

Falcataria moluccana (Miq.) root distribution seedlings in response to nitrogen concentrations and tillage

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Abstract

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Falcataria moluccana (Miq.) Barnaby & Grimes is an important species for forest plantation programmes in Malaysia and is widely used in the wood industry. However, its root interactions have not been widely investigated due to the limited methodologies and information about the root distribution of trees and crops in forest plantation and agroforestry systems. This study was conducted to determine the rhizotron-scale root interactions of *F. moluccana* at different tillage and nitrogen concentrations under four different treatments: control, tillage, fertiliser, and tillage with fertiliser. The rhizotron-scale experiment was conducted at a greenhouse where *F. moluccana* (Batai) seedlings were transplanted in transparent rhizotron tubes (one-metre-high transparent polycarbonate solid sheet) using topsoil and river sand to simulate natural growing conditions. Root Intensity (RI), Root Length Density (RLD), Specific Root Length (SRL), dried shoot biomass and root biomass were recorded. Root biomass and SRL were notably higher (25–50 cm depth) at 6 WAT (Weeks After Transplanting), and the shoot biomass (tillage + fertiliser) was significantly higher at 14 WAT. However, plants treated with different tillage and nitrogen concentrations showed no significant impact on the RI and RLD. Fertiliser treatment only, and tillage with fertiliser treatment, showed greater root distribution at the rhizotron scale. These findings contribute to forest plantation and natural forest rehabilitation efforts by helping optimise the soil resources within ecosystems for sustainable forest management.

Keywords

Falcataria moluccana, fast growing, root competition, root, distribution perennial

Introduction

Malaysia selected eight plant species for inclusion in the forest plantation programme under a compensatory plantation project (NORDAHLIA et al., 2014), including *Hevea brasiliensis* (rubber-wood), *Acacia* spp., *Tectona grandis*

(Teak), *Azadirachta excelsa* (Sentang), *Khaya* spp., *Neolamarckia cadamba* (Laran), *Falcataria moluccana* (formerly known as *Paraserianthes falcataria*) (Batai) and *Octomeles sumatrana* (Binuang) (HASHIM et al., 2015). One of the recommended species, *F. moluccana*, is a fast-growing tree species that belongs to the Mimosoide-

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ae subfamily in Leguminosae (also known as Fabaceae) (KRISNAWATI et al., 2011). Legume trees influence nutrient status within the root zone of nearby plants via nitrogen (N) biological fixation, nutrient recovery from soil layers underneath the pasture root zone, nutrient loss reduction through erosion and leaching, and increased soil nutrient availability due to the rising soil organic matter mineralisation (DUBEUX et al., 2014).

Known as Batai in Malaysia, Sengon in Indonesia and white albizia in Papua New Guinea, *F. moluccana* is native to Indonesia, Papua New Guinea, Solomon Islands and Australia (KRISNAWATI et al., 2011; HASSAN and RAHMAN, 2019). *F. moluccana* is one of the most popular multi-purpose legume trees that has been planted widely in Southeast Asia (HASSAN and RAHMAN, 2019; ISKANDAR and ELLEN, 2000). This species was most extensively planted in Sabah state, with 12,049 hectares, followed by Peninsular Malaysia, with about 1,530 hectares planted in several states, and finally Sarawak (175.5 hectares) (KRISHNAPILLAY et al., 1998).

Since 2000, the effect of resource availability is among the five significant themes in root studies, and requires an in-depth understanding of plant interactions (SCHENK, 2006). Water and mineral nutrient availability in the soil and absorption by the roots are critical for plant growth and productivity (KUDOYAROVA et al., 2015). Although roots cannot actively grow towards the source of nutrition, they will spread out when they come into contact with areas that are particularly rich in nitrogen and phosphorus (CROW, 2005). Additionally, nutrient deficiencies rarely result in plant death, but they can inhibit plant growth and yield (UNGER and KASPAR, 1994). Soil fertility and plant nutrition are critical elements for plant development required to achieve specific management goals.

In most cases, 17 elements – consisting of macro- and micronutrients – are considered essential to meeting the requirements of plant metabolism and enabling a plant to complete its life cycle (MCGRATH et al., 2014). Maintaining sufficient soil nutrients, such as nitrogen, is critical to any crop production system (AHMAD et al., 2010). Nitrogen is the most limiting factor in crop production, influencing the root growth in plants, soil fertility and crop yield (TAMIRU et al., 2017). Hence, fertiliser application had a profound effect on nitrogen content in the soil (SADEJ and PRZEKWAŚ, 2008) and replenishes other nutrients essential for plants. Fertiliser application rate is a crucial factor for increasing agricultural production (ONASANYA et al., 2017). In particular, application of organic nitrogen is essential during the initial growth stage, leading to increased biomass production along with higher growth rates (LIM et al., 2022).

Tillage is the mechanical manipulation of soil for crop production, and significantly impacts soil characteristics, including water conservation, temperature, infiltration and evapotranspiration (BUSARI et al., 2015). The implementation of appropriate tillage practices is essential for enhancing both soil structure and crop productivity. According to AGBEDE (2010), it has been suggested that tillage practices may have a positive impact on plant performance. However, research on this topic is limited due to the fragile nature of tropical soil, where adequate and

integrated soil management is needed to maintain high soil and crop productivity. However, it should be noted that tillage practices can also have negative impacts on the soil, since they have the potential to disrupt the hardpan layer of the subsoil in the long run. This affects the bulk density of soil as well as the soil nutrient status, reducing the productivity of the crop (SHAHID et al., 2016). Minimising the tillage can reduce mineralisation and nitrification rates, increasing the N immobilisation and denitrification rates and decreasing N availability (VAN KESSEL and HARTLEY, 2000).

To date, studies on *F. moluccana* have been limited to assessing its growth performance using soil media of different origin in the nursery (PARAMITHA and MARDJI, 2015) or different types of transplanting media (SANTOSA, 2016), and improving its growth performance using bio-char application (RINIARTI et al., 2020) or bioplastic pots made from starch (LIEW et al., 2016), as well as monitoring the early growth performance of different clones (SHU et al., 2017).

However, knowledge on the interaction between the root traits based on tillage practices as well as on root lodging susceptibility is limited (BIAN et al., 2016), and how the roots interact under different tillage application has not been studied yet for *F. moluccana*. The results of this study can have great importance for silvicultural management of forest rehabilitation. To this end, this study employed *F. moluccana* as a plant model to investigate root interactions under different tillage and nitrogen concentration conditions at the rhizotron scale.

Materials and method

Site description

This experiment was carried out in an open area near the greenhouse located in the Faculty of Tropical Forestry at Universiti Malaysia Sabah (6°02'0.7" N, 116°07'34.9" E). The rainfall distribution range throughout the year is 5.4–197.4 mm (Malaysian Meteorological Department, 2020) with a temperature range of 23–33 °C, and 82% humidity.

Experimental description

A rhizotron tube (transparent polycarbonate solid sheet) (1 m height × 150 mm × 150 mm) was used in this study. It was enclosed in a net to prevent water logging in the tubes and to hold back the soil from flowing down, providing good aeration in the soil. Each tube was filled with river sand (at a depth of 25–100 cm) and topsoil (at a depth of 0–25 cm) compacted using a steel compactor at every five centimetres' depth to simulate the natural conditions in the field. After that, each tube was watered with 5 litres of water to wash out the nutrients from the soil before *F. moluccana* seedlings were transplanted. Then, the rhizotron tubes were arranged upright on a wooden stand. The tubes were wrapped using non-transparent plastic sheets to avoid light exposure of the soil and roots.

A total of four (4) treatments were conducted: control (T1), tillage without fertiliser (T2), fertiliser without tillage (T3) and both tillage and fertiliser (T4). Liquid

Table 1. Mean values of soil physical and chemical properties of the topsoil after the experiment. The mean values were not significantly different using ANOVA and Tukey's HSD ($P > 0.05$), $n = 3$.

Properties	Control (T1)	Tillage only (T2)	Fertilizer only (T3)	Tillage and Fertilizer (T4)
Sand (%)	64.7 ± 2.9	63.7 ± 3.4	67.5 ± 4.8	62.8 ± 3.9
Silt (%)	6.98 ± 4.5	7.21 ± 4.58	5.3 ± 3.2	7.5 ± 4.21
Clay (%)	28.32 ± 6.01	29.09 ± 5.35	27.2 ± 6.3	29.7 ± 6.8
Textural class	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
Moisture content (%)	8.58 ± 4.14	9.44 ± 4.45	8.1 ± 4.38	8.73 ± 4.45
pH	3.61 ± 0.32	3.44 ± 0.45	3.24 ± 0.13	3.7 ± 0.15
Total N (%)	0.11 ± 0.02	0.12 ± 0.026	0.14 ± 0.016	0.14 ± 0.018
P (ppm)*	0.11 ± 0.02	0.13 ± 0.01	0.20 ± 0.06	0.22 ± 0.07
K (ppm)*	0.28 ± 0.11	0.26 ± 0.11	0.30 ± 0.15	0.30 ± 0.07

*(aqua regia extractable).

fertiliser was used during the study. Each treatment was replicated three (3) times, and a total of 36 experimental units of rhizotron were arranged in a Completely Randomised Design (CRD). Tillage was applied manually using a 50-cm long steel rod to break up the compacted soil and aerate the soil (ESMERALDO, 2017). For fertiliser treatment, 30 ml of diluted AG Leader 954 (2 ml of liquid fertiliser mixed with 600 ml of water) was applied in each tube, corresponding to 50 kg N ha⁻¹ and containing macronutrients, micronutrients, amino acids, gibberellic acids (GA3), hormone betaines and seaweed. Each treatment was applied once before the transplanting of *F. moluccana* seedlings was performed. Table 1 shows the soil properties between the treatments after the experiment.

The soil was analysed using a Vario Macro cube CHNS elemental analyser (Elemental Analysensysteme, Langensfeld, Germany) for total nitrogen, and Inductively Coupled plasma–Optical Emission spectroscopy (ICP-OES) for available phosphorus (P) and exchangeable potassium (K). P and K were extracted with aqua regia (AHMADPOUR et al., 2015; KERACA, 2004). A total of 0.5 g of dry soil was weighed. Aqua regia was made by (3 HCL : 1 HNO₃) solution, 4 mm of it were added to the soil samples, which were then digested at 110 °C. Following the cooling process, 10 ml of 1.2% HNO₃ was added and heated for 30 minutes at 80 °C. Next, deionised water was added to the solution to make it slightly under 20 ml, followed by heating it at 80 °C for an additional 30 minutes. Next, deionised water was added again until the solution reached 20 ml. Lastly, each sample was thoroughly mixed and filtered through filter paper (Whatman no. 42) before being placed into an Eppendorf tube. The pipette method was used for determining soil texture (OLESIK, 1991), while a gravimetric technique was used to determine soil moisture content (SHUKLA et al., 2014). Soil pH was determined using a 1:2.5 (soil : water suspension) ratio method (HOSSAIN et al., 2012).

Data collection

Root Intensity (RI), Specific Root Length (SRL), Root Length Density (RLD), Shoot Biomass (SB) and Root Biomass (RB) were all measured throughout the experiment. RI was measured once every week from 3–14 weeks after

transplanting (WAT). Root counting was conducted using root images captured with a root camera (Sony DSC-W830). Before root imaging, calibration with a grid line size of 20 × 20 mm was established. All sides in each rhizotron tube were divided into four quarterly depths (namely 0–25, 25–50, 50–75 and 75–100 cm). Root counting was conducted by intercepting the grid lines in the photos taken, and the values for the number of roots were transformed to RI (intersections m⁻¹ grid line) (HASSAN et al., 2021b).

To observe the root growth of *F. moluccana*, SB, RB, RLD and SRL were measured at 3 different harvesting times through destructive sampling. During root harvest, RB and SB were cut, washed, and oven-dried at 75 °C for 48 hours. After that, root-soil samples were removed and cut into four (4) interval depths. Next, roots were washed using a root washing technique (HASSAN et al., 2021a) to separate roots from soil and organic matter. Roots were then collected and kept in a 50 ml Eppendorf tube with 40% ethanol (HASSAN et al., 2021b) before further measurement. For RLD (cm cm⁻³) determination, roots were put in a tray with distilled water before being scanned using WinRHIZO Root Scanner. The SRL (cm g⁻¹) was measured afterwards, whereby roots from subsamples were measured in length and divided by their weight (g) before this figure was scaled up to the size of the entire root sample (HASSAN et al., 2021b).

Statistical analysis

All measured parameters underwent statistical analysis in the Statistical Package for the Social Sciences (SPSS) version 24.0, including to determine their means. A one-way ANOVA and post hoc pairwise comparisons, utilising Tukey's HSD, were employed to compare the RI, SRL, RLD, RB and SB of *F. moluccana* under various tillage treatments and nitrogen concentrations for all the sampling dates, maintaining a 5% probability level.

Results

Root intensity of *F. moluccana*

The Root Intensity (RI) of *F. moluccana* under different treatments at three selected harvest times, and root images

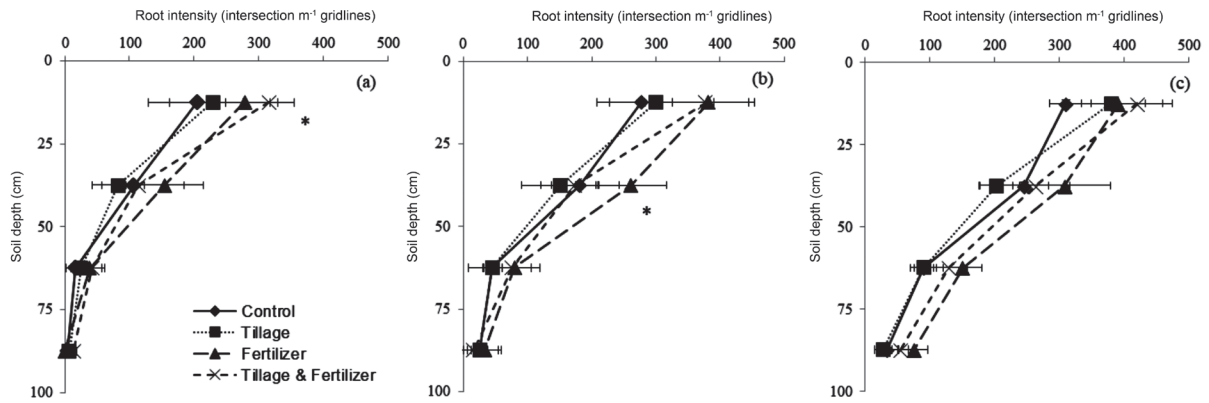


Fig. 1. *F. moluccana* seedling Root Intensity (with intersection m^{-1} gridlines) at various treatment parameters (i.e., control, fertilizer, tillage, and tillage + fertilizer), at various soil depth levels under 1 m at rhizotron scale for three independent root measurement dates; 6 weeks after transplanting (WAT) (a), 11 WAT (b) and 14 WAT (c). The mean figures were assessed with ANOVA and the Tukey's HSD. All mean figures had a statistically significant difference $*(P < 0.05)$. The error bars reflect the standard deviation values of the mean, $n = 36$ (6 WAT), $n = 24$ (11 WAT), as well as $n = 12$ (14 WAT).

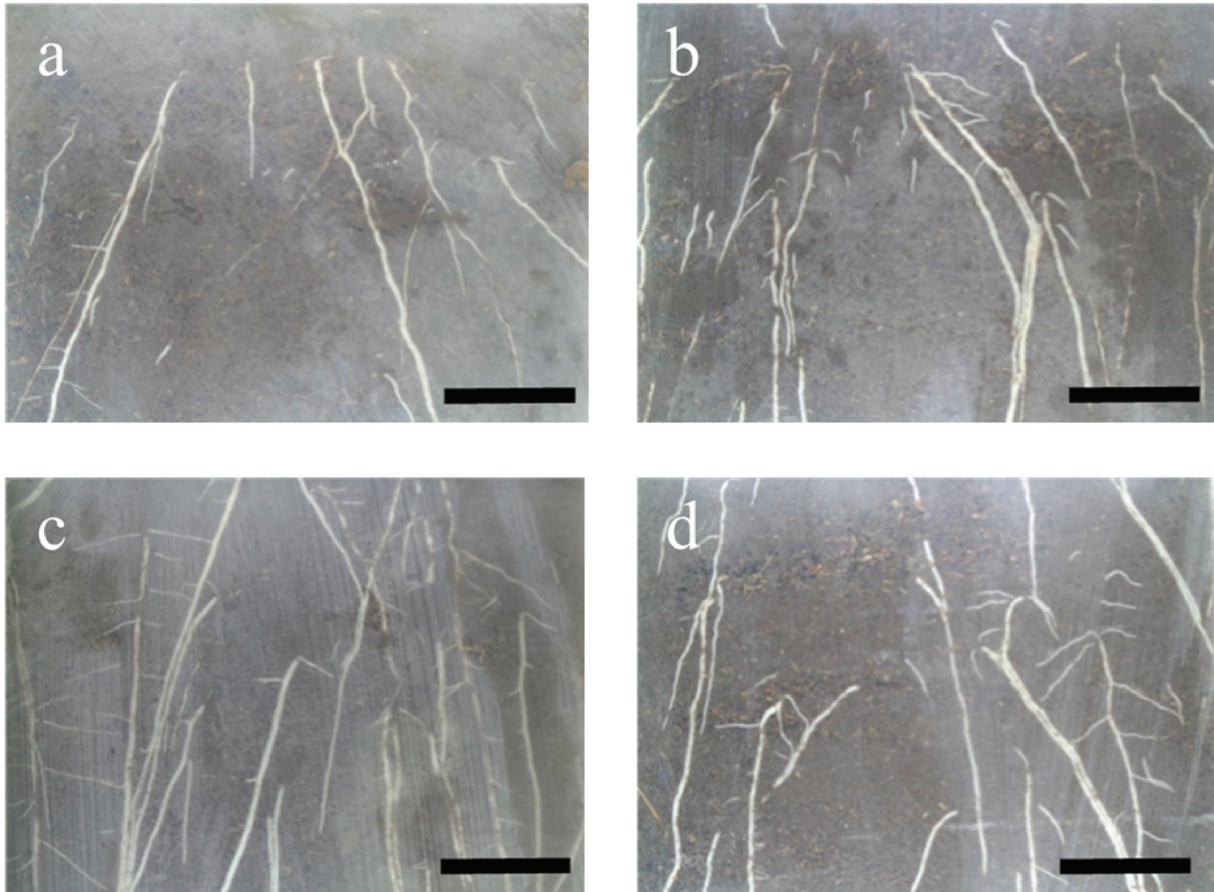


Fig. 2. Root images sample of *F. moluccana* seedling from each treatment with scale bars denoting 10 mm. (a) Control; (b) Fertilizer; (c) Tillage; (d) Tillage + fertilizer). (The size of each image is 37.8 mm \times 50.4 mm).

from each treatment, are presented in Figures 1 and 2, respectively. The RI of the plants was similar ($P > 0.05$) for all treatments (control, solely tillage, solely fertiliser, and mixed treatment (tillage + fertiliser)), at different depths and dates (6 WAT (Fig. 1a), 11 WAT (Fig. 1b) and 14 WAT (Fig. 1c)). However, there was a statistically significant

difference ($P < 0.05$) at 6 WAT at 0–25 cm soil depth under the mixed treatment. At 11 WAT, the RI of *F. moluccana* demonstrated a significant difference ($P < 0.05$) under solely fertiliser treatment at a soil depth of 25–50 cm. No significant difference ($P > 0.05$) of RI was detected in terms of all treatments at 14 WAT (Fig. 1c).

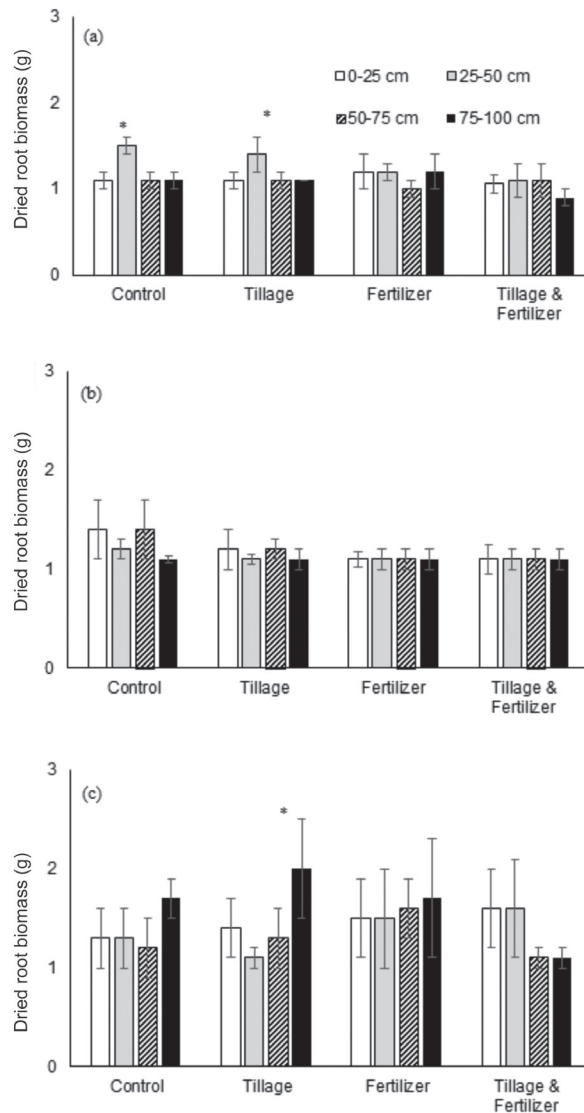


Fig. 3. *F. moluccana* seedling Dried Root Biomass (g) at various treatments (control, fertilizer, tillage and tillage + fertilizer) at various soil depth levels under the 1 m at rhizotron scale, at three independent root measurement dates; 6 weeks after transplanting, WAT (a), 11 WAT (b) and 14 WAT (c). The mean figures were assessed with ANOVA and Tukey's HSD. A statistically significant difference was seen between mean depths in the treatment $(P < 0.05)$. The error bars reflect the mean standard deviations, $n = 36$ (6 WAT), $n = 24$ (11 WAT) and $n = 12$ (14 WAT).

Dried root biomass for *F. moluccana*

Figure 3 depicts the root biomass (g) of *F. moluccana* seedlings under different treatments and at different soil depths of under 1 m at the rhizotron scale, measured at 6 WAT (a), 11 WAT (b) and 14 WAT (c). At 6 WAT (Fig. 3a), there was no significant difference for solely fertilizer and mixed treatments (tillage + fertiliser). Nevertheless, at a soil depth of 25–50 cm, the control and solely tillage treatment (i.e. both with no presence of fertiliser) demonstrated significant differences ($P < 0.05$) in root biomass. No significant difference ($P > 0.05$) in the root biomass of

F. moluccana was detected for all treatments at 11 WAT (Fig. 3b). On the other hand, in 14 WAT, for solely tillage treatment at a soil depth of 25–50 cm, the root biomass demonstrated a significant difference ($P < 0.05$) compared to the other treatments (Fig. 3c).

Root length density

Figure 5 depicts the RLD of *F. moluccana* measured at three different harvest times: 6 WAT (Fig. 5a), 11 WAT (Fig. 5b) and 14 WAT (Fig. 5c). Overall, no significant difference ($P > 0.05$) in RLD was detected for all the treatments. It was observed that the RLD of *F. moluccana* under the solely fertiliser and mixed treatments decreased at 14 WAT (Fig. 5c).

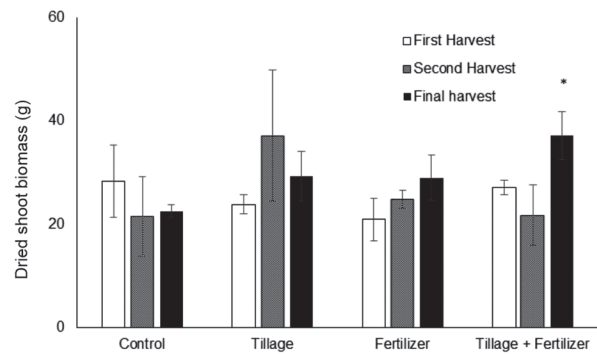


Fig. 4. *F. moluccana* seedling Dried Shoot Biomass (g) at various treatments (control, fertilizer, tillage and tillage + fertilizer), at various soil depth levels under the 1 m at rhizotron scale at three independent root measurement dates; 6 weeks after transplanting, WAT (first harvest), 11 WAT (second harvest) and 14 WAT (final harvest). The mean figures were assessed with ANOVA and Tukey's HSD. The mean figures of tillage + fertilizer were significantly different $(P < 0.05)$. The error bars reflect mean standard deviations, $n = 36$ (6 WAT), $n = 24$ (11 WAT) and $n = 12$ (14 WAT).

Specific root length of *F. moluccana*

Figure 6 showed the specific root length (SRL) of *F. moluccana* under different tillage treatments and nitrogen concentrations, measured at three independent harvest times: 6 WAT (Fig. 6a), 11 WAT (Fig. 6b) and 14 WAT (Fig. 6c). For 6 WAT, at a soil depth of 25–50 cm, a significant difference was found in the control and solely tillage treatment. However, no difference in SRL was found between treatments of *F. moluccana* at 11 WAT and 14 WAT, as shown in Fig. 6b and 6c, respectively.

Discussion

Root intensity using direct visual determination (e.g. root camera) and root biomass were used in the present study. The advantages and disadvantages of these methods have been explained well in previous studies (MAEGHT et al., 2013; HASSAN et al., 2021c). Direct visual determination is difficult and uncertain because both live and dead roots

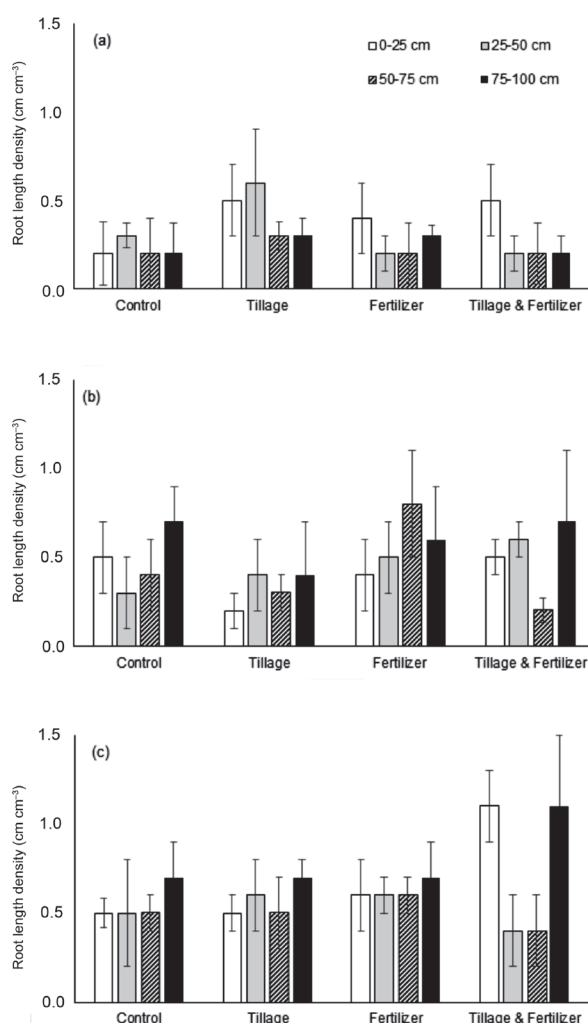


Fig. 5. *F. moluccana* seedling Root Length Density (cm cm^{-3}) at various treatments (control, fertilizer, tillage, and tillage + fertilizer), at various soil depth levels under the 1 m at rhizotron scale at three independent root measurement dates; 6 weeks after transplanting, WAT (a), 11 WAT (b) and 14 WAT (c). The mean figures were assessed with ANOVA and Tukey's HSD. The mean figures were not significantly different among treatments ($P > 0.05$). The error bars reflect mean standard deviations, $n = 36$ (6 WAT), $n = 24$ (11 WAT) and $n = 12$ (14 WAT).

are measured in this process. Similarly, root biomass measurement is problematic because not only are dead roots measured, but the fine roots are lost during the root washing procedure (HASSAN et al., 2021a). However, both methods are still able to quantify root growth, although without very high precision.

The results indicated that more fine roots were found in mixed treatment at two different harvesting times (Fig. 1). Tillage application modified the soil structure to speed up root penetration at the beginning of the study, because tillage greatly affects the soil characteristics, including water conservation, temperature level, infiltration and evapotranspiration (BUSARI et al., 2015), and enhances the

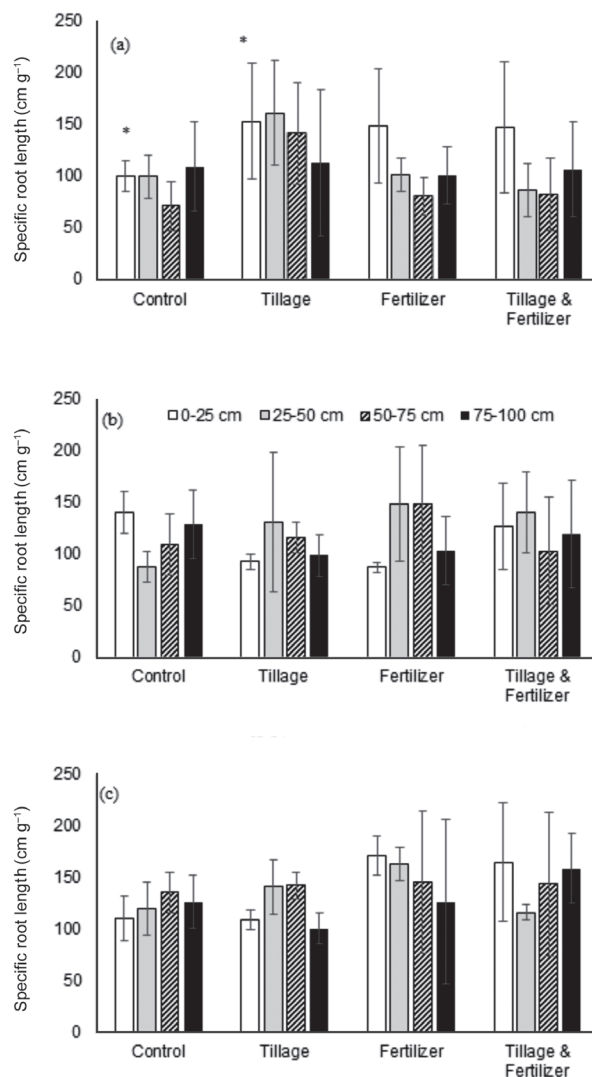


Fig. 6. *F. moluccana* seedling Specific Root Length (cm g^{-1}) at various treatments (control, fertilizer, tillage, and tillage + fertilizer), at various soil depth levels under the 1 m at rhizotron scale at three independent root measurement dates; 6 WAT (a), 11 WAT (b) and 14 WAT (c). The mean figures were assessed using ANOVA and Tukey's HSD. The mean values of tillage and control in a soil depth of 25–50 cm were significantly different $*(P < 0.05)$. Bars reflect mean standard deviations, $n = 36$ (6 WAT), $n = 24$ (11 WAT) and $n = 12$ (14 WAT).

performance of the plant (AGBEDE, 2010). The additional nutrients from the fertilizer help the root penetrate deeper in the soil (MEMON et al., 2013; AHMAD et al., 2010).

The mean root biomass in the control and solely tillage treatments at 6 WAT and 14 WAT was greatest at a soil depth of 25–50 cm and 75–100 cm, respectively. Improving soil physical properties causes root growth to strongly react to the soil environment, resulting in plasticity in the size and form of the root system (SCARPARE et al., 2019). The findings of the present study are in line with RAMIREZ-GARCIA et al. (2015), where the nitrogen within the soil, especially in the top layer, had no noticeable effect on the results due to harvests being performed during the

early development phase. Increased root mass is an essential plant response to the availability of nutrients, and the root mass fraction is crucial to the increase in total root length, especially of fine roots (BARGAZ et al., 2017).

The shoot biomass of the *F. moluccana* seedlings was slightly affected in the mixed treatment at 14 WAT. Shoot biomass is associated with root performance in the soil. Plant biomass has been shown to be most significantly affected by species identity and by fertilisation (CAHILL and LAMB, 2002). Changes in biomass allocation caused by a lack of below-ground resources, differences in light, soil nutrients and water may also contribute to this variation (ZHOU et al., 2018).

The SRL showed significant differences at the beginning of the harvest that were absent in the middle and final harvests. However, at 14 WAT, the highest SRL values were recorded under the fertiliser treatment even though there was only a slight difference under tillage with fertiliser treatment on the upper layer of soil. This is because fertiliser acts as additional nutrition (as it contains salts and other organic chemicals such as nitrogen (N), potassium (K) and phosphorus (P)) needed by plants when the soil cannot provide sufficient nutrients (ALNAAS et al., 2021)). According to a previous study by COOK and TRLICA (2016), fertiliser treatment contributes to raising the amount of organic matter (OM) primarily on the first layer of soil, and both tillage with fertiliser and solely tillage treatments were almost indistinguishable in this regard. Root proliferation occurs as soil is loosened by tilling action, and this soil loosening contributes to greater root presence in the top soil layer as long as there is adequate nutrient supply and water uptake (OGBAN and BABALOLA, 2009). Unlike the control treatment in the present study, eliminating the act of soil cultivation leads to drying and warming soil, encouraging the upper layer of soil to accumulate more organic carbon (KRASKA et al., 2021).

On the other hand, root washing causes damage and loss of fine roots, impacting the plants' ability to compete for resources and the ratio of root length, respectively (POIRIER et al., 2018; STOKES et al., 2009; ROUMET et al., 2016), which slightly affected the SRL and RLD obtained in this study. However, to overcome this problem, an alternative method (using a sieve) was applied. Briefly, washing roots involved running water over a fine mesh sieve (0.2–1 mm) gently to eliminate fine heavy particles like sand and coarse heavy particles like pebbles, and extracting debris with forceps to remove impurities of the same size and density as the roots of interest (PEREZ-HARGUINDEGUY et al., 2013). Despite taking these precautions, however, root loss of 20–40% may still occur (JUDD et al., 2015).

Based on the present study, tillage can be employed as a silvicultural technique without – or with less – fertiliser input to boost the root growth in deeper soil layers for soil resources in forest plantations, especially after soil degradation by logging activity or forest harvesting. Long-term and comprehensive research is essential – particularly in field conditions, where soil heterogeneity plays a more significant role – to better understand the root interactions of plant species under various environmental conditions.

Conclusion

The results indicate that the combination of tillage and fertiliser application promotes the root growth of *Falcataria moluccana* at the rhizotron scale. In terms of aboveground interactions, the shoot biomass in the mixed treatment at the final stage surpassed that of all other treatments. Long-term research on the root distribution of this species in actual field conditions is crucial. The findings from this study could provide valuable insights for future forest rehabilitation and appropriate silvicultural treatments.

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