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Soil microarthropods in reforested area of dipterocarpus seedlings at different stages

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Abstract : Early stages of reforestation are critical due to the low survivorship of seedlings, and inoculation with ectomycorrhiza fungi may improve the survivorship of the seedlings. Therefore, this study compared the diversity and abundance of soil microarthropods in 1-year and 2-year reforested areas planted with dipterocarpus seedlings and a non-reforested area in Chulalongkorn University Area, Saraburi Province, Thailand. Soil samples were collected monthly from October 2014 – October 2015. Soil microarthropods were extracted with Berlese-Tullgren funnels. Nine major groups of soil microarthropods were collected. Mites and collembolans were the most abundant soil microarthropods, with the highest abundance observed in the 2-year reforestation area, followed by the 1-year reforestation area and the non-reforested area, respectively. Soil moistures, soil temperatures, herbaceous cover and water holding capacity were significantly different among the reforestation and non-reforested areas.
Keywords: diversity, abundance, microarthropod, reforestation

Introduction

Most of soil microarthropods are detritivores, and therefore assist in decomposition of organic matter (Kardol *et al*., 2011) and can assist the movement of fungal spores through soil. Soil microarthropods was dominated by springtails and mites in several habitats, such as montane spruce forest (Jitka *et al*., 2014), hill evergreen forest, agricultural area (Cortet *et al*., 2002 and Tabaglio *et al*., 2009), and even green roofs (Heather and Alan, 2013). They are commonly found in fertile soils, so they can be used as a biological indicator for soil quality and changes of reforested area. At present, deforestation in Thailand has continually deteriorated soil ecosystems. We hypothesized that diversity and abundance of soil microarthropods increased with increasing tree age. Chulalongkorn University began a reforestation effort using dipterocarpus seedlings inoculated with ectomycorrhizae to promote the growth and survivability of tree seedlings at Chulalongkorn University Area, Kangkhoi District, Saraburi Province in 2012. However, the effects of dipterocarpus seedlings inoculated with ectomycorrhizae on soil microarthropods have not been assessed.

The aims of this study were to evaluate the diversity and abundance of soil microarthropods in reforested area of dipterocarpus seedlings inoculated with ectomycorrhizae at different stages and to study the relationships of physical and biological factors with the diversity and abundant of soil microarthropods. This study will help in monitoring, planning, and managing, and recovery of forest ecosystems.

Methodology

1. Study sites

The study was conducted at the Chulalongkorn University Center of Learning Network for the Region (CU-CLNR), Kangkhoi District, Saraburi Province. The study area was divided into non-reforested area, 1-year reforested area (planted in 2013), and 2-years reforested area (planted in 2012) with two replicates per each treatment. The pond rim was then plug-planted with Dipterocarp seedlings, usually a mixture of *Hopea odorata*, *Dipterocapus alatus*, *Shorea roxburghii*, *Shorea obtuse* and *Shorea siamensis*, in proportions of approximately 3: 8:

2: 2: 3, respectively. The seedlings were inoculated with ectomycorrhizal fungi of mixed species, such as *Russula* spp., *Lactarius* sp. and possibly others from the local soils. Soil sample was obtained from pond excavation during which 3 meter depth of soil was inverted.

2. Sampling

Six 1-m^2 quadrats were placed at random in each plot. Soil samples were taken from 20×20×10 cm^3 subplots within the quadrats (Figure 1). Samples were collected once a month for 13 months from October 2014 to October 2015. The soil samples were weighed to determine wet weight, and microarthropods were extracted with Berlese-Tullgren funnels for three days (Sakchoowong *et al*., 2007) and were stored in 70% ethanol until sorted and identified to major groups (Class, Subclass or Order) and morphospecies (Borror *et al*., 1976 and Dindal, 1990).

3. Biological factors

Percent herbaceous cover was estimated by eye with the aid of 1×1 m^2 quadrat (Braun-Blanquet, 1932 and Heather and Alan, 2013). Additionally, the aboveground cover herbaceous material was collected from 0.25×0.25 m^2 subplot and oven-dried to measure biomass. Organic matter was oven-dried and weighed (Zhao, 2008).

4. Physical factors

Soil temperatures were measured during monthly samplings using a thermometer placed at 10 cm depth in the soils. Soil moisture was measured by drying 100 g of the soil samples at 105 ± 5 °C for 24 hours (Zhao, 2008 and Jitka *et al*., 2014). Water holding capacities (WHC) of the soils were also tested. Soil pH was measured by mixing soil samples with distilled water at 1:1 ratio and tested with pH meter. In addition, dried soil samples 200g were mixed with distilled water in 1,000 ml cylinder and the thickness of layer precipitated was measured for soil texture (Jinu Eo, 2008 and Zhao, 2008). Monthly average rainfalls were obtained from the Office of Hydrology Irrigation Center for Central Region-RID. Furthermore, the soil samples were sent two times in wet and dry seasons to Central Laboratory and Greenhouse Complex (Kasetsart University Kamphaeng Saen Campus) for nitrogen (KCl extraction and Distillation), phosphorus (Bray II extraction and Spectroscopy) and potassium (NH_4OAc extraction and Atomic spectroscopy).

5. Statistical analysis

All statistical tests were performed in SPSS 17.0. Differences between biological and physical factors in the 3 study areas were tested using one-way ANOVA. Relationships between microarthropods with the biological and physical factors were examined using Pearson's correlation. Diversity was measured and compared using the Shannon-Wiener index, Simpson's index, evenness index, species richness (Margalef Index) (Krebs, 1999) based on morphological species.

Results and Discussion

1. Biological and physical factors

The soil texture was classified as sandy loam in all the three areas. There was no significant difference of soil composition among all the study areas with the range of 30–50 % sand, 30–55 % silt and 7–19 % clay (Table1). Soil moisture was significantly different between the three areas whereas soil temperature was significantly different between the non-reforested and the 1-year dipterocarpus reforestation areas with the 2-year dipterocarpus reforestation areas. Moreover, plant biomass estimates were significantly different between the non-reforested and 2-year dipterocarpus reforestation areas, but the plant cover, organic matter, available N, P and K were not

significantly different between the three areas. In addition, phosphorus levels were lower in the 2-year dipterocarpus reforestation areas, possibly due to the phosphorus uptake in the seedlings were also improved by ECM infection (Yazid *et al*., 1994).

2. Soil microarthropods

Soil microarthropods were collected monthly from October 2014 – October 2015 belonged to 9 major groups (Figure 2). Thirty, thirty-one and thirty–four morphospecies were found in the non-reforested area, the 1–year and the 2–year dipterocarpus reforestation areas, respectively. The total abundance of the soil microarthropods was highest in the 2–year dipterocarpus reforestation area (3,710 ± 486 ind./m^2), followed by the 1–year reforestation area (3,000 ± 336 ind./m^2) and the non-reforested area (2,557 ± 335 ind./m^2), respectively (Figure 3). However, the abundance of the soil microarthropods significantly differed between the 2–year dipterocarpus reforestation area and the non-reforested area (P=0.05). Acari were the most abundant groups in the three areas, followed by Collembola. Symphyla, Protura, Diplura, Araneae, Spirobolida, Geophilomorpha and Pseudoscorpionida had the lowest abundance, which was 0.01 to 2.2 % of the total abundance. Furthermore, the abundance of Protura, Symphyla, Spirobolida and Geophilomorpha were highest in the dipterocarpus reforestation areas, while Pseudoscorpionida was highest in the non-reforested area (Figure 4).

Shannon's diversity index was highest in the 2-year dipterocarpus reforestation area (0.96±0.03) than in the non-reforested area (0.90±0.03), while 1–year dipterocarpus reforestation area had the lowest diversity (0.76±0.03) (Table 2). Shannon's diversity index was significantly different between the 1-year dipterocarpus reforestation area with the non-reforested and the 2–year dipterocarpus reforestation areas. Furthermore, the Simpson's diversity index, evenness index and morphospecies richness were not significantly different in all three areas. The high Sorensen's similarity index (based on morphospecies) (92 to 95%) showed that the soil microarthropods composition was very similar among the study areas. This might due to dispersal by wind, water and actively by migration (Ojala and Huhta, 2001) from the surrounding area.

3. Relationship between environmental factors and abundance of soil microarthropods

Soil microarthropods were positively correlated with soil moisture in the non-reforested area (r = 0.752, P-value = 0.003). Kardol *et al.* (2011) and Shao *et al.* (2015) reported that the high soil moisture supported the abundance of soil microarthropods. Acari and Collembola were the most abundant of soil microarthropods in this study. However, Collembola abundance was negatively reported to be affected by high temperature and low soil moisture (Heather and Alan, 2013). Furthermore, low abundance of Protura, Symphyla, Spirobolida and Geophilomorpha in the non-reforested area may be from low soil moisture and high soil temperature in the non-reforested area. Increasing age of the seedlings affected several environmental factors, particularly soil moisture and soil temperature, which affected in an increasing trend of soil microarthropods abundance. The growth of plants through increased canopy induced shifts in soil microarthropod abundance and other compositions, particularly soil moisture and soil temperature in the area.

Conclusion

The reforestation with dipterocarpus seedlings inoculated with EMC fungi had a higher abundance of soil microarthropods than the non-reforested area and showed significant difference among non-reforested and the dipterocarpus reforestation areas. The dominant groups were Acari and Collembola. Increasing age of seedling affected several environmental factors, particularly soil moisture and soil temperature, which affect in an increasing trend of soil microarthropods abundance.

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Table 1 Biological and physical factors (mean ± SE) measured in the dipterocarpus reforestation areas at the 1–year (RF1) and 2–year (RF2) stages and the non-reforested plot (RF0). ANOVA with LSD (parametric), same superscript letters means no significant difference at $p \leq 0.05$.

Factor (Mean ± SD)	Study sites		
	RF0	RF1	RF2
Soil texture	sandy loam	sandy loam	sandy loam
Sand (%)	50	45	39
Silt (%)	39	39	48
Clay (%)	11	16	13
Soil pH	6.4–7.2	6.7–7.1	6.7–7.1
Available N (mg/kg)	9.77 ± 1.3^{a}	11.55 ± 1.3^{a}	9.77 ± 1.3^{a}
Available P (mg/kg)	6.3 ± 2.32^{a}	9.67 ± 2.19^{a}	5.62 ± 1.19^{a}
Available K (mg/kg)	164.73 ± 14.77^{a}	238.14 ± 12.42^{b}	197.45 ± 13.43^{a}
Soil temperature (°C)	29.78 ± 0.24^{a}	29.38 ± 0.32^{a}	29.02 ± 0.20^{b}
Soil moisture (%)	9.23 ± 0.43^{a}	12.06 ± 0.46^{b}	12.65 ± 0.57^{c}
Water holding capacity (%)	62.16 ± 1.10^{a}	59.41 ± 0.79^{b}	59.36 ± 1.47^{b}
Soil organic matter (%)	0.73 ± 0.14^{a}	0.85 ± 0.12^{a}	0.73 ± 0.22^{a}
Biomass of herbaceous (g/m^2)	14.46 ± 2.52^{a}	16.52 ± 2.24^{ab}	17.43 ± 2.11^{b}
Herbaceous cover (%)	49.55 ± 6.62^{a}	64.49 ± 5.50^{a}	55.16 ± 3.81^{a}

Table 2 Morphospecies richness (Margalef Index), Simpson diversity index, Shannon diversity index and Shannon-Wiener's Evenness Index of soil microarthropods communities in the dipterocarpus reforestation areas at the 1–year (RF1) and 2–year (RF2) stages and the non-reforested area (RF0). Same superscript letters mean no significant difference at $p \leq 0.05$.

	Richness	Simpson	Shannon	Evenness
RF0	2.14 ± 0.14^{a}	0.18 ± 0.01^{a}	0.90 ± 0.02^{a}	1.66 ± 0.41^{a}
RF1	2.37 ± 0.19^{a}	0.15 ± 0.01^{a}	0.76 ± 0.03^{b}	1.14 ± 0.17^{a}
RF2	2.33 ± 0.17^{a}	0.16 ± 0.01^{a}	0.96 ± 0.03^{a}	1.39 ± 0.16^{a}

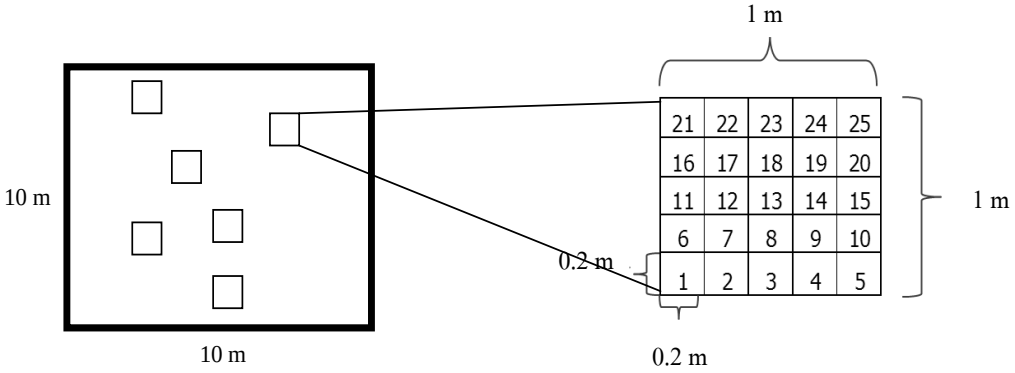

Figure 1 Sampling plots to take soil sample and factors

Figure 2 Subclass Acari (a), Order Collembola (b), Order Pseudoscorpionida (c), Order Diplura (d), Order Protura (e), Order Spirobolida (f), Order Araneae (g), Order Geophilomorpha (h) and Class Symphyla (i).

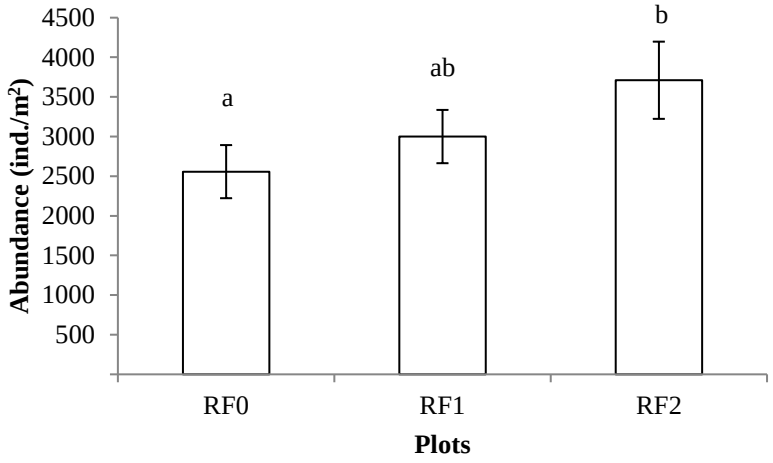

Figure 3 The abundance of soil microarthropods in the dipterocarpus reforestation areas at the 1–year (RF1) and 2–year (RF2) stages and the non-reforested area (RF0). Similar letter means not significant difference at $p < 0.05$.

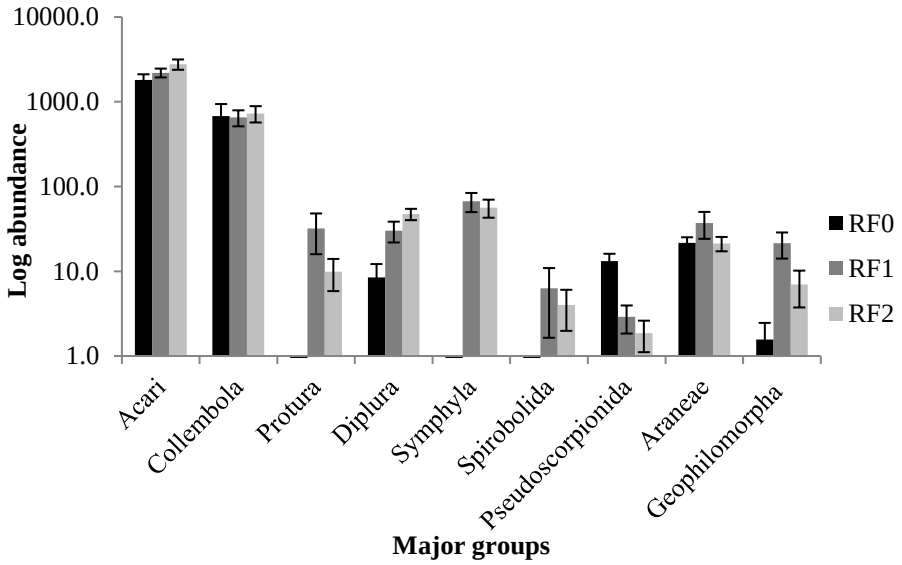

Figure 4 The abundance in each major group in the dipterocarpus reforestation areas at the 1–year (RF1) and 2–year (RF2) stages and the non-reforested area (RF0).