cinnamomum* stand, it increased from 10-yr old to 24-yr old stand. And in 10-20 cm soil layer of *Pinus* stand, N stock increased from 10-yr old to 24-yr old stand then stayed stable. While all the other soil layers didn't suggest significant difference along chronosequence within stand type (Fig.2 and 3).

3.2 Admixing effects on soil OC and N stocks

The relative soil OC and N stocks were calculated to determine the additive or non-additive effects in different soil layers in mixed forest stands at different ages. For mineral soil OC stock, mixing planting always exerted positive effects, as all the relative soil sequestered OC and N stock values were positive in all the soil profiles in mixed stands. But almost all the CIs did not cross $y=0$, suggesting these positive admixing effects were strongly non-additive, except the relative OC sequestration in 20-30 cm soil layers of 10-yr old stand. In the 10-yr old stand, the over-performance of soil OC sequestration was mainly attributed to the top and subsoil layers, and these positive effects were stable in the first two soil layers, then decreased in the deepest layer. In the 24-yr old stand, this value increased with soil depth. In the 45-yr old stand, the fractions were enhanced in the deepest soil layer, and the two upper layers were similar in the fraction. Along the chronosequence, the positive effects for N stock increased with stand ages. However, the admixing effects for soil OC were highest in the 24-yr old stands, and then 45-yr old stands and in the 10-yr old (Fig. 4).

4 Discussion

4.1 soil organic carbon and nitrogen stocks

The soil organic carbon stocks under three stand types always followed the order: mixed *Pinus- Cinamonum* stand > *Cinamonum* stand \geq *Pinus* stand, except for those in the 10-20 and 20-30cm soil layer of 10-yr old stand with slight



differences. Many researchers reported the species effects on carbon stock in mineral soil (Berger et al., 2002; Wang et al., 2013). In the previous study of this site, the annual litterfall was also the highest in the mixed *Pinus-Cinamonum* stand, followed the *Pinus*, and *Cinamomum* was the lowest (Xu et al., 2013). Therefore, the higher soil organic carbon under mixed stands may be attributed to the higher annual litterfall in mixed stands. While the higher soil OC in *Cinamonum* stand compared to *Pinus* is likely connected with the litter decomposition rate, as the needle leaves of *Pinus* accumulated on the forest floor with lower composition rate, and the higher litter input may not necessarily increase soil carbon content (Fontaine et al., 2004). With increasing stand age, the soil OC stock under *Cinamomum* exceeded *Pinus* stand became more pronounced in whole soil profile. Furthermore, when compared with broadleaf tree species, pine trees always tended to allocate much more total organic matter production to aboveground growth, which features them as fast growing tree species and caused lower direct carbon input to soil (Cuevas et al., 1991). In our results, the highest OC stock presented in 24-yr old stands, while under *Cinamomum* stands, though not significant, carbon stock still had an increasing trend from 24 to 45 years old stands. And in mixed stands, though there being relative smaller partition of *Cinamomum*, the trend of soil OC stock is similar with *Cinamomum* stands but not in *Pinus* stand. These confirmed that broadleaf tree species are prior to conifers in the long-term growth.

For soil total nitrogen stock, mixed stand exhibit priority for almost all the cases, showing higher soil N stocks in mixed *Pinus-Cinnamomum* stands compared to corresponding monocultures. And compared to the mean value of counterpart monoculture at the same ages, the soil N stock in mixed stands increased by 8.30%, 11.17% and 31.45%, respectively, suggesting these admixing effects were more pronounced with stand ages along chronosequence. In monocultures, *Pinus* stand showed priority in earlier stage while *Cinnamomum* in the later stage. All above presented the same trend as soil organic carbon do, but less pronounced. A large amount of soil nitrogen stored in soil organic matter, and with the decomposition of soil organic matter, nitrogen will be released and able to be uptaken by plants or leaching. So soil nitrogen concentration positively correlated to soil organic carbon concentration as reported in our previous study (Wen et al., 2014), thereby the soil nitrogen stocks, to some degree, shared the similar patterns of soil OC stocks under the same circumstances. And as Hume et al (2016) discussed, the accumulation of soil nitrogen may be slower than carbon because nitrogen is progressively locked-up in live biomass, which increases with stand age, the soil nitrogen stock changed less significantly among stand ages and stand types compared with soil OC stock.

In the uppermost soil layer, soil organic carbon stocks under three stand types are all significantly increased from 10-yr old stands to 24-yr old stands, then became stable thereafter, but that of *Pinus* stands slightly declined from 24-yr old to 45-yr old stands without significance. This trend are inconsistent with (Turner and Lambert, 2000), which reported a decline of soil organic carbon in the topsoil (0-10cm) in about 5 years following a stable stage within 25 years. But it's consistent with that detected in a Chinese fir plantation, which increased with increasing stand ages from about 10 to 20 years old, then, stabilized (Chen et al., 2013). Most of the similar researches that focus on the changes of organic carbon stock along chronosequence suggested that soil organic carbon stock in top soil would reach a stable level after approximately 20 years



(Turner and Lambert, 2000; Tremblay et al., 2006; Chen et al., 2013), that can be interpreted to the formation of the balance which depends on the carbon input and organic decomposition (Hume et al., 2016). While, in the initial stage of afforestation, the changes of organic carbon storage along increasing stand age differed. The plantations converted from a natural native forests will always present a decrease of soil organic carbon in topsoil within 10 years, and then, some will rebound and increase until reach the equilibrium level (Chen et al., 2013), the others will keep decreasing to that level (Turner and Lambert, 2000). In our results the significant differences of soil organic carbon stocks can only be detected in the topsoil, and the deepest soil under mixed stand which obeys the trend of the top (Fig.2a). Top soils under forests are always most susceptible to disturbance, and it can directly impacted by the carbon input by litters and fine roots which always decline along soil depth (Wang et al., 2016), and our data in the same sites(data not published) also suggested fine roots mostly(around 50%, except for *Pinus* whose shallow fine roots decreased along chronosequence) assembled in upper soil. Also, in our previous study (Wen et al., 2014), the ratio of soil microbial biomass/soil organic carbon was lowest in topsoil, which indicates lower mineralization rate, then make the organic matter harder to be decomposed compared with that the deeper layers. So the differences of OC stocks in topsoil along chronosequence are more pronounced than the deeper layer.

4.2 Admixing effect along chronosequence

In the topsoil, admixing effect on soil OC was more pronounced along chronosequence. This may be accounted for the increasingly intensive interactions between two species along increasing stand age. And in the pure stands, the intraspecific competitions also become more intensive along chronosequence, which will block carbon sequestration because of nutrient and water limits and favor in mixed forests where the interspecific competition is generally less intensive. The additive effect shown in 20-30cm soil layer under 10-yr old mixed stand, the observations were almost equal to the expected values, confirmed that the room occupied by below-and above-ground tree biomass was not big enough to exert significant interaction when the stands are relatively young. Our results also showed, to some degree, higher admixing effects in deeper soil layer that may attribute to the lower expected values in deeper soil layer, which makes the (observed-expected)/expected more sensitive to the increment of observed values.

The admixing effects of soil N stock in whole profile suggested a consistent increasing trend in whole soil profile. Fine roots of mixed stands compared with pure stands' are always supposed to exploit deeper soil (Cremer et al., 2016), so they may assemble more nitrogen from deeper soil (even deeper than our samples) to upper soil. Also, Lei et al. (2012) reported higher fine root turnover caused by higher fine root production of mixed stands which potentially increased nitrogen input in mixed stands. In addition, mixed stands are more resistant to disturbance like windthrow and insect calamities that may result in nitrate leaching. All those discussed above point to a higher nitrogen stock under mixed stands and these kinds of effects may increase along chronosequence.



5. Conclusions

The tree species and composition as well as stand age significantly affect soil organic carbon and nitrogen stock. Converting pure stands into mixed stands can significantly enhance soil OC and N stock. And this positive admixing effect becomes more pronounced along chronosequence for OC only in top soil while inconsistent trend presents in to deeper soil. However, for soil N stock, it becomes more pronounced along chronosequence in whole soil profile. Besides, the tree species identification also affects the soil OC sequestration and N stock. In topsoil *Cimamomum* stands always contain higher soil OC and N stock than *Pinus* stands due to the different strategies of carbon and nutrient allocation and different rate of organic decomposition, but these are not pronounced in deeper soil.

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Table 1 Stand characteristics in pure species *Pinus* stands, pure *Cinnamomum* stands and mixed *Pinus-Cinnamomum* stands at age of 10, 24 and 45 years.

Stand	Age	Species	Density (n ha ⁻¹)	Diameter at breast height (cm)	Height (cm)	Basal area (m ² ha ⁻¹)
<i>Pinus</i> stands	10	<i>Pinus</i>	2592	9.38	5.28	20.06
	24	<i>Pinus</i>	2050	14.18	12.86	35.37
	45	<i>Pinus</i>	600	21.40	12.47	22.84
<i>Cinnamomum</i> stands	10	<i>Cinnamomum</i>	2708	7.77	5.99	14.26
	24	<i>Cinnamomum</i>	900	17.02	13.71	23.46
	45	<i>Cinnamomum</i>	800	21.06	13.24	30.63
Mixed <i>Pinus-Cinnamomum</i> stands	10	<i>Pinus</i>	902	7.64	4.73	4.37
		<i>Cinnamomum</i>	1689	8.14	7.2	9.83
	24	<i>Pinus</i>	267	18.88	12.35	7.80
		<i>Cinnamomum</i>	592	15.27	11.41	12.45
	45	<i>Pinus</i>	250	19.69	12.37	7.91
		<i>Cinnamomum</i>	325	21.94	13.75	12.91



Table 2 The effects of plantation stand, stand age and soil depth on soil OC concentration, N concentration, OC and N stock. Values shown are ANOVA F values and P values. Bold font indicates significant differences at $p < 0.05$.

Factor	OC concentration		N concentration		OC stock		N stock	
	F value	P	F value	P	F value	P	F value	P
stand	24.30	0.0000	11.89	0.0000	36.73	0.0000	15.17	0.0000
age	0.00	0.9942	60.53	0.0000	35.99	0.0000	0.54	0.5814
depth	699.57	0.0000	205.33	0.0000	489.87	0.0000	65.62	0.0000
stand × age	2.07	0.1283	10.50	0.0000	2.87	0.0236	3.92	0.0042
stand × depth	8.61	0.0002	2.39	0.0937	5.53	0.0003	1.15	0.3354
age × depth	2.00	0.1588	0.05	0.8163	16.79	0.0000	4.18	0.0027
stand × age × depth	0.15	0.8579	0.73	0.4834	1.03	0.4142	0.67	0.7217



Figure Captions

Fig. 1 Soil organic carbon (OC) concentration and nitrogen (N) concentration in pure *Pinus*, *Cinnamomum* and mixed *Pinus-Cinnamomum* stands in 0-10 cm, 10-20 cm and 20-30 cm soil depth at the age of 10, 24 and 45 years. Error bars indicate standard errors. Different letters indicate significant differences among different stands within the same soil profile and age stages ($p < 0.05$).

Fig. 2 Total soil organic carbon (OC) and nitrogen(N) stock in pure *Pinus*, *Cinnamomum* and mixed *Pinus-Cinnamomum* stands in 0-30 cm soil depth at the age of 10, 24 and 45 years. Error bars indicate standard errors. Different lower case letters indicate significant differences among different stands within the same ages ($p < 0.05$).

Fig. 3 Soil organic carbon (OC) and nitrogen(N) stock in pure *Pinus*, *Cinnamomum* and mixed *Pinus-Cinnamomum* stands in 0-10 cm, 10-20 cm and 20-30 cm soil depth at the age of 10, 24 and 45 years. Error bars indicate standard errors. Different letters indicate significant differences among different stands within the same soil profile and age stages ($p < 0.05$).

Fig. 4 Investigation of additive or non-additive interactions for soil OC stock (a) and N stock (b) in mixed *Pinus-Cinnamomum* stands in 0-10 cm, 10-20 cm and 20-30 cm soil depth at the age of 10, 24 and 45 years. Observed values were compared to expected values calculated as the average value in monocultures of *Pinus* and *Cinnamomum*. Error bars represent 95% CI, and mixtures for which the CIs do not cross $y = 0$ are considered to be significantly non-additive.



Fig. 1

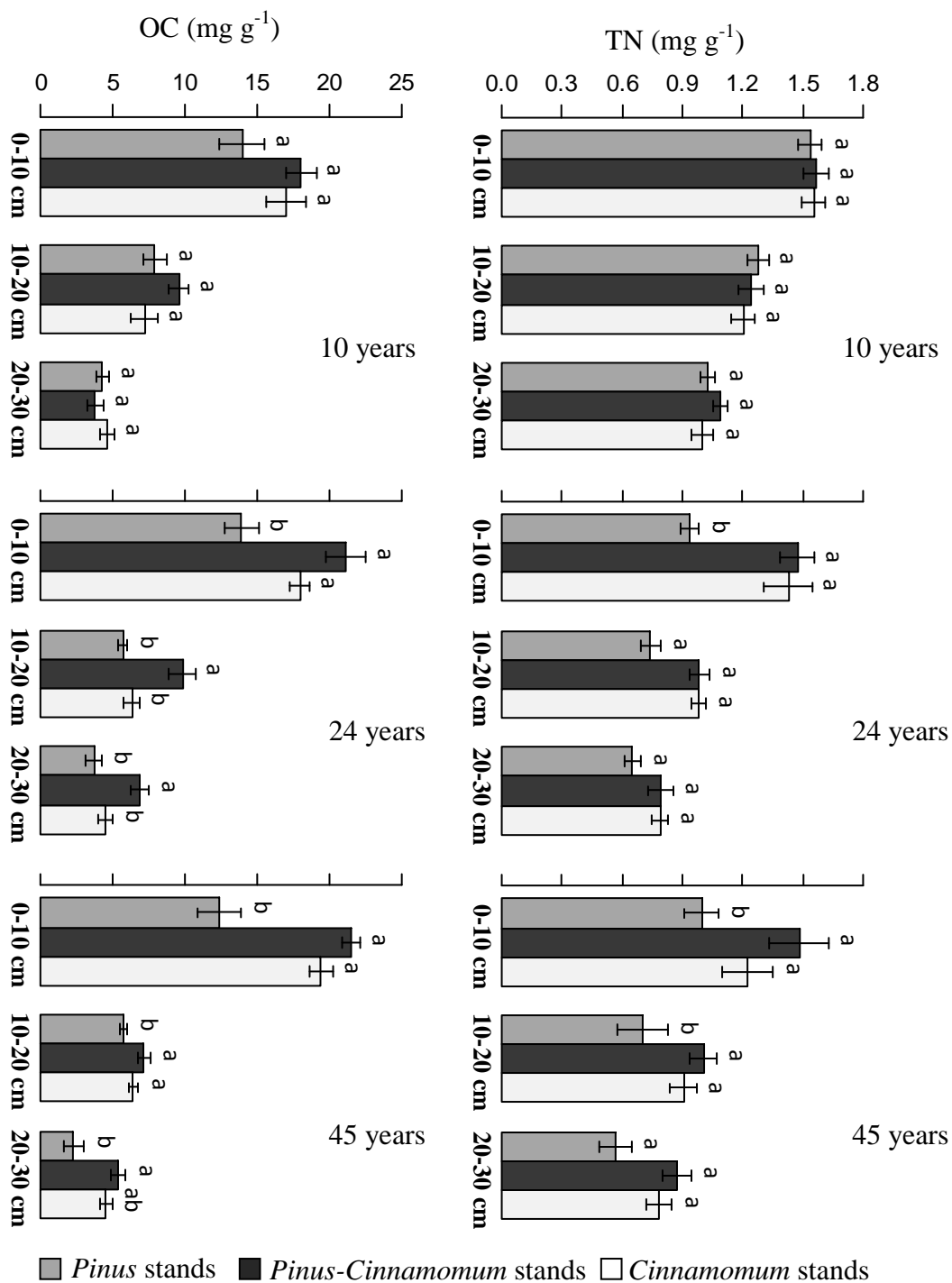




Fig. 2

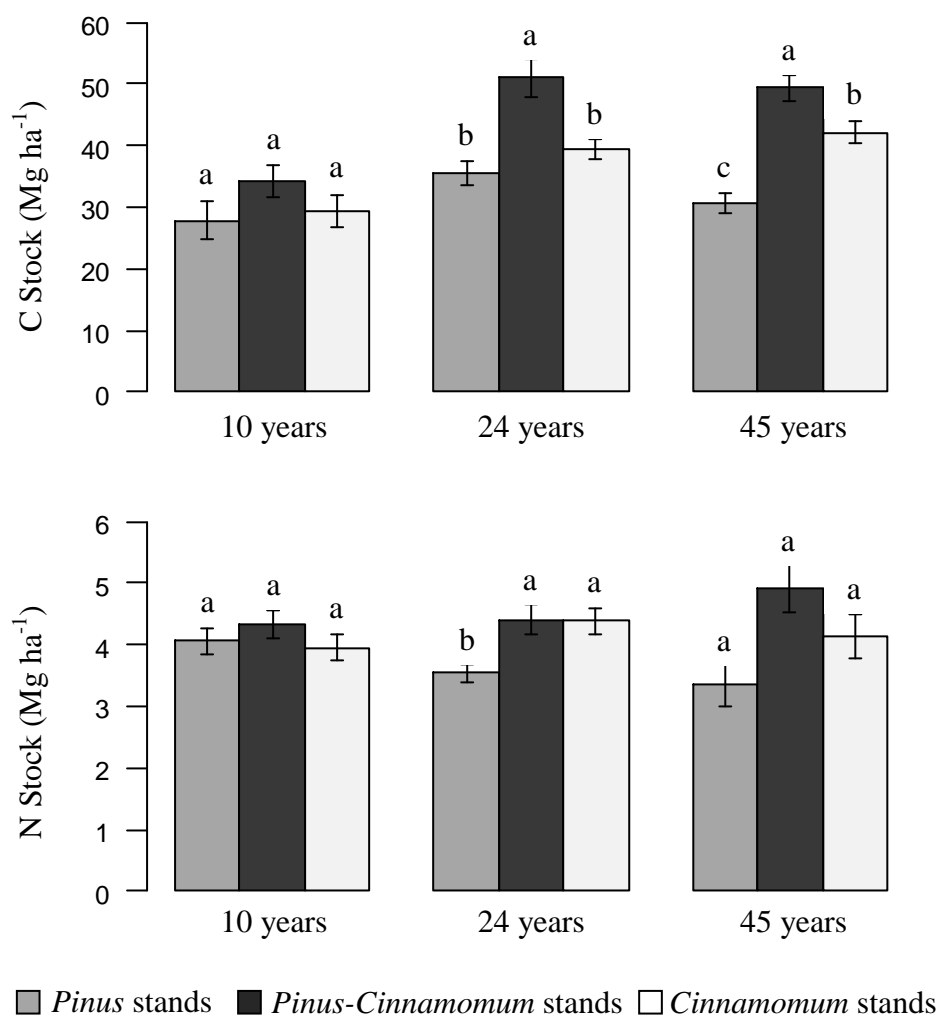




Fig. 3

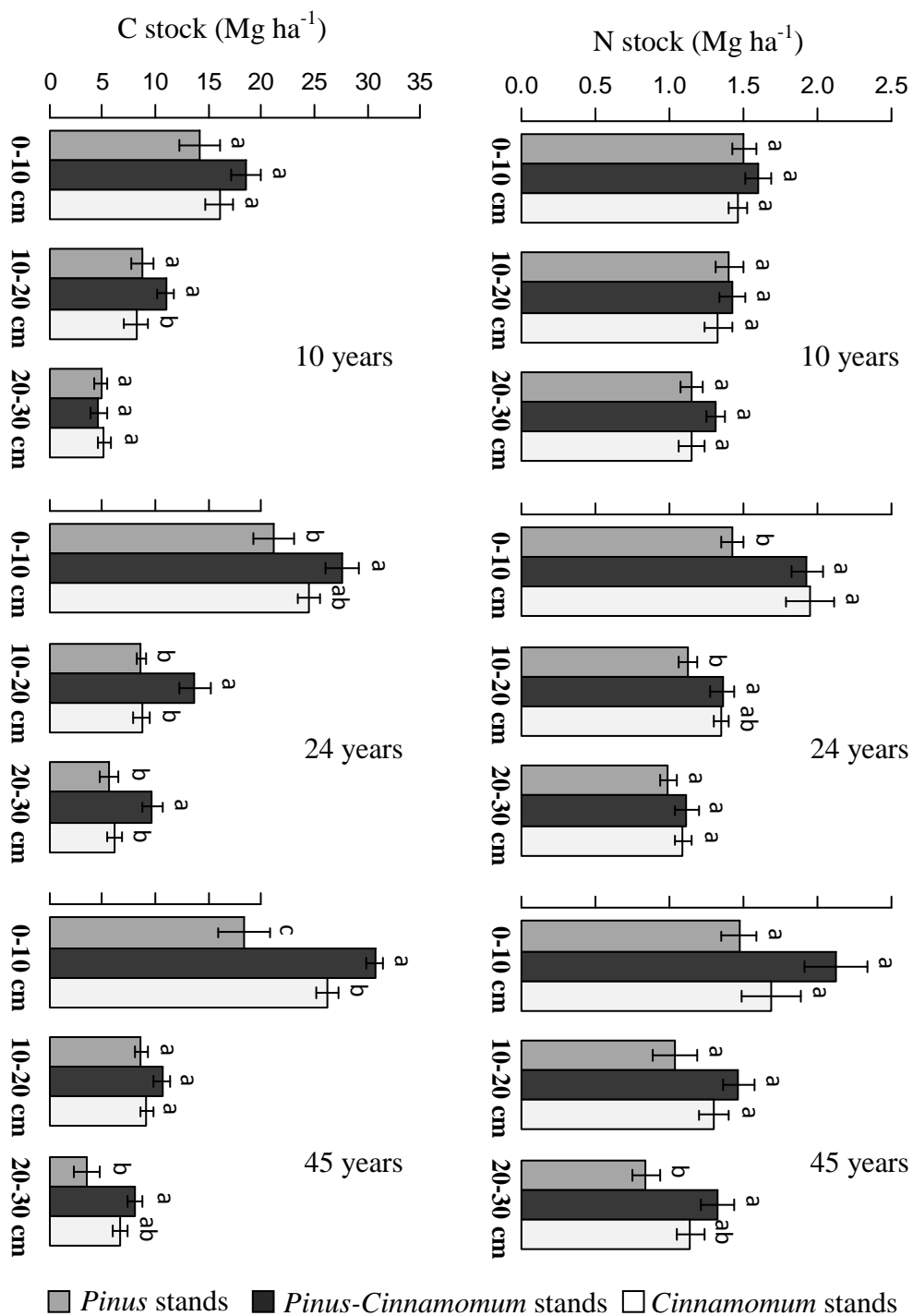




Fig. 4

