



## Soil-litter Collembolan Diversity in an Arabica Coffee-Benguet Pine-Based Agroforestry System

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### Abstract

*The study aimed to determine the effect of Arabica coffee-Benguet pine-based agroforestry system on soil-litter Collembolan diversity. Under this agroforestry system, two coffee production systems were identified in this study, the Agroforestry Coffee System (ACS) and the Lone Coffee System (LCS). The ACS had coffee plants growing under Benguet pine trees while the LCS had coffee plants that were not under any shade trees. Shannon diversity index ( $H'$ ), Margalef's richness index ( $D_{mg}$ ), Soil temperature ( $ST$ ), and Soil Moisture Content ( $SMC$ ) data were gathered and subjected to linear regression with correlation analysis, and student  $T$ -test. The result of this study revealed a higher species richness of Collembola under the ACS ( $D_{mg} = 3.52 \pm 0.47$ ) than the LCS ( $D_{mg} = 1.75 \pm 0.36$ ). Similarly, the ACS had higher diversity index ( $H' = 1.68 \pm 0.66$ ) than the LCS ( $H' = 0.90 \pm 0.49$ ). The ACS  $ST$  and  $SMC$  were  $18.60 \pm 0.21$  °C and  $68.34 \pm 12.22$ %, while LCS  $ST$  and  $SMC$  were  $21.24 \pm 1.31$  °C and  $55.38 \pm 5.52$ %.  $ST$  had significant negative association with diversity and  $SMC$  had positive association with diversity. However, only  $D_{mg}$  had significant correlation with  $SMC$ . In regression analysis, 18.7% of the total variation in  $H'$  was explained by  $ST$ . While for  $D_{mg}$ , 56.6% and 21.7% of its total variations were explained by  $ST$  and  $SMC$ , respectively. These results showed that the ACS can conserve Collembolan diversity because it creates a microclimatic condition favorable for the Collembolans. This finding could serve as basis for endeavors to promote and develop agroforestry systems.*

**Key Words:** agroforestry system, Arabica coffee, Collembolan diversity, soil moisture content, soil temperature

### Introduction

Soil-litter Collembola are small insects that play a crucial role in soil fertility; and in this era when we are facing climate change issues, their diversity is threatened. Henceforth, conserving them is needed or else, there would be an ecological imbalance which might affect us all. To conserve Collembolan diversity, the environmental conditions of their habitat should be maintained. One way to do this is to have an ecosystem that provides a stable microenvironment favorable to them. It is a common viewpoint that diversity is quite appreciable in forest ecosystems. Though maintaining forests is a good thing, it might not be economically sensible especially when it comes with the expense of food

and livelihood. Hence, it is wiser to look for candidate ecosystem in the agricultural sector. One suitable agroecosystem would be the agroforestry systems because it produces crops, provides livelihood, and retains its forest characteristics.

Collembolans are bioindicators of soil quality (Machado et al., 2019; Filho et al., 2016), land use intensification impacts (Ponge et al., 2003), and soil pollution (Liu et al., 2018; Fiera, 2009). It could also be a sentinel species of the soil fauna (Caro & O' Doherty, 1999; Gobat et al., 2004) which means, if the Collembolan diversity is threatened, the soil fauna community is threatened as well. Collembola and other soil fauna render important ecological services to the soil, particularly in soil fertility (Ertiban, 2019). Soil fertility involves complex ecological processes that are mediated by the soil organism like the soil-litter collembola. Their contribution to soil fertility maintenance is its involvement in organic matter decomposition specifically comminution and soil humification processes (Bagyaraj et al., 2016; Zimmer, 2002). In addition to that, many Collembolans graze on soil microbiota which stimulates microbial mineralization (Bayaraj et al., 2016) increasing mobilization of available nutrients such as calcium and nitrogen (Filser, 2002).

Agroforestry is a dynamic, ecologically-based, natural resource management system that integrates trees on farms and agricultural landscape, leading to diversified and sustainable production for increased social, economic, and environmental benefits for land users at all levels (Food and Agriculture Organization of the United Nations, 2015). Agroforestry is generally known to conserve a great deal of biodiversity (Bardhan et al., 2012; Udawatta et al., 2019; Vallejo- Ramos et al., 2016) because the system can buffer the extremities of the climate (Lin, 2007) that threatens biodiversity. In particular, this system may have the potential to conserve the diversity of soil-litter collembola. However, this generalization may only be applicable to above ground system when it comes to soil biodiversity because the system itself was mainly based on the above ground diversity of flora and fauna. Hence, to ascertain the effect of agroforestry on soil biodiversity, studies must be conducted. Therefore, this study was conducted to determine whether soil microclimate created by the agroforestry system components would be able to conserve the soil-litter Collembolan community living on it. The study was conducted in an Arabica coffee (*Coffea arabica* Linnaeus)-pine (*Pinus kesiya* Royle ex Gordon) agroforestry system in the mountain province of the Philippines.

## Materials and Methods

### Description of the Study Site

The study was conducted in an Arabica coffee plantation that is located at Bektey, Longlong, La Trinidad, Benguet, Philippines (16°26'42" N; 120°34'02" E). The coffee plantation is owned by Benguet State University (BSU) and is managed through its Institute of Highland Farming System and Agroforestry (IHFSA). Arabica coffee plants in the study are more than 43 years old, rejuvenated, and almost all of them are growing under shade by trees dominated by the Benguet pine trees. The climate in the area is rainy during May to October and dry for the rest of the year.

The study site was selected because most of the Arabica coffee plants in the province are growing under agroforestry systems, and it is important to understand their effect on soil-litter Collembolan community because these systems have low input of synthetic fertilizers, making the coffee plants rely mostly for their nutrition on the system's efficiency to cycle nutrients and be liberated for absorption of plants.

Since the study focused on the effect of microclimate on soil-litter Collembolan diversity, two Arabica coffee production systems were identified in the plantation. These were the Agroforestry Coffee System (ACS) and the Lone Coffee System (LCS). Coffee plants in the ACS are growing under shaded trees in a sloping terrain while for LCS, the coffee plants are not shaded and are growing in

terraces (Figures 1-2). Using the estimates of the Google earth pro software, the ACS lies in an elevation range from 1,436 to 1,484 meters above sea level, while the LCS lies within 1,401 to 1,434 meters above sea level.

**Figure 1**

*Agroforestry Coffee System Area Showing the Coffee Plants (Red Arrow) Growing Under the Benguet Pine Trees (Yellow Arrow)*



**Figure 2**

*Lone System Area (LCS) Showing the Coffee Plants (Red Arrows) Growing in the Terraces and Without Benguet Pine Trees Shading Them*



## Stratification of the Coffee Systems

In this study, the stratified sampling method (Leather & Watt, 2005; Naranjo, 2008) was utilized. The ACS and LCS were divided into three strata. To partition the ACS, a string measuring 33 m was set along the slope in a straight line then divided into three. The lower division of the slope was designated as lower stratum, while the middle and upper portions of the slopes were set as middle and upper strata, respectively. Slope of each stratum was measured using the level method (Critchley et al., 1991). The mean slope of lower stratum as measured was 40.52% or 22.03°, middle stratum was 72.73% or 35.77°, and the upper stratum 54.56% or 28.56°. For the LCS, it was stratified according to the terraces. The lower terrace of the LCS was set as the lower stratum, the higher terrace was the upper stratum, and the terraces in between the two was the middle stratum.

## Determining the Experimental Plot and Sampling Quadrats

In each stratum, a 100 m<sup>2</sup> experimental plot was determined (Rojas et al., 2009), and in each experimental plot, three sampling quadrats measuring 50 x 50 cm was randomly allocated (Dash & Dash, 2009).

## Sampling Method

Soil-litter Collembolan sampling was done once a month and it was done in October and November, 2018. A 50 x 50 x 5 cm metal frame was placed within the sampling area, and all litters within the frame were collected and contained in a black polyethylene bag. After that, the frame was inserted into the soil at five-centimeter depth (Palacios-Vargas et al., 2007; Bird et al., 2000) and those soils within the frame were collected using a self-lock plastic bag. Sealed and tagged soil and litter samples from each quadrat were brought to the laboratory as soon as possible for Collembola extraction.

## Extraction, Collection, and Identification of Collembola

Soil and litter samples were fed in the Berlese (Tullgren) funnels (Figure 3) separately to extract the Collembolans. In each funnel, a 40-watt light bulb (Auerbrach & Crossley Jr., 1960) was suspended 10 cm above (Da Silva Moco et al., 2009) to increase temperature within the samples. At the bottom, a vial containing 70% alcohol with a drop of glycerin was attached to collect the falling Collembolans. The samples were continuously exposed to the light for 48 hrs (Bano & Roy, 2016) and the Collembolans trapped in the vials were collected twice; first was after 24 hours of exposure to light and second was after 48 hours. The collembola individuals extracted from soil and litter samples from each quadrat were collected separately and stored in individual vials filled with 95% ethyl alcohol and a drop of glycerin.

For each vial, Collembola individuals were poured into petri dish, focused under dissecting and compound microscopes alternately to sort and group them based on their morphological similarities. After that, a representative of each group was taken and identified up to genus level using published taxonomical keys for Collembola.

Figure 3

*Berlese Funnels with Substrates Inside Lighted by a Bulb and the Bottom with the Catching Vials Filled with 70% Ethyl Alcohol and a Drop of Glycerin*



### Collection of Biological Samples

After the Collembolans were identified, their community abundance, relative abundance, and genera richness were determined. Community abundance referred to the total abundance of the Collembola individuals at each coffee system. Relative abundance referred to the population of the Collembola individuals per genus in each coffee system. Genera richness (*S*) referred to the total number of Collembola genera at each coffee system.

### Collection of Microclimatic Data

The microclimatic data gathered were soil temperature and soil moisture content (SMC) through the use of soil thermometer and gravimetric method (Johnson, 1962). They were taken on dates coinciding with the schedule for soil and litter sampling.

After the litters were collected from each quadrat, the soil temperature was taken at five-centimeter depth. A soil thermometer was inserted into the soil four times and each temperature reading was recorded then the mean temperature at each quadrat was computed.

The SMC of each quadrat was measured using the gravimetric method (Johnson, 1962). A soil core sample measuring 5 cm in diameter and 5 cm in length was taken beside each sampling quadrats. Using a 5 cm diameter by 8.5 cm length core sampling metal and rubber mallet, the soil core samples of each stratum were taken and placed immediately in individual soil moisture containers with a determined weights. After that, the fresh weight of the soil core samples was taken then oven dried at 100 to 110°C for 24 hours (Shepard & Addison, 2008; Black 1996). After oven drying, the dry weight of the soil core samples was taken and the SMC ratio of the soil sample were computed using the formula (Reynolds, 1970):

$$\%SoilMoistureContent = \frac{(FW - SMCW) - DW}{DW} \times 100$$

Where, FW is the fresh weight of the soil; SMCW is the soil moisture container weight; and DW is the dry weight of the soil after oven drying.

## Data Analysis

The taxonomic diversity and richness of the Collembola at each coffee production system was determined using the Margalef's species richness index ( $D_{mg}$ ), and Shannon diversity index ( $H'$ ). The formula of these diversity indices is presented in Table 1.

Means of the diversity indices ( $H'$ , and  $D_{mg}$ ) and microclimatic data from the ACS and LCS were compared through student T-test statistics. After that, the diversity indices and microclimatic data were subjected to Pearson production moment correlation and simple linear regression. All statistical analysis was done using the STAR statistical software created by the International Rice Research Institute (IRRI).

**Table 1**

*Formula of the Diversity Indices Used in the Study*

Diversity Index	Formula <sup>1</sup>	Reference
Margalef's species richness ( $D_{mg}$ )	$D_{mg} = \frac{(S - 1)}{\ln N}$	Mangurran (2004)
Shannon-Wiener index ( $H'$ )	$H' = -\sum p_i \ln p_i$ $p_i = \frac{n_i}{N}$	Gonçalves and Pereira (2012), Kinasih et al. (2016), Yeom and Kim (2018)

<sup>1</sup>  $S$  – total number of species;  $N$  – total population of those species in the community;  $p_i$  – proportion of the individuals belonging to the  $i^{th}$  genus of the total sample; and  $n_i$  – number of individuals in the  $i^{th}$  genus (Gonçalves & Pereira, 2012; Kinasih et al., 2016; Mangurran, 2004)

## Results and Discussion

The total number of genera collected from the study was 35, of that, 34 genera were present in the ACS, while only 23 genera were present in the LCS. Obtained  $H'$  and  $D_{mg}$  values were significantly higher in the ACS than in the LCS (Table 2). The statistical analysis of the soil temperature and SMC showed that the ACS has significantly lower soil temperature, and higher SMC compared to the LCS (Table 2). Results of the Pearson correlation showed that soil temperature had a negative linear association with the diversity indices, while SMC had a positive linear association. SMC had significant correlation with  $D_{mg}$  but had weak association with  $H'$  (Table 3). The obtained coefficient of determination showed that 18.72% of the total variation or changes in  $H'$  was explained by the regression model containing only soil temperature as the predictor variable. SMC almost did not account for any variation in  $H'$ . For  $D_{mg}$ , 56.78% and 21.78% of its total variations or changes were explained by soil temperature and SMC, respectively (Table 3).

**Table 2**

*Diversity Indices and Soil Microclimatic Conditions of the ACS and LCS*

Production System	$H'$ <sup>1</sup>	$D_{mg}$ <sup>1</sup>	Soil Temperature, °C	SMC, %
ACS	1.68±0.66 <sup>a</sup>	3.52±0.47 <sup>a</sup>	18.60±0.21 <sup>a</sup>	68.34±12.22 <sup>a</sup>
LCS	0.90±0.49 <sup>b</sup>	1.75±0.36 <sup>b</sup>	21.24±1.31 <sup>b</sup>	55.38±5.52 <sup>b</sup>

<sup>1</sup> Values are mean ± SD. Means in a column having the same letters of superscript are not significantly different at 5% level of significance using the T-test statistic

The higher diversity and genera richness in the ACS as shown by the diversity indices values implies that the system can conserve Collembolan community. This result conforms with the findings of Kinasih et al. (2016) and Sopsop and Lit (2015) that agroforestry systems conserves soil-litter arthropod diversity. The ACS can support Collembolan community because it creates a soil microclimatic condition that is favorable for the Collembola. The soil microclimatic analysis showed that the ACS has a cooler and more humid environment, and this tends to be favored by the Collembolan community as shown by the  $R^2$  values, and the trends of relationships, where, as the soil temperature decreases and SMC increases, the diversity of the Collembola increases. This favorable soil microclimatic condition in the ACS could be due to the shade trees, higher vegetation diversity, and more litter biomass. Trees are taller plants and with greater canopies that can affect the ground temperature (Myers-Smith & Hik, 2013; Martius et al., 2004). The study of Lozano-Parra, Pulido, Lozano-Fondon and Schnabel (2018) on the effects of vegetation cover on soil temperatures in the Drylands of Mediterranean Regions showed that daytime daily maximum average soil temperatures under tree canopies were 7.1 °C lower than the atmospheric temperature. On the other hand, soil temperatures in the grassland were 4.2 °C higher than in the air.

**Table 3**

Pearson Correlation Coefficient (R) Showing the Direction and Magnitude of Association Between the Diversity Indices ( $H'$ ,  $D_{mg}$ ) and Soil Microclimatic Elements; and Coefficient of Determination ( $R^2$ ) Showing How Much Variation in the Diversity Indices is Explained by the Soil Microclimatic Elements

Soil microclimatic Elements	$H'$		$D_{mg}$	
	R	$R^2$	R	$R^2$
Soil Temperature	-0.43*	18.72%*	-0.75*	56.78%*
SMC	0.07 <sup>ns</sup>	0.49% <sup>ns</sup>	0.47*	21.78%*

\*Significant at 5% level of significance using the T-test (for R), and ANOVA (for  $R^2$ ) statistics

<sup>ns</sup>Not significant at 5% level of significance using the T-test (for R), and ANOVA (for  $R^2$ ) statistics

Agroforestry systems could conserve a great amount of plant diversity. Bhagwat et al. (2008) reported that agroforestry systems can have species richness equivalent to more than 60% of that of the natural forests. Also, Vallejo-Ramos et al. (2016) found that 26% to 90% of wild species of plants could be conserved by agroforestry systems. In the ACS, more species of plants were present as compared to the LCS. This could be due to systems microenvironment and the production management practices where interventions in the ACS are lesser compared to the LCS. There was lesser plant diversity in the LCS because while the area is producing coffee, it is also used for production of citrus and semiannual crops where the production practices include vegetation-decreasing methods such as weeding, tilling, as well as application of synthetic and organic fertilizer and pesticides. Greater plant diversity could have been the reason for the more abundant litter biomass observed in the ACS because diversity provide more source of litter biomass that has off-varying decomposition rates. Quantity of litter biomass in agroforestry systems are second to forests and higher than the litter biomass accumulated in monoculture systems (Tongkaemkaew et al., 2018; Hairiah et al., 2006; Schroth et al., 2002). Litter biomass is important in soil hydrology, because they catch and retain water from rainfall (Li et al 2018; Zhou et al 2018) then gradually release it to the soil. Moreover, litter biomass also cools soil temperature and reduces soil water evaporation (Xing et al., 2018; Facelli & Pickett, 1991).

## Conclusion

Agroforestry systems such as the Arabica coffee- Benguet pine agroforestry system can promote and maintain the species richness and diversity of soil-litter Collembola because agroforestry system presents a microenvironment favorable for the Collembola. This finding of the study could serve as basis and encouragement to government organizations, non-government organizations and/or enthusiasts who endeavors themselves in biodiversity conservation to promote and develop agroforestry systems because not only agroforestry systems help in global warming and climate change mitigation but also, it conserves the Collembola and other soil-litter fauna that plays a crucial role in nutrient cycling and soil humification. Maintaining the efficiency of Collembola and other soil organisms to render their ecological services is crucial to crops growing under agroforestry systems because inputs of synthetic fertilizers in agroforestry systems are low.

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