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Collembola Inoculation in Soil Incorporated Mango Leaf Litter Enhances Decomposition for Organic Matter Building and Nutrient Banking

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Abstract: ***Leaf litter accumulation in the understory of a mango orchard is a potential source of soil nutrients and organic matter, but farmers recognize it as waste material that needs to be discarded from the system. Most of the common management practices in dealing with such problem will be burning. This farmer's practice will not only cause environmental problems but also degrade the fertility condition of the soil. The purpose of this study is to provide an alternative agricultural practice in improving soil health condition that is both economical and sustainable. This can be done by enhancing the decomposition of mango leaf litter through soil micro-arthropod Collembola inoculation and incorporation of the leaf litter in the soil. It promotes physical decomposition of the leaf litter, further increasing the accumulation of soil organic particulate matter and promoting nutrient banking. In this study, mass loss of the decomposing mango leaf litter, soil organic matter addition, and N, P, K soil nutrient banking were determined through litterbag experiment in the microcosm environment. Treatments include non-inoculated and non-incorporated leaf litter (T1), non-inoculated but incorporated leaf litter (T2), inoculated but non-incorporated leaf litter (T3) and inoculated and incorporated leaf litter (T4). The results reveal that inoculation of Collembola enhances the decomposition of mango leaf litter by 56% to 70% (mass reduction) which promotes the soil organic matter accumulation. Soil nutrient banking is also promoted by increasing the nutrient accumulation by 29% higher for nitrogen and four times higher for potassium. Soil phosphorus, regardless of soil treatment facilitates nutrient banking by the nutrient releases of decomposing mango leaf litter. The study concludes that soil inoculation of Collembola enhances the decomposition of soil incorporated mango leaf litter by 40%.***

1. Introduction

Leaf litter fall in any woodland ecosystem is a phenomenon that opens the opportunity to bring nutrients taken up by the trees back to the soil (Ndakara, 2012). The decomposition of accumulated leaf litters in the soil is not only valuable for increasing the availability of nutrients but also for promoting the addition of soil organic matter (SOM) (Hasanuzzaman et al., 2014) hence, improving fertility status. The decomposition of plant litter, mineralization of nutrient-bound element in the organic matter and humus accumulation in the soil are significant and major ecological processes in sustaining and stabilizing the living component in an ecosystem (Berg and McClaugherty, 2008). Nutrient releases and organic matter addition in the soil pool through litter decomposition is governed by factors that directly affect the degradation process. These are climate, litter quality and soil fauna (Blair, 1988; Aerts, 1997; Xuluc-Tolos, 2003; Gonzales and Seastedt, 2001;). High concentration of lignin (Couteaux et al., 2006) and CN ratio (Yang et al., 2012) are litter components that contribute to low degradation rate. According to Cornwell et al. (2008), such litter traits control the decomposition rate within biomes. Nevertheless, Aerts(1997) stated that climate effect on litter quality and the decomposers in the decomposition is directly due to temperature and moisture. Also, litter decomposition undergoes a two-stage process (Aerts, 1997); the involvement of detritivores and the facilitation of soil microorganisms (fungi and bacteria). The physical decomposition of freshly added organic materials in the soil such as leaf litter is carried out by soil microarthropods such as mites and Collembola. These soil fauna is mainly responsible in the fragmentation of leaf litter which results the addition of particulate organic matter (POM) in the soil (Gonzales and Seastedt, 2001). The mineralization of nutrients from the chemically degrading POM is facilitated by soil microorganisms. Thus, leaf litter decomposition will promote addition of soil organic matter as well as nutrient banking that will sustain the fertility condition of the soil.

Amongthe most common and abundant soil microarthropod identified with leaf litters are the Colembolans (Fountain et al., 2005, and Chahartaghi et al., 2005). It has an average population density ranging from 10^4 to 10^5 individuals in a square meter area in most terrestrial ecosystem (Hopkins, 1997; Kampichler and Bruckner, 2009). Their population dominates in the soil (Hopkins, 1997). It feeds primarily on soil plant liters and microorganisms (Ruess et al., 2007) particularly on senescent fungal hyphae which contribute to the physical fragmentation of leaf litters. Thus, soil microarthropod Collembola function as the physical decomposers of soil accumulated plant litter (Illig et al., 2008) enhancing the decomposition process of soil organic debris, and its nutrient mineralization (Larsen, 2007).

The continuous incorporation of plant debris into the soil improves not only the physical property of the soil, such as structure and porosity, but also increases the food availability for microorganisms (Kautz et al., 2005). This makes the soil more conducive for the collembolans to increase their population. Thus, the abundance of their

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population in the soil is dependent primarily in the soil-litter cover.

In fruit tree plantation such as orchards, utilization of leaf litter fall as source of SOM and nutrients can improve the fertility status of the soil (Musvoto et al., 2000) creating an opportunity to increase soil productivity. However, in such ecosystem, burning of leaf litter understory is one of the most common sanitation practices to dispose plant waste material caused by high accumulation on the orchard floor (Curtis, 1924 as cited by Holb, 2006). Such management in the orchard plantation environment causes not only health problems (Sannigrahi, 2009) but also lead to the depletion of soil organic matter as well as nutrient losses in the soil (Graham et al., 2002). The application of commercial fertilizer materials may supply crop nutrient requirement to augment the inability of the soil, to provide plant nutrients. Nevertheless, nutrient augmentation through the application of commercial fertilizer is not only costly but it does not actually neither improve nor maintain the long term fertility status of the soil (Steiner et al., 2007). Approximately 40% of the cost of production in mango orchard is attributed to fertilizer inputs (Noor Mmemon, 2015) and continuous application of N-fertilizers can cause soil acidification (Guo et al., 2010), and atmospheric and underground water pollution specifically in the soil with low organic matter content (Yang et al., 2004; Zhu et al., 2005).

In the Philippines, mango production is considered one of the major sources of income for orchard growers. According to the 2011 report of the International Trade Center, Philippines ranked 6th among the mango exporting countries in the world with 4.2% share in world export amounting to USD 44, 000 in 2010. Mango is thereby considered as among the four major exporting fruit crops together with banana, pineapple and coffee in the country (Philippine Statistics Authority-SSA, 2014). As of 2014, mango orchard covers ~23% (187.8 ha.) of total land area for fruit crop production (Philippine Statistics Authority-SSA, 2014). This vast resource of leaf litter can be used to enhance organic matter accumulation in the soil and promoting nutrient banking. SOM enhancement in orchard floor management significantly contributes not only to the health condition of the trees (Rowley, 2011) but also for rehabilitating the soil fertility status for the benefit of intercropping (Agreda et al., 2006).

Despite the opportunity to increase organic matter content as well as to bring back nutrients in the soil, burning of leaf litter in the mango orchard is a common practice. The low decay rate under natural decomposition of mango leaf litter is due to its resistant chemical property which is high cellulose component and carbon to nitrogen ratio (Musvoto et al., 2000). Despite the low decay rate of mango leaf litter, contribution to enhance soil organic matter content is still highly significant (Mubarak et al., 2008 and Musvoto et al., 2000). The chalenges is to find means to enhance the decomposition rate of mango leaf litter that is both economical and with minimal impact to the environment.

Conceptualization of this study was drafted due to the fact that waste material such as leaf litter accumulation in the soil is an opportunity to enhance soil fertility of mango orchard system through enhanced decomposition strategy rather than it can cause sanitation problems. This experiment hypothesizes that the decomposition of accumulated soil leaf litter will be further enhanced by mechanical leaf fragmentation through soil incorporation by cultivation and inoculation of soil micro-arthropod *Collembola*. Specifically it aims to promote (i) the addition of SOM content by increasing the rate of decay by mechanical and biological fragmentation, and; (ii) nutrient mineralization during the early stages of the decomposition period (3 months) for soil nutrient banking.

The significance of this study is to provide environmentally sound agricultural practices particularly in waste disposal of accumulated leaf litter in the understory of the orchard floor that will improve soil fertility condition in agroforestry ecosystem particularly fruit tree orchards in the Philippines. Enhancing the decomposition of leaf litter rather than burning will effectively promote the accumulation of the organic matter as well as nutrient deposition in the soil. The output of this study is also significant in engaging into intercropping practices within orchard agroecosystems ensuring soil fertility condition and the sustainability of its productivity. Early releases of nutrients in the soil through mineralization from the decaying mango leaf litter can be utilized by intercrop which may provide additional income for orchard farmers as well as encourages green manuring for the fertility of the soil.

2. Materials and Methods

2.1 Experimental Design

The decomposition of mango leaf litter was quantified through litterbag experiment in soil microcosm setup. The isolated and combined effect of leaf litter soil incorporation and inoculation of microarthropod Collembola to enhance the decomposition of mango leaf litter was tested. Soil nutrient banking and organic matter addition through the decomposing leaf litter were also determined. Plant tissue and soil samples used were analysed for its nutrient content prior to experimentation. Soil microarthropod Collembola from the leaf litter and soil samples were extracted, identified and reared as an inoculants. The experiment evaluated four (4) treatments representing without soil inoculation of Collembola in non-incorporated leaf litter in the soil (T1) and incorporated leaf litter T2); and, with Collembola inoculation in non-incorporated leaf litter in the soil (T3) and incorporated leaf litter (T4). The decomposing leaf litter and the soil in microcosms in each treatments were analysed every after 14-days for the span of three (3) months. The treatments were replicated trice producing a total of 72 microcosms. The experiment was carried out using a Randomized Complete Block (RCB) design and treatment means were compared using unplanned multiple range test (DMTR).

2.2 Site Description

Soil and leaf litter samples are collected at the mango orchard project of the College of Agriculture, Central Luzon State University. The orchard project is in the middle of the extensive land area coverage of paddy rice production of the

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university. It is situated geographically 15° 44' 9.82" N latitude and 120° 56' 24.10" E longitude. Climate in this region is tropical mainly influenced by the southwest monsoon that has seasonal rainfall distribution occurs from May to October. The average annual temperature is 32 °C (max) and 23.2 32 °C (min) . May is the warmest month of the year and January is the coolest season averages to 29.8°C and 25.9°C, respectively. Mean annual precipitation is about 2,205.4 mm wherein most of it occurs during rainy season starting May to October (PAGASA-CLSU, 2017).

The orchard is established in a 90m x 60m land area with a planting distance of 7m x 10m which had a tree population of about 75 mango trees. The orchard is on its mature age wherein active fruit bearing stage started for more than a decade and still productive at present time. The soil type in this area is classified as Maligaya series (Ustic Epiaquaerts) with a clay loam texture (BSWM, 2010 and Phil Rice, 2008). Most of the soil understory is covered with fresh to merely decomposed leaf litter with a thickness of about 2 cm. However, most inter-row areas are covered with patches of grasses. The average annual leaf litter fall production of the orchard is 11.2 tons/ha/yr.

2.3 Soil and Leaf Litter Collection

Composite soil samples and leaf litter material were collected at the Mango Orchard project. Ten sampling points were selected randomly within the orchard using a $1m^2$ quadrat in every sampling point.

Freshly-fallen leaves which accumulated on the surface of the soil were collected and used in the litterbag while leaf litter samples that were currently decomposing were used for Collembola extraction using a Berlese funnel. The fresh senescent leaf litters were dry-cleaned using hand brush to remove soil particles and other materials that adhere to its surfaces. Samples were cut into 5cm x 5cm size and placed in litterbag with a mesh size of 2mm.

Soil samples, on the other hand, were obtained by scraping the upper 5cm of the soil column and were used in the microcosm experiment. Samples were air dried for 5 days and pulverized thereafter. Non-soil particles were discarded by sieving the soil samples in 2 mm sieve mesh. Processed soil samples were defaunated by freeze-thaw procedure (Yang et al., 2012). Soil defaunation was done to ensure the removal of the remaining soil fauna after air drying and sieving the samples.

2.4 Rearing of the Extracted *Collembola sp*.

The extracted specimens were collected using distilled water to preserve its living condition. Isolation of the Collembola species that was reared from the mixtures of extracted soil fauna was done by flotation technique (Hale, 1964). Manual separation and isolation was done using dissecting microscope. Selected and isolated Collembola specimen were transferred in the formulated food-based media composed of defaunated humus, ground charcoal and baker's yeast with the ratio of 2:1:1, respectively. The formulated media was based on the food preferences of Collembola (Lensing et al, 2005; Gonzales & Seastedt, 2001; Chahartaghi et al., 2005 and Yang et al., 2012). The effectivity of the formulated food-based media was tested by incubating the introduced Collembola specimens for 14 days. After the incubation period, Collembola introduced in the media proliferated. Population density of reared Collembola from the stock culture (food-based media) was determined

2.5 Soil Inoculation of *Collembola*

For the preparation of soil inoculants, approximately 15 grams from the stock culture (food-based media) was transferred in each of the 50 plastic cup container and incubated for another 7 days to determine the survival of the population of Collembola in the inoculum. At the end of the 7-day incubation period, average population density of *Collembola* in the inoculum was determined.

Inoculation of Collembola was done at once for all inoculated treated treatments. Each treated microcosms were inoculated by the number of population of Collembola grown per 15-gram of inoculant. Inoculation was done by extracting the Collembola from the prepared inoculant using floatation technique.

2.6 Litterbag in Soil Microcosms

A total of 72 soil microcosms were prepared and used in the study. A 15 cm tall plastic canister with a diameter size of 14 cm was used as soil microcosm. The top lid of the canisters was perforated and secured with fine mesh to facilitate aeration but also to isolate the Collembola and limit their movement out of the microcosms. Three hundred fifty gram of defaunated soil samples was transferred and contained in the soil microcosm. The weight of the soil sample used was calculated to simulate the weight of the soil in a hectare basis using the average and common soil bulk density of 1.33g/cc.

On the other hand, litterbag was constructed and fabricated using 2mm nylon mesh to contain the mango leaf litter samples. The diameter size of the litterbag is 13cm which is sufficiently enough to cover the entire surface of the soil in the microcosms. The pre-cut leaf litter samples were placed inside the fabricated litterbag at the rate of 3.7g/litterbag

2.7 Leaf Litter Decomposition

Prepared litterbag of mango leaf litter was then introduced into the soil microcosms following the different treatment formulation (Table 1). Leaf litter soil incorporation was done by covering the mango litterbag with the soil at a depth of 2cm while non-incorporated treatment, litterbag was just placed on top of the soil within the microcosm. For soil inoculation treatment, all microcosms with litterbags that fall within the treatment formulation were inoculated with *Collembola* from the prepared inocula.

The decomposition of mango leaf litter in the microcosms were subjected under ambient air condition in the laboratory. Incubation period lasted for 3 months but destructive sampling for the assessment was done every 14-days.

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The determination for mass loss of the incubated mango leaf litter in the litterbag-microcosm experiment was done every after destructive sampling for the duration of 84-day period of the decomposition study. Litterbags in each of the microcosms were removed and placed in the Berlese funnel for *Collembola* extraction procedure. After 24 hours of incubation in the Berlese funnel, mango leaf litter samples in the litterbag were submerged in distilled water for 1 hour to separate soil particles inside the litterbag. Leaf litter samples were air dried for 24 hours and then oven driedfor another 12 hour at 40 ^{o}C. After removing the samples from the analytical dryer, leaf litter samples were exposed again in ambient air condition for at least 2 hours before weighing the samples in the analytical balance.

Biomass of the samples from the previous analysis was deducted from the biomass obtained from the recent samples over its previous weight. Such procedure was used to calculate percent (%) mass loss of the mango leaf litter that undergoes decomposition process. The decay rate, however, was calculated using the single exponential decay function by Olson (1963) as cited by Mubarak et al., (2008)

3. Results and Discussion

Collembola Population. With the initial average population count of 127 (±16) Collembola in the inoculum, a significant change of population was observed in soil inoculated treatments during the 84-day period of decomposition (Figure 1). Non-inoculated treatments show no variation of *Collembola* population during the incubation period due to the effect of soil defaunation procedure. The increase of population of Collembola in inoculated microcosms is due to the proliferation of the initial population of the inoculum. The availability of food and the conduciveness of the environment for the completion of their life cycle cause the population to grow (Hopkins, 1997).

During the 84-day period of the decomposition of mango leaf litter, the population count of Collembola in inoculated treatments started to increase from day-14 to day -42 (T3 = 2,361 individuals and T4 = 2697 individuals). Thereafter, the population started to decrease until day-84 with population count of 967 individual for T3 and 1,229 individuals for T4.

Aside from the effect of temperature and moisture condition, the reproduction capability of Collembola based on their life cycle as well as the availability of food for their consumption are the most possible factors that affected population (Ferguson and Joly, 2002). However, with the increasing temperature and the decreasing moisture condition, Collembola population decreases. The increasing population of inoculated Collembola in the microcosm from the day of inoculation until reaching its peak population at day-42 is mainly attributed to the availability of food to sustain their life cycle. Since the experiment is conducted under laboratory condition, soil temperature and moisture does not have any significant variation. The average life span of tropical species of Collembolans, 87 days for males and 65 days for females, oviposition (egg laying) at 14 days after hatching (Mari-Mutt and Soto-Adames, 1987). The eggs of Collembola are not simultaneously hatched but take 16 days to maximize hatchings. Thus, with the ample food supply and the conduciveness of its environment, the population of inoculated Collembola peaked at 48 days after inoculation. With the continuous supply of food and without predators, the population of the inoculated Collembola is expected to increase. Nevertheless, their population started to decrease possibly due to reaching the unbalanced condition of population-to-food ratio, causing food limitation. According to Milton and Kaspari (2007), Collembola feeds from the senescent fungal hyphae that accumulate on the leaf litters. If the production of senescent fungal hyphae is outnumbered by the population increase of the inoculated Collembola, the food supply of the Collembola will be limited.

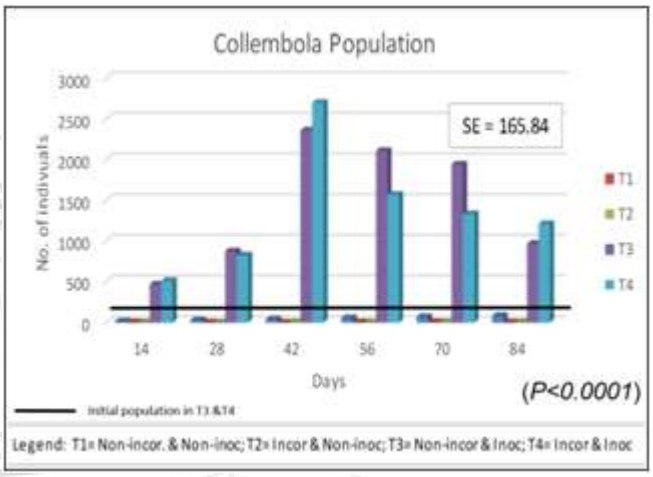

Figure 1: Population count of *Collembola* during the three month decomposition period of mango leaf litter

Mass Loss of Mango Leaf Litter. The percent mass loss of mango leaf litter in each treatment during the decomposition period is shown in Figure 2. Unincorporated leaf litter with inoculation has the highest mass loss percentage of 70% compared to other treatments; 56% for T4, 40% for T1 and 44% for T2.

Results also show that when mango leaf litter was incorporated into the soil, the physical decomposition of the substrate is reduced: 20% reduction in Collembola inoculated treatments (T3 and T4), and 9% reduction in non-inoculated treatments (T1 and T2). However, the 56% mass loss obtained from the treatment inoculated with Collembola but incorporated with mango leaf litter (T3) is 29% higher than the treatment with the same litter but uninoculated with Collembola (T2).

The significance of this result under field condition is that, ploughing the soil understory in an orchard system for intercropping management will incur 30% increase in leaf litter decomposition with Collembolainoculation compared to no inoculation. This result is also substantiated by the observations of Kaneko and Salamangca (1999) in their study, wherein enhancement of mass loss corresponds to the abundance of soil microarthropods. Yang et al. (2012) also revealed that the mass loss of leaf litter with collembola is 10% higher as compared to the degradation caused by microorganisms alone.

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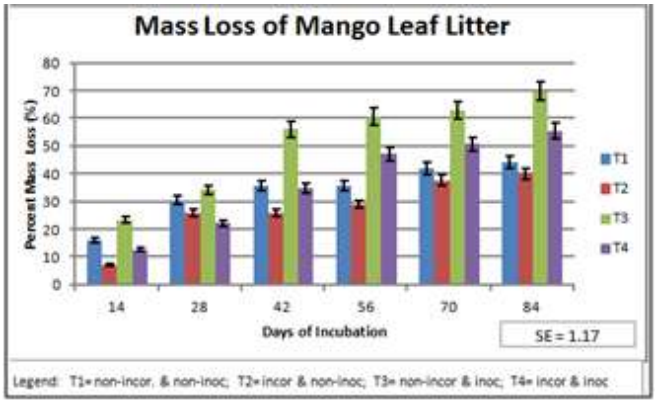

Figure 2: Percent mass loss of mango leaf litter during the 84-day period of decomposition.

Soil Organic Matter. The organic matter content of the soil is calculated directly through the carbon content of the soil by a conversion factor of 1.72 (Thiagalingam, 2000). The changes of organic matter content of the soil during the 84-day period of incubation is presented in Figure 3.

Trend lines from the plotted values of SOM content from the different treatments reveal no accumulation in T1 and T2. In contrast, SOM accumulation was observed in T3 and T4, and decrease on the 84th day. Even with such reduction, the organic matter building in the soil is still significant. From the initial SOM content of 2.99%, it increased to 3.59% (20% increase) in T4. In T3, the SOM slightly increased to 3.51% (17% increase).

The results show that soil organic matter building is facilitated by the increase of physical fragmentation of mango leaf litter during decomposition process. The contribution of Collembola in building SOM is demonstrated in high mass reduction of the mango leaf litter from 50% up to 70%. The grazing behavior of Collembola on the fungal hyphae that colonized on the leaf litter surfaces enhances the physical decomposition which promote addition of POM that builds SOM. Base on the study of Marriott and Wander (2006), the addition of small debris from physical fragmentation of organic material adds up to the concentration of particulate organic matter (POM) in the soil. Rovira (2010) also stated that coarse fraction of POM (2 mm) that enters into the soil becomes part of the labile pool of SOM.

Soil Nutrient Banking. Changes in the physical structure as well as in the chemical composition of the substrate will start to occur as decomposition process takes over. Figure 4 shows the changes in the nutrient (N, P and K) content in the mango leaf litters and in the soil.

The overall decreasing trend of mango leaf litter nutrient content, for all treatments, indicates the chemical degradation happening during the decomposition process. Losses of nutrient concentrations in mango leaf litter during the decomposition process signify releases in the soil environment. Nutrients from the decomposing leaf litter open the opportunity for nutrient to re-enter again into the soil (Ndakara, 2012). This serves as a pool of available nutrients for the utilization of soil organisms. Releases of N, P and K from the decomposing mango leaf litter in different treatments promote nutrient banking in the soil.

In the soil, N, P, K show different linear trends: decreasing N, increasing P, and both increasing and decreasing K (Figure 12). For N, the overall diminishing concentration in the soil for all treatments despite the releases shown in the litter samples is possibly due to several factors such as leaching, crop removal, soil erosion, volatilization and denitrification (Hermanson et al., 2000 and Brady, 1984). Of all the factors contributing to N-losses, denitrification is the most probable cause of N decrease in the soil samples. Due to the unsterilized soil sample condition, the presence of denitrifiying microorganisms in the soil is highly possible. On a weekly basis, however, there are significant variabilites from day-0 to day-14; there is an abrupt increase in N in all treatments. Among the treatments, the highest increase (0.25%) is found in T4. The average of such increase from day-0 to day-70 is 0.21% N, which is 29% higher than the initial N-content of the soil samples. These results show that soil nitrogen banking is high from day-14 to day-70 which is advantageous for short duration plants, particularly legumes, for intercropping purposes (Agreda et al., 2006 and Usherwood, 1998).

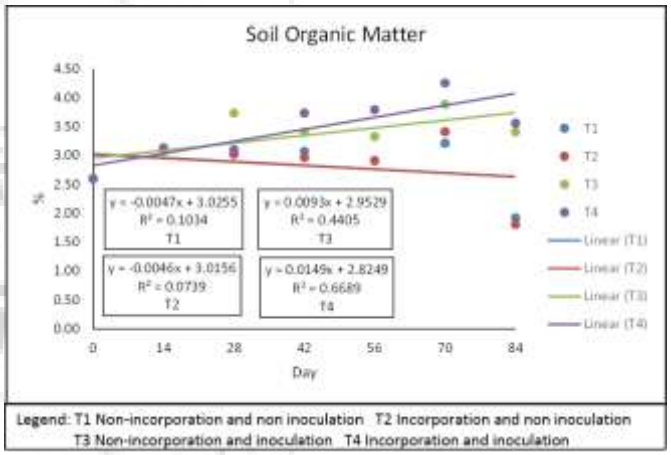

Figure 3: Changes of soil organic matter content of mango leaf litter decomposition.

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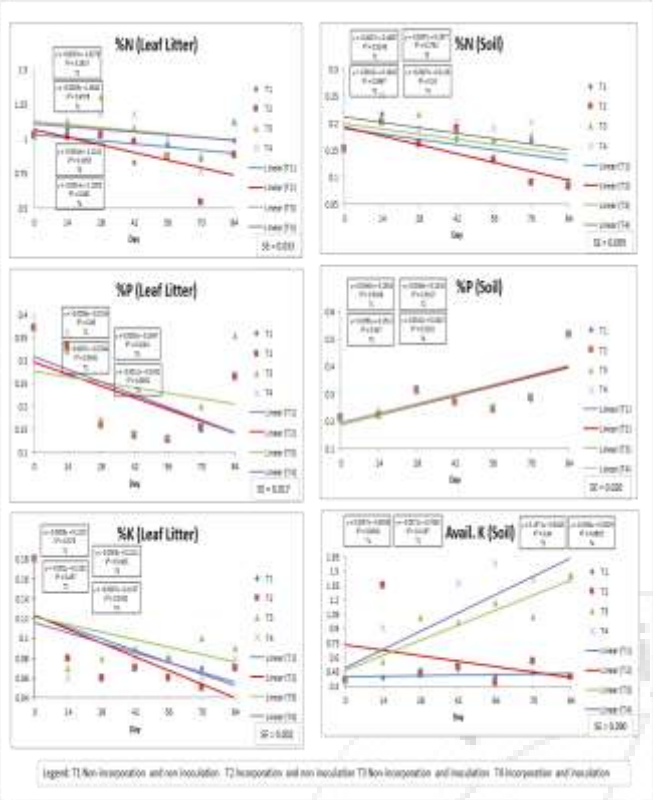

Figure 4: Changes in N, P and K in the mango leaf litters and soil during the 84-day incubation period.

Soil-P shows an increasing trend for all treatments, which peaked on the 84th day (Figure 12). Regardless of the inoculation of Collembola, soil-P content increase in the late stage of the decomposition. Such increase in P concentration can be attributed to the increase of bacterial population which is highly correlated in the addition of cellulose from organic materials and due to soil incubation effect (Hedley et. al., 1982). Since Collembola is a fungivorous microarthropod, bacterial population wss not affected in Collembola inoculated treatments (T3 and T4).

For available K in soil, T3 and T4 show an increasing trend.In T4, significant increase started at day-42 to day-70 and peaked at day-56 with 1.58 me/100g soil. Such increase is ~ 4x higher than the initial K content of the soil samples. The significant addition of 50% to 70% POM from fragmented mango leaf litter through physical decomposition by *Collembola* inoculation causes the appreciable increase of soil available K.

As promoted by inoculation of *Collembola*, the decomposition of mango leaf litter enhances soil nutrient banking of N, P and K. The increase of soil-N starting from the early period (day-14) of decomposition process accompanied by the late deposition of soil-P at day-70 and K at day-42 onwards is very timely for planting short-term crops under intercropping management approach in orchard systems.

4. Conclusion

The study provides a beneficial alternative to the practice of leaf litter burning in orchard understory by enhancing the decomposition rate of mango leaf litter through soil inoculation of microarthropod Collembola. Increasing the decay rate of mango leaf litter to build SOM and promote nutrient banking in the soil will not only sustain the orchard itself but also facilitate intercropping management of short duration cash crops. Nutrient release from the substrate is but a natural phenomenon that happens during the decomposition, however, the timing of peak additions into the soil should also be considered. The inoculation of Collembola does not only promote nutrient banking in the soil. Its peak accumulation coincides with the temporal nutrient requirement of short duration cash crops that is recommended for orchard intercropping management.

Incorporating the litter samples in the soil reduces the percent mass loss by 9%. However, soil inoculation enhances mass loss of decaying incorporated leaf litter by 40%, having a 56% mass loss at the end of 84-day period of decomposition process. The organic debris loss in the substrate (56%) adds up to the soil system that promotes building of SOM from 2.99% to 3.60%. Inoculation of Collembola in incorporated leaf litter also promotes soil nutrient banking. Peak concentrations of N, P and K in the soil during the decomposition process are in accordance to the temporal nutrient requirement of intercrop variety. Soil-N and K of inoculated and incorporated treatment (T4) is 29% and 4x higher compared to uninoculated treatment (T2). For soil-P, regardless of soil inoculation, nutrient banking is facilitated by the nutrient releases of decomposing mango leaf litter.

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