

Dear editor

Please find below our responses to the comments of the reviewers and explanations how we addressed the critical points. We are grateful for the very constructive suggestions, which certainly helped to improve our manuscript.

*Below, you will find our responses in **italics** to the respective comments.*

With best regards,

Pifeng Lei

On behalf co-authors

Responses to Reviewer # 1 ##

This manuscript shows the effects of mixed forests on the soil organic carbon and nitrogen stocks. This paper underline the benefits of mixed forests on the ecosystem functioning, and more precisely on a belowground compartment, which is largely underestimate. Currently, ecosystem services by soil, like carbon sequestration, are a hot topic and this paper bring some interesting results. The results are novel and very relevant for the future. I think the paper requires minor revision.

This study pointed the need to more information about the effect of mixed forest on soil C storage. The message is clear and concise and the manuscript is overall well written. The methods and statistical analyses seem appropriate. However, there are several critical issues should be further justified and/or addressed.

General comments: - About the chronosequence. In the materials and methods, your description is too short. We need more information about the soil type and soil management in different station. The conditions required for a site to be included in the chronosequence study were that, with the exception of time, all soil-forming factors had remained constant since forest establishment. Could you add a table with all these soil informations? - About deep soil. Could you justify the soil depth of your experimentation? Some recent papers underline that the carbon could be sequestered

in a deeper soil layer, more than 50 cm, and in a very stable form: humified. In page 2 line 22, you didn't show any results of studies with soil deeper than 20 cm. – About roots. Page 7 line 9, you wrote “..in the same site (data not published) also suggested fine roots.....”. I think it will be a clear advance of this study to publish your data of roots here. With these data, you could confirm your pattern and highlight the distribution of carbon in the vertical and temporal ways. Roots data is quite difficult to obtain and it's a very relevant information. - What about the effect of more species? Could you add perspectives about that? And also the time effect after 45-yr ?

RE: Thank very much for the comments and suggestions, which is quite helpful to improve our manuscript. The soil conditions remained constant without disturbance after planting owing to either the foundation of Botanic Garden or the young age of the forests. The soil types at two stations were similar, but there were differences between them before the forest establishment. As the field in Taolin forestry station was used for nursery and abandoned for three years before planting. We used this site just due to the scarcity of young plantations in Hunan Botanic Garden. More importantly, our purpose here is to evaluate the admixing effects on soil OC and N stocks by comparing mixed forests and corresponding monocultures over time, not to estimate the soil carbon and stock in mineral soil, which is also quite important of course. Therefore, what we considered was to make sure the three parallel forests at the same age are located in one site. See P3, L20-25.

Here we collected the soil samples down to 30 cm depth as the soils in this area were susceptible to the environmental variations and sensitive to the carbon input by litter and fine roots, which matches the purposes to assess the admixing effects on soil OC and N stock over time (Wang et al., 2013; Cremer et al., 2016). Of course, it is also quite interesting to know the C sequestration in the deeper soils. See P4, L1-3.

Regarding the fine root data, we have developed a manuscript for that and submitted to BMC Ecology recently. See P8, L1-3.

Here we only compared the two species mixtures with corresponding monocultures. It

is quite interesting to know the diversity effects on soil C and N stocks when containing more species. But to find this kind of the field sites for this purpose are really difficult, even more difficult to find when the time effects were also considered. It is quite good suggestion adding some sentences about the perspectives of diversity effects containing more species and time effects after 45-yr. See P8, L24-25.

Responses to the specific comments:

P2, L11. Yes, but could you add the recent paper of Grossiord et al 2014 “Tree diversity does not always improve resistance of forest ecosystems to drought” doi: 10.1073/pnas.1411970111

Re: *added as suggested. See P2, L11.*

P4, L13-15. It’s not clear

Re: *Here we rephrased as “Then these expected values of OC or N stock were compared with observed values for each individual sample that were measured experimentally in mixed stands with formula: (observed-expected)/expected. For soil OC and N stock in specific soil depth at given stand ages, 95% confidence intervals (CI) were calculated with above formula. If the CIs for mixtures did not cross $y = 0$, the admixing effect was considered non-additive (Ball et al., 2008)”. See P4, L22-26*

P7, L7. Which soil depth?

Re: *Here, we deleted that sentence and replace with “In our results the soil OC and N stocks decreased significantly with increasing soil depth. However, the magnitude of over-performance in the Pinus-Cinnamomum mixed stands over monocultures increased with soil depth (Fig 3 and Fig. 4)”, since the added analysis of significant differences in Fig.3 and Fig.4 as suggested makes the results clearer. See P7, L31-33.*

P7, L17. Need more information about “intraspecific competition”. Competition for light and/or water? Do you have some results about that? There are some harvesting in pure stands during the chronosequence in order to decrease this competition? Roots

data, could be nice also in this context.

Re: *We do not have data for that, so far. As there was no harvesting or thinning for all the plantations here, we stated that as general phenomenon as the partitioning use of light and soil resources by different species, which induce overyielding and higher input of litter and fine roots to soil. We also added one reference here. see P2, L13. Regarding the roots data, please see P8, L1-3.*

Table 1. Could you add +/- SE (standard errors) in each value?

Re: *Here we added \pm standard deviation.*

Table 2. P value = 0.0000, it's not realistic. I prefer P value < 0.0001 –

Re: *Corrected as suggested.*

Fig 3. Could you analyze also differences among soil layers within the same stand?

Re: *Analyzed and marked in the Figure as suggested.*

Fig 4. Could you add letters for significant differences?

Re: *Added as suggested. Here our purpose was to see the magnitude trend of admixing effect over time. So we added the significant differences among different ages within the same soil layers.*

Responses to Reviewer # 2 ##

(1) The experimental design compares stand composition effects with one site being 200 km away from the other two and provides not justification how we can assume that the main difference in SOC and n can be attributed to stand age and not the difference in site conditions. Mean climate conditions may be the same but soils are never the same as well as aspect, slope etc. Moreover I did not find any information about previous land use in these sites. The authors discuss that there are significant trends in SOC development with time depending on former land use, so this is very pertinent information to include and discuss. We would need much more detail about

all these site conditions to be convinced that the two sites are “similar”.

RE: *You are right about this experimental design. It would be nice to do this experiment in one site. Here we used two sites due to the scarcity of young plantations in Hunan Botanic Garden. Here we do need to justify the site selection in the method section to point out specifically that our purpose in this study is to evaluate the admixing effects on soil OC and N stocks over time by comparing mixed forests and corresponding monocultures at three forest development stages, not to compare the absolute values of SOC and N stock along forest development in each forest types. For this purpose, we strictly selected three paired plantations within the same age in one site, consisting of pure Pinus, Cinamonum plantation and Pinus-Cinamonum mixed plantation. The principle of selection field site we used was to make sure three plantation types at the same age in one site. We stick to this principle as well during our analysis. Accordingly, we compared the magnitude of the admixing effects on the SOC and N over by using **the relative values (e.g. percentages of over-performance in mixtures over monocultures, (observed-expected)/expected)** at different stand ages, instead to comparing the absolute SOC and N stocks in one specific forest type with stand development. This is how we did to avoid the assumption of “the main difference in SOC and n can be attributed to stand age and not the difference in site conditions”.*

(2) I have problems to understand the experimental design at each of the three sites, but it is relatively clear to me that there cannot be any proper replication of stand age as there is just one site per stand age. It is also unclear whether there were three subplots in each stand which were used as replicates in the statistical analysis – or if the individual cores were used as replicates in the analysis? In any event the statistical analyses must be flawed as we cannot separate the stand age effect from the site effect. Even within the site these subsamples within stands of a certain age are not real replicates for a statistical analysis.

RE: *In this part, we apologize for the confusion. As we have one previous publication (Wen et al. 2014) as reference, we did not make it clear in our previous MS. We*

selected three stands, including pure Pinus, Cinamonum and Pinus-Cinamonum mixed plantations in each development stage (10, 24 and 45 years old). And three plots were established in each forest stands at each development stage as three replicates. Thereby this experiment included 27 plots consisting of Pinus-Cinnamomum mixed plantations and corresponding monocultures at age of 10, 24 and 45 years old. In each plot, 4 soil cores were sampled, sliced to three layers and treated each of them as individual sample for SOC and N analysis. I hope this explanation is clearer and sound. See P3, L7-14 and P3, L29-30.

(3) Lastly the authors performed a three-way analysis of variance with all possible interactions. This indicates use of pseudoreplicates, but apart from this they also include “depth” as a factor. This is highly problematic as the three layers are not independent. For instance, if a 0-10 cm layer has a high C concentration then the 10-20 cm layer and 20-30 cm layers underneath are highly likely to also have higher C concentrations. If depth is included in the statistical model, the authors need to account for this correlative structure in their data. This is similar to accounting for e.g. repeated measures analysis when analyzing a time series of data.

RE: *Yes, Here we treated all the measurement as pseudoreplicates here simply to detect the effect of forest types, age and depth on SOC and N.*

(4) The most recent literature is not well referenced and addressed in the Introduction and the Discussion. For instance, Guckland et al. (2009) studied beech dilution gradients (J. Plant Nutr. Soil Sci. 172: 500-511) and more recently, Dawud et al. (2016, 2017) studied effects of tree species diversity gradients on soil C and N stocks in mature European stands (Dawud et al. 2016, Ecosystems 19: 645–660 ; Dawud et al. 2017, Funct. Ecol. 31: 1153-1162).

RE: *They are very good references, we cited and combined them in our revised version. See P6, L19-22.*

(5) The data basis is a bit thin (only C and N concentrations and stocks in mineral soil), and the authors could leave out C and N concentration from the main

manuscript with no major loss of information. Instead, the paper would have been much stronger with inclusion of forest floor C and N stocks. Recent literature has shown that there are also clear dynamics in forest floor C and N stocks as a result of tree species mixtures. In addition the authors also mention a previous study of litterfall in the same sites, and unpublished root biomass data. I strongly suggest these data be included and discussed for a more coherent and strong publication (if the manuscript can be reworked for publication based on revision of the above-mentioned flaws).

RE: It would be more informative to have more data, for example, the forest floor C and N stocks, although it is quite thin in these site that we used here. This is also typical in our area in subtropical area. Regarding the litterfall data, it was measured for other experiment and the data is not complete if we use it for our purpose as they only measured these three forests of 24 year old, instead of all the three age classes. For the fine root data, we recently developed one MS and submitted it to BMC Ecology recently.

(6) The language of the manuscript needs substantial linguistic checking by a native English speaker. The wording is not correct in many places and several sentences are hard to understand.

RE: We checked the linguistic errors and revised our manuscript intensively. I hope this version is clear and acceptable.

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 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.

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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 atmospheric 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; Grossiord et al., 2014). The former is explained by inducing facilitation or ease interspecific competition and enhancing resource use efficiency by niche partitioning, and the latter by increasing 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, recycling from deeper mineral soil via root mortality, and even atmospheric N deposition (Hume et al., 2016). However, the changes of soil organic carbon and nitrogen stocks at different stand ages are rarely quantified and poorly understood, and it is especially true, in the context of comparison of pairwise mixed forests with 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;

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

2 Materials and methods

2.1 Study site

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. Therein, some *Pinus elliotii* were spotted in between and we treated it as *P. massoniana* because of the similarity of growth and biological characteristics of them (we grouped them as "*Pinus*", thereafter). Three 20 m×20 m plots were established in each of the forest stand, including mixed *Pinus-Cinnamomum* stands and corresponding pure species stands (*Pinus* and *Cinnamomum*), at age of 24 and 45 years old in March 2013 in the Botanical Garden in Hunan Province, China (28°06'N, 113°02'E), amounting to 18 plots. Due to the lack of young stands in the Botanical Garden, we selected another site located in Taolin forestry station (28°55' N, 113°03' E), Hunan Province, China. This site was used as nursery and abandoned since 2000, and then planted in 2003. Three plots in each of the three plantations types at 10 years old were set up with same method mentioned above, but with size of 12m×12m constrained by the smaller patches there. In total, our study included 27 plots consisting of mixed *Pinus-Cinnamomum* plantations and corresponding monocultures at age of 10, 24 and 45 years old. These two sites are of 200 km 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 and pH value ranges from 4.12 to 4.86. The forests remained unmanaged and all soil-forming factors had remained constant since forest establishment owing to either the foundation of Botanic Garden or the young age of the forests. Here we used two sites for this experiment due to the difficulties of finding one field site with all these plantations with gradient of forest ages. The principle of field site selection here was to put three plantation types at the same age in one site. Therefore, we think these two sites are suitable for our experiment since our purpose here was to evaluate the admixing effects on soil C and N stocks by comparing mixed forests and corresponding monocultures at the same age rather than comparing soil C and N in forests at different ages. More detailed information about the experimental site and soil condition referred to Wen et al. (2014).

2.2 Soil sampling and laboratory analyses

Four soil samples were randomly collected in each plot using a metal corer (10 cm in diameter) from three depths: 0-10, 10-20, 20-30 cm and each of them were treated as one individual sample, then air-dried and sieved through 0.25mm sieve.

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Here we collected the soil samples down to 30 cm depth as the soils in this area were susceptible to the environmental variations and sensitive to the carbon input by litter and fine roots, which matches the purposes to assess the admixing effects on soil OC and N stock over time (Wang et al., 2013; Cremer et al., 2016). 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).

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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 Ba_{p,mix} is the basal area of *Pinus* in the mixed stand, Ba_{p,pure} is the basal area of *Pinus* in the pure *Pinus* stand, the Ba_{c,mix} is the basal area of *C. camphora* in the mixed stand, Ba_{c,pure} is the basal area of *Cinnamomum* in the *Cinnamomum* pure stand, Stock_{p,pure} is the soil OC or N stock under pure *Pinus* stand and Stock_{c,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 for each individual sample that were measured experimentally in mixed stands, with formula: (observed-expected)/expected. For soil OC and N stock in specific soil depth at given stand ages, 95% confidence intervals (CI) were calculated with above formula. If the CIs for mixtures did not cross y = 0, the admixing effect was considered non-additive (Ball et al., 2008). 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).

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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 the specific 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 soil OC stocks decreased significantly with increasing soil depth and the similar pattern that highest OC stock in mixed stand over monocultures were observed within given soil layers at given stand ages. 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 showed 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 10-20 cm soil layers, both 10-yr-old and 45-yr-old stands exerted no significant differences in soil OC stock among three stand types while in 24-yr-old stands mixture had significantly higher soil OC stock than the monocultures; in the 20-30 cm soil layers mixed stands showed similar soil OC stock pattern when compared with corresponding monocultures and in 24-yr-old stands mixture showed significant higher soil OC stock than two pure *Pinus* and *Cinnamomum* stand.

Soil depth and stand type, but not stand age exerted significant effects on soil N stock in mineral soil (Table 2). Within the same stand, the soil N stocks decreased with soil depth, and among them, the superficial soil layers (0-10 cm) exhibited significantly higher soil N stocks than the other two deeper soil layers (Fig. 3). Only in 0-10 cm and 10-20 cm soil layers of 24-yr old stands and 20-30 cm 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 along chronosequence for all stands, except in *Cinnamomum* stand, which marginally decreased from 24-yr old to 45-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 exert significant difference along

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chronosequence within stand type (Fig.2 and 3).

3.2 Admixing effects on soil OC and N stocks

The relative performance of soil OC and N stocks in the *Pinus-Cinnamomum* mixed stands were calculated to determine the additive or non-additive effects for each stand at different soil layers and different stand ages by comparing observed soil OC or N stock values with expected values based on counterpart monocultures. In our study the mixing planting always exerted positive effects, as all the relative values of soil sequestrated OC and N stock 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 two upper soil layers, and then decreased in the deepest layer. In the 24-yr old stand, it increased with soil depth. In the 45-yr old stand, the relative percentage was the highest in the deepest soil layer (20-30 cm). Overall, the percentage of over-performance in mixture was significant higher in 24-yr-old stand than that in 10-yr-old stand and then, it marginally decreased to 45-yr-old stand. For soil N stock, however, the results showed consistent pattern that the positive effects increased with increasing stand ages (Fig. 4).

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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 > *Cinamomum* 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 demonstrated the species diversity or species identity effects on soil OC, and N accumulation in forests and revealed that species diversity and/or abundance of dominant tree species exerted effects on OC and N stocks through carbon input by litter and fine roots (Berger et al., 2002; Guckland et al., 2009; Wang et al., 2013; Dawud et al., 2016). 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). although many previous studies also showed larger carbon stocks under coniferous species than broad-leaved species (Kasel and Bennett, 2007; Schulp et al., 2008; Wang et al., 2013). With increasing stand age, the gap of soil OC stock between *Cinamomum* and *Pinus* stand became more pronounced in whole soil profiles investigated. Furthermore, when compared with broadleaf tree species, conifer species tended to allocate much more total organic matter

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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 this study, the highest OC stock presented in 24-yr old stands, while in 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 N stock, mixed stand exhibited priority for almost all the cases, revealing 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. But whether this enhanced admixing effect on soil N accumulation will continue needs further investigation. In monocultures, *Pinus* stand showed priority in earlier stage while *Cinnamomum* in the later stage in terms of soil N stocks, which is similar to the trend of soil OC stocks, 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 enable to be uptaken by plants or leaching, as reported in our previous study that soil nitrogen concentration positively correlated to soil organic carbon concentration, (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 likely is the explanation that the soil nitrogen stock changed less significantly with stand ages and between different stand types compared with soil OC stock here.

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 significant differences. This trend are inconsistent with previous study, which reported a decline of soil organic carbon in the topsoil (0-10cm) in certain period of time after plantation establishment, (Turner and Lambert, 2000). But it's consistent with the result from a Chinese fir plantation, where the soil OC stocks 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 (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 conversion of plantations into 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 lowest level (Turner and Lambert, 2000). In our results the soil OC and N stocks decreased significantly with increasing soil depth. However, the magnitude of over-performance in the Pinus-Cinnamomum mixed stands over monocultures increased with soil depth (Fig 3 and Fig. 4). Top soils under forests are always most susceptible to disturbance, and it can be directly

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impacted by the carbon input through litter, and fine roots which always decline along soil depth (Wang et al., 2016), and our data in the same sites also suggested fine roots mostly assembled in upper soil and fine-root overyielding occurred in the topsoil layer (0-10 cm) (data not published). Also, in our previous study (Wen et al., 2014), the ratio of soil microbial biomass/soil organic carbon was lowest in topsoil, which may suggest lower mineralization rate in deeper soil and make the the accumulation of OC and N stocks in deeper soil along chronosequence more pronounced over topsoil layer. Here we only collected the soil down to 30 cm depth, effects of species diversity and species identity on the deeper soil merits further investigation to improve model parameter of soil OC and N processes in deep soil profile.

4.2 Admixing effect along chronosequence

In the two topsoil layers (0-10 cm and 10-20 cm), admixing effect on soil OC was more pronounced along chronosequence. This is likely 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 the mixed forests where the interspecific competition is relatively less intensive in general (Lei et al., 2012a). 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 or the period of soil OC accumulation processes. Our results also showed, to some degree, higher admixing effects in deeper soil layer that may attribute to the lower expected values in monocultures 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. (2012b) 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 environmental disturbances and are more capable of N retention and prevent leaching in soil (Tilman et al., 1996), This merits further studies in more diverse communities in forests to concur this pattern of higher nitrogen stock under mixed stands over monocultures and these positive effects increased along chronosequence.

5. Conclusions

The tree species and composition as well as stand age significantly affect soil organic carbon and nitrogen stocks. 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 the deeper soil. However,

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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 more soil OC and N stocks than *Pinus* stands due to the different strategies of carbon and nutrient allocation and different rate of organic decomposition, but these differences are less pronounced in deeper soil layers.

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5 Acknowledgements

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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. (mean \pm standard deviation)

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 \pm 3.26	5.28 \pm 3.97	20.06
	24	<i>Pinus</i>	2050	14.18 \pm 4.34	12.86 \pm 6.52	35.37
	45	<i>Pinus</i>	600	21.40 \pm 5.30	12.47 \pm 1.88	22.84
<i>Cinnamomum</i> stands	10	<i>Cinnamomum</i>	2708	7.77 \pm 2.60	5.99 \pm 1.25	14.26
	24	<i>Cinnamomum</i>	900	17.02 \pm 6.52	13.71 \pm 2.74	23.46
	45	<i>Cinnamomum</i>	800	21.06 \pm 6.73	13.24 \pm 2.29	30.63
Mixed <i>Pinus-Cinnamomum</i> stands	10	<i>Pinus</i>	902	7.64 \pm 1.82	4.73 \pm 0.82	4.37
	24	<i>Cinnamomum</i>	1689	8.14 \pm 2.81	7.2 \pm 0.73	9.83
	45	<i>Pinus</i>	267	19.88 \pm 5.06	12.35 \pm 1.64	7.80
	24	<i>Cinnamomum</i>	592	15.27 \pm 5.92	11.41 \pm 3.13	12.45
	45	<i>Pinus</i>	250	19.69 \pm 4.10	12.37 \pm 2.60	7.91
		<i>Cinnamomum</i>	325	20.94 \pm 8.54	13.75 \pm 2.79	12.91

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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	<0.0001	11.89	<0.0001	36.73	<0.0001	15.17	<0.0001
age	0.00	0.9942	60.53	<0.0001	35.99	<0.0001	0.54	0.5814
depth	699.57	<0.0001	205.33	<0.0001	489.87	<0.0001	65.62	<0.0001
stand×age	2.07	0.1283	10.50	<0.0001	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	<0.0001	4.18	0.0027
stand×age×depth	0.15	0.8579	0.73	0.4834	1.03	0.4142	0.67	0.7217

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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 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 small letters indicate significant differences among different stands within the same soil profile and age stages ($p < 0.05$). Different capital letters indicate significant differences among different soil layers within the same stand at given stand age ($p < 0.05$).

Fig. 4 Investigation of additive or non-additive interactions for soil OC stock (a) and N stock (b) in *Pinus-Cinnamomum* mixed 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. Different letters indicate significant differences among different stand ages within the same soil depth profile.

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Fig. 1

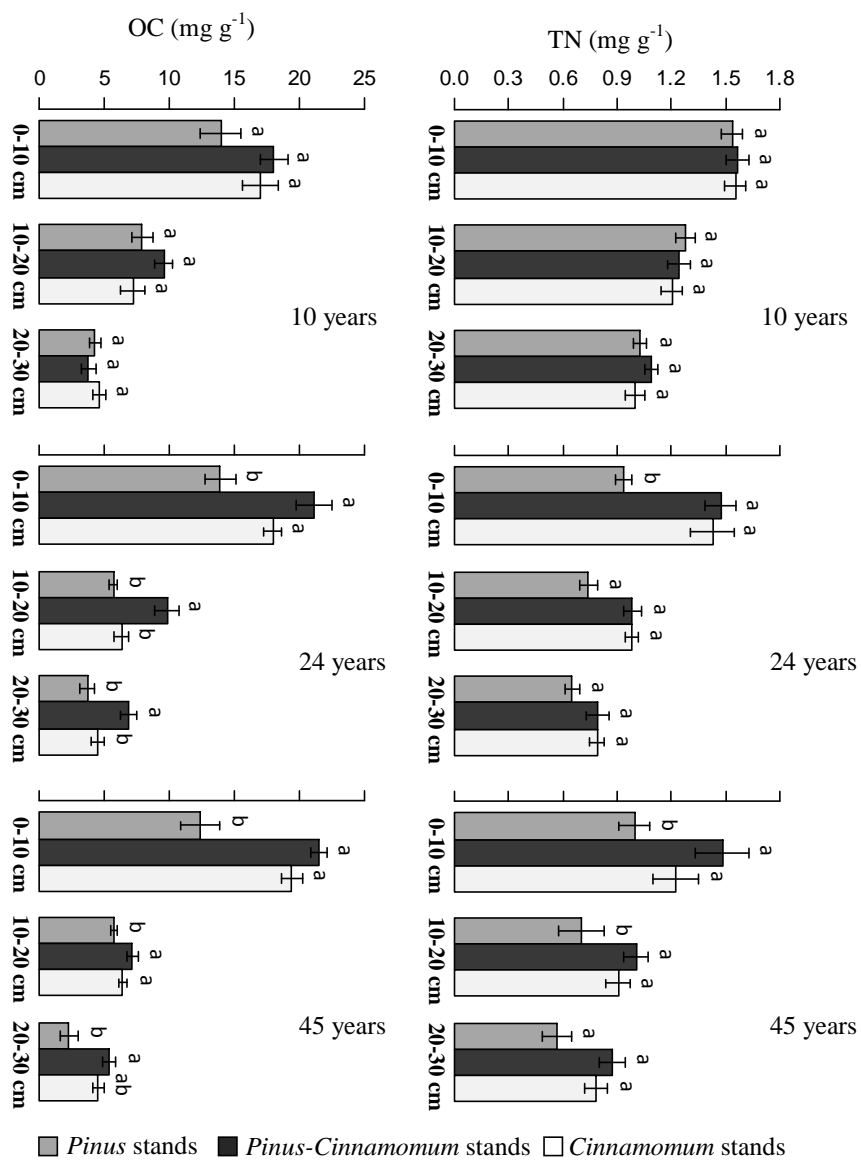


Fig. 2

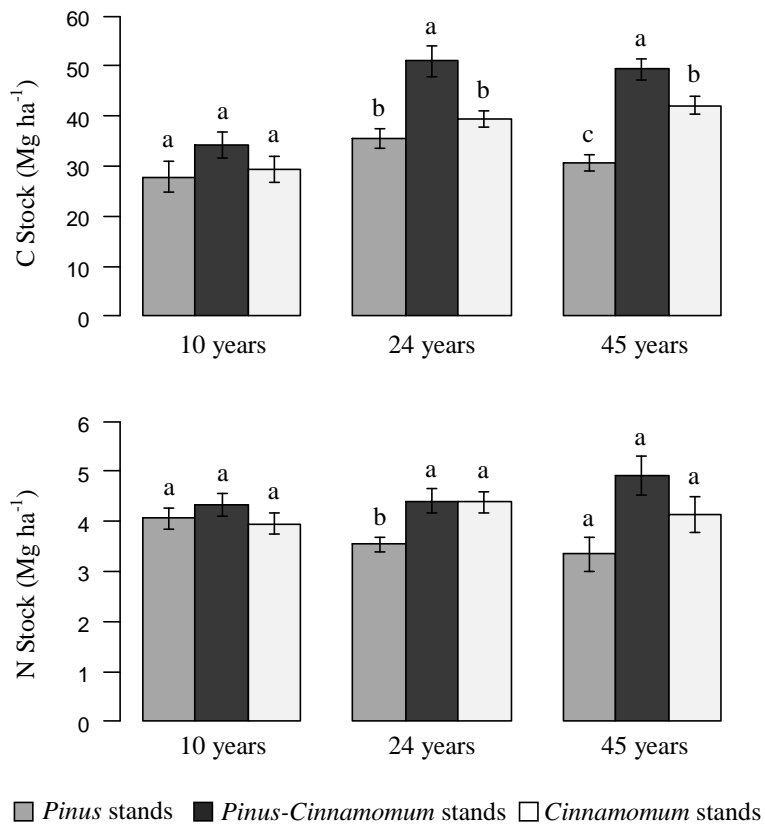


Fig. 3

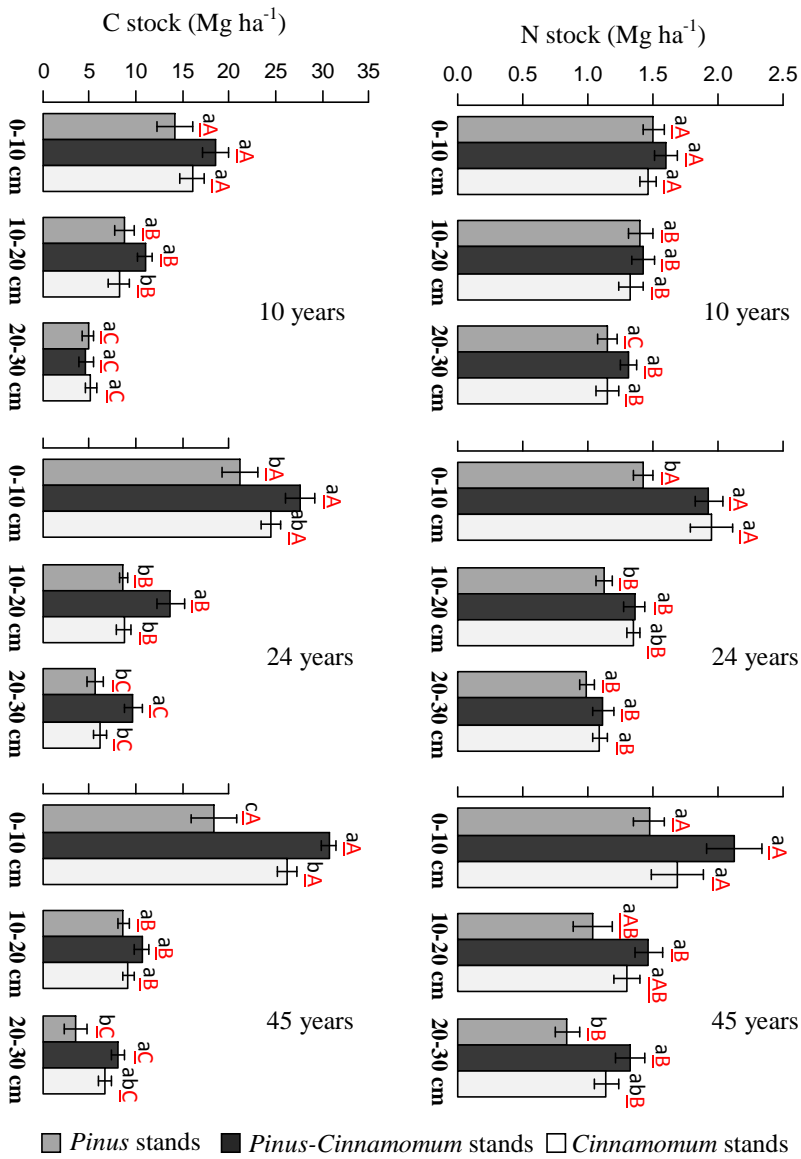


Fig. 4

