

Effect of long-term Tillage and Nitrogen Fertilization Residue on Soil Biochemical Properties and Cowpea Yield

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Received April 22, 2021; Revised May 20, 2021; Accepted 02 September 2021

ABSTRACT

Sustainable soil management practices must enhance or maintain soil quality and crop yields. The objective of this experiment was to study the effect of long-term (32 years) tillage system and nitrogen fertilization residue on soil biochemical properties and cowpea (*Vigna unguiculata*) yields. This research was conducted using four replications, a factorial experiment arranged in a randomized block design. The first factor was tillage systems consisting of intensive tillage (T₁) and no-tillage (T₂) and the second factor was nitrogen fertilization residue (N) consisting of N₁ (0 kg N ha⁻¹) and N₂ (200 kg N ha⁻¹). Data were analyzed using analysis of variance; if there was a significant difference will be continued with the Least Significant Difference (LSD) test at 5%. Principal Component Analysis (PCA) determined the relationship among observed variables. The results showed that no-tillage could give better results on soil respiration and soil bacteria and fungi population than intensive tillage. Lower soil pH was found at 200 kg N ha⁻¹ fertilizer residue than control (without N fertilizer). The cowpea growth and yields were high at long-term N₁T₂ and N₂T₁ treatment. Principal component analysis showed an interrelationship among soil biochemical properties, growth, and yield of cowpea.

Keywords: Bacteria, fungi, long-term tillage, nitrogen fertilization residue, respiration

INTRODUCTION

Improper long-term soil tillage has an impact on soil degradation and crop yield. Ramos *et al.* (2011) suggested that long-term intensive tillage can cause a decrease in soil physical, chemical, and biological properties and causes a decrease in land productivity. A conservation tillage system can be applied to maintain soil quality. The conservation tillage system consists of a no-tillage and minimum tillage system. According to Aziz *et al.* (2013), Mathew *et al.* (2012), and He *et al.* (2011), a no-tillage system can improve the physical, chemical, and biological soil qualities, causing better plant growth and yields compared to conventional (intensive) tillage system.

A long-term (30 years) no-tillage system containing 200 kg ha⁻¹ of N fertilizer residue can

increase macro and micronutrient uptake and higher corn yield than intensive tillage (Yupitasari *et al.* 2020). In addition, humic acid content, better aggregate quality, and soil microorganism biomass higher in no-tillage treatment compared to the intensive tillage treatment (Andita *et al.* 2019; Suryani 2020; and Miura *et al.* 2016).

The impact of no-tillage on soil biological properties can be studied by measuring the abundance of bacteria and fungi and soil respiration. Bacteria and fungi are soil microorganisms that have an essential role in soil. Singh and Gupta (2018) explained that the diversity and population of soil microorganisms have a role in maintaining the sustainability of the ecosystem. According to Prayudyaningsih *et al.* (2015), if the soil contains various kinds of microorganisms, it can be said that the soil has high fertility. Each microorganism has a specific role in supporting plant growth and yields. In addition, plants can also affect the activity and diversity of soil microorganisms (Buyer *et al.* 2002 and Paterson 2003).

Plants need nutrients taken by roots from the soil to support growth and development. One of the essential nutrients for plants is nitrogen (N). If the N in the soil cannot meet the needs of plant nutrients, then the growth and development of plants will be disturbed (Erawan *et al.* 2013). This long-term study with the rotation pattern of cereals and legumes is expected to reduce the need for N nutrients because N fertilization is only carried out on cereal crops, and then the residue from N fertilization in cereals is used as a *starter* for N by *fixing Rhizobium* in legumes.

This study aimed to determine the effect of the long-term (32 years) tillage system and nitrogen fertilization residues on soil chemical and biological properties, cowpea yields, and the relationship between several soil properties and cowpea yields.

MATERIALS AND METHODS

The Experimental Site

This research was long-term research since 1987 until now (32 years) in the experimental laboratory of the Politeknik Negeri Lampung, which is located at 105 ° 13'45.5" – 105 ° 13'48.0" east and 05° 21'19.6" – 05°21'19.7" South Latitude, with an elevation of 122 meters above sea level. The crop rotation pattern applied during long term study was cereal crops (maize/upland rice) – legumes (soybeans/ cowpea/ green beans) – fallow (Utomo 2015). Analysis the diversity of soil bacteria and fungi and soil respiration was carried out at the Soil Biology Laboratory; while, the total N content, C-Organic, and soil pH analysis were carried out at the Soil Chemistry Laboratory, Faculty of Agriculture, University of Lampung.

Experimental Design

The research was design in a Randomized Block Design (RBD), arranged in a 2×2 factorial with four replications. The first factor was the tillage system consisting of intensive tillage (T_1) and no-tillage (T_2). The second factor was nitrogen fertilization residue (N) consisting of N_1 (0 kg N ha⁻¹) and N_2 (200 kg N ha⁻¹). Fertilizing N is intended for cereal crops, while for the 32nd planting season, cowpea (legumes) are used only to utilize residues of N fertilization from the previous growing season.

Research Implementation

In a no-tillage plot, not treated at all, weeds were controlled using a herbicide with an active

ingredient of glyphosate at a dose of 3-5 liters per hectare, and weeds from plant residues were used as mulch. In intensive tillage plots, the soil was hoe twice to a depth of 0-20 cm before planting and weeds were removed from the experimental plots. The land was divided into 36 experimental plots with the size of each plot 4 m × 6 m, and the distance between the experimental plots was 0.5 m. We were planting cowpea seeds of local new variety by directly planting two cowpea seeds to each planting hole with a distance of 40 cm × 20 cm.

Plants cultivated in the previous growing season were cereals (maize) and, in this 32nd planting season, were planted with legumes (coffee beans) so that they only utilized residues of nitrogen fertilization from the previous season. As basic fertilizer, SP-36 fertilizer at a dose of 100 kg ha⁻¹, and KCl at a dose of 50 kg ha⁻¹ were applied. Soil samples were taken at harvest time in a composite manner at five points using a soil drill in each experimental plot. Soil samples obtained were then put into plastic and labeled, which were then stored in the refrigerator for analysis in the laboratory.

Soil Respiration Measurement

Measurement of soil respiration was carried out using a modified Verstraete method (Anas 1989) which was 100 g of dry soil weight, and two film tubes containing 10 ml of 0.2 N KOH and 10 ml of distilled water were put into a jar measuring 1L. The jars were closed until airtight and incubated at room temperature in the dark for ten days. The blank (control) was not filled with soil. The analysis was performed to determine the amount of CO₂ bound by the KOH solution determined by titration.

Soil Bacteria and Fungi Observation

The method used to calculate the total soil bacteria and fungi was to grow bacteria and fungi from dilution in a petri dish using the *spread plate count* method (Hastuti and Ginting 2007). The soil extraction was carried out using the dilution *Plating Method* (Pelczar and Chan 2006).

Observation of total bacterial colonies on NA (*Nutrient Agar*) media counts the population of bacteria. Observations were made from the first day after incubation until day 4. While the observation of fungi on PDA (*Potato Dextrose Agar*) was carried out by counting the population or number of fungi that grew from the fifth to the seventh day of incubation. Bacteria were counted only from Petri dishes with 30-300 colonies, while fungi were counted from Petri dishes with 10-100 colonies. The

formula for calculating the population of bacteria and fungi (Hastuti and Ginting 2007) :

$$\text{Total Population (CFU) } g^{-1}\text{soil} = \frac{\text{total colony}}{\text{dillution factor} \times \text{soil dry weight}}$$

Data Analysis

Data were analyzed using analysis of variance. If there was a significant difference, the Least Significant Difference (LSD) test continued at 5% level. A correlation test was conducted to determine the relationship between variables. Analysis of the magnitude of the influence between observational variables and their relationship was carried out using *Principal Component Analysis (PCA)* using R and R Studio *Software* (Tharwat 2016).

RESULTS AND DISCUSSION

The results of effects long-term tillage systems and residues of N fertilization on the soil properties and the growth and yields of cowpea are presented in Tables 1 and 2. It shows that the application of the tillage system had a significant effect on the

population of bacteria and fungi as well as soil respiration, Total-N, and the number of cowpea leaves. The interaction between the tillage system and long-term N fertilization residues significantly affected the C-Organic content of the soil, plant height, and yields of cowpea. Then, the treatment of residual N fertilization significantly affected soil pH. It has long been known that the role of soil organisms in ecosystems is significant in the nutrient cycle and the carbon cycle (Bender *et al.* 2016). Each 1 g of soil is estimated to contain up to 1 billion bacterial cells consisting of tens of thousands of taxa, 200 m fungal hyphae, and various kinds of nematodes, earthworms, and arthropods (Bardgett *et al.* 2014).

The results on the effect of the tillage system and the long-term residue of N fertilization showed that the application of the tillage system had a significant effect on the population of soil bacteria and fungi (Table 1). The population of soil bacteria and fungi, respiration, total-N content, and the number of cowpeas leaves with the application of a no-tillage system were higher than those with an intensive tillage system (Figure 1). Previous studies showed that the land with a no-tillage system contains

Table 1. Soil biological and chemical properties after 32 years of cropping as affected by tillage systems and N fertilization residue.

Treatments	PB	PF	R	C	N	T	pH
Intensive Tillage	5.54	3.62	5.54	1.49	0.09	28.00	6.06
Intensive Tillage + 200 kg ha ⁻¹ N residue	5.50	3.67	5.5	1.39	0.08	27.25	5.61
No-tillage	5.61	3.78	5.61	1.45	0.12	27.50	5.96
No-tillage + 200 kg ha ⁻¹ N residue	5.62	3.75	5.62	1.69	0.13	28.00	5.76
Analysis of variance							
Tillage system (T)	*	*	*	ns	*	ns	ns
N fertilizer residue (N)	ns	ns	ns	ns	ns	ns	*
T × N	ns	ns	ns	*	ns	ns	ns

Note: PB = total soil bacteria, PF = total fungi, R = soil respiration, C = organic C, N = Total N, T = temperature.

Table 2. Cowpea growth and yield after 32 years of cropping as affected by tillage systems and N fertilization residue.

Treatments	Plant height (cm)	Total leaf	100 seeds weight (g)	Yield (Mg ha ⁻¹)
Intensive Tillage	20.83	19.85	11.45	0.74
Intensive Tillage + 200 kg ha ⁻¹ N residue	16.22	18.90	11.30	1.16
No-tillage	25.42	22.75	11.14	1.26
No-tillage + 200 kg ha ⁻¹ N residue	25.82	22.63	11.20	1.26
Analysis of variance				
Tillage system (T)	*	*	ns	*
N fertilizer residue (N)	*	ns	ns	*
T × N	*	ns	ns	*

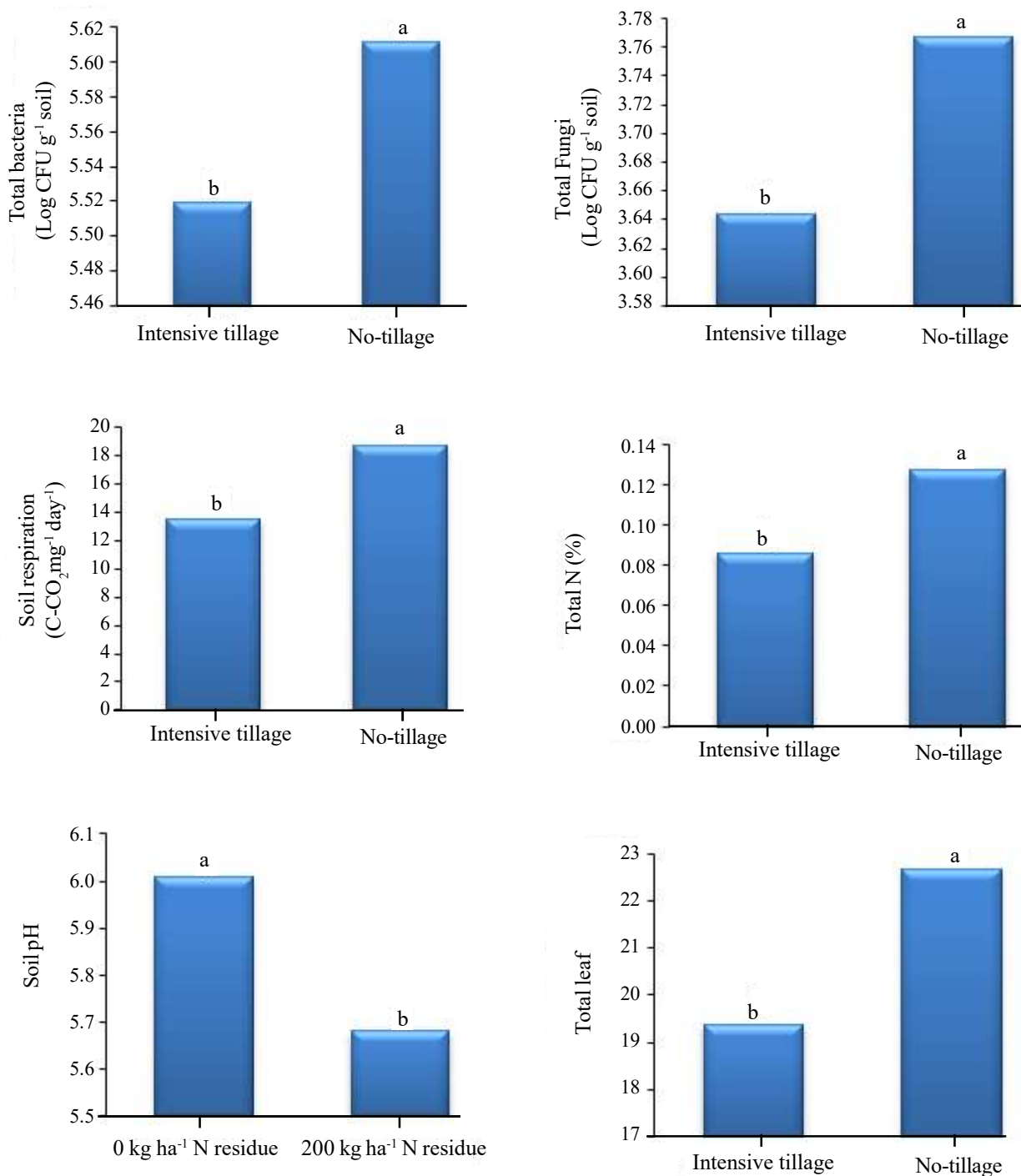


Figure 1. Total soil bacteria, fungi, respiration, pH and total leaf of cowpea after 32 years of cropping as affected by tillage systems and N fertilization residue.

microbial biomass and higher abundances of fungi and bacteria than intensive tillage systems (Utomo *et al.* 2013 and Miura *et al.* 2016). The no-tillage system resulted in higher soil respiration than the intensive tillage system (Table 2). The results of this study follow Gajda (2010), Gajda and Przewoka (2012), and Gajda *et al.* (2013), who stated that the application of no-tillage and conservation tillage causes higher soil biological activity than conventional

tillage. Suin (2012) stated that one factor affecting the population density of organisms is organic matter. The higher the organic matter content, the higher the population density of organisms.

In no-tillage systems, weeds and crop residues were previously used as mulch. In the decomposition process, plant residues and weeds in a no-tillage system become mulch as a source of organic matter for soil organisms. Thereby it could increase the

Table 3. Total soil organic carbon, plant high, and cowpea yield after 32 years of cropping as affected by tillage systems and N fertilization residue.

N fertilizer residue	Tillage											
	Intensif tillage		No tillage		Intensif tillage		No tillage					
	Soil Organic Carbon (%)		Plant High (cm)		Cowpea Yield (ton ha ⁻¹)							
0 kg ha ⁻¹	1.49	a	1.45	a	20.83	a	25.42	a	0.74	b	1.26	a
	A		A		B		A		B		A	
200 kg ha ⁻¹	1.39	a	1.69	a	16.22	b	25.82	a	1.16	a	1.26	a
	B		A		B		A		A		A	
LSD 5%	0.24				2.31				0.15			

Note: The numbers followed by the same letters in the same column or row are not significantly different from the LSD test at 5% level. Lowercase letters are read vertically. capital letters are read horizontally.

nutrient availability, such as N needed for plants growth which included the increase in the plant height and leaves a number. So that although there was no N residue from the previous N fertilization, the soil in the no-tillage system contained N from the decomposition of soil organic matter. In the soil, some microorganisms play a role in the decomposition process of organic matter, immobilization, and mineralization reactions that play a role in supplying plant nutrients, helping plants tolerate abiotic stresses, and strengthening plant defenses against pathogens and insects. As Antonius *et al.* (2018), the number of soil microorganisms is strongly influenced by organic matter because the more organic matter, the greater the energy source used by soil organisms. According to Widyati (2013), Perez-Jaramillo *et al.* (2015), Mendes *et al.* (2011), and Pieterse *et al.* (2014), stable soil organisms can support plant growth and yield.

The system without tillage increased soil organic C and plant height when there was a residue of N fertilization 200 kg ha⁻¹. In addition, the tillage system, even without residue N fertilization, could produce cowpea plants with higher production than the intensive tillage system (Table 3). According to Utomo *et al.* (2012), this can be caused by the excellent process of decomposition of litter or plant residues previously in a system without tillage, causing plants to grow and produce higher production compared to intensive tillage. Meanwhile, in the intensive tillage system, the soil is fully processed, and mulch is not applied so that nothing covers the soil surface, which can lead to no addition of organic C from previous crop residues and cause loss of soil organic C through erosion and decomposition.

The residue of 200 kg ha⁻¹ N fertilization decreased soil pH (Figure 1). By the results of previous studies, increasing the dose of N fertilizer

significantly lowered the pH. Soil acidity with N fertilization 200 kg N ha⁻¹ was significantly lower than the treatment without N fertilization (Andita *et al.* 2019).

Based on *Principal Component Analysis (PCA)* (Figure 2), the relationship between two variables is closed when the angle formed is less than 90°; on the other hand, if the angle formed is more than 90°, then the relationship between the two variables can be said to be less close. There was a close correlation or correlation between cowpea production, soil respiration, fungal population, and soil organic C content. In addition, the four variables were also correlated with the number of leaves, plant height, bacterial population, and total N content of the soil. The higher the respiration rate reflects the high activity and number or population

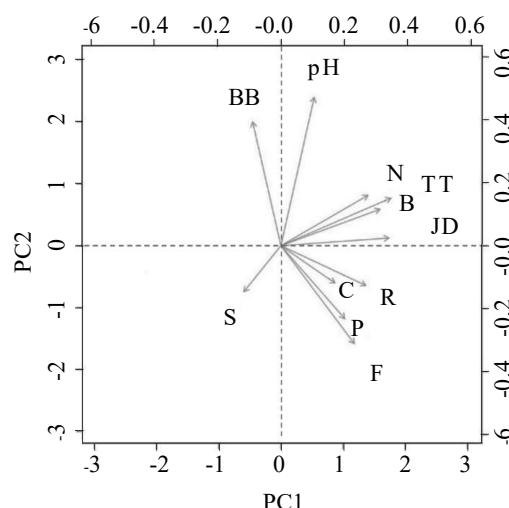


Figure 2. *Principal Component Analysis (PCA)* from soil chemical and biological properties, growth and yield of cowpea after 32 years of cropping as affected by tillage systems and N fertilization residue.

of soil microorganisms, the higher the population of soil bacteria and fungi. In addition, the higher the organic C content of the soil impacts the high population of soil bacteria and fungi because soil organic C reflects the amount of soil organic matter that can be used as a substrate or energy source for bacteria and fungi. In addition, this is also supported by the opinion of Blanco-Canqui *et al.* (2013), which stated that the high content of soil organic C affects the biomass and activity of soil microorganisms because it can provide a stable habitat in the form of soil aggregates. Although plant production has a good correlation with bacterial and fungal populations, the correlation between plant production and bacterial populations is more closely related to the fungal population. According to Stursova *et al.* (2012), bacteria utilize a more specific substrate, namely cellulose, while fungi are better able to utilize a wider variety of litter constituents.

Plant growth includes height and number of leaves and plant yields interrelated with soil respiration, bacterial and fungal populations of the soil. Plants can affect the community of soil microorganisms due to organic compounds released by plants through the roots or called root exudates. Root exudates that accumulate in the area around the surface of plant roots can be utilized as an energy source by soil microorganisms. Van Dam and Bouwmeester (2016) stated that root exudates are a significant organic carbon source in the soil. The number of root exudates released by plants can reach 50% of the total products of photosynthesis. In addition, according to Mommer *et al.* (2016) and Philippot *et al.* (2013), the area around plant roots is an area that is rich in nutrients so that it becomes a habitat for microorganisms such as bacteria, fungi (including arbuscular mycorrhizae), oomycetes, viruses and archaea in carrying out their metabolic processes.

The number of leaves affects the population of bacteria and fungi and plant yields because the leaves are the part of the plant where photosynthesis takes place. It has been explained previously that the carbon produced by the photosynthesis process released by plants through the roots can reach 50% of the total carbon produced in the photosynthesis process. The root exudates are released by plants in the form of sugars, organic acids, amino acids, phenolic compounds, or terpenoids (Van Dam and Bouwmeester 2016).

The soil acidity or soil pH level was not correlated with soil respiration rate, fungal population, and cowpea yields, but soil pH was

correlated with soil bacterial population. These results agree with Rousk *et al.* (2010), which stated that the effect of soil pH on the soil bacterial community was higher than that of the soil fungi community.

CONCLUSIONS

Long-term no-tillage (32 years) has higher soil respiration, bacterial and fungal populations, and cowpea yields than the intensive tillage system. The residue of long-term N fertilization (32 years) did not affect soil respiration, bacteria, and fungi population but caused a decrease in soil pH. There was an interaction between the tillage system and long-term N fertilization residue on soil organic C content, plant height, and cowpea yield. There is a reciprocal relationship or mutual influence between the growth and yield of cowpea with soil chemical (total N and organic C) and biological (population of bacteria and fungi and soil respiration) properties.

ACKNOWLEDGEMENT

Our deeply thanks to Politeknik Negeri Lampung for the facilities and experimental plots and especially to Prof. Dr. Ir. Muhajir Utomo, M.Sc. as the initiator of this long-term research.

REFERENCES

- Anas I. 1989. *Biologi tanah dalam praktek*. Pusat Antar Universitas Bioteknologi. Institut Pertanian Bogor. Bogor. 161 p. (in Indonesian).
- Andita RA, Sarno, M Utomo and AK Salam. 2019. Pengaruh sistem olah tanah dan pemupukan nitrogen jangka panjang terhadap kandungan asam humat dan asam fulvat pada tanaman jagung (*Zea mays* L.) Tahun ke-29 di lahan politeknik negeri lampung. *J Agrotek Tropika* 7: 361-370. (in Indonesian).
- Antonius S, RD Sahputra, Y Nuraini and TK Dewi. 2018. Manfaat pupuk organik hayati, kompos dan biochar pada pertumbuhan bawang merah dan pengaruhnya terhadap biokimia tanah pada percobaan pot menggunakan tanah ultisol. *J Biologi Indonesia* 14: 243-250. (in Indonesian).
- Aziz I, T Mahmood and KR Islam. 2013. Effect of long-term no-till and conventional tillage practices on soil quality. *Soil Tillage Res* 131: 28-35
- Bardgett RD and WH van der Putten. 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515: 505-511.
- Bender SF, C Wagg and MG van der Heijden. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends Ecol Evol* 31: 440-452.

- Blanco-Canqui H, CA Shapiro, CS Wortmann, RA Drijber, M Mamo, TM Shaver and RB Ferguson. 2013. Soil organic carbon: the value to soil properties. *J Soil Water Conserv* 68: 129A-134A.
- Buyer JS, DP Roberts and E Russek-Cohen. 2002. Soil and plant effects on microbial community structure. *Can J Microbiol* 48: 955-964.
- Erawan D, WO Yani and A Bahrun. 2013. Pertumbuhan dan hasil tanaman sawi (*Brassica juncea* L.) pada berbagai dosis pupuk urea. *J Agroteknos* 3: 19-25. (in Indonesian).
- Hastuti RD and RCB Ginting. 2007. Enumerasi Bakteri, Cendawan, dan Aktinomisetes. *Metode analisis biologi tanah*. Bogor. Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan, Badan Penelitian dan Pengembangan Pertanian, Departemen Pertanian, pp. 13-22. (in Indonesian).
- He J, H Li, RG Rasaily, Q Wang, G Cai, Y Su, Q Xiaodong and L Liu. 2011. Soil properties and crop yields after 11 years of no-tillage farming in wheat maize cropping system in north china plain. *Soil Tillage Res* 113: 48-54.
- Mathew RP, Y Feng, L Githinji, R Ankumah and KS Balkcom. 2012. Impact of no-tillage and conventional tillage systems on soil microbial communities. *Appl Environ Soil Sci* 2012: 548620.
- Mendes R, M Kruijt, I de Bruijn, E Dekkers, M van der Voort, JHM Schneider, Y Piceno, TZ DeSantis, GL Andersen, PAHM Bakker and JM Raaijmakers. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332:1097-1100.
- Miura T, K Owada, K Nishina, M Utomo, A Niswati, N Kaneko and K Fujie. 2016. The effects of nitrogen fertilizer on soil microbial communities under conventional and conservation agricultural management in a tropical clay-rich ultisol. *Soil Sci* 181: 68-74.
- Mommer L, P Hinsinger, C Prigent-Combaret and EJ Visser. 2016. Advances in the rhizosphere: stretching the interface of life. *Plant Soil* 407:1-8.
- Paterson E. 2003. Importance of Rhizodeposition in the Coupling of Plant and Microbial Productivity. *Eur J Soil Sci* 54: 741-750.
- Pelczar MJ and ECS Chan. 2006. *Dasar-dasar mikrobiologi jilid 2*. UI Press. Jakarta. (in Indonesian).
- Philippot L, JM Raaijmakers, P Lemanceau and WH Van Der Putten. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11: 789-799.
- Pieterse CMJ, C Zamioudis, RL Berendsen, DM Weller, SC Van Wees and PA Bakker. 2014. Induced systemic resistance by beneficial microbes. *Annu rev phytopathol* 52: 347-375.
- Ramos ME, AB Robles, A Sanchez-Navarro and JL Gonzalez-Rebollar. 2011. Soil responses to different management practices in rainfed orchards in semiarid environments. *Soil Tillage Res* 112: 85-91.
- Rousk J, E Bååth, PC Brookes, CL Lauber, C Lozupone, JG Caporaso, R Knight and N Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in arable soil. *ISME J* 4: 1340-1351.
- Singh JS and VK Gupta. 2018. Soil microbial biomass: a key soil driver in management of ecosystem functioning. *Sci Total Environ* 634: 497-500.
- Suin MN. 2012. *Ekologi Hewan Tanah*. Bumi Aksara. Bandung. 189 hlm. (in Indonesian).
- Suryani WM. 2020. Pengaruh sistem olah tanah dan residu pemupukan nitrogen jangka panjang tahun ke-32 terhadap distribusi agregat pada pertanaman kacang tunggak (*Vigna unguiculata* L.) di politeknik negeri lampung. *Skripsi*. Fakultas Pertanian Universitas Lampung. (in Indonesian).
- Tharwat A. 2016. Principal component analysis - a tutorial. *Int J Appl Pattern Recognit* 3: 197-240.
- Utomo M. 2015. *Tanpa olah tanah, teknologi pengolahan pertanian lahan kering*. Graha Ilmu. Yogyakarta. (in Indonesian).
- Utomo M, IS Banuwa, H Buchari, Y Anggraini and Berthiria. 2013. Long-term tillage and nitrogen fertilization effects on soil properties and crop yields. *J Trop Soils* 18: 131-139.
- Utomo M, H Buchari, IS Banuwa, LK Fernando and R Saleh. 2012. Carbon storage and carbon dioxide emission as influenced by long-term conservation tillage and nitrogen fertilization in a corn-soybean rotation. *J Trop Soils* 17: 75-84.
- Van Dam NM and HJ Bouwmeester. 2016. Metabolomics in the rhizosphere: tapping into belowground chemical communication. *Trends Plant Sci* 21: 256-265.
- Widyati E. 2013. Pentingnya keragaman fungsional organisme tanah terhadap produktivitas lahan. *Tekno Hutan Tanaman* 6: 29-37. (in Indonesian).
- Yupitasari M, M Utomo, A Karyanto and AK Salam. 2020. Pengaruh sistem olah tanah jangka panjang, pemupukan N dan residu N terhadap serapan hara mikro dan produksi tanaman jagung (*Zea mays* l) setelah pengolahan lahan kembali. *J Trop Upland Res* 2: 24-35. (in Indonesian).