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Effect of the 32nd-year Soil Tillage and Nitrogen Fertilization on the Population and Biomass of Earthworm under *Zea mays* L.

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ABSTRACT

The abundance and biomass of earthworms are affected by soil tillage and fertilization. This research aimed to study long-term 32nd-year soil tillage and nitrogen fertilization on the population and biomass of earthworms under Zea mays L. plantation. The research was conducted using a randomized block design (RBD) which consisted of two factors. The first factor was the soil tillage that was Intensive Tillage (IT), Minimum Tillage (MT), and No-Tillage (NT). The Second factor was nitrogen fertilization that was N0 = 0 kg N ha^{-1} and N1= 200 kg N ha^{-1}. Enumeration of earthworms was conducted by hand-sorting methods. Data of earthworm population and biomass were tested using analysis of variance and continued with the least significant difference (LSD) test at the 95% significant level. The population and biomass of earthworms at MT or NT were higher than that IT. The population and biomass of earthworms in the plots without fertilization were higher than those at 200 kg N ha^{-1}. There was an interaction between the soil tillage and N fertilization on earthworm biomass observed 60 days after planting at a 0-10 cm depth. There was a positive correlation between soil pH and earthworm population and biomass before the tillage phase.

Keywords: Corn, earthworms, fertilization, soil tillage

INTRODUCTION

Soil organisms such as soil fauna have an essential role in providing nutrients in the soil. The abundance of soil fauna can be a bioindicator in assessing soil quality. Earthworms, one of the macrofauna groups, can be used as bio-indicators to assess soil quality (Nurhidayati *et al.* 2012). According to Fründ *et al.* (2011), earthworms are closely related to good soil quality. Earthworms can be considered a soil quality indicator because they are sensitive to changes in soil properties (Peres *et al.* 2018), for example, due to fertilization and continuous use of pesticides.

Fertilization, intensive tillage (IT), and burning of crop residues are conventional agricultural cultivation practices applied by most farmers. According to Utomo (2015), the intensive tillage is carried out using a plow or hoe so that the soil becomes loose and plant roots can develop well with a depth of tillage that can reach 20-40 cm. There is a negative impact due to intensive tillage practice. It can reduce soil health, marked by a rapid decrease in soil organic matter, decreased soil biota biodiversity, reducing productivity (Hairiah *et al.* 2003; Nurhidayati *et al.* 2012), including earthworms. The application of the conservation tillage (CT) is considered capable of maintaining soil quality by maintaining carbon stocks in the soil, the activity of organisms on the soil surface (Cookson *et al.* 2008), biomass, and diversity of earthworms (Pelosi *et al.* 2014). The conservation tillage is carried out by leaving most of the plant residue on the soil surface to affect soil temperature, moisture content, bulk density, soil porosity, and soil aggregates (Sessiz and Gursoy 2010).

In addition to soil cultivation, the application of inorganic fertilizers can also affect the activity and population of earthworms. Although inorganic fertilizers can increase plant production or growth, they can indirectly decrease earthworm abundance if organic matter is not considered. Application of inorganic fertilizers containing ammonium can be toxic to earthworms because it can increase soil acidity (Marhan and Scheu 2005). Intensive and long-term application of inorganic fertilizers can reduce the rate of reproduction and 30% of the earthworm population (Lalthanzara and Ramanujam 2010).

The research aimed to study the effect of long-term 32nd-year tillage and N fertilization systems on the population and biomass of earthworms under *Zea mays* L. plantation.

MATERIALS AND METHODS

The Experimental Site

The research is a long-term that has been conducted since 1987 and is currently entering its 32nd year. This research was conducted from December 2019 to April 2020, located in the experimental field belonging to the Lampung State Polytechnic. Earthworm identification and analysis of soil samples were carried out at the Laboratory of Soil Biology and Laboratory of Soil Science, Faculty of Agriculture, Lampung University.

Research Design

The research was conducted using a factorial randomized block design (RBD) with two factors. The first factor was the tillage system, which consisted of T1 = Intensive tillage (IT), T2 = Minimum tillage (MT), T3 = No-tillage (NT). The second factor was long-term nitrogen fertilization, which consisted of N0= 0 kg N ha^{-1} and N1= 200 kg N ha^{-1}. The land was divided into 24 experimental plots, with each plot of 4 m × 6 m, and the distance between the experimental plots was 1 m. The maize seeds were planted in 75 cm × 25 cm; every hole consisted of 2 seeds.

Earthworm Sampling and Measurement

Earthworm samples were taken by making a monolith with a 25 cm × 25 cm size at a depth of 0-10 cm and 10-20 cm. The earthworm population was observed by hand sorting method (counting by hand). Worm and cocoon obtained in each layer is inserted into the bottle containing soil and labeled according to the treatment. After being brought to the laboratory, earthworms were cleaned, and the biomass was weighed. After being weighed, adult earthworms were put into bottles containing 70% alcohol to identify the type under a stereomicroscope. Estimation of the population and biomass of earthworm and soil sampling was carried out in 3 observation times, namely before tillage (BT), maximum vegetative or 60 days after planting (DAP), and harvest (103 DAP). Population and biomass of earthworm measured following this formula:

$$Earthworm\ population\ (Individual\ m^{-2}) = \frac{\sum(Adult\ esrthworm + juvenile\ biomass + cocoon\ biomass)}{Sampling\ area\ (m^{-2})}$$

After the earthworm population was calculated then it was weighed to get the earthworm biomass with the formula :

$$Earthworm\ biomass\ (g\ m^{-2}) = \frac{\sum(Adult\ earthworm\ Biomass + juvenile\ biomass + cocoon\ biomas)}{Sampling\ area\ (m^{-2})}$$

Selected Soil Properties Analysis

Some soil properties were analyzed to support the primary interpretation variable. The parameters measured were Organic-C (%) using a Walkley and Black method, pH H2O using an Electrometric method, soil temperature (oC) using a thermometer, and soil water content (%) using a Gravimetric method.

Statistical Analysis

The data obtained were tested for the homogeneity of the variety using the Bartlett test and continued by the Tukey test for testing the non-additivity. After the assumptions are fulfilled, the data was processed with analysis of variance and followed by the Least Significant Difference (LSD) test at the 95% confidence level ($P<0.05$). A correlation test was performed to determine the relationship between selected soil properties on the population and biomass of earthworms.

RESULTS AND DISCUSSION

Earthworm Population

The analysis of variance in Table 1 shows that the soil tillage significantly affects the earthworm population at a depth of 0-10 and 10-20 cm at each observation time. Nitrogen fertilization significantly affected the earthworm population in the observations of 60 DAP and 103 DAP at a 0-10 cm depth. There was no interaction between the soil tillage and nitrogen fertilization (T × N) on the earthworm population.

The results of the LSD test (Table 2) show that the earthworm population at BT, 60 DAP, and 103 DAP at a 0-10 cm depth in intensive tillage was significantly lower than the minimum without tillage. At each observation, the earthworms' population at a 10-20 cm depth at no-tillage and minimum tillage were significantly higher than intensive till-

Table 1. The effect of 32nd-year soil tillage and nitrogen fertilization on earthworm populations.

Variance	Observation time					
	BT		60 DAP		103 DAP	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
	F-value dan Significance					
T	4.99*	4.11*	25.32*	12.67*	99.56*	6.45*
N	2.95 ns	0.03 ns	5.14*	0.01ns	13.35*	2.40 ns
TN	0.21 ns	0.51 ns	0.75 ns	0.08 ns	0.49 ns	0.15 ns

Note: BT = before tillage; DAP= days after planting; T = soil tillage; N = nitrogen fertilization; TN = the interaction between the tillage system and nitrogen fertilization; ns = no significant effect at the 95% level; *= significant effect at the 95% level

Table 2. The effect of 32nd-year soil tillage on earthworm populations.

Treatment	Earthworm population (individuals m^{-2})					
	BT		60 DAP		103 DAP	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 c
T1	44 b	10 (3) a	86 b	8 (2) b	124 c	16 b
T2	70 a	18 (4) ab	142 a	40 (6) a	236 a	40 a
T3	70 a	28 (5) b	136 a	46 (7) a	196 b	44 a
LSD 0.05	14	1	13	1	12	13

Note : BT = before tillage; DAP = day after planting; T1 = IT; T2 = MT; T3 = NT; the numbers followed by the same letter are not significantly different from the LSD test at the 95% confidence level. The numbers in parentheses are the result of the transformation with ($\sqrt{x}$ + 0.5)

Table 3. The effect of 32nd-year N fertilization on the earthworm populations at a 0-10 cm depth.

Treatments	Earthworm population (individuals m^{-2})	
	60 DAP	103 DAP
N0	129 a	197 a
N1	113 b	173 b
LSD 0.05	11	10

Note: DAP = day after planting; N0 = 0 kg N ha^{-1}; N1 = 200 kg N ha^{-1}; the numbers followed by the same letter are not significantly different from the LSD test at the 95% confidence level.

age. The results of the LSD test in Table 3 show that at 60 DAP and 103 DAP at a 0-10 cm depth, the earthworm population in N0 treatment was significantly higher than N1.

Table 2 shows that minimum tillage and no-tillage could produce a higher earthworm population than intensive tillage. The result was in line with Sharma *et al.* (2017), who stated that intensive soil tillage can damage the soil structure and disrupt the activity of soil organisms so that the availability of organic material is low. Intensive soil tillage produces more soil pores, and it will be filled with oxygen which can accelerate the decomposition of organic matter and reduce soil moisture (Utomo 2012). In addition, long-term intensive tillage can increase the bulk density of the soil which at the same time reduces the earthworm population (Niswati *et al.* 2018).

Earthworm Biomass

Based on the results of the analysis of variance in Table 4, the soil tillage has a significant effect on earthworm biomass during BT observations at a 0-10 cm depth and on observations of 60 DAP and 103 DAP at a 0-10 cm and 10-20 cm depth. Nitrogen fertilization treatment significantly affects

BT and 60 DAP at a 0-10 cm depth and 103 DAP at a 10-20 cm depth. There was an interaction between the soil tillage with nitrogen fertilization (TxN) on the earthworm biomass at 60 DAP observations at a 0-10 cm depth.

The results of the LSD test in table 5 show that the biomass of earthworms in the intensive

Table 4. The effect of 32nd-year soil tillage and nitrogen fertilization on the earthworm biomass (g m^{-2}).

Variance	Observation Time					
	BT		60 DAP		103 DAP	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
	F-value and Significance					
T	7.91*	0.71 ns	18.95*	13.84*	73.34*	11.18*
	7.79*	0.99 ns	4.72*	0.23 ns	0.51 ns	9.93*
TN	1.96 ns	0.88 ns	4.91*	0.31 ns	0.14 ns	0.55 ns

Note: BT = before tillage; DAP = days after planting; T = tillage system; N = nitrogen fertilization; TN = the interaction between the tillage system and nitrogen fertilization; ns= not significant effect at the 95% level; *= significant effect at the 95% level.

Table 5. The effect of 32nd-year soil tillage on the earthworm biomass at a 0-10 cm depth.

Treatment	Earthworm Biomass (g m^{-2})		
	BT	60 DAP	103 DAP
T1	0.20 (0.82) b	0.96 c	1.50 c
T2	0.8 (1.11) a	1.74 a	2.88 a
T3	0.8 (1.11) a	1.44 b	2.32 b
LSD 0.05	0.13	0.19	0.17

Note : BT = before tillage; DAP = day after planting; T1 = IT; T2 = MT; T3 = NT; the numbers followed by the same letter are not significantly different from the LSD test at the 95% confidence level. The numbers in parentheses are the result of the transformation with ($\sqrt{x}$ + 0.5).

Table 6. The effect of 32nd-year soil tillage on the earthworm biomass at a 10-20 cm depth at 60 DAP and 103 DAP.

Treatments	Earthworm Biomass (g m^{-2})	
	60 DAP	103 DAP
T1	0.08 (0.76) c	0.18 (0.82) b
T2	0.50 (1.00) a	0.54 (1.01) a
T3	0.30 (0.89) b	0.56 (1.02) a
LSD 0.05	0.07	0.07

Note: DAP = days after planting; T1 = IT; T2 = MT; T3 = NT; the numbers followed by the same letter are not significantly different from the LSD test at the 95% confidence level. The numbers in parentheses are the result of the transformation with ($\sqrt{x}$+ 0.5).

Table 7. The effect of 32nd-year nitrogen fertilization on the earthworm biomass at a 0-10 cm depth at BT and 60 DAP

Treatment	Earthworms Biomass (g m^{-2})	
	BT	60 DAP
N0	0.8 (1.11) a	1.49 a
N1	0.4 (0.91) b	1.27 b
LSD 0.05	0.11	0.16

Note: DAP = days after planting; N0 = 0 kg N ha^{-1}; N1 = 200 kg N ha^{-1}; the numbers followed by the same letter are not significantly different from the LSD test at the 95% confidence level. The numbers in parentheses are the result of the transformation with ($\sqrt{x}$ + 0.5).

tillage at a 0-10 cm depth was significantly lower than that of minimum tillage and no-tillage at each observation time. Table 6 shows that at the observation time of 60 DAP and 103 DAP at a 10-20 cm depth, earthworm biomass in the intensive tillage was much lower than the minimum tillage and without tillage. The results of the LSD test at 95% level in table 7 show that the biomass of earthworms at plot without fertilizer was significantly higher than fertilization treatment at BT and 60 DAP at a 0-10 cm depth. Furthermore. Table 8 shows that the biomass of earthworms when observing 103 DAP with a 10-20 cm depth in N0 treatment was significantly higher than N1.

Population and earthworm biomass at Nitrogen fertilization treatment with a dose of 0 kg ha^{-1} (N0) were higher than nitrogen fertilization at 200 kg ha^{-1} (Tables 3, 7, and 8). The land used is a long-term research area so that the application of nitrogen fertilizers has an impact on increasing soil acidity and reducing soil organisms, especially earthworms, compared to without nitrogen fertilization. According to Tang *et al.* (2002), the continuous addition of N fertilizers can impact increasing soil acidity through the oxidation of NH_4^+ to NO_3^- by contributing H^+ to the soil. Earthworms are organisms that are sensitive to changes in soil pH. Population, diversity, and ability to live of earthworms tend to be lower in acid soil conditions (Moore *et al.* 2013).

Based on Table 4, there is an interaction between the tillage system and nitrogen fertilization

Table 8. The effect of 32nd-year nitrogen fertilization on the earthworm biomass at a 10-20 cm depth at 103 DAP.

Treatment	Earthworm Biomass (g m^{-2})
N0	0.55 (1.01) a
N1	0.31 (0.89) b
LSD 0.05	0.06

Note: N0 = 0 kg ha^{-1}; N1 = Fertilization N 200 kg ha^{-1}; the numbers followed by the same letter are not significantly different from the LSD test at the 95% confidence level. The numbers in parentheses are the result of the transformation with (√x+ 0.5).

Table 9. Interaction effect of 32nd-year soil tillage and nitrogen fertilization on the earthworm biomass at a 0-10 cm depth at 60 DAP.

Nitrogen	Soil tillage		
	T1	T2	T3
	………..Earthworm Biomass (g m^{-2})…………		
N0	1.00 c	2.08 a	1.40 b
	A	A	A
N1	0.92 b	1.40 a	1.48 a
	A	B	A

Note: T1= IT; T2= MT; T3= NT; N0= 0 kg N ha^{-1}; N1= 200 kg N ha^{-1}; The numbers followed by the same letters, lowercase letters for rows and uppercase letters for columns, do not different according to the 5% LSD test.

Table 10. The effect of 32nd-year soil tillage and nitrogen fertilization on soil properties at BT, 60 DAP, and 103 DAP.

Variance	Water content (%)			Temperature (°C)			
	SOT	60 DAP	103 DAP	SOT	60 DAP	103 DAP	SOT
						F-value and Significance	
T	0.16^{ns}	0.73^{ns}	3.73*	0.37^{ns}	14.55*	3.18^{ns}	1.27^{ns}
N	0.13^{ns}	0.85^{ns}	1.67^{ns}	2.82^{ns}	70.00*	102.27*	13.39*
TN	0.31^{ns}	1.01^{ns}	0.97^{ns}	0.09^{ns}	3.30^{ns}	1.36^{ns}	1.74^{ns}

Note: BT = before tillage; DAP = days after planting; T = tillage system; N = nitrogen fertilization; TN = the interaction between the tillage system and nitrogen fertilization; ns= not significant effect at the 95% level; *= significant effect at the 95% level at 60 DAP observations at a 0-10 cm depth, so a two-way LSD test is carried out in Table 9. The results showed that the combination of no-fertilization with the MT produces the highest earthworm biomass compared to NT and IT. The combination of fertilization N 200 kg ha^{-1} with MT or NT had the same earthworm biomass but was significantly higher than IT.

Effect of Soil Physical and Chemical Properties on the Population and Biomass of Earthworm

In this study, the physical properties of the soil observed were soil moisture content and soil temperature, while the soil chemical properties observed were soil organic-C and soil pH. The analysis of variance (Table 10) shows that the soil tillage has a significant effect on the soil water content at 103 DAP and soil temperature at 60 DAP. Nitrogen fertilization significantly affected the temperature at 60 DAP and 103 DAP and the soil pH before tillage, 60 DAP, and 103 DAP. There is no interaction between the soil tillage (T) and nitrogen fertilization (N) on the soil's physical and chemical properties.

A correlation test was carried out on the soil's physical and chemical properties based on the population and total earthworm biomass (0-20 cm depth) data. Correlation test results in Table 10 show that soil pH has a significant correlation with the population and biomass of earthworms before tillage

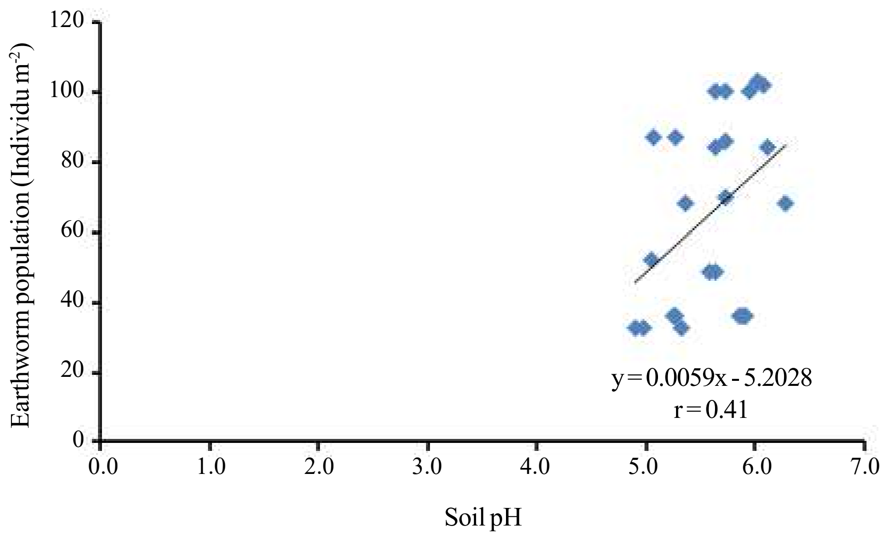

Figure 1. Correlation between soil pH and earthworm populations at before tillage.

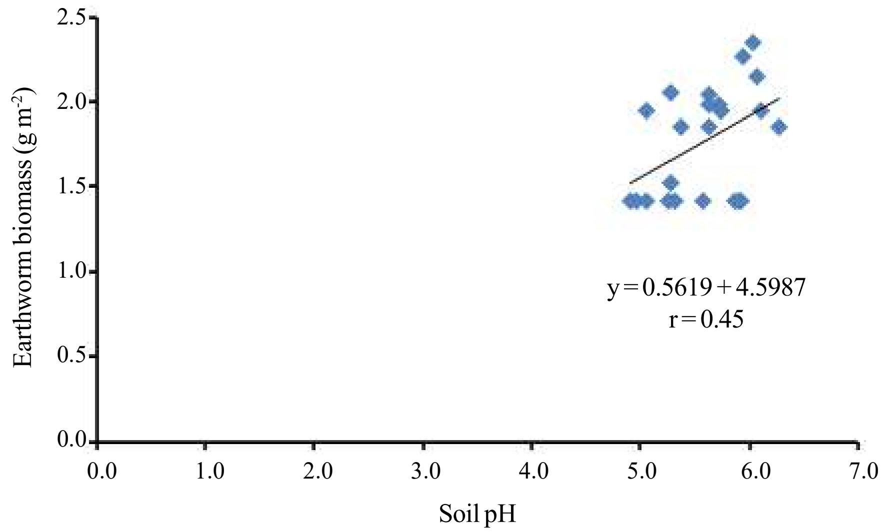

Figure 2. Correlation between soil pH and earthworm biomass at before tillage.

observation. The pH and biomass of earthworms have a positive correlation, namely the increasing the pH value, the population (Figure 1) and biomass (Figure 2) of earthworms in the soil will increase. The results of the LSD test, the effect of 0 and 200 kg N fertilization on soil pH, explained that

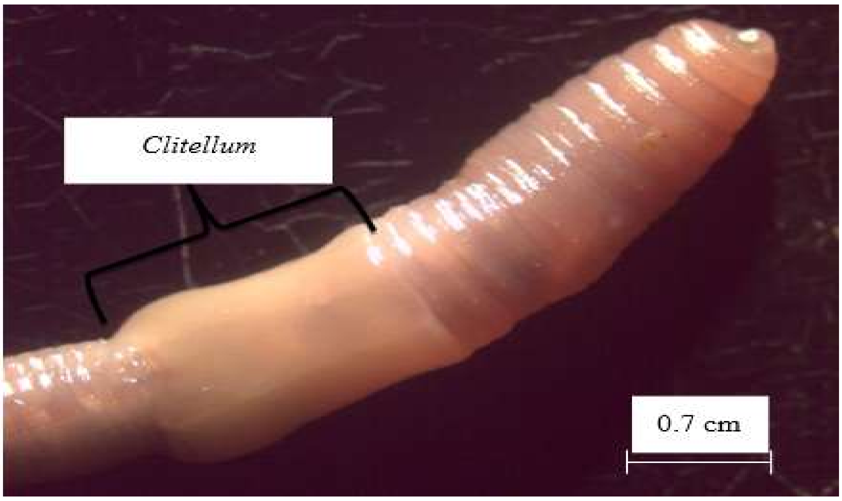

Figure 3. Earthworms clitellum (reproductive organs) at magnification (12.5x).

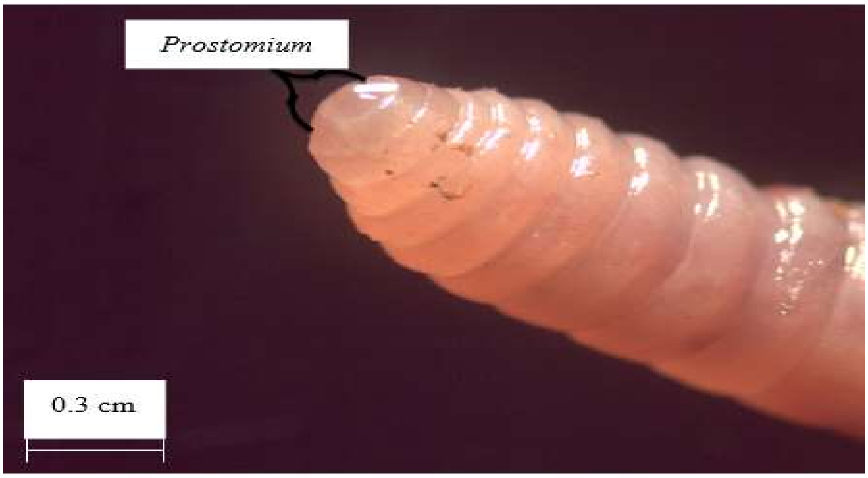

Figure 4. Earthworm prostomium type epilobus at magnification (12.5x).

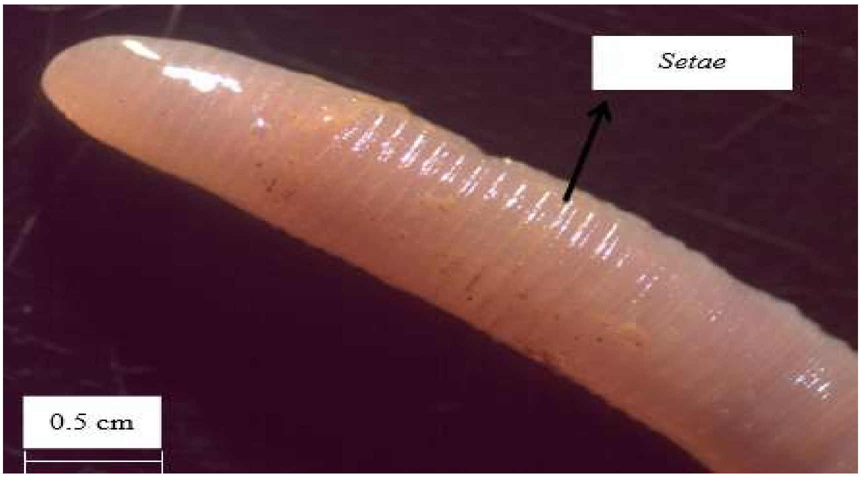

Figure 5. Earthworm setae with perisetin type at magnification (12.5x).

before tillage, the soil pH values were 5.83 and 5.36 respectively and tended to decrease to 5.70 and 5.31, respectively at 103 DAP.

Because in the observations before tillage, the land used had not been given fertilizer treatment, so there had been no change in the pH value, and there were still plant residues on the soil surface as a source of food for earthworms. There was a positive correlation between soil pH and earthworm abundance, meaning that the closer to the neutral pH, the abundance of earthworms would also increase (Duddigan *et al.* 2021). Based on research by Wu *et al.* (2019), earthworms are very sensitive to changes in soil pH. Earthworms can still reproduce and grow at acidic pH, but cocoon production will be hampered if the soil pH is less than 5.2.

Earthworm Identification

Earthworm identification used a collection of adult earthworms obtained in the field based on the identification instructions or earthworm determination keys (Edward and Lofty 1977). Based on the results of the identification of earthworms, there was only one type of earthworm with characteristics, the location of the clitellum is starting at segment 15 (Figure 3), prostomium type is epilobus (Figure 4), and setae (fine hairs) has patterned perisetin (Figure 5).

The identification of earthworms in this study indicates that the types of earthworms obtained belong to the genus *Pheretima* under the family of Megascolicida. Based on Ratnawati *et al.* (2019), the earthworms genus *Pheretima* sp. has an anterior blackish brown color, posterior dark brown, dorsal dark brown, and ventral light brown whitish. The clitellum is located in segments 12-16. Prostomium type is epilobus seen between a circle of grooves as a complete separation and the prostomium with a clear protrusion. *Pheretima* sp. can survive and reproduce with relatively wide environmental conditions and locations (Jayanthi 2014), as geophagus species (live on the surface of the ground), able to live in low pH and organic matter, such as ultisols (Anwar 2009). According to Yusnaini *et al.* (2008), the *Pheretima* sp. is most often found at a 0-20 cm soil depth, but the population was higher in layers 0-10 cm than layer 10-20 cm.

CONCLUSIONS

The population of earthworms in the conservation tillage (no-tillage or minimum tillage) was higher than the intensive tillage at a 0-10 cm depth at 60 DAP and 103 DAP observations, while the earthworm biomass at conservation tillage was higher at each observation. The population of earthworms without fertilizer was higher than fertilization of 200 kg N ha^{-1} at a 0-10 cm depth at 60 DAP and 103 DAP, while the biomass of earthworms without fertilizer was higher than the observations before tillage and 60 DAP. Soil pH has a positive correlation with earthworm population and biomass before tillage.

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