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Original Article

Monitoring seasonal fine root dynamics of *Hevea brasiliensis* clone RRIM 600 in Southern Thailand using minirhizotron technique

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Abstract

Rubber tree (*Hevea brasiliensis*) plantations in various environments are affected by climate variability, and the fine root dynamics of rubber trees significantly respond to environmental factors. Therefore, rubber clone RRIM 600 was used to investigate the fine root dynamics in southern Thailand using minirhizotron imaging at 0-60 cm soil depths from February 2014 to January 2015. Dynamics of fine root production and mortality were greater in the rainy season than in the dry season. The fine root distribution peaked around 20-30 cm and decreased over 40-60 cm soil depths. The maximal leaf area index (April 2014) was found 2 months after leaf flushing, while the fine roots showed a slight increase after leaf development for 1 month (May 2014). Both rainfall and soil moisture had significant (p<0.01) positive correlations with fine roots of which there were more in the rainy season than in the dry season.

Keywords: RRIM 600, fine root dynamics, minirhizotron, *Hevea brasiliensis*, season

1. Introduction

Rubber tree (*Hevea brasiliensis*) is a well-known important source of natural rubber latex, used as a raw material for example by the vehicle industry. Over the last decades, the increasing demand for natural rubber has led to an expansion of rubber plantations that increase rapidly in South-East Asia (Fox, Castella, Ziegler, & Westley, 2014). The global consumption of natural rubber is expected for continue growth with rising prices in the future. However, the rubber plantations in sub-optimal regions with non-traditional cultivation areas (drier and colder) suffer from climate effects on growth and latex yield (Carr, 2012) causing slow girth increment and delayed open tapping (Priyadarshan *et al.*, 2005). There is potential to improve the productivity of low yielding rubber plantations (Warren-Thomas, Dolman, & Edwards, 2015).

In Thailand, the leading producer of natural rubber, the rubber plantations are expanding to higher elevations and dry areas where yields are affected by environmental conditions. Reducing the environmental impacts on rubber trees would improve the efficiency of rubber production. Understanding of the role and functional root traits of a plant is highly significant, also as regards the whole ecosystem. The mechanisms controlling fine root dynamics, e.g., root production and root mortality, remain to be examined for rubber trees.

Fine-root production is well-known to indicate terrestrial net primary production (McCormack *et al.*, 2015). The patterns of fine root dynamics in various plant species are associated with the above and below ground parts, and with the whole-plant traits (Withington, Reich, Oleksyn, & Eissenstat, 2006). During a rubber tree's lifespan, the development of fine roots fluctuates due to varying environ-mental conditions. Vulnerability to environmental stresses affects fine root production throughout the growing season.

The study of fine root dynamics in a rubber plantation by Gonkhamdee, Maeght, Do, and Pierret (2009) indicates that fine root growth peaks at soil depths 0-150 cm in May and June. Chairungsee *et al.* (2013) used field rhizotron to estimate the effects of seasonality on fine root dynamics in a mature rubber plantation, and strong

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stimulation of fine root growth by rain was found with the first rainfall. Also, the fine root growth was depressed by tapping to harvest latex, demonstrating that tapping disturbed the carbon dynamics in the whole tree.

Special techniques are required to investigate the long-term root dynamics hidden in the soil and involving complex mechanisms (Ephrath & Eizenberg, 2010). Non-destructive minirhizotron technique is widely used because of its minimal soil disturbance compared with traditional methods, such as soil coring or trench profiling (Tingey, Phillips, & Johnson, 2000). Moreover, minirhizotron technique provides more realistic data on the fine-root dynamics than sequential soil coring, as it outputs camera images (Johnson, Tingey, Phillips, & Storm, 2001) or scanner images (Villordon, Labonte, & Solis, 2011) that can be subjected to appropriate image analysis.

For sustainable modern rubber cultivation, understanding the fine-root dynamics is important for improving agricultural management (timing of fertilization and irrigation). Therefore, seasonal fine-root dynamics were investigated as related to soil environmental conditions, leaf phenology and latex harvesting.

2. Materials and Methods

2.1 Experimental sites and growth environments

A field trial was conducted at a farmer's plantation located at Ban Rai Ooi (6° 59' N, 100° 22' E), Chalung, Hat Yai district, Songkhla province, southern Thailand. The plantation of rubber clones RRIM 600 (3x7 m spacing) was 16 years old and had been tapped for 9 years. The soil texture of the plantation area was clay loam. Mean contents of clay, silt and sand varied from 37.9, 37.7 and 24.3 % at 0-30 cm soil depth, respectively, to 40.6, 37.5 and 21.8 % at 60-100 m soil depth.

Year 2014 had a strictly dry season during February-April 2014, while May 2014-January 2015 was a rainy season (sourced from Thai Meteorological Department). Fertilizer application and weed management were performed following the instructions from the Rubber Authority of Thailand (RAOT).

2.2 Minirhizotron measurement

A minirhizotron was used for monitoring rubber fine root growth, and six acrylic tubes (1000 mm length, 100 mm diameter, and sealed at the bottom) were installed, and then each tube was covered with a PVC (polyvinyl chloride) cap, and layers of black plastic bags were wrapped on top to prevent water from running into the tubes and to protect against penetration of sunlight into the tubes (Taylor, Upchurch, & McMichael, 1990). The minirhizotron tubes were installed in October 2013 at 45° angles and at 1 m distances on soil surface from the rubber tree bases (2 tubes per tree) (Bragg, Govi, & Cannell, 1983). For each tree two minirhizotrons were installed on opposite sides of the tree, in north and west directions from it. Measurements were done in January 2014 – January 2015 (recorded at 30-day intervals), while after installation 2 months were allowed for root re-equilibration with the minirhizotron installation and to improve soil-tube contact surface (Nawong, 2015).

Images were recorded by a custom-made mini-rhizotron camera assembled using a high-quality web camera (Logitech HD C905) with macro focusing feature. The camera module was mounted on a ball screw driven shaft and supported with linear slide ball bearing shaft, stepping motor driver and remote controller, for precise control of camera movement inside a minirhizotron tube. Image calibration used a photo of graph paper placed on minirhizotron surface, taken with the minirhizotron camera before start of actual image recording. The camera was operated via a USB cable connected to a laptop. Digital root images had 640 x 480 pixel resolution. Images (frame 35 x 50 mm) were taken at 24 locations for a total area of 42,000 mm^2 per tube .

All the root images were processed using Rootfly (Clemson University, Clemson, SC, USA) software with manual tracing, and outputting the root length per area. Changes in the fine roots by season were assessed. New and existing roots in each image were monitored every month. The validity of fine root production and mortality were evaluated as described by Hendrick and Pregitzer (1993): the white and light-yellow roots were considered live roots, while the dark brown and black were considered dead.

2.3 Soil moisture and leaf area index

Soil moisture content was measured monthly (January 2014 - January 2015) at 10 - 60 cm depths using a soil profile probe; PR2 (Delta-T Devices, England). Leaf area index was estimated using the hemispherical photography taken with a Nikon Coolpix 8400 camera with a Nikon FC-E8 fish-eye lens, with the images captured vertically upward from beneath the canopies (6 images per month). A calibration was conducted with the equation proposed by Sopharat and Sdoodee (2008). All the pictures were analyzed using the GLA software (Frazer, Canham, & Lertzman, 1999). LAI variations were observed once a month at shedding and flushing periods during the growing season.

2.4 Rubber production

Percentage of dry rubber content (%DRC) and dry rubber yield (gram per tree per tapping: g/t/t) were calculated from latex yield on every tapping day during 1. January 2014 – 3. February 2014 and 26. March 2014 - January 2015 (204 tapping days). The tapping system was a third spiral and downward cut (S/3, d/2).

2.5 Statistical analysis

The data were collected using Microsoft Excel to examine seasonal effects on the appearance of live roots and on fine root distribution at different soil depths. Pearson correlation coefficients were analyzed.

3. Results

3.1 Climatic data and soil moisture

Rainfall distribution in the dry season was monitored in February-April 2014 and for rainy season it was monitored in May 2014-January 2015. The total cumulative rainfalls during dry and rainy seasons were 108 and 1658 mm, respectively, and the maximum monthly rainfall occurred in December 2014 (685 mm). Also, the numbers of the rainy days in the dry and rainy seasons were 13 and 146 days, respectively. The daily evapotranspiration was the highest in March 2014 (6.64 mm) and gradually decreased until December 2014 (2.61 mm) (Figure 1a). Volumetric soil moisture content measured at 10, 20, 30, 40, 60, and 100 cm soil depths varied within 11-52 % during the experimental period. Soil moisture content at 10 cm depth had large fluctuations, more so than at the lower depths with less soil moisture during the dry season (February-April 2014), and the summer rainfall occurred in late April 2014 increasing the soil water in May 2014. However, decrease of soil moisture appeared during June-July 2014 due to low rainfall. In August 2014 soil moisture content gradually increased until the end of the study in December 2014. However, at 60 and 100 cm soil depths, soil moisture content reached its highest values (25-52 % vol) in the late rainy season (November-December 2014) (Figure 1b).

3.2 Leaf area index (LAI) and total fine root length density

LAI observations showed annual defoliation in late January-February 2014, followed by flushing of new leaves in March 2014 until the maximum LAI in April 2014. However, the LAI slightly decreased from September 2014 to December 2014 (3.40 to 2.40). The total fine root length density (fine root production and mortality) (mm mm^{-2}) monitored by minirhizotron imaging showed constant fine roots over the three months (January 2014 to March 2014) in the beginning of this study, and a slight increase to the first peaking stage (1.65 mm mm^{-2}) in May 2014 that was 1 month later than the maximum LAI. Then, the total fine root length density decreased again in June 2014, followed by increase to the maximum total fine root length density (3.25 mm mm^{-2}) in October 2014. Then it gradually decreased to the end of December 2014 (Figure 2).

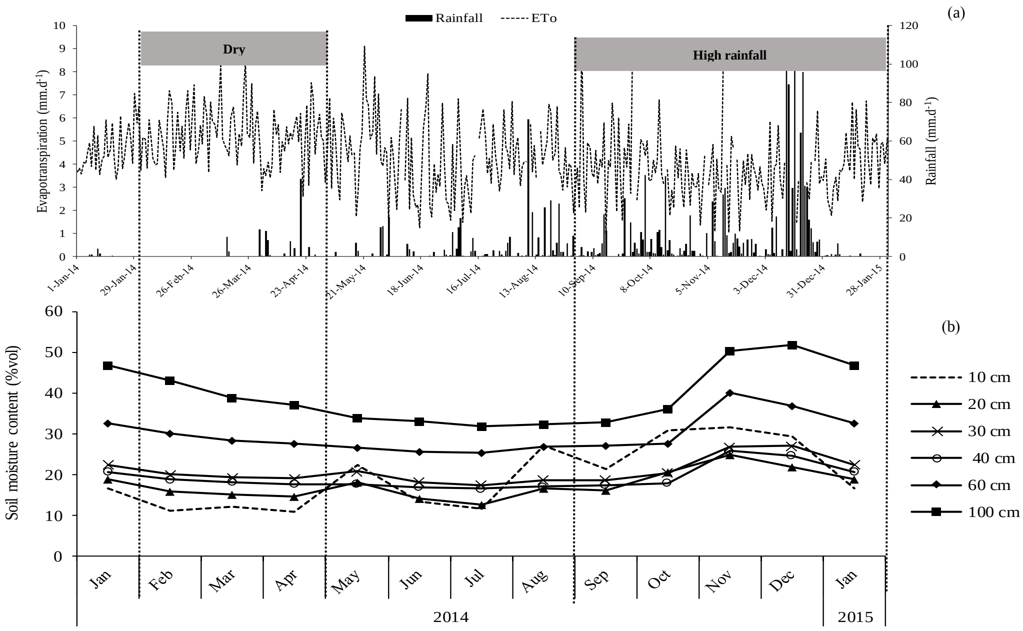

Figure 1. Seasonality in time traces of daily rainfall (mm d^{-1}) and daily evapotranspiration; ETo (mm d^{-1}) (a), and (b) soil moisture content (% vol) measured at 10,20,30,40, 60, and 100 cm depths during January 2014-January 2015. Vertical dashed line separates dry season from the high rainfall period.

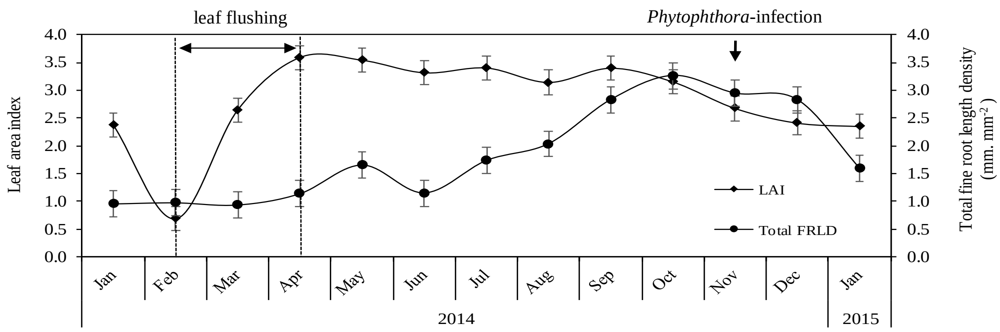

Figure 2. Variations of leaf area index estimated from hemispherical photography, and monthly means of total fine root length density monitored during January 2014-January 2015. Leaf flushing stage occurred during February 2014-April 2014, and *Phytophthora*-infection was found in September 2014.

3.3 Seasonal fine root dynamics

The monthly fine root production was greater in the rainy season (May 2014-December 2014) than in the dry season (February 2014-April 2014). Fine root production increased after the summer rainfall in late April 2014 at 10-40 cm soil depths, whereas the fine root production decreased in June 2014. Subsequently, the fine root production resumed quickly in July 2014 and showed similar pattern at all soil depths. However, in October 2014 the fine root production at 20-30 cm reached its maximum (0.96 and 0.86 mm mm^{-2}) at the soil surface layer (10-20 cm). Fine root production gradually decreased until the end of observations in January 2015 (0.06 and 0.38 mm mm^{-2}) (Figure 3a).

In addition, the dry season had very low of monthly fine root mortality until June 2014. The major fine root mortality was found at 30 cm soil depth in August 2014 (0.13 mm mm^{-2}), with high appearance of root senescence and subsequently the highest root mortality. Therefore, in September 2014-December 2014, the fine root mortality at 20-50 cm depth with slight increase until the end of this study (Figure 3b). Figure 4 shows that there was low total fine root length density in March, thus it tended to increase in June, leading to very high proliferation in October 2014.

Vertical total fine root length density at 20-30 cm soil depths was significantly higher than at other soil depths. The total fine root length density decreased with depth, except in 0-10 cm surface soil (Figure 5).

3.4 Latex yield and dry rubber content

Percentage of dry rubber content (%DRC) and rubber dry weight (g/tree/tapping) were recorded during January 2014-January 2015 as shown in Figure 6. The lowest approximately 25 g/tree/tapping was found in late March 2014 after re-opening tapping. From May 2014, rubber dry weight slowly increased until September 2014 and then fluctuated until the end of January 2015. In contrast, the percentage of dry rubber content did not show much fluctuations (an average 33.6 g/tree/tapping) over the year (Figure 6).

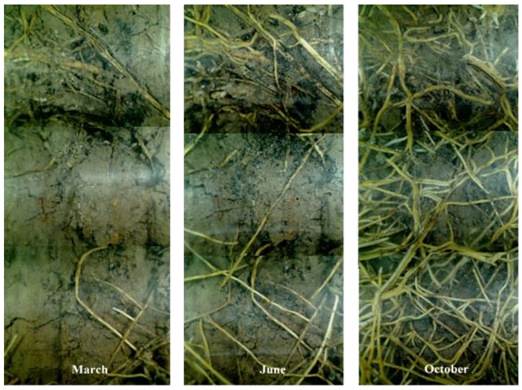

Figure 4. Minirhizotron images of rubber tree's fine roots. These images were captured at 10-20 cm soil depth in March, June and October 2014.

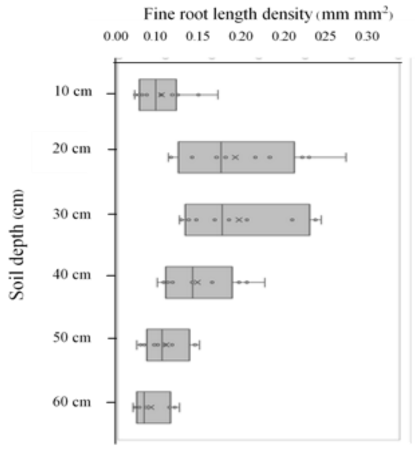

Figure 5. Box-whisker plot of fine root length density (mm.mm^{-2}) observed at 10 cm depth intervals (10-60 cm). The center vertical line in each box indicates the median value. Upper and lower edges (hinges) of the box indicate 25th and 75th percentiles. The whiskers extend to maximum and minimum values.

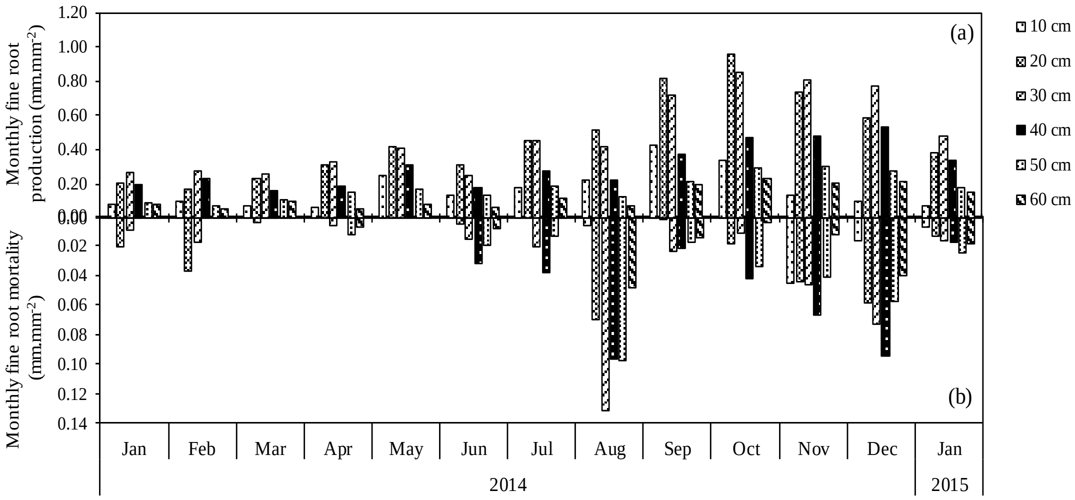

Figure 3. Monthly fine root production (a), and mortality (b) of rubber trees at 10-60 cm soil depth during January 2014 - January 2015.

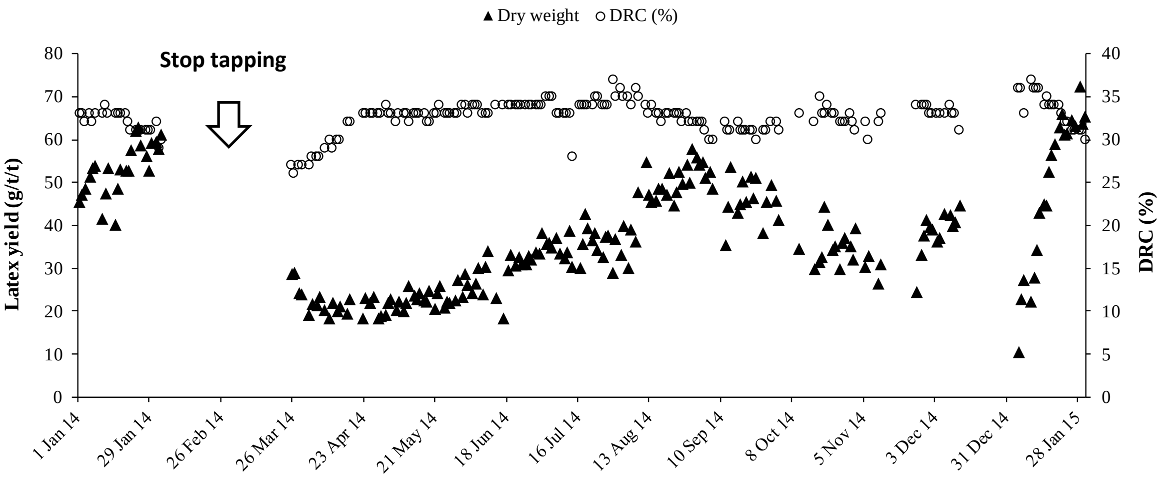

Figure 6. Dry rubber content (%) and latex yield (g/t/t) recorded during January 2014- January 2015.

3.5 Correlation coefficients among environmental parameters, latex yield, leaf area index, and fine root dynamics

Table 1 shows Pearson correlations among environmental conditions, dry rubber content, rubber dry weight, and fine root length density. The dates were grouped into dry and rainy seasons. In the dry season (February 2014-April 2014), rainfall, evapotranspiration, soil moisture, leaf area index, and rubber dry weight were not significant correlated with fine root dynamics, except that evapotranspiration had a negative correlation with the fine root dynamics at all 10-50 cm soil depths.

In addition, during the rainy season (May 2014-January 2015) soil moisture significantly correlated with the fine root dynamics at 30, 60 cm and at 40-50 cm soil depths. However, Pearson coefficients for all seasons showed that fine root dynamics at 40-60 cm soil depth, rainfall and soil moisture had a significant positive correlation. Evapotranspiration showed a negative correlation with fine root dynamics at 20-60 cm soil depth. Nevertheless, there was no correlation between fine root dynamics, latex yield, dry rubber content, and leaf area index.

4. Discussion

4.1 Seasonal fine root dynamics

Rainfall and evapotranspiration during the study indicated that the dry season occurred between February 2014 to April 2014, with the accumulated rainfall less than in the previous year (357 mm in 2013 and 109 mm in 2014). Normally, southern Thailand experiences the Southeast Asian monsoon with rainfall from April to December, as the monsoon is a large-scale seasonal reversal of the wind regime (Loo, Billa, & Singh,

2015). The production and mortality of fine roots tend to occur simultaneously in most woody ecosystems (Dilustro, Day, Drake, & Hinkle, 2002). Fine root growth in the dry season was below that in the rainy season, because low amounts of soil water incurred poor nutrient availability.

Similarly, Jiménez, Moreno, Nuela, Pat No, and Lloyd (2009) reported that the rate of fine root growth was low during the dry season and increased during the rainy season, and this might especially impact the fine root production in forest ecosystems. Moreover, fine root production and mortality were greater in the rainy season than in the dry season. This indicates that the fine root dynamics might be dominantly controlled by soil water content and other endogenous factors (carbohydrate supplies and phenological signals) (Misson *et al.*, 2006).

During summer rainfall, the top soil had the greatest changes in moisture content, simultaneously with the leaf re-foliation inducing a strong increase of total fine root length. The increase of fine roots in shallow depth soil was stimulated by summer rainfall, which substantially influenced the total fine root length. In the rainy season, high frequency of rainfall decreased the leaf area index due to *phytophthora*-infections affecting dry matter production, thus reducing growth and causing low productivity.

Besides, decreased leaf area index reduces radiation interception capacity that affects dry matter and latex production (Leong, Leong, & Yoon, 1982). When fine root length in the subsoil started to increase, it was supported by carbohydrate reserve (Silpi *et al.*, 2007) that played a role as the major source for production of new leaves and latex, particularly during leaf shedding and flushing. The highest fine root production in this study was found at 20-30 cm soil depth, consistent with what has been reported previously. Maeght *et al.* (2015) found the highest rates of root emergence about 3 months after onset of the rainy season, in the first two meters of soil profile and particularly in the shallow depths. In the dry period, summer rainfall occurred in April 2014 and the fine root production was simultaneous, along with new-leaf flushing.

The results show that seasons and soil moisture affect live and dead root dynamics. The appearance of dead roots was greatest during the rainy season, higher than in the dry season. The reason for this pattern seems to be related to the internal cycling of carbohydrates and nutrients, whereas tree age could affect root longevity or root turnover during their seasonal variations. However, this study did not assess

Table 1. Correlation coefficients (Pearson) of climatic parameters, experimental site parameters, and rubber tree characteristics related to fine root length density (FRLD) from soil surface to 60 cm depth during February 2014-January 2015.

Dry season	FRLD10	FRLD20	FRLD30	FRLD40	FRLD50	FRLD60
Rainfall (mm)	-0.506	0.660	-0.660	-0.720	0.790	0.660
ETo (mm)	0.900	-0.801	0.801	0.749	-0.674	-0.801
Soil moisture (%vol)	0.233	-0.413	0.413	0.486	-0.578	-0.413
Dry weight (g/t/t)	0.225	-0.405	0.405	0.479	-0.571	-0.405
(%) DRC	-0.972	0.910	-0.910	-0.873	0.815	0.910
LAI	-0.133	0.318	-0.318	-0.395	0.491	0.318
Rainy season						
Rainfall (mm)	0.002	0.546	.697*	0.703*	0.762*	0.633
ETo (mm)	-0.480	-0.759*	-0.742*	-0.734*	-0.711*	-0.690*
Soil moisture (%vol)	0.323	0.868**	0.876**	0.760*	0.884**	0.814**
Dry weight (g/t/t)	-0.018	0.239	0.389	0.464	0.423	0.378
(%) DRC	-0.337	-0.387	-0.269	-0.033	-0.217	-0.147
LAI	-0.070	-0.691*	-0.875**	-0.962**	-0.940**	-0.876**
All seasons						
Rainfall (mm)	0.226	0.654*	0.757**	0.754**	0.792**	0.705*
ETo (mm)	-0.626*	-0.839**	-0.796**	-0.765**	-0.839**	-0.745**
Soil moisture (%vol)	0.419	0.824**	0.854**	0.770**	0.801**	0.814**
Dry weight (g/t/t)	0.061	0.213	0.321	0.390	0.288	0.279
(%) DRC	0.310	0.429	0.383	0.411	0.552	0.370
LAI	0.181	0.015	-0.115	-0.190	-0.002	-0.153

* Correlation is significant at $p<0.05$
** Correlation is significant at $p<0.01$

details of carbohydrate allocation, which relates to canopy phenology and likewise to fine root dynamics (Righi & Bernardes, 2008).

Other factors associated with fine root functioning could include deep roots traits that can influence shallow fine root growth (Pierret *et al.*, 2016). Kuruppuarachchi, Seneviratne, and Madurapperuma (2013) reported that an increased production of fine roots and a simultaneous leaf flush on the canopy with a green-up and fine root growth during the dry season generally allowed the trees to absorb more water under water-stress. Furthermore, rainfall and temperature are among climatic factors that might affect fine root production in different ecosystems.

4.2 Minirhizotron approaches

The results show that monitoring fine roots in a mature rubber plantation by minirhizotron imaging is suitable for a long-term field study. Non-destructive observations by minirhizotrons are widely accepted for continuous root tracking over their lifespan and permits observing fine root production, mortality, and turnover. Moreover, the minirhizotron technique is less labor intensive than conventional soil coring.

Results of this study suggest that an optimum distance for minirhizotron tube setup in not less than 1 m from the rubber tree. Whenever the minirhizotron setup is at an angle, care is needed to avoid gaps in soil-tube contact that might cause underestimating roots in the upper soil layer. Therefore, recording root images can be started a few months after minirhizotron tube installation, depending on the experimental conditions of each case. For further study, it is suggested that multiple years of root observations under field conditions will be needed to investigate the patterns in root dynamics in combination with plant carbon balance and climatic conditions, especially the rainfall patterns.

5. Conclusions

Monitoring seasonal fine root dynamics using minirhizotron technique, it was found that fine root production gradually increased after the onset of rainy season, peaking in shallow soil depths (20-30 cm). High fine root mortality occurred during the rainy season at 20-60 cm soil depth. Fine roots at deeper soil depths (40-60 cm) showed less fluctuations than at soil surface because of adequate soil water content. This preliminary result indicates that responses in fine root dynamics were induced by climatic factors. The complex interactions between latex yield and fine root dynamics are crucial for agriculture applications. Also, studies on seasonal carbon partitioning between above ground and below ground need to be performed, to integrate managing the fine root system into sustainable rubber production.

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