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RESEARCH ARTICLE

Vertical distribution of soil extractable organic C and N contents and total C and N stocks in 78-year-old tree plantations in subtropical Australia

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Abstract Few studies have focused on the effects of long-term forest plantations on the soil profile of carbon (C) and nitrogen (N) stocks. In this study, we selected 78-year-old tree plantations that included three coniferous tree species (i.e., slash pine, hoop pine and kauri pine) and a *Eucalyptus* species in subtropical Australia. We measured soil extractable organic C (EOC) and N (EON) contents and total C and N stocks under different tree species on the forest floor and along a soil profile to 100 cm depth. The results showed that *Eucalyptus* had significantly higher soil EOC contents (3.3 Mg ha^{-1}) than the other tree species (EOC of 1.9–2.3 Mg ha^{-1}) and had significantly higher EON (156 kg ha^{-1}) contents than slash pine (107 kg ha^{-1}). *Eucalyptus* had significantly higher soil C (58.9 Mg ha^{-1}) and N (2.03 Mg ha^{-1}) stocks than the other tree species (22.3–27.6 Mg C ha^{-1} and 0.71–1.23 Mg N ha^{-1}) at 0–100 cm depth. There were no differences in soil C stocks at the 0–100 cm depth among the coniferous tree species. Forest floor C stocks had stronger effects on mineral soil total N stocks than fine root biomass, whereas fine root biomass exerted stronger effects on soil total C stocks at the 0–100 cm depth than forest floor C and N stocks. Our results addressed large differences in soil C and N stocks under different tree species, which can provide useful information for local forest management practices in this region.

Keywords Extractable organic C · Extractable organic N · Total C · Total N · Tree species · Soil profile

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Introduction

In recent years, increasing concerns have been felt by government policy-makers and scientists regarding increased global carbon dioxide (CO_2) emissions caused by deforestation. Ecosystem functions such as C sequestration resulting from afforestation have attracted an increasing amount of attention (Lal 2005). Worldwide, extensive areas of non-forest land have been planted to forest, which has been recognized as a management tool for stocking C in soils, thereby helping to mitigate climate change (Fuchs et al. 2013).

Forest farmers usually select certain tree species such as coniferous tree species as they consider demands for timber of certain tree species and aim to optimize the production of stemwood (Lu et al. 2012; Vesterdal et al. 2013). The effects of afforestation with different tree species on soil C and N stocks have been widely studied (Berthrong et al. 2009; Guo and Gifford 2002; Nave et al. 2013; Paul et al. 2002; Vesterdal et al. 2013). Most of these studies have focused on changes in soil C and N stocks arising after less than 30 years of afforestation. Studies on the effects of long-term plantations with different tree species on soil C and N stocks are scarce, particularly along a vertical profile.

It has been known that differences in soil C and N stocks under different tree species result from differences in root and litter input as well as decomposition rates (Guo and Gifford 2002). However, few studies have focused on the relative contribution of fine root and litter decomposition to soil C and N stocks. Recently, researchers have found that macrofaunal activity contributes greatly to mineral soil C stocks, thus making the process more complex than expected (Vesterdal et al. 2013). Compared to surface soil C and N stocks, subsurface soil C and N stocks at > 20 cm depth have been largely ignored though most subsoil horizons contribute more than half of the total soil C and N stocks, so it is necessary to consider them in studies of global C and N cycling (Rumpel and Kögel-Knabner 2011).

Compared with soil total C and N stocks, soil extractable organic C (EOC) and N (EON) contents act as an active part of soil C and N stocks and are the focus of management practices (Lu et al. 2012; Zhou et al. 2013). Soil EOC and EON contents play a vital role in biochemical cycling in terrestrial ecosystems, thus influencing ecosystem productivity and sustainability (Chen and Xu 2008; Müller et al. 2009). Soil EOC and EON contents have been widely used as an indicator of the lability of soil organic matter, although they generally comprise only a small fraction of soil organic matter (Chen and Xu 2008).

In subtropical Queensland, local forest farmers plant large areas of conifer species because of the higher economical interest. We selected a 78-year-old tree plantation in this region to investigate the effects of different tree species on soil total C and N stocks. The objectives of this study were (1) to compare soil EOC and EON contents as well as total C and N stocks and (2) to investigate the relative contributions of the forest floor and fine roots to soil C and N stocks along a profile to 100 cm under different tree species.

Materials and methods

Experimental site

We selected a 78-year-old forest plantation with different tree species that was established in 1935 on a site that was originally a banana farm (*Musa acuminata* Colla). The forest plantation site is located at Cooloola, Tin Can Bay, Southeast Queensland, Australia (25°56′49″S, 153°5′27″E). The altitude is 43 m above sea level with a mean annual rainfall of 1287 mm. Winter temperatures range from 7 to 23 °C over June to August, and summer temperatures range from 18 to 30 °C over December to February (Zhou et al. 2017). Four tree species were selected, including an exotic coniferous species (slash pine (*Pinus elliottii* Engelm. Var. *elliottii*)) and two native conifer species (hoop pine (*Araucaria cunninghamii* Ait) and kauri pine (*Agathis robusta* C. Moore)), as well as a *Eucalyptus* species (*Eucalyptus grandis* W Hill ex Maiden). All of them were planted adjacently on a broad, gently undulating plain with a gentle slope of less than 5°. The plot size of each tree species was 1.087, 0.308, 0.428 and 0.60 ha, respectively (Zhou et al. 2017). Four subplots of 10 m × 20 m in each tree plantation were selected using a diagonal sampling pattern (i.e., one point at each corner) for forest floor and soil sampling, resulting in a total of 16 subplots. These subplots were at least 20 m away from the other tree plantations to avoid edge effects. We acknowledge that these subplots were pseudoreplicates. However, given that soil texture and soil moisture content (Table S1), soil extractable organic C and N contents (Table 1) and soil total N stocks (Table S2) were similar at the 60–100 cm depth across the tree species stands, we consider these subplots to be real replicates. The thicknesses of the litter and fermentation layers were 5–6 and 1–2 cm for slash pine, respectively, whereas the corresponding values were 4–5 and 1–2 cm for the hoop pine and kauri pine plots, respectively. The *Eucalyptus* plot had a thicker litter layer of 8–10 cm and a similarly thick fermentation layer of 1–2 cm. Soil texture in this region is more than 90% sandy soil.

Sampling of forest floor layers and profile soils

Forest floor litter and fermentation layers were collected in August 2013 within a quadrat of 25 cm × 25 cm using a diagonal sampling pattern (i.e., one point at each corner and one in the center of each subplot). After that, soil samples were collected using a soil auger (8 cm in diameter) at vertical depths of 0–10, 10–20, 20–40, 40–60 and 60–100 cm within each quadrat. The soil cores were immediately mixed thoroughly within each subplot and kept in a cooler (4 °C). After passing the samples through a 2-mm sieve to remove roots and stones, the soil samples were stored at 4 °C prior to analysis. Bulk density for each soil depth layer was calculated according to the volume of the core and the soil mass of the layers. A portion of each fresh sample was stored at 4 °C for analysis of soil moisture, pH, and EOC and EON contents. The other portion was air-dried and stored at room temperature for analysis of soil total C and N after being finely ground. Soil moisture content was determined after the sample was oven-dried at 105 °C overnight. Forest floor layer and soil C and N contents were determined using an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyzer (Isoprime-EuroEA 3000) (Zhou et al. 2016).

Measurement of soil EOC and EON contents

Soil pH was measured at a 1:2.5 dry soil/water ratio. Soil NH_4^+-N, NO_3^--N, EOC and EON concentrations were determined in hot water extracts (Zhou et al. 2013). Briefly, field soil samples (5 g) were extracted with 50 mL of hot water and

Table 1 Differences in extractable organic C (EOC) and N (EON) (mean ± standard errors) at the forest floors and along the soil profile to 100 cm with hot water extraction under different tree species

Profile	EOC (kg ha^{-1})				EON (kg ha^{-1})				EOC:EON			
	Slash pine	Hoop pine	Kauri pine	*Eucalyptus*	Slash pine	Hoop pine	Kauri pine	*Eucalyptus*	Slash pine	Hoop pine	Kauri pine	*Eucalyptus*
L-layer	218 ± 15ab	237 ± 17a	154 ± 6.3b	233 ± 52a	3.6 ± 0.4ab	5.0 ± 0.7a	3.3 ± 0.3b	3.1 ± 0.5b	61.4 ± 4.0ab	48.4 ± 3.3b	48.8 ± 5.6b	73.9 ± 9.1a
F-layer	205 ± 34ab	136 ± 7b	195 ± 21ab	218 ± 54a	4.6 ± 1.2	2.9 ± 0.1	4.8 ± 0.8	3.6 ± 0.9	47.8 ± 4.7b	47.1 ± 4.7b	42.1 ± 2.7b	59.9 ± 1.9a
0–10 cm	340 ± 41b	341 ± 31b	360 ± 30b	625 ± 77a	14.7 ± 2.9b	18.4 ± 1.9ab	23.1 ± 1.5a	22.4 ± 1.8a	24.2 ± 1.8a	18.8 ± 1.5b	15.7 ± 0.9b	27.6 ± 1.6a
10–20 cm	237 ± 23b	341 ± 54b	361 ± 26b	693 ± 95a	11.3 ± 1.1b	19.8 ± 2.7a	22.5 ± 1.4a	25.6 ± 2.8a	21.1 ± 1.6b	17.2 ± 1.0bc	16.2 ± 1.4c	27.2 ± 3.0a
20–40 cm	394 ± 33b	592 ± 52a	643 ± 40a	732 ± 70a	23.9 ± 1.8b	33.2 ± 4.8ab	44.1 ± 3.7a	36.5 ± 4.6a	16.5 ± 0.6ab	18.5 ± 1.6ab	15.1 ± 1.99b	20.2 ± 1.2a
40–60 cm	366 ± 23b	407 ± 44b	383 ± 36b	584 ± 53a	22.5 ± 2.6	26.9 ± 3.4	32.6 ± 1.6	31.9 ± 5.7	16.7 ± 1.3a	15.5 ± 1.2a	11.7 ± 0.7b	18.9 ± 1.7a
60–100 cm	519 ± 66	617 ± 86	523 ± 33	703 ± 63	35.1 ± 7.2	42.4 ± 2.9	35.8 ± 2.9	39.8 ± 4.6	15.4 ± 1.3	14.5 ± 1.6	14.9 ± 1.4	17.9 ± 1.5
0–100 cm	1858 ± 155c	2301 ± 167b	2271 ± 104b	3338 ± 146a	107 ± 12b	141 ± 13ab	158 ± 8a	156 ± 16a	17.5 ± 0.6b	16.5 ± 1.1b	14.5 ± 1.1b	21.6 ± 1.3a

Different letters within the same row indicate significant differences at $P < 0.05$ among the treatments

L-layer litter layer, *F-layer* fermentation layer

incubated at 70 °C for 16 h. After that, the Falcon tubes were rotated in an end-to-end shaker at 120 rpm for 1 h, and then, the supernatant was filtered through Whatman no. 42 paper. The concentration of inorganic N (sum of NH_4^+-N and NO_3^--N) was measured on a SmartChem 200 Discrete Analyzer (WESTCO Scientific Instruments Inc.). Soil EOC and total soluble N in soil extracts were determined using a SHIMADZU TOC-VCPH/CPN analyzer (fitted with a total N unit). Soil EON was calculated by subtracting extractable inorganic N from total soluble N for every soil sample.

Calculation of soil C and N stocks

Forest floor C content was calculated by multiplying C concentration by the mass of the forest floor samples. The forest floor mass was calculated after drying the samples at 65 °C for 2 days. Changes in soil C and N stocks between two sampling times were evaluated in terms of C concentrations. The bulk density calculation for each soil layer (i) was calculated according to the following formula:

$$BD_i = \frac{W}{Vol_{auger} - Vol_{roots}}, \quad (1)$$

where BD_i indicates the bulk density in g cm^{-3}, W is the dry weight of the soil samples (most of our samples were sandy with a particle diameter of less than 2 mm, so the weight of the > 2 mm aggregate fraction in the sample is negligible) (Vesterdal et al. 2008), Vol_{roots} is the volumes (cm^3) of the roots and Vol_{auger} is the volume (cm^3) of the soil auger. Soil total C (in Mg ha^{-1}) was then calculated as:

$$Total\_C = BD_i \times D_i \times C_i, \quad (2)$$

where BD_i is the bulk density of the layer in g cm^{-3}, D_i is the soil layer depth in cm and C_i is the C concentration in mg g^{-1}. Soil total N was calculated using the formula but substituting N for C.

Statistics

We calculated soil mineral C and N stocks at the 0–20 cm depth and soil EOC and EON contents as well as total C and N stocks at the 0–100 cm depth. We used one-way analysis of variance (ANOVA) to determine the effects of tree species on EOC, EON, EOC:EON ratios, total C, total N, C:N ratios as well as soil C and N stocks at forest floor and along the soil profile among the treatments. Means and the least significant difference at 5% level were calculated to separate the difference among the tree species. We established an analysis of covariance of model (ANCOVA) to investigate the levels of soil pH along the gradient of soil C stocks and to test whether there are different responses of soil pH to changes in soil C stocks among the different tree species. Pearson's correlation coefficients among the soil properties were also calculated. All ANOVA, ANCOVA and correlation analyses were performed using R v.3.2 software (R Core Team 2015).

Structural equation modeling was performed using R software with the *lavaan* package to quantify the relative contributions of fine roots and forest floor C and N contents to soil total C and N stocks along the profile. We considered that path analysis was most appropriate for data sets with large sample sizes but that the number of samples for the variables in every model was too small (n = 16). However, small sample sizes generally result in conservative fitting estimates (Shipley 2000). In structural equation modeling, a χ^2 test is used to determine whether the covariance structures implied by the model adequately fit the actual covariance structures of the data. A non-significant χ^2 test ($P > 0.05$) indicates an adequate model fit. The coefficients of each path, taken as the calculated standardized coefficients, were determined by analyzing the correlation matrices. Paths in this model were considered to be significant at $P < 0.05$. These coefficients indicate by how many standard deviations the effect variable would change if the causal variable was changed by one standard deviation (Shipley 2000).

Results

Soil moisture contents and pH under different tree species

In general, *Eucalyptus* had a higher soil moisture content than the other three coniferous species at the 0–10, 10–20 and 20–40 cm depths (Table S1). There were no differences in soil moisture content among the three coniferous tree species across the depths except for the 20–40 cm depth. Most measured soil pH within the plantations was below 6.0 (Table S1), indicating that the soil is acidic at this site. In general, *Eucalyptus* had lower soil pH than the other tree species, and kauri pine had higher soil pH than slash pine and hoop pine across all soil depths.

Forest floor and soil profile EOC and EON contents

In general, *Eucalyptus* had higher EOC contents and EOC:EON ratios, but it had lower EON contents than the other tree species at the forest floor, including in the litter and fermentation layers (Table 1). Hoop pine had higher EOC and EON contents, but it had lower EOC:EON ratios than slash pine and kauri pine in the litter layer, whereas there were no significant differences in the EOC, EON and EOC:EON ratios among the three coniferous tree species in the fermentation layer.

In general, *Eucalyptus* had significantly higher soil EOC and EON contents as well as EOC:EON ratios than the other tree species across all the soil depths (Table 1). Hoop pine and kauri pine had higher soil EOC and EON contents, but they had lower EOC:EON ratios than slash pine across all soil depths (Table 1).

Total C and N stocks at the forest floors and along the soil profile

Eucalyptus had significantly higher forest floor C than hoop pine and kauri pine (Fig. 1a), whereas kauri pine had significantly higher total N stocks than hoop pine (Fig. 1b). There were no differences in forest floor N stocks among *Eucalyptus*, slash pine and kauri pine. Slash pine had higher C:N ratios than the other tree species in the litter layer, whereas *Eucalyptus* had higher C:N ratios than the other tree species in the fermentation layer.

In general, *Eucalyptus* had higher soil C and N stocks than the other three coniferous species across all depths (Table S2). Hoop pine and kauri pine had higher soil C and N stocks, but they had lower C:N ratios among the three coniferous species across the profile. When calculating soil C and N stocks along the soil profile, we found that *Eucalyptus* had significantly higher mineral soil C and N stocks at the 0–20 cm depth (Fig. 1) and soil total C and N stocks at the 0–100 cm depth than the other tree species (Fig. 2). There were no differences in soil C and N stocks among the three coniferous tree species at 0–20 cm depth, but slash pine had significantly lower soil N stock than the other coniferous tree species at 0–100 cm depth (Fig. 2).

Proportion of soil C and N stocks for each layer

The forest floor accounted for 17–22 and 19–22% of total C (Fig. 3a) and N stocks (Fig. 3b), respectively, for all species, with the highest proportions under hoop pine and the lowest values under *Eucalyptus*. Mineral soils at the 0–20 cm depth accounted for 29–44 and 34–51% of total C and N stocks among the tree species, with the lowest values found under slash pine. Slash pine had the highest proportion of total C (52%) and total N (45%), whereas the other tree species had similar proportions, ranging across 37–38% for total C and 27–31% for total N at 20–100 cm depth.

Relationships among forest floor C and N stocks and fine root biomass and soil C and N stocks

We examined tree species-specific relationships among soil pH and soil C stocks and soil C:N ratios and EOC:EON ratios for each tree species (Fig. 4). We found that there were significantly negative relationships between soil pH and soil total C stocks under slash pine and under *Eucalyptus* (both $P < 0.05$).

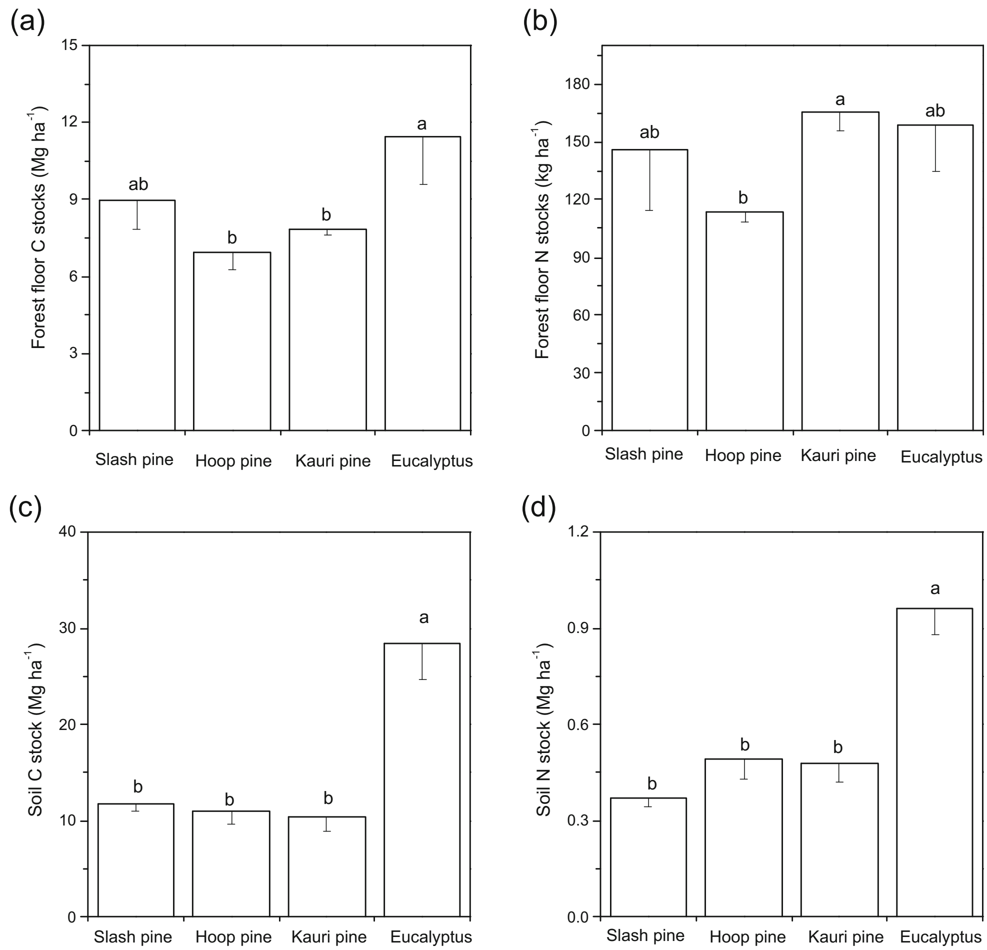

Fig. 1 Differences in forest floor C (**a**) and N (**b**) stocks, mineral soil C (**c**) and N (**d**) stocks at the 0–20 cm depth under different tree species. Different letters indicate significant differences at $P < 0.05$ among the treatments

There were no relationships between C:N and EOC:EON for each tree species (all $P > 0.05$). On the other hand, we established a covariance model to investigate whether there are different responses of soil pH along the gradient of soil C stocks for any tree species. The covariance model shows that there are no covariance relationships between soil pH and soil C stocks among the tree species ($P = 0.39$) (Table S3). Therefore, we pooled all soil pH values together and found that there were significantly negative relationships between soil total C and pH ($P < 0.001$, $n = 80$).

Given that there was a limited number ($n = 16$) of soil C stock data, we cannot investigate the tree species-specific relationships between soil C and N stocks, fine root biomass and forest floor C and N stocks. Therefore, we pooled all these data together and used structural equation modeling to calculate the relative contributions of the forest floor and fine roots to soil C and N stocks among all tree species. We found that forest floor total N stocks had significant effects on soil EON contents and total N stocks at the 0–20 cm depth (Fig. 5), which was supported by a significantly positive relationship between soil EOC:EON at the 0–20 cm depth and forest floor EOC:EON, as well as a positive relationship between soil EOC:EON at the 0–20 cm depth and forest floor C:N ratios (Fig. S1). However, we found that fine root biomass had significantly negative effects on soil EOC contents and C stocks at the 0–100 cm profile (Fig. 6).

Discussion

Afforestation is a useful management practice for mitigating anthropogenic CO_2 emissions. During the past few decades, a number of studies have quantitatively reviewed changes in soil C and N stocks after afforestation (Berthrong et al. 2009; Laganière et al. 2010; Nave et al. 2013; Shi et al. 2013). The majority of these studies focused on global patterns of soil C and N stock changes with afforestation. However, only limited data are available on the effect of afforestation on soil C and N stocks in Australia, especially in long-term forest plantations. We acknowledge that apart from tree species, it has been established that soil C and N stocks are influenced by many abiotic factors such as soil parent material and climate (Baritz et al. 2010; Callesen et al. 2003). Here, we selected adjacent planted forests with a relatively flat terrain, the same soil parent material and the same progression of soil succession, where abiotic factors might

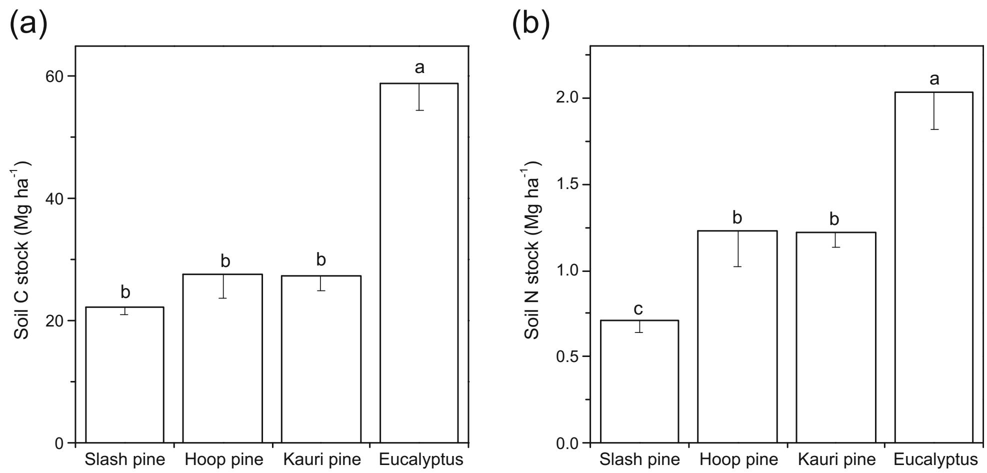

Fig. 2 Soil total C (**a**) and N (**b**) stocks at the 0–100 cm depth under different tree species. Different letters indicate significant differences at $P < 0.05$ among the treatments

have negligible effects on any differences in soil C and N stocks among the four tree species.

Soil EOC and EON contents under different tree species

Soil EON contents are very important for forest management practices since they serve as a short-term reservoir of nutrients for soil microorganisms and plants and are highly correlated with plant productivity (Chen and Xu 2008; Curtin et al. 2006). *Eucalyptus* is a broadleaf hardwood tree species that has been reported to have higher soil EOC than coniferous tree species (Wang and Wang 2011). In this study, we found that *Eucalyptus* had higher soil EOC than the other coniferous tree species (see Table 1). This could be attributed to higher litter biomass decomposition (Xing et al. 2010), which was supported by the higher litter biomass found under *Eucalyptus* (10.86 Mg ha^{-1}) relative to the coniferous species (6.97–8.96 Mg ha^{-1}). Similarly, hoop pine and kauri pine had higher soil EOC and EON contents along the profile to 100 cm (Table S2). On the other hand, *Eucalyptus* had lower soil pH, which could inhibit microbial activity and subsequently enhance soil C stocks under *Eucalyptus* which was supported by the significantly negative relationship between soil pH and soil total C stocks (see Fig. 4). We found that *Eucalyptus* had higher soil EON contents than slash pine and hoop pine (Table 1), which is consistent with previous studies showing large differences in soil EON between coniferous and broadleaf trees (Xing et al. 2010).

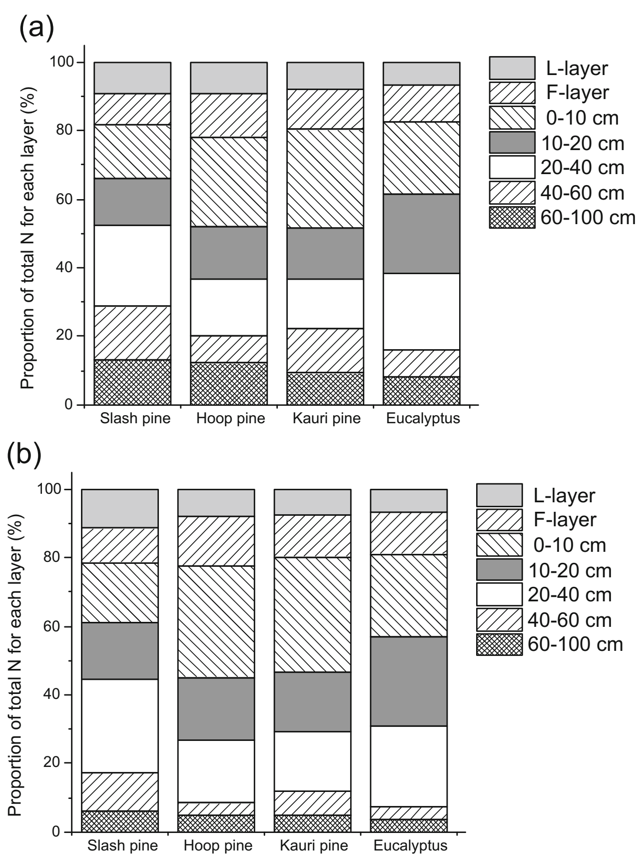

Fig. 3 Proportions of C (**a**) and N (**b**) stocks within each layer at the forest floor and along the soil profile to 100 cm depth under different tree species. *L-layer* litter layer, *F-layer* fermentation layer

Importance of forest floor C and subsurface C stocks

A number of researchers have suggested that forest floor C largely contributes to mineral soil C stocks (Cotrufo et al. 2013; Vesterdal et al. 2013). In this study, we found that forest floor C, including the litter and fermentation layers, accounted for 17–22% of total C stocks within the soil profile across the sites (see Fig. 3). We also found that litter quality (as indicated by the C:N and EOC:EON ratios) greatly affected mineral soil EOC:EON ratios (see Fig. S1). However, no significant positive relationship was found between forest floor C stocks and soil C stocks at the 20–100 cm depth ($P > 0.05$), indicating that the forest floor mainly influences soil C stocks at the 0–20 cm depth, but not soil C stocks at the 20–100 cm depth.

By comparison, subsurface soil layers at the 20–100 cm depth contributed to 37–38% of the profile's C stocks (see

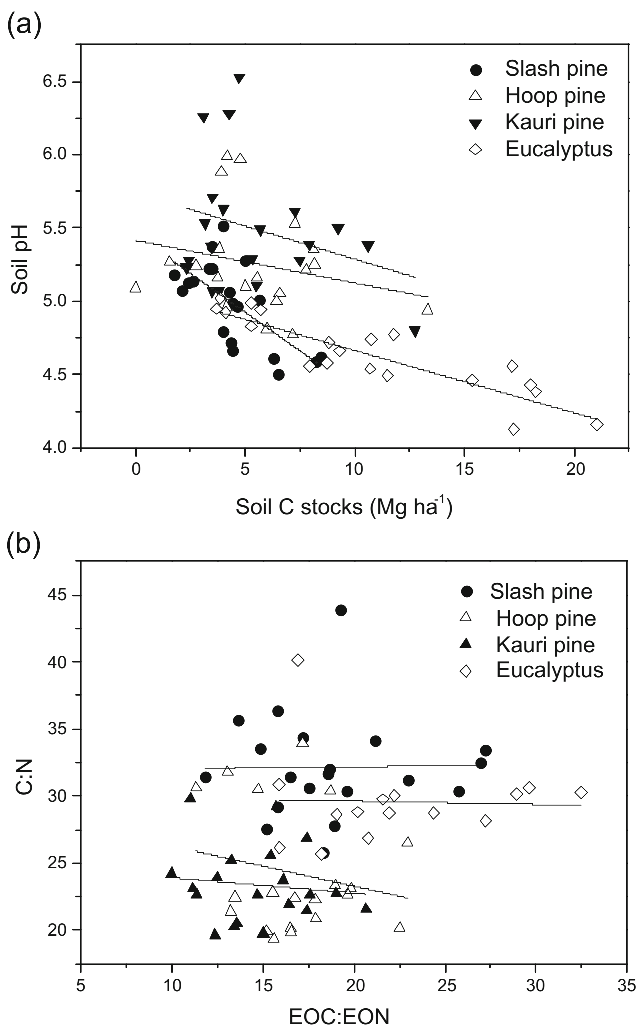

Fig. 4 Relationships between soil C stocks and pH (**a**) and between soil C:N and EOC:EON (**b**) at 0–100 cm depth under different tree species. Linear fitting results of soil C stocks and pH: slash pine $y = 5.45 - 0.11x$, $r = 0.42$, $P < 0.05$; hoop pine $y = 5.41 - 0.03x$, $r = 0.01$, $P = 0.32$; kauri pine $y = 5.74 - 0.05x$, $r = 0.04$, $P = 0.20$; *Eucalyptus* $y = 5.09 - 0.04x$, $r = 0.76$, $P < 0.001$. Linear fitting results of soil C:N and EOC:EON: slash pine $y = 31.91 + 0.01x$, $r = -0.05$, $P = 0.95$; hoop pine $y = 29.26 - 0.30x$, $r = -0.01$, $P = 0.41$; kauri pine $y = 25.09 - 0.12x$, $r = -0.04$, $P = 0.61$; *Eucalyptus* $y = 30.12 - 0.02x$, $r = -0.07$, $P = 0.89$

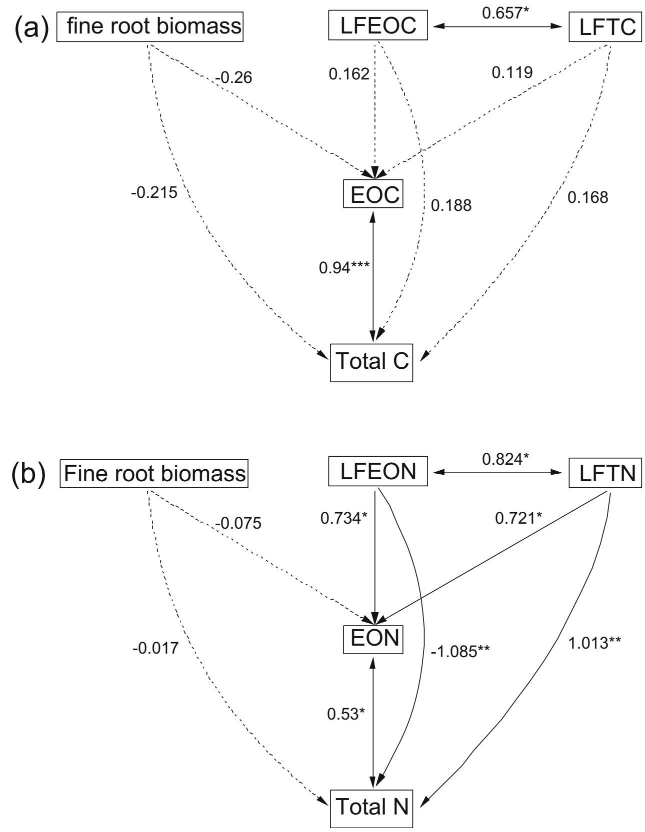

Fig. 5 Path diagrams representing the final model showing the contributions of fine roots and forest floor litter to the soil's total C (**a**) and total N stocks (**b**) at the 0–20 cm depth under different tree species. Numbers associated with single- or double-headed arrows are partial regression coefficients of multiple regressions. *EOC* extractable organic C, *EON* extractable organic N, *LFEOC* EOC in the litter and fermentation layers, *LFEON*, EON in the litter and fermentation layers, *LFTC and LFTN*, total C and total N in the litter and fermentation layers, respectively. *Solid arrows* denote the directions and effects that were significant ($P < 0.05$); the numbers on these pathways are the coefficients. *Dashed arrows* represent the directions and effects that were not significant ($P > 0.05$). *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Fig. 3), which is consistent with previous findings that despite their lower C content, subsurface soil layers may contribute to more than half of the total soil C stocks (Rumpel and Kögel-Knabner 2011). Large differences in total C were found among the treatments and the extent of these differences decreased with increasing soil depth except for the 60–100 cm depth (see Figs. 3 and 4). These results indicate that it requires a longer period of time to observe large differences in total C at deeper soil layers. The soil surface layer is directly subjected to C inputs from foliar materials, twigs and branches via litter decomposition. C inputs from the uppermost layers may be incorporated into deeper soil layers through soil fauna (Don et al. 2008) or leaching of EOC from forest floors (Hansson et al. 2010). These mechanisms work with C inputs via root exudates to contribute to an accumulation of C stocks in the long term (Cotrufo et al. 2013).

Relationships among forest floor C and N stocks, fine root biomass and soil C and N stocks

In general, *Eucalyptus* had significantly higher soil total C and N stocks than the other coniferous tree species (Fig. 2). Given that the pools of soil total C and N stocks under tree species are derived from two main processes (i.e., forest floor litter decomposition and root exudation and root residue decomposition (Finzi et al. 1998; Grüneberg et al. 2014; Rumpel and Kögel-Knabner 2011; Vesterdal et al. 2008)), we found that forest floor N stocks had stronger effects on soil total N at the 0–20 cm depth than fine root biomass (see Fig. 5), whereas

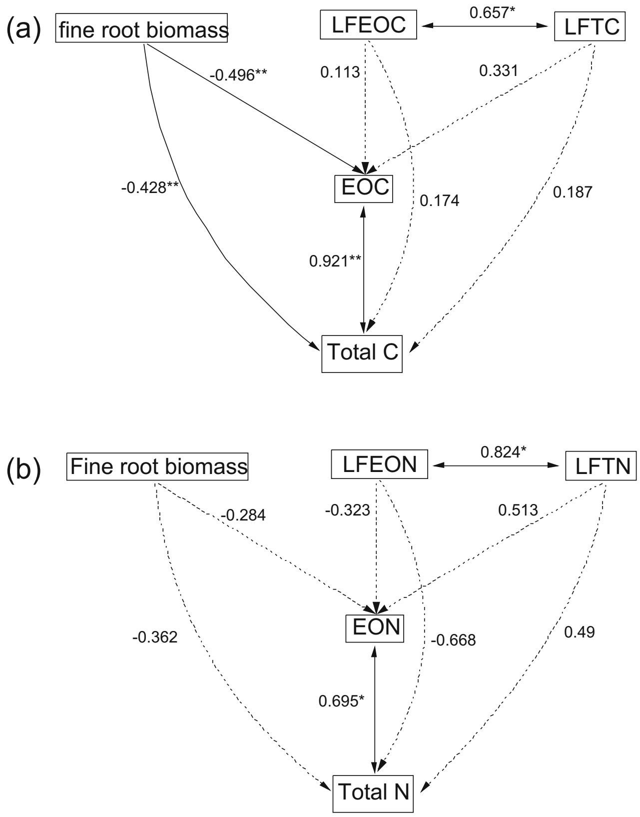

Fig. 6 Path diagrams representing the final model showing the contributions of fine roots and forest floor litter to the soil's total C (**a**) and total N stocks (**b**) at 0–100 cm under different tree species. Numbers associated with single- or double-headed arrows are partial regression coefficients of multiple regressions. *EOC* extractable organic C, *EON* extractable organic N, *LFEOC* EOC in the litter and fermentation layers, *LFEON* EON in the litter and fermentation layers, *LFTC and LFTN* total C and total N in the litter and fermentation layers, respectively. *Solid arrows* denote the directions and effects that were significant ($P < 0.05$); the numbers on these pathways are the coefficients. *Dashed arrows* represent the directions and effects that were not significant ($P > 0.05$). *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

fine root biomass had stronger effects on soil total C stocks at the 0–100 cm depth than forest floor C stocks (see Fig. 6). As *Eucalyptus* had a lower fine root biomass (Fig. S2), to exclude the *Eucalyptus*-specific effects on soil C and N stocks, we re-calculated these relationships without *Eucalyptus* and found that fine root biomass had a negative influence on soil EOC contents and C stocks at the 0–100 cm depth as well, although the differences did not reach significant levels (Fig. S3). Moreover, we noticed that there were significantly negative relationships between fine root biomass and soil N stocks (Fig. S3). All these results are in contrast to what we thought and previous studies showing that there are significantly positive relationships between fine root biomass and soil C stocks (Dawud et al. 2016; Rumpel and Kögel-Knabner 2011). The reason for this could be attributed to competition by plants for higher N for growth with soil microorganisms under tree species, which was supported by the negative relationship between fine root biomass and soil N stocks (Fig. 6), which was also found when re-calculating the relationship without *Eucalyptus* (Fig. S3). Given that there are stronger relationships between soil C and N stocks ($r = 0.956$, $P < 0.001$, $n = 80$), the negative relationships between fine root biomass and soil N stocks may account for negative relationships between fine root biomass and soil C stocks among the tree species. On the other hand, as the soil is very poor with over 95% having a sandy texture in this region, nutrient leaching could influence the relationships between fine root biomass and soil C and N stocks. More research is needed to clarify the tree species-specific effects on soil N cycling, plant–soil microorganism interactions and N leaching in this region.

Conclusion

Long-term *Eucalyptus* plantations had higher soil C and N stocks than the other tree species, but there were no differences in soil C stocks at the 0–100 cm depth among the coniferous tree species. Subsurface soil layers contributed nearly half of the total C stocks, highlighting the importance of deep soil C stocks. Forest floor N stocks had stronger effects on mineral soil total N stocks than fine root biomass, whereas fine root biomass exerted significantly negative effects on soil total C stocks at the 0–100 cm depth than forest floor C stocks. The reason for this could be attributed to competition by plants for higher N for growth with soil microorganisms under tree species. We acknowledged that the subplots in this study were pseudoreplicates, but given that these forest plantations are adjacent with a relatively flat terrain and similar soil texture and soil characteristics, we consider these subplots to be real replicates. Our results addressed large differences in soil C and N stocks under different tree species, which can provide useful information for local forest management practices in this region.

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