Effect Of Lime Application On Indigenous Nitrogen-Fixing Bacteria In Tidal Soils Managed For More Than 30 Years

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah, serta menganalisis dampak interaksi tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah. Pengambilan sampel tanah di lahan pasang surut Desa Mulya Sari dan Banyu Urip Kabupaten Banyuasin, Sumatera Selatan. Kemudian sampel diteliti di Laboratorium Mikrobiologi, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sriwijaya, Indralaya, selama bulan Januari-Mei 2017. Berdasarkan hasil Tipologi lahan pasang surut tidak mempengaruhi populasi bakteri penambat nitrogen indigen, tetapi berpengaruh terhadap pH tanah. Jumlah total bakteri terbanyak pada minggu kedua pada tipologi tanah D, yaitu $6{,}0 \times 10^7$ CFU/ml dan pH tertinggi 5,18 pada tipologi B pada minggu ketiga. Pemberian dolomit mempengaruhi populasi bakteri penambat nitrogen indigen, dimana pada tanah yang diberi dolomit populasi bakteri lebih sedikit dibandingkan tanah yang tidak diberi perlakuan dolomit, dengan populasi bakteri terbanyak pada $9{,}3 \times 10^5$ CFU/ml pada minggu kedua dan pemberian dolomit menigkatkan pH tanah, dengan pH tanah tertinggi 4,93 pada minggu kedua. Interaksi antara tipologi lahan dan pemberian dolomit hanya memberi pengaruh terhadap populasi bakteri penambat nitrogen indigen dengan populasi terbanyak $3{,}5 \times 10^7$ CFU/ml pada tipe tanah D pada minggu kedua. pH tertinggi 5,33 pada tipologi tanah B pada minggu keempat.

ABSTRACT

This research purposes to know the effect of soil typology and lime on the population of indigenous nitrogen-fixing bacteria and soil pH, and to analyze the effect of soil typology interaction and lime application on population of indigenous nitrogen-fixing bacteria and soil pH. Sampling of the soil was in tidal land of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. Then the samples be inspected in Microbiology Laboratory at Biology Department of Faculty of Science,Sriwijaya University, Inderalaya, during January-May 2017. Based on the results tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected the soil pH. The highest total number of bacteria at the second week on the soil typology D, ie 6.0×10^7 CFU / ml and the highest pH of 5.18 on typology B in the third week. Application of dolomite affected the population of indigenous nitrogen-fixing bacteria, which in dolomite-treated soil populations were fewer than dolomite un-treated soil, with the largest bacterial population at 9.3×10^5 CFU / ml during the second week and dolomite increased soil pH, with pH the highest ground of 4.93 at the second week. The interaction between land typology and dolomite application only affected the population of indigenous nitrogen-fixing bacteria with the most populations of 3.5×10^7 CFU / ml on soil typology D in the second week. The highest pH on soil typology B of 5.33 was at the fourth week.

Keywords: nitrogen-fixing bacteria, tidal land, lime treatment

INTRODUCTION

Tidal swamps of Mulya sari and Banyu Urip villages, South Sumatra, showed that soil pH was below 5.0 after opening of 30 year old with pH values ranging from 4.0-4.59 (very acid to acid) in all land typologies in combination with high H^+ and Al^{3+} exchangeability. The values of H^+ and Al^{3+} were from 0.65-1.0 cmol $^{(+)}$ / kg and 1.77-4.49 cmol $^{(+)}$ / kg (Budianta et al, 2016).

Villages of Banyu Urip and Mulya Sari, the location of soil sampling with respectively has been guided NPK fertilization for more than 30 years. It is known that the application of chemical fertilizers has side effect compared to natural fertilizers. According to Lokasari (2011), NPK's unfavorable nature is continuously that NPK is not ionizing in soil solution so it is easy to wash, since it can not be absorbed by the soil colloids. To be absorbed by plants had to undergo amonification and nitrification process first. The rapid and slow amide-form changes of the urea to the form of N compounds can be absorbed by the plant depending largely on several factors including population, microorganism activity, soil moisture content, soil temperature and amount of Urea fertilizer provided.

Tidal land of Banyu Urip and Mulya Sari villages were used as the location of soil sampling because the area can represent four typologies of tidal land with low soil pH. According to Budianta et. Al., (2016), soil pH measurements in four soil typologies in Banyu Urip and Mulya Sari villages obtained results, were namely in type A with pH 4.05; Type B with pH 4.59; Type C with pH 4.44; And type D with a pH of 4.55, where the lowest pH in the highest H^+ and Al^{3+} exchanged combinations was found in land typology A after rice growth. It was caused by the nitrification process of nitrogen fertilization causing a decrease in soil pH.

Lime is a source of ameliorant material widely used to improve soil fertility. Lime is an important s ource of Ca nutrients that support plant growth, increase soil pH, reduce soil Al and Mn content (Scott and Fisher, 1989; Mora et al., 2002 ; Koesrini et al., 2015). According to Saputri et al. (2016), the addition of dolomite is needed to regulate the acidity (pH) of growing media, calcium and carbon elements can enrich the mineral content of the media, and function as enzyme activators. According to Winarno (2004) lime also serves as an activator of various types of enzymes that are related in the metabolism of proteins and carbohydrates.

According to Kuswandi (1993), with the increaseof the pH of the soil, the supply of Mg and Ca nutrients can shift the position of H + on the colloid surface to neutralize the soil acidity. Calcification also aims to reduce the risk of aluminium effects, increase the availability of P elements as a result of P extraction from Al-P and Fe-P bonds, increase N fixation and N mineralization to increase CEC, and assist in the completion of the reform with accompanying nutrient release from organic materials and microbial bodies.

MATERIALS AND METHOD

This research was conducted from January to June 2017. Sampling of soil samples was taken in Tidal area of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. The research was conducted in Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya.

Equipment used in the field were sack, soil tester and a shovel. The tools used in the laboratory were autoclave, colony counter, pH meter, erlemeyer ml, hot plate, incubator, ose needle, konduktormeter, bunsen lamp, mixer, mortarporselein, petri dish, 1 ml micropipette, plastic and test tube. Materials needed were LGI medium, aquadest, and dolomite.

This research was used Factorial Complete Random Design, with treatment factor, as follows:

Factor 1: Dolomite Dose (K)

K0 = 0 (without treatment)

K1 = 2 Ton ha^{-1}

Factor 2: Tidal Swamp Land Typology (L)

L1 = Typology A

L2 = Typology B

L3 = Typology C

L4 = Typology D

The number of combinations each repeated 3 times, so it would produce 24 units of experiment.

Soil samples from tidal swamps were types A, B, C and D with aseptic method at 3 soil sampling points and homogenised on each typology. after having homogenized then the samples were inserted into a sterilized sac with different codes according to their respective topological type, then stored in cool box. The soil samples taken from layers of cultivation with a depth of about 0-20 cm in geographical coordinates are located about S 02^0 41'24 ", E $104^0$45'25" for A, S $02^0$39'26 "E $104^0$44'27" for B,S $02^0$39 '44 ", E $104^0$44'027" for C, and S $02^0$38'37 ", E $104^0$43'16" for typology D

Soil with different typology sieved with the sieve size of 0.5 mm hole diameter, then weighed as much as 1000 g, then dried up the ground, after the soil was inserted into polybag, the moisture was measured by using Soil tester. If the soil moisture content is less than 85% (optimum humidity for bacterial growth) first soil has to be given water using 500 ml water bottle poured slowly on the soil, and the humidity increases (Anonymous, 2017). Then dolomite was applicated to each soil in polybags with different dolomite amounts according to the dosage, for 1000 g of soil volume 1.84 g for dose 2 ton ha^{-1} and

on control not application of dolomite. Then it is calculated and observed and measured pH every 1 week for 28 days.

Variables observed were: (1) Total population of bacteria; (2) pH; (3) C-Orgaanic; (4) N-Total; (5) Al-exc

1. Number of Bacterial Populations

The calculation of bacterial cell count was performed by diluting 5 g samples taken from treated soil into 45 mL sterile aquades, made up to a dilution level of 10-6. The result of dilution from 10-4 - 10-6, was taken each 1 mL then put into a sterile petri dish that did not contain the medium of LGI, after inserted 1 ml of dilution, it is the medium of LGI was put wrapped. Then it was incubated at 37^{o}C for 24 hours. The number of bacteria that grow on counter colonies was Calculated (Modified Munawar, 1999).

2. Soil Reaction (pH)

Soil pH measurements were performed before dolomite given, then it was measured again after dolomite was given once a week for 28 days by means of soil weighed as much as 10 g twice, each was inserted into a shake bottle, and 50 ml of aquades were added to the bottle first (pH H_2O). with a ratio of 1: 5. Then 50 ml KCl 1 M is for second bottle (pH KCl). Then it was shaken with a shaker for 30 minutes. The soil suspension was measured with a calibrated pH meter using a buffer solution of pH 7.0 and pH 4.0. Then the writer recorded the pH value in 2 decimal places. (Balai Penelitaian Tanah, 2005)

3. C-organic

Dry soil wind weighed as much as 1 gram, then it was put into the erlenmeyer flask and then added 5 ml of K2Cr2O7 and H_2SO_4. The liquid was cooled and diluted with added aquadest up to 200 ml. Then *diphenylamine* solution and titrated with *ammonium ferro sulfate* solution until the suspense turned green. Primary solution (peniter) was recorded and made blank without using soil samples, then it was calculated.

4. N-Total

The clear extract solution of the destructed product was 25 ml each into boiling flask and boiling stone was added, then it was diluted with aquades to 100 ml, plus 20 ml of 30% NaOH and boiling flask was immediately closed. Then boiling flask was connected to a distillation apparatus to distill the removed N and accommodated by an erlenmeyer containing 10 ml of 1% boric acid and three drops of Conway indicator (red). Distillation was carried out until the volume of the 60 ml container solution was

green. The distillation solution was then subjected to H2SO4 (0.05 N) until the green color turns pink. A blank solution was then prepared and calculated. (Usman, 2012).

5. Al-exc

Soil samples were weighed 5 gr, then it was put into a 250 ml erlenmeyer flask, added 50 ml 1 N KCl and covered the erlenmeyer flask with plastic and shaken the erlenmeyer flask with a shaking machine ± 15 minutes. After being shaken, filtered soil solution that has been shaken by Whatman sieve was accommodated by plastic bottles. Then 25 ml of the filter was put into a 100 ml erlenmeyer flask and added 5 drops of Fenolphtalein indicator. It was titrated with 0.1 N NaOH solution with 10ml burette to a light pink solution. After that, 1 drop of 0.01 N HCl was added to the erlenmeyer and the erlenmeyer was until shaken the solution was clear white. Then added a solution of 4% NaF was as much as 10 ml, if the solution contains Al then the solution would turn pink again. Then was the solution was re-titrated using 0.01 N HCl until the solution color turned clear white.

The soil reaction (pH), C-Organic, N-total, Al-exc and bacteria population data were analyzed with Anova at α = 0,05. If Anova results show a real difference, the data would processed with Tukey's HSD (Honest Significant Difference) test using statistics program 7.0.

RESULTS AND DISCUSSION

Characteristics Soil of Soil Chemistry In Four Land Typologies

Differences of soil chemical properties on various land typologies prior to lime application based on a 5% Tukey's Honest Significant Difference (HSD) test were presented in Table 1:

Table 1. Characteristics soil of soil chemistry in four land typologies

Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
A	$3{,}87^{b}$	$51{,}97^{a}$	$1{,}68^{a}$	$4{,}49^{b}$
B	$4{,}21^{a}$	$62{,}56^{a}$	$1{,}87^{a}$	$2{,}27^{a}$
C	$4{,}24^{a}$	$44{,}11^{a}$	$1{,}48^{a}$	$2{,}03^{a}$
D	$4{,}11^{a}$	$59{,}43^{a}$	$1{,}68^{a}$	$1{,}77^{a}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Based on the significant difference test of Tukey's Honest Significant Difference (HSD) presented in Table 1, it was known that pH and Al-exc on typology A were significantly different from pH and Al-excon typology B, C and D. This is because the soil A pH was lower than soil typology B, C and D. A typology soil was very acidic because it had Al-exc high. Which was known that Aluminum content in soil could be exchanged generally on acid soils. This was supported by the statement by Ewin et al (2015) that the increase of Al-exc content of aluminum saturation soils will also increase the soil pH and also related to the interchangeable aluminum content and aluminum saturation of soil. While C-organic and N-total on typology A, B, C and D showed no significant difference.

Based on Table 1 above it was known that C-organic, N total, pH and Al-exc of the four typologies of tidal lands were not application lime treatment. In the table above the value of C-organic shows the number 44.11 to 62.56, where the amount has shown that the C-organic content in the four typologies of the land were high. It was known that the high amount of C-organic in the soil may decrease the pH of the land. In addition, high C-organic content also becomes a nutrient for microbial life on soils, especially non-symbiotic nitrogen fixing bacteria.

Effect Of Four Land Typologies And Lime Application On The Population Of Indigenous Nitrogen Fixing Bacteria

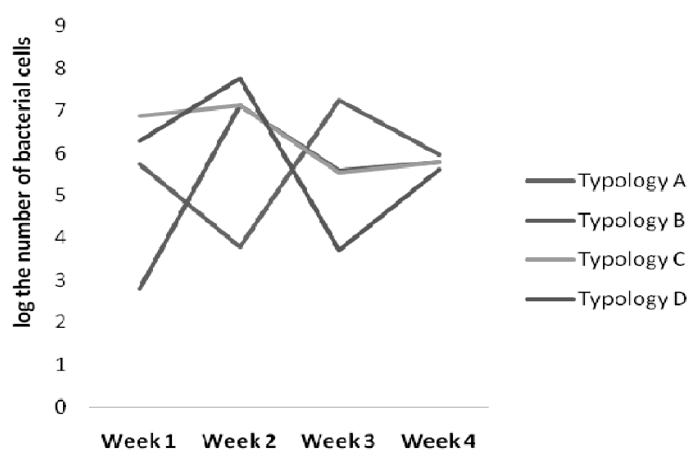


Figure 1. Effect of four land typologies on population of indigenous nitrogen fixing bacteria

Figure 1 above was presented based on the table of the effect of four typologies of land on the number of bacterial populations. According to the figure above the bacterial population at the highest was shown in the second week in the soil typology D, whereas the soil typology A in the second week showed the lowest population. In the fourth week the lowest of bacterial population for all typologies. The figure above showed the distance

between the top of the population of bacteria in typology A and D was quite far away, it was significant difference, but the Anova test results did not show was significant differently. It is caused by the variation of data on the amount of bacteria obtained was very diverse. When viewed as the highest number of populations during the second week in all land typologies, and decreased in the third and the fourth week, it was thought that in the second week is the optimal growth phase in bacteria so that if extended at week three and four many bacteria die, the bacterial population decreased in those weeks.

The lowest bacterial populations obtained in typology B in the first week, when viewed by the amount of nutrients in typology B has very high C-organic and N-total content compared with typology A, C and D (Table 1). High N-total can provide nutrients for bacterial growth, but high N-total results in nitrogen fixing bacteria unable to perform nitrogen fixation, supported by the statement of Imas et al. (1989), nitrogen-fixing bacteria are capable of using nitrogen compounds in the form of ammonium, nitrate, and organic nitrogen compounds. It becomes the most effective nitrogen fixing inhibitor.

Table 2. Effect of lime application on the population of indigenous nitrogen fixing bacteria

Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
	Week 1	Week 2	Week 3	Week 4
ND	$2{,}4x10^{5}$	$8{,}3x10^{6}$	$1{,}2x10^{7}$ b	$1{,}2x10^{7}$ b
D	$3{,}0x10^{5}$	$9{,}3x10^{5}$	$3{,}0x10^{3}$ a	$3{,}0x10^{5}$ a

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Overall, the population of bacteria is more common in unregulated limestone soils. It was thought that limiting certain amounts of lime can inhibit the growth of the indigenous nitrogen fixing bacteria living on the land, according to Havlin et. Al, (1999) the provision of lime to increase soil pH in the tropics often decreases the production due to over liming. Therefore, calcification should be aimed at eliminating the toxic effects of Al^{3+} ions.

Based on Table 2 above, it was found that the population of nitrogen fixing bacteria treated in the third week was a decrease in the bacterial population, while in the fourth week the bacterial population increased and the population was as same as the population in the first week. It was suspected that lime application on over a period of time affects the life of the indigenous nitrogen fixing bacteria in the soil. While the population of nitrogen-fixing bacteria on the soil not application of lime from week to week increased the population of nitrogen-fixing bacteria. It was suspected that the bacteria grew on the soil is an indigenous nitrogen-fixing bacteria that has adapted itself to acid soils.

It was recommended for farmers to plant on soil that has been given a blur so that the plants are not poisoned by pyrite compounds, in the first and second weeks after treatment, because at that time the population of live nitrogen-fixing bacteria still sufficient to supply

free nitrogen through non-symbiotic nitrogen fixation process, it was supported by the statement of Purwaningsih and Saefudin (2012), plant that has high protein content indicates that the plant requires a lot of nutrients, especially nutrients N for growth

Table 3. Interaction effects of four typologies of soil and lime application on the population of indigenous nitrogen fixing bacteria

Soil Typologies	Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
		Week 1	Week 2	Week 3	Week 4
A	ND	$1,3x10^{5}$	$1,3x10^{7\ a}$	$3,3x10^{7}$	$1,8x10^{7}$
	D	$2,0\ x10^{5}$	$3,0\ x10^{2\ b}$	$1,3\ x10^{7}$	$4,0\ x10^{5}$
B	ND	$2,0x10^{2}$	$1,9x10^{7\ a}$	$1,1x10^{7}$	$1,5x10^{7}$
	D	$2,6\ x10^{3}$	$9,0\ x10^{5\ ab}$	$1,3\ x10^{4}$	$2,0\ x10^{5}$
C	ND	$3,3x10^{7}$	$2,2x10^{7\ a}$	$5,1x10^{6}$	$1,4x10^{7}$
	D	$2,0\ x10^{5}$	$8,0\ x10^{5\ ab}$	$2,2\ x10^{4}$	$2,0\ x10^{5}$
D	ND	$5,0x10^{5}$	$8,8\ x10^{5\ ab}$	$1,3\ x10^{7}$	$8,0x10^{5}$
	D	$8,1\ x10^{5}$	$3,5\ x10^{7\ a}$	$1,9\ x10^{2}$	$2,0\ x10^{5}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types in tidal land, ND = treatment without dolomite, D = treatment with dolomite)

In the first, third and fourth weeks of the population on the interaction of the four soil typologies and lime treatment and no lime showed no significant difference. The second week showed significant differences in bacterial populations on the soil typology A given lime and soil typology A which was not given lime, but the population of bacteria on calcified soil typology A was not significantly different from the soil typology B, C, D which are lime application or not. In the second week on soil typology A, B, and C bacterial populations were higher in the treatment without lime, whereas in typology D the number of bacterial populations was higher in the treatment of lime.

Effects Of Four Typologies Of Soil And Lime Application On Soil Reactions (pH)

Analysis of Varian (ANOVA) on the effects of four typologies of land to soil reaction (pH) in the first week was obtained $p = 0,119964$ ($p > 0,05$) at α 5%. It showed that in the first week of soil typology has no significant effect on pH, but in the second week, the third until the fourth of the anova test results showed that the effect of land typology on pH, in which $p = 0,00$ ($p < 0,05$) at α 5%.

Table 4. The effect of four land typologies on soil reactions (pH)

Soil Typologies	Soil Reactions (pH)			
	Week 1	Week 2	Week 3	Week 4
A	4,33	4,43 ^{a}	4,50 ^{ab}	4,42 ^{a}
B	4,82	5,13 ^{b}	5,18 ^{c}	5,12 ^{a}
C	4,60	4,42 ^{a}	4,43 ^{a}	4,50 ^{a}
D	4,55	4,52 ^{a}	4,57 ^{b}	4,53 ^{b}

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Table 4 above after 5% Tukey's Honest Significant Difference (HSD) test showed that the first week of pH on soil typology A, B, C and D was not significantly different, however in the second, the third and the fourth week pH on typology A, B , C and D showed the significantly different. In the second week the pH of typology B was significantly different from typology A, C and D, but the three typologies were not significantly different from each other. It is in contrast to the third week. The pH of typology A was not significantly different from typology C and D, but it was significantly different from typology B. In contrast, soil typology B was significantly different from soil typology A, C and D. In soil typology C was significantly different from soil typology D.

Based on the data in Table 4, it may be recommended for local farmers to plant on typology A and B fields during the third week after lime treatment, since typology A and B at that time have been a good time for planting with high increase in pH. While for typology C and D, planting by farmer should be done in the first week after lime treatment, because in typology C and D, the highest pH increase reaction occurs in the first week after lime treatment.

Table 5. The effect of lime application on the soil reaction (pH)

Treatment	Soil Reaction (pH)			
	Week 1	Week 2	Week 3	Week 4
ND	4,42 ^{a}	4,86 ^{a}	4,45 ^{a}	4,45 ^{a}
D	4,73 ^{b}	4,93 ^{b}	4,89 ^{b}	4,83 ^{b}

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on the 5% Tukey's Honest Significant Difference (HSD) test indicated in Table 5 above that lime application had a significant effect on pH altitude. It was supported by Basuki (2007), one of the goals of lime on acid soil is to increase the soil pH through hydrolysis of $CaCO_3$ so as to affect the dissolution of Al or Fe and prevent poisoning in plants. In the second week the highest pH value was compared to the first, third or fourth week.

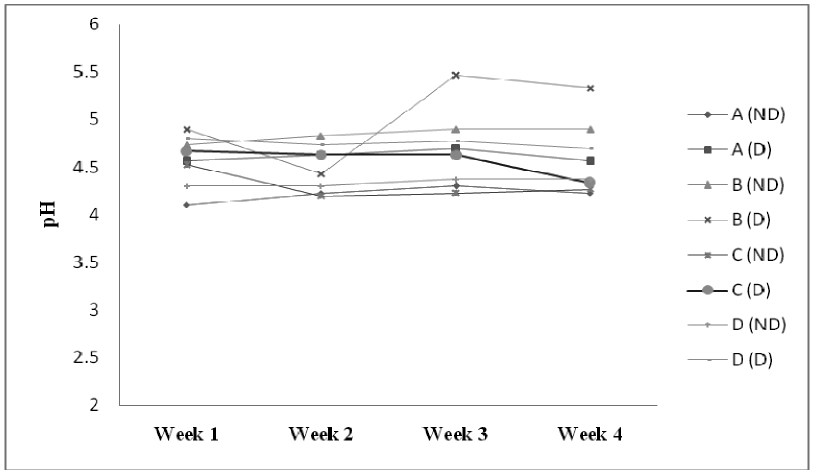


Figure 2. Interaction effects of four land typologies and lime application on soil reactions (pH) (A, B, C and D = tidal soil type, ND = treatment without dolomite, D = treatment with dolomite)

Figure 2 above, based on the Table of effects of four land typologies to pH, in the first week the highest soil pH was found in the soil typology B was treated with lime, soil typology B treated with lime from the first week showed the highest pH ie, range 5, but in the second week was decreased. In typology A, C and D either treated with lime or not, it did not show significant differences in soil pH from the first week to the fourth week.

Soil Chemical Properties in Four Typologies After Soil Treatment

Table 6. Soil chemical properties in four typologies after soil treatment

No.	Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
1.	A	4,42 [a]	58,12 [b]	3,24 [b]	4,43
2.	B	5,12 [a]	27,26 [a]	1,61 [a]	2,55
3.	C	4,50 [a]	26,23 [a]	1,67 [a]	5,21
4.	D	4,53 [b]	27,13 [a]	1,61 [a]	4,11

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

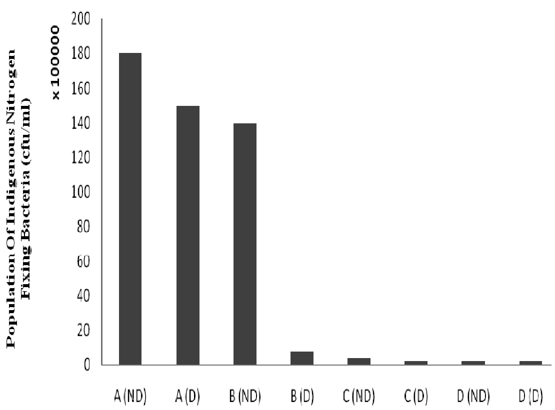
When compared to the initial soil chemical properties measurement of pH values, C-organic and Al-exc on average increased in measurement of soil chemical properties the four typologies after soil treatment. N-total increased significantly only in typology A, compared with typology B, C and D. According to Prasetyo and Suradikarta (2006), high Al saturation value is found in Ultisol soils of sediment and granite (> 60%), and Low value on Ultisol soil from andesitic and volcanic volcanic materials (0%). The ultisole of the tufa material has a low Al saturation in the upper layer (5-8%), but it is high in the bottom layer (37-78%). It seems that Al saturation on Ultisol soils related to soil pH.

Table 7. Soil chemical properties in four typologies after soil treatment at four weeks

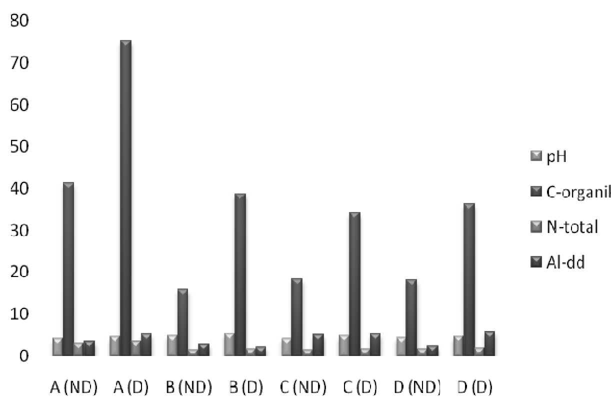
Treatment	pH	C-organic (g)	N-total (g)
ND	4,45 [a]	23,35 [a]	1,92
D	4,83 [b]	46,01 [b]	2,22

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on Table 7 according to the 5% Tukey's Honest Significant Difference (HSD) test, it was found that at pH and C-organic significantly affected lime treatment. At lime-treated pH was significantly different from pH which was not given lime. It also occurred in C-organics that was lime application on significantly different from those of not application of lime on C-organic.According to Bot and Benites (2005), the value of C-organic values in soil can show the content of organic matter in the soil which is an important benchmark for management on agricultural soils and C-organic was believed to be the key to resistance to drought and sustainability of food production. In addition, C-organic was also effected by the type of vegetation on soil.



(a)



(b)

Figure 3. Interaction effects of four land typologies and lime application on the population of indigenous nitrogen fixing bacteria (a) and Soil chemical properties in four typologies after soil treatment [pH, C-organic, N-total, Al-exc] (b). [A, B, C and D = typologies of tidal land], [ND = treatment without dolomite, D = treatment with dolomite]

Based on Figure 3 it can be seen the number of bacterial population (a) decreased the number of bacteria in the four typologies of soil with lime. At pH (b) there was no significant increase and decrease in the interaction of the four typologies of soil and lime

treatment. In C-organic and N-total increases in the soil typology A given lime. Aldd had no significant effect on the interaction of the four typologies of soil and lime treatment.

Given the initial calculation of soil chemical properties prior to the application of lime amounts of soil C-organic soils in the four high land typologies, it is thought to be one of the causes of soil acidity due to the presence of organic compounds. Lime appication on a certain amount can increase the soil pH but indirectly proportional to the population of growing bacteria. It is suspected that lime application can inhibit the organic acids needed by the living bacteria of the soil. According to Winarso (2005), organic matter in the soil can be defined as the remains of plants and animals in the soil in various weathering, consisting of both living and death. Organic matter in the soil can function or improve on chemical, physical and biological properties of the soil.

CONCLUSION

Based on the research that has been done, then it can be concluded that tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected soil pH. The highest total number of bacteria in the second week on the soil typology D, was $6.0 x 10^7$ CFU / ml and the highest pH of 5.18 on typology B was in the third week. Application of dolomite affected the population of indigenous nitrogen fixing bacteria, where in dolomite application on the soil populations was fewer than untreated dolomite, with the largest bacterial population at $9.3 x 10^5$ CFU / ml in the second week and dolomite increased the soil pH, with the highest soil pH of 4.93 in the second week. And the interaction between land typology and dolomite application on only affected the population of indigenous nitrogen-fixing bacteria with the most populations of $3.5 x 10^7$ CFU / ml on soil typology D in the second week. The highest pH of 5.33 on soil typology B was in the fouth week .

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Effect Of Lime Application On Indigenous Nitrogen-Fixing Bacteria In Tidal Soils Managed For More Than 30 Years

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah, serta menganalisis dampak interaksi tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah. Pengambilan sampel tanah di lahan pasang surut Desa Mulya Sari dan Banyu Urip Kabupaten Banyuasin, Sumatera Selatan. Kemudian sampel diteliti di Laboratorium Mikrobiologi, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sriwijaya, Indralaya, selama bulan Januari-Mei 2017. Berdasarkan hasil Tipologi lahan pasang surut tidak mempengaruhi populasi bakteri penambat nitrogen indigen, tetapi berpengaruh terhadap pH tanah. Jumlah total bakteri terbanyak pada minggu kedua pada tipologi tanah D, yaitu $6{,}0x10^7$ CFU/ml dan pH tertinggi 5,18 pada tipologi B pada minggu ketiga. Pemberian dolomit mempengaruhi populasi bakteri penambat nitrogen indigen, dimana pada tanah yang diberi dolomit populasi bakteri lebih sedikit dibandingkan tanah yang tidak diberi perlakuan dolomit, dengan populasi bakteri terbanyak pada $9{,}3x10^5$ CFU/ml pada minggu kedua dan pemberian dolomit menigkatkan pH tanah, dengan pH tanah tertinggi 4,93 pada minggu kedua. Interaksi antara tipologi lahan dan pemberian dolomit hanya memberi pengaruh terhadap populasi bakteri penambat nitrogen indigen dengan populasi terbanyak $3{,}5x10^7$ CFU/ml pada tipe tanah D pada minggu kedua. pH tertinggi 5,33 pada tipologi tanah B pada minggu keempat.

ABSTRACT

This research purposes to know the effect of soil typology and lime on the population of indigenous nitrogen-fixing bacteria and soil pH, and to analyze the effect of soil typology interaction and lime application on population of indigenous nitrogen-fixing bacteria and soil pH. Sampling of the soil was in tidal land of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. Then the samples be inspected in Microbiology Laboratory at Biology Department of Faculty of Science,Sriwijaya University, Inderalaya, during January-May 2017. Based on the results tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected the soil pH. The highest total number of bacteria at the second week on the soil typology D, ie $6.0x10^7$ CFU / ml and the highest pH of 5.18 on typology B in the third week. Application of dolomite affected the population of indigenous nitrogen-fixing bacteria, which in dolomite-treated soil populations were fewer than dolomite un-treated soil, with the largest bacterial population at $9.3x10^5$ CFU / ml during the second week and dolomite increased soil pH, with pH the highest ground of 4.93 at the second week. The interaction between land typology and dolomite application only affected the population of indigenous nitrogen-fixing bacteria with the most populations of $3.5x10^7$ CFU / ml on soil typology D in the second week. The highest pH on soil typology B of 5.33 was at the fourth week.

Keywords: nitrogen-fixing bacteria, tidal land, lime treatment

INTRODUCTION

Tidal swamps of Mulya sari and Banyu Urip villages, South Sumatra, showed that soil pH was below 5.0 after opening of 30 year old with pH values ranging from 4.0-4.59 (very acid to acid) in all land typologies in combination with high H^+ and Al^{3+} exchangeability. The values of H^+ and Al^{3+} were from 0.65-1.0 cmol $^{(+)}$ / kg and 1.77-4.49 cmol $^{(+)}$ / kg (Budianta et al, 2016).

Villages of Banyu Urip and Mulya Sari, the location of soil sampling with respectively has been guided NPK fertilization for more than 30 years. It is known that the application of chemical fertilizers has side effect compared to natural fertilizers. According to Lokasari (2011), NPK's unfavorable nature is continuously that NPK is not ionizing in soil solution so it is easy to wash, since it can not be absorbed by the soil colloids. To be absorbed by plants had to undergo amonification and nitrification process first. The rapid and slow amide-form changes of the urea to the form of N compounds can be absorbed by the plant depending largely on several factors including population, microorganism activity, soil moisture content, soil temperature and amount of Urea fertilizer provided.

Tidal land of Banyu Urip and Mulya Sari villages were used as the location of soil sampling because the area can represent four typologies of tidal land with low soil pH. According to Budianta et. Al., (2016), soil pH measurements in four soil typologies in Banyu Urip and Mulya Sari villages obtained results, were namely in type A with pH 4.05; Type B with pH 4.59; Type C with pH 4.44; And type D with a pH of 4.55, where the lowest pH in the highest H^+ and Al^{3+} exchanged combinations was found in land typology A after rice growth. It was caused by the nitrification process of nitrogen fertilization causing a decrease in soil pH.

Lime is a source of ameliorant material widely used to improve soil fertility. Lime is an important s ource of Ca nutrients that support plant growth, increase soil pH, reduce soil Al and Mn content (Scott and Fisher, 1989; Mora et al., 2002 ; Koesrini et al., 2015). According to Saputri et al. (2016), the addition of dolomite is needed to regulate the acidity (pH) of growing media, calcium and carbon elements can enrich the mineral content of the media, and function as enzyme activators. According to Winarno (2004) lime also serves as an activator of various types of enzymes that are related in the metabolism of proteins and carbohydrates.

According to Kuswandi (1993), with the increaseof the pH of the soil, the supply of Mg and Ca nutrients can shift the position of H + on the colloid surface to neutralize the soil acidity. Calcification also aims to reduce the risk of aluminium effects, increase the availability of P elements as a result of P extraction from Al-P and Fe-P bonds, increase N fixation and N mineralization to increase CEC, and assist in the completion of the reform with accompanying nutrient release from organic materials and microbial bodies.

MATERIALS AND METHOD

This research was conducted from January to June 2017. Sampling of soil samples was taken in Tidal area of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. The research was conducted in Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya.

Equipment used in the field were sack, soil tester and a shovel. The tools used in the laboratory were autoclave, colony counter, pH meter, erlemeyer ml, hot plate, incubator, ose needle, konduktormeter, bunsen lamp, mixer, mortarporselein, petri dish, 1 ml micropipette, plastic and test tube. Materials needed were LGI medium, aquadest, and dolomite.

This research was used Factorial Complete Random Design, with treatment factor, as follows:

Factor 1: Dolomite Dose (K)

K0 = 0 (without treatment)

K1 = 2 Ton ha^{-1}

Factor 2: Tidal Swamp Land Typology (L)

L1 = Typology A

L2 = Typology B

L3 = Typology C

L4 = Typology D

The number of combinations each repeated 3 times, so it would produce 24 units of experiment.

Soil samples from tidal swamps were types A, B, C and D with aseptic method at 3 soil sampling points and homogenised on each typology. after having homogenized then the samples were inserted into a sterilized sac with different codes according to their respective topological type, then stored in cool box. The soil samples taken from layers of cultivation with a depth of about 0-20 cm in geographical coordinates are located about S 02^0 41'24 ", E $104^0$45'25" for A, S $02^0$39'26 "E $104^0$44'27" for B,S $02^0$39 '44 ", E $104^0$44'027" for C, and S $02^0$38'37 ", E $104^0$43'16" for typology D

Soil with different typology sieved with the sieve size of 0.5 mm hole diameter, then weighed as much as 1000 g, then dried up the ground, after the soil was inserted into polybag, the moisture was measured by using Soil tester. If the soil moisture content is less than 85% (optimum humidity for bacterial growth) first soil has to be given water using 500 ml water bottle poured slowly on the soil, and the humidity increases (Anonymous, 2017). Then dolomite was applicated to each soil in polybags with different dolomite amounts according to the dosage, for 1000 g of soil volume 1.84 g for dose 2 ton ha^{-1} and

on control not application of dolomite. Then it is calculated and observed and measured pH every 1 week for 28 days.

Variables observed were: (1) Total population of bacteria; (2) pH; (3) C-Orgaanic; (4) N-Total; (5) Al-exc

1. Number of Bacterial Populations

The calculation of bacterial cell count was performed by diluting 5 g samples taken from treated soil into 45 mL sterile aquades, made up to a dilution level of 10-6. The result of dilution from 10-4 - 10-6, was taken each 1 mL then put into a sterile petri dish that did not contain the medium of LGI, after inserted 1 ml of dilution, it is the medium of LGI was put wrapped. Then it was incubated at 37^oC for 24 hours. The number of bacteria that grow on counter colonies was Calculated (Modified Munawar, 1999).

2. Soil Reaction (pH)

Soil pH measurements were performed before dolomite given, then it was measured again after dolomite was given once a week for 28 days by means of soil weighed as much as 10 g twice, each was inserted into a shake bottle, and 50 ml of aquades were added to the bottle first (pH H_2O). with a ratio of 1: 5. Then 50 ml KCl 1 M is for second bottle (pH KCl). Then it was shaken with a shaker for 30 minutes. The soil suspension was measured with a calibrated pH meter using a buffer solution of pH 7.0 and pH 4.0. Then the writer recorded the pH value in 2 decimal places. (Balai Penelitaian Tanah, 2005)

3. C-organic

Dry soil wind weighed as much as 1 gram, then it was put into the erlenmeyer flask and then added 5 ml of K2Cr2O7 and H_2SO_4. The liquid was cooled and diluted with added aquadest up to 200 ml. Then *diphenylamine* solution and titrated with *ammonium ferro sulfate* solution until the suspense turned green. Primary solution (peniter) was recorded and made blank without using soil samples, then it was calculated.

4. N-Total

The clear extract solution of the destructed product was 25 ml each into boiling flask and boiling stone was added, then it was diluted with aquades to 100 ml, plus 20 ml of 30% NaOH and boiling flask was immediately closed. Then boiling flask was connected to a distillation apparatus to distill the removed N and accommodated by an erlenmeyer containing 10 ml of 1% boric acid and three drops of Conway indicator (red). Distillation was carried out until the volume of the 60 ml container solution was

green. The distillation solution was then subjected to H_2SO_4 (0.05 N) until the green color turns pink. A blank solution was then prepared and calculated. (Usman, 2012).

5. Al-exc

Soil samples were weighed 5 gr, then it was put into a 250 ml erlenmeyer flask, added 50 ml 1 N KCl and covered the erlenmeyer flask with plastic and shaken the erlenmeyer flask with a shaking machine ± 15 minutes. After being shaken, filtered soil solution that has been shaken by Whatman sieve was accommodated by plastic bottles. Then 25 ml of the filter was put into a 100 ml erlenmeyer flask and added 5 drops of Fenolphtalein indicator. It was titrated with 0.1 N NaOH solution with 10ml burette to a light pink solution. After that, 1 drop of 0.01 N HCl was added to the erlenmeyer and the erlenmeyer was until shaken the solution was clear white. Then added a solution of 4% NaF was as much as 10 ml, if the solution contains Al then the solution would turn pink again. Then was the solution was re-titrated using 0.01 N HCl until the solution color turned clear white.

The soil reaction (pH), C-Organic, N-total, Al-exc and bacteria population data were analyzed with Anova at $\alpha = 0,05$. If Anova results show a real difference, the data would processed with Tukey's HSD (Honest Significant Difference) test using statistics program 7.0.

RESULTS AND DISCUSSION

Characteristics Soil of Soil Chemistry In Four Land Typologies

Differences of soil chemical properties on various land typologies prior to lime application based on a 5% Tukey's Honest Significant Difference (HSD) test were presented in Table 1:

Table 1. Characteristics soil of soil chemistry in four land typologies

Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
A	$3{,}87^{b}$	$51{,}97^{a}$	$1{,}68^{a}$	$4{,}49^{b}$
B	$4{,}21^{a}$	$62{,}56^{a}$	$1{,}87^{a}$	$2{,}27^{a}$
C	$4{,}24^{a}$	$44{,}11^{a}$	$1{,}48^{a}$	$2{,}03^{a}$
D	$4{,}11^{a}$	$59{,}43^{a}$	$1{,}68^{a}$	$1{,}77^{a}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Based on the significant difference test of Tukey's Honest Significant Difference (HSD) presented in Table 1, it was known that pH and Al-exc on typology A were significantly different from pH and Al-excon typology B, C and D. This is because the soil A pH was lower than soil typology B, C and D. A typology soil was very acidic because it had Al-exc high. Which was known that Aluminum content in soil could be exchanged generally on acid soils. This was supported by the statement by Ewin et al (2015) that the increase of Al-exc content of aluminum saturation soils will also increase the soil pH and also related to the interchangeable aluminum content and aluminum saturation of soil. While C-organic and N-total on typology A, B, C and D showed no significant difference.

Based on Table 1 above it was known that C-organic, N total, pH and Al-exc of the four typologies of tidal lands were not application lime treatment. In the table above the value of C-organic shows the number 44.11 to 62.56, where the amount has shown that the C-organic content in the four typologies of the land were high. It was known that the high amount of C-organic in the soil may decrease the pH of the land. In addition, high C-organic content also becomes a nutrient for microbial life on soils, especially non-symbiotic nitrogen fixing bacteria.

Effect Of Four Land Typologies And Lime Application On The Population Of Indigenous Nitrogen Fixing Bacteria

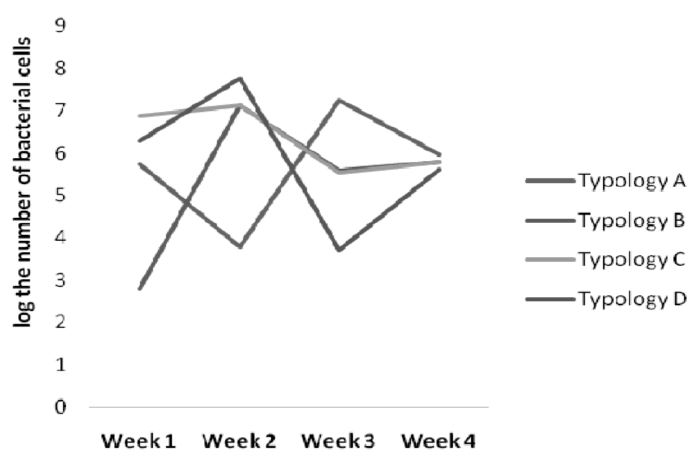


Figure 1. Effect of four land typologies on population of indigenous nitrogen fixing bacteria

Figure 1 above was presented based on the table of the effect of four typologies of land on the number of bacterial populations. According to the figure above the bacterial population at the highest was shown in the second week in the soil typology D, whereas the soil typology A in the second week showed the lowest population. In the fourth week the lowest of bacterial population for all typologies. The figure above showed the distance

between the top of the population of bacteria in typology A and D was quite far away, it was significant difference, but the Anova test results did not show was significant differently. It is caused by the variation of data on the amount of bacteria obtained was very diverse. When viewed as the highest number of populations during the second week in all land typologies, and decreased in the third and the fourth week, it was thought that in the second week is the optimal growth phase in bacteria so that if extended at week three and four many bacteria die, the bacterial population decreased in those weeks.

The lowest bacterial populations obtained in typology B in the first week, when viewed by the amount of nutrients in typology B has very high C-organic and N-total content compared with typology A, C and D (Table 1). High N-total can provide nutrients for bacterial growth, but high N-total results in nitrogen fixing bacteria unable to perform nitrogen fixation, supported by the statement of Imas et al. (1989), nitrogen-fixing bacteria are capable of using nitrogen compounds in the form of ammonium, nitrate, and organic nitrogen compounds. It becomes the most effective nitrogen fixing inhibitor.

Table 2. Effect of lime application on the population of indigenous nitrogen fixing bacteria

Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
	Week 1	Week 2	Week 3	Week 4
ND	$2{,}4x10^5$	$8{,}3x10^6$	$1{,}2x10^{7\ b}$	$1{,}2x10^{7\ b}$
D	$3{,}0x10^5$	$9{,}3x10^5$	$3{,}0x10^{3\ a}$	$3{,}0x10^{5\ a}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Overall, the population of bacteria is more common in unregulated limestone soils. It was thought that limiting certain amounts of lime can inhibit the growth of the indigenous nitrogen fixing bacteria living on the land, according to Havlin et. Al, (1999) the provision of lime to increase soil pH in the tropics often decreases the production due to over liming. Therefore, calcification should be aimed at eliminating the toxic effects of Al^{3+} ions.

Based on Table 2 above, it was found that the population of nitrogen fixing bacteria treated in the third week was a decrease in the bacterial population, while in the fourth week the bacterial population increased and the population was as same as the population in the first week. It was suspected that lime application on over a period of time affects the life of the indigenous nitrogen fixing bacteria in the soil. While the population of nitrogen-fixing bacteria on the soil not application of lime from week to week increased the population of nitrogen-fixing bacteria. It was suspected that the bacteria grew on the soil is an indigenous nitrogen-fixing bacteria that has adapted itself to acid soils.

It was recommended for farmers to plant on soil that has been given a blur so that the plants are not poisoned by pyrite compounds, in the first and second weeks after treatment, because at that time the population of live nitrogen-fixing bacteria still sufficient to supply

free nitrogen through non-symbiotic nitrogen fixation process, it was supported by the statement of Purwaningsih and Saefudin (2012), plant that has high protein content indicates that the plant requires a lot of nutrients, especially nutrients N for growth

Table 3. Interaction effects of four typologies of soil and lime application on the population of indigenous nitrogen fixing bacteria

Soil Typologies	Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
		Week 1	Week 2	Week 3	Week 4
A	ND	$1,3x10^5$	$1,3x10^{7\ a}$	$3,3x10^7$	$1,8x10^7$
	D	$2,0\ x10^5$	$3,0\ x10^{2\ b}$	$1,3\ x10^7$	$4,0\ x10^5$
B	ND	$2,0x10^2$	$1,9x10^{7\ a}$	$1,1x10^7$	$1,5x10^7$
	D	$2,6\ x10^3$	$9,0\ x10^{5\ ab}$	$1,3\ x10^4$	$2,0\ x10^5$
C	ND	$3,3x10^7$	$2,2x10^{7\ a}$	$5,1x10^6$	$1,4x10^7$
	D	$2,0\ x10^5$	$8,0\ x10^{5\ ab}$	$2,2\ x10^4$	$2,0\ x10^5$
D	ND	$5,0x10^5$	$8,8\ x10^{5\ ab}$	$1,3\ x10^7$	$8,0x10^5$
	D	$8,1\ x10^5$	$3,5\ x10^{7\ a}$	$1,9\ x10^2$	$2,0\ x10^5$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types in tidal land, ND = treatment without dolomite, D = treatment with dolomite)

In the first, third and fourth weeks of the population on the interaction of the four soil typologies and lime treatment and no lime showed no significant difference. The second week showed significant differences in bacterial populations on the soil typology A given lime and soil typology A which was not given lime, but the population of bacteria on calcified soil typology A was not significantly different from the soil typology B, C, D which are lime application or not. In the second week on soil typology A, B, and C bacterial populations were higher in the treatment without lime, whereas in typology D the number of bacterial populations was higher in the treatment of lime.

Effects Of Four Typologies Of Soil And Lime Application On Soil Reactions (pH)

Analysis of Varian (ANOVA) on the effects of four typologies of land to soil reaction (pH) in the first week was obtained $p = 0,119964$ ($p > 0,05$) at α 5%. It showed that in the first week of soil typology has no significant effect on pH, but in the second week, the third until the fourth of the anova test results showed that the effect of land typology on pH, in which $p = 0,00$ ($p < 0,05$) at α 5%.

Table 4. The effect of four land typologies on soil reactions (pH)

Soil Typologies	Soil Reactions (pH)			
	Week 1	Week 2	Week 3	Week 4
A	4,33	4,43 [a]	4,50 [ab]	4,42 [a]
B	4,82	5,13 [b]	5,18 [c]	5,12 [a]
C	4,60	4,42 [a]	4,43 [a]	4,50 [a]
D	4,55	4,52 [a]	4,57 [b]	4,53 [b]

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Table 4 above after 5% Tukey's Honest Significant Difference (HSD) test showed that the first week of pH on soil typology A, B, C and D was not significantly different, however in the second, the third and the fourth week pH on typology A, B , C and D showed the significantly different. In the second week the pH of typology B was significantly different from typology A, C and D, but the three typologies were not significantly different from each other. It is in contrast to the third week. The pH of typology A was not significantly different from typology C and D, but it was significantly different from typology B. In contrast, soil typology B was significantly different from soil typology A, C and D. In soil typology C was significantly different from soil typology D.

Based on the data in Table 4, it may be recommended for local farmers to plant on typology A and B fields during the third week after lime treatment, since typology A and B at that time have been a good time for planting with high increase in pH. While for typology C and D, planting by farmer should be done in the first week after lime treatment, because in typology C and D, the highest pH increase reaction occurs in the first week after lime treatment.

Table 5. The effect of lime application on the soil reaction (pH)

Treatment	Soil Reaction (pH)			
	Week 1	Week 2	Week 3	Week 4
ND	4,42 [a]	4,86 [a]	4,45 [a]	4,45 [a]
D	4,73 [b]	4,93 [b]	4,89 [b]	4,83 [b]

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on the 5% Tukey's Honest Significant Difference (HSD) test indicated in Table 5 above that lime application had a significant effect on pH altitude. It was supported by Basuki (2007), one of the goals of lime on acid soil is to increase the soil pH through hydrolysis of $CaCO_3$ so as to affect the dissolution of Al or Fe and prevent poisoning in plants. In the second week the highest pH value was compared to the first, third or fourth week.

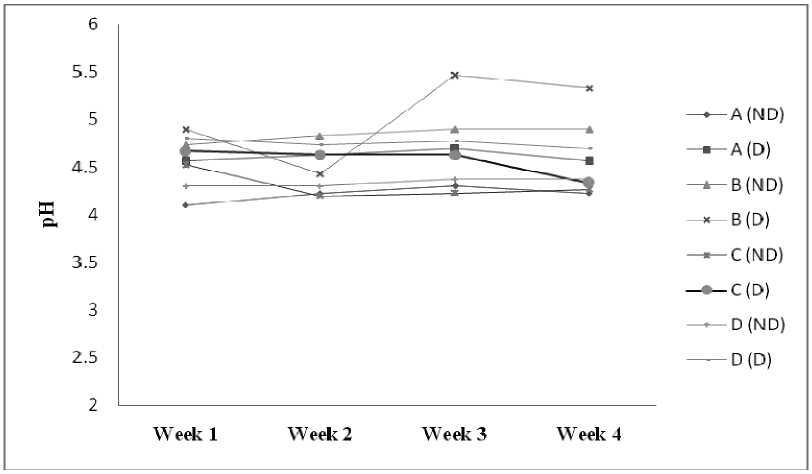


Figure 2. Interaction effects of four land typologies and lime application on soil reactions (pH) (A, B, C and D = tidal soil type, ND = treatment without dolomite, D = treatment with dolomite)

Figure 2 above, based on the Table of effects of four land typologies to pH, in the first week the highest soil pH was found in the soil typology B was treated with lime, soil typology B treated with lime from the first week showed the highest pH ie, range 5, but in the second week was decreased. In typology A, C and D either treated with lime or not, it did not show significant differences in soil pH from the first week to the fourth week.

Soil Chemical Properties in Four Typologies After Soil Treatment

Table 6. Soil chemical properties in four typologies after soil treatment

No.	Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
1.	A	4,42 ^{a}	58,12 ^{b}	3,24 ^{b}	4,43
2.	B	5,12 ^{a}	27,26 ^{a}	1,61 ^{a}	2,55
3.	C	4,50 ^{a}	26,23 ^{a}	1,67 ^{a}	5,21
4.	D	4,53 ^{b}	27,13 ^{a}	1,61 ^{a}	4,11

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

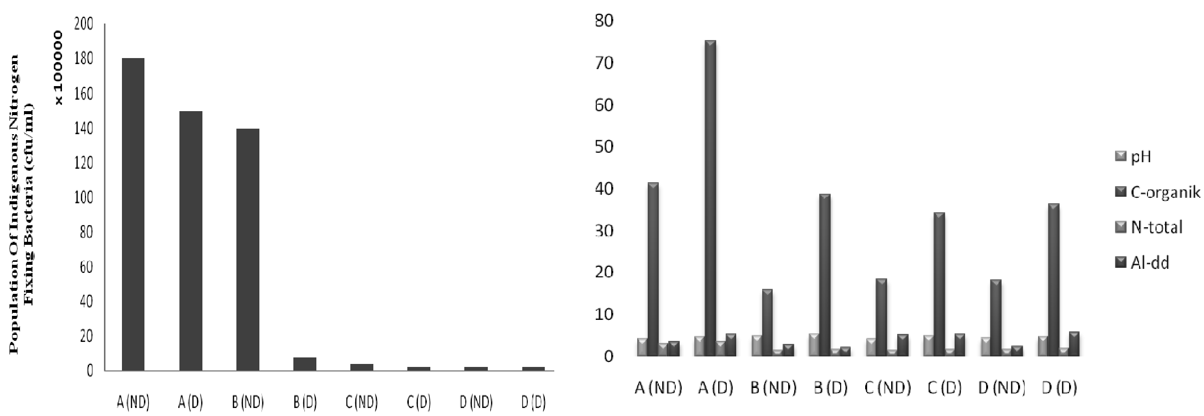
When compared to the initial soil chemical properties measurement of pH values, C-organic and Al-exc on average increased in measurement of soil chemical properties the four typologies after soil treatment. N-total increased significantly only in typology A, compared with typology B, C and D. According to Prasetyo and Suradikarta (2006), high Al saturation value is found in Ultisol soils of sediment and granite (> 60%), and Low value on Ultisol soil from andesitic and volcanic volcanic materials (0%). The ultisole of the tufa material has a low Al saturation in the upper layer (5-8%), but it is high in the bottom layer (37-78%). It seems that Al saturation on Ultisol soils related to soil pH.

Table 7. Soil chemical properties in four typologies after soil treatment at four weeks

Treatment	pH	C-organic (g)	N-total (g)
ND	4,45 [a]	23,35 [a]	1,92
D	4,83 [b]	46,01 [b]	2,22

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on Table 7 according to the 5% Tukey's Honest Significant Difference (HSD) test, it was found that at pH and C-organic significantly affected lime treatment. At lime-treated pH was significantly different from pH which was not given lime. It also occurred in C-organics that was lime application on significantly different from those of not application of lime on C-organic.According to Bot and Benites (2005), the value of C-organic values in soil can show the content of organic matter in the soil which is an important benchmark for management on agricultural soils and C-organic was believed to be the key to resistance to drought and sustainability of food production. In addition, C-organic was also effected by the type of vegetation on soil.



(a) (b)

Figure 3. Interaction effects of four land typologies and lime application on the population of indigenous nitrogen fixing bacteria (a) and Soil chemical properties in four typologies after soil treatment [pH, C-organic, N-total, Al-exc] (b). [A, B, C and D = typologies of tidal land], [ND = treatment without dolomite, D = treatment with dolomite]

Based on Figure 3 it can be seen the number of bacterial population (a) decreased the number of bacteria in the four typologies of soil with lime. At pH (b) there was no significant increase and decrease in the interaction of the four typologies of soil and lime

treatment. In C-organic and N-total increases in the soil typology A given lime. Aldd had no significant effect on the interaction of the four typologies of soil and lime treatment.

Given the initial calculation of soil chemical properties prior to the application of lime amounts of soil C-organic soils in the four high land typologies, it is thought to be one of the causes of soil acidity due to the presence of organic compounds. Lime appication on a certain amount can increase the soil pH but indirectly proportional to the population of growing bacteria. It is suspected that lime application can inhibit the organic acids needed by the living bacteria of the soil. According to Winarso (2005), organic matter in the soil can be defined as the remains of plants and animals in the soil in various weathering, consisting of both living and death. Organic matter in the soil can function or improve on chemical, physical and biological properties of the soil.

CONCLUSION

Based on the research that has been done, then it can be concluded that tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected soil pH. The highest total number of bacteria in the second week on the soil typology D, was $6.0x10^7$ CFU / ml and the highest pH of 5.18 on typology B was in the third week. Application of dolomite affected the population of indigenous nitrogen fixing bacteria, where in dolomite application on the soil populations was fewer than untreated dolomite, with the largest bacterial population at $9.3x10^5$ CFU / ml in the second week and dolomite increased the soil pH, with the highest soil pH of 4.93 in the second week. And the interaction between land typology and dolomite application on only affected the population of indigenous nitrogen-fixing bacteria with the most populations of $3.5x10^7$ CFU / ml on soil typology D in the second week. The highest pH of 5.33 on soil typology B was in the fouth week .

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Effect Of Lime Application On Indigenous Nitrogen-Fixing Bacteria In Tidal Soils Managed For More Than 30 Years

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah, serta menganalisis dampak interaksi tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah. Pengambilan sampel tanah di lahan pasang surut Desa Mulya Sari dan Banyu Urip Kabupaten Banyuasin, Sumatera Selatan. Kemudian sampel diteliti di Laboratorium Mikrobiologi, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sriwijaya, Indralaya, selama bulan Januari-Mei 2017. Berdasarkan hasil Tipologi lahan pasang surut tidak mempengaruhi populasi bakteri penambat nitrogen indigen, tetapi berpengaruh terhadap pH tanah. Jumlah total bakteri terbanyak pada minggu kedua pada tipologi tanah D, yaitu $6{,}0x10^7$ CFU/ml dan pH tertinggi 5,18 pada tipologi B pada minggu ketiga. Pemberian dolomit mempengaruhi populasi bakteri penambat nitrogen indigen, dimana pada tanah yang diberi dolomit populasi bakteri lebih sedikit dibandingkan tanah yang tidak diberi perlakuan dolomit, dengan populasi bakteri terbanyak pada $9{,}3x10^5$ CFU/ml pada minggu kedua dan pemberian dolomit menigkatkan pH tanah, dengan pH tanah tertinggi 4,93 pada minggu kedua. Interaksi antara tipologi lahan dan pemberian dolomit hanya memberi pengaruh terhadap populasi bakteri penambat nitrogen indigen dengan populasi terbanyak $3{,}5x10^7$ CFU/ml pada tipe tanah D pada minggu kedua. pH tertinggi 5,33 pada tipologi tanah B pada minggu keempat.

ABSTRACT

This research purposes to know the effect of soil typology and lime on the population of indigenous nitrogen-fixing bacteria and soil pH, and to analyze the effect of soil typology interaction and lime application on population of indigenous nitrogen-fixing bacteria and soil pH. Sampling of the soil was in tidal land of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. Then the samples be inspected in Microbiology Laboratory at Biology Department of Faculty of Science,Sriwijaya University, Inderalaya, during January-May 2017. Based on the results tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected the soil pH. The highest total number of bacteria at the second week on the soil typology D, ie $6.0x10^7$ CFU / ml and the highest pH of 5.18 on typology B in the third week. Application of dolomite affected the population of indigenous nitrogen-fixing bacteria, which in dolomite-treated soil populations were fewer than dolomite un-treated soil, with the largest bacterial population at $9.3x10^5$ CFU / ml during the second week and dolomite increased soil pH, with pH the highest ground of 4.93 at the second week. The interaction between land typology and dolomite application only affected the population of indigenous nitrogen-fixing bacteria with the most populations of $3.5x10^7$ CFU / ml on soil typology D in the second week. The highest pH on soil typology B of 5.33 was at the fourth week.

Keywords: nitrogen-fixing bacteria, tidal land, lime treatment

INTRODUCTION

Tidal swamps of Mulya sari and Banyu Urip villages, South Sumatra, showed that soil pH was below 5.0 after opening of 30 year old with pH values ranging from 4.0-4.59 (very acid to acid) in all land typologies in combination with high H^+ and Al^{3+} exchangeability. The values of H^+ and Al^{3+} were from 0.65-1.0 cmol $^{(+)}$ / kg and 1.77-4.49 cmol $^{(+)}$ / kg (Budianta et al, 2016).

Villages of Banyu Urip and Mulya Sari, the location of soil sampling with respectively has been guided NPK fertilization for more than 30 years. It is known that the application of chemical fertilizers has side effect compared to natural fertilizers. According to Lokasari (2011), NPK's unfavorable nature is continuously that NPK is not ionizing in soil solution so it is easy to wash, since it can not be absorbed by the soil colloids. To be absorbed by plants had to undergo amonification and nitrification process first. The rapid and slow amide-form changes of the urea to the form of N compounds can be absorbed by the plant depending largely on several factors including population, microorganism activity, soil moisture content, soil temperature and amount of Urea fertilizer provided.

Tidal land of Banyu Urip and Mulya Sari villages were used as the location of soil sampling because the area can represent four typologies of tidal land with low soil pH. According to Budianta et. Al., (2016), soil pH measurements in four soil typologies in Banyu Urip and Mulya Sari villages obtained results, were namely in type A with pH 4.05; Type B with pH 4.59; Type C with pH 4.44; And type D with a pH of 4.55, where the lowest pH in the highest H^+ and Al^{3+} exchanged combinations was found in land typology A after rice growth. It was caused by the nitrification process of nitrogen fertilization causing a decrease in soil pH.

Lime is a source of ameliorant material widely used to improve soil fertility. Lime is an important s ource of Ca nutrients that support plant growth, increase soil pH, reduce soil Al and Mn content (Scott and Fisher, 1989; Mora et al., 2002 ; Koesrini et al., 2015). According to Saputri et al. (2016), the addition of dolomite is needed to regulate the acidity (pH) of growing media, calcium and carbon elements can enrich the mineral content of the media, and function as enzyme activators. According to Winarno (2004) lime also serves as an activator of various types of enzymes that are related in the metabolism of proteins and carbohydrates.

According to Kuswandi (1993), with the increaseof the pH of the soil, the supply of Mg and Ca nutrients can shift the position of H + on the colloid surface to neutralize the soil acidity. Calcification also aims to reduce the risk of aluminium effects, increase the availability of P elements as a result of P extraction from Al-P and Fe-P bonds, increase N fixation and N mineralization to increase CEC, and assist in the completion of the reform with accompanying nutrient release from organic materials and microbial bodies.

MATERIALS AND METHOD

This research was conducted from January to June 2017. Sampling of soil samples was taken in Tidal area of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. The research was conducted in Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya.

Equipment used in the field were sack, soil tester and a shovel. The tools used in the laboratory were autoclave, colony counter, pH meter, erlemeyer ml, hot plate, incubator, ose needle, konduktormeter, bunsen lamp, mixer, mortarporselein, petri dish, 1 ml micropipette, plastic and test tube. Materials needed were LGI medium, aquadest, and dolomite.

This research was used Factorial Complete Random Design, with treatment factor, as follows:

Factor 1: Dolomite Dose (K)

K0 = 0 (without treatment)

K1 = 2 Ton ha^{-1}

Factor 2: Tidal Swamp Land Typology (L)

L1 = Typology A

L2 = Typology B

L3 = Typology C

L4 = Typology D

The number of combinations each repeated 3 times, so it would produce 24 units of experiment.

Soil samples from tidal swamps were types A, B, C and D with aseptic method at 3 soil sampling points and homogenised on each typology. after having homogenized then the samples were inserted into a sterilized sac with different codes according to their respective topological type, then stored in cool box. The soil samples taken from layers of cultivation with a depth of about 0-20 cm in geographical coordinates are located about S 02^0 41'24 ", E $104^0$45'25" for A, S $02^0$39'26 "E $104^0$44'27" for B,S $02^0$39 '44 ", E $104^0$44'027" for C, and S $02^0$38'37 ", E $104^0$43'16" for typology D

Soil with different typology sieved with the sieve size of 0.5 mm hole diameter, then weighed as much as 1000 g, then dried up the ground, after the soil was inserted into polybag, the moisture was measured by using Soil tester. If the soil moisture content is less than 85% (optimum humidity for bacterial growth) first soil has to be given water using 500 ml water bottle poured slowly on the soil, and the humidity increases (Anonymous, 2017). Then dolomite was applicated to each soil in polybags with different dolomite amounts according to the dosage, for 1000 g of soil volume 1.84 g for dose 2 ton ha^{-1} and

on control not application of dolomite. Then it is calculated and observed and measured pH every 1 week for 28 days.

Variables observed were: (1) Total population of bacteria; (2) pH; (3) C-Orgaanic; (4) N-Total; (5) Al-exc

1. Number of Bacterial Populations

The calculation of bacterial cell count was performed by diluting 5 g samples taken from treated soil into 45 mL sterile aquades, made up to a dilution level of 10-6. The result of dilution from 10-4 - 10-6, was taken each 1 mL then put into a sterile petri dish that did not contain the medium of LGI, after inserted 1 ml of dilution, it is the medium of LGI was put wrapped. Then it was incubated at 37^oC for 24 hours. The number of bacteria that grow on counter colonies was Calculated (Modified Munawar, 1999).

2. Soil Reaction (pH)

Soil pH measurements were performed before dolomite given, then it was measured again after dolomite was given once a week for 28 days by means of soil weighed as much as 10 g twice, each was inserted into a shake bottle, and 50 ml of aquades were added to the bottle first (pH H_2O). with a ratio of 1: 5. Then 50 ml KCl 1 M is for second bottle (pH KCl). Then it was shaken with a shaker for 30 minutes. The soil suspension was measured with a calibrated pH meter using a buffer solution of pH 7.0 and pH 4.0. Then the writer recorded the pH value in 2 decimal places. (Balai Penelitaian Tanah, 2005)

3. C-organic

Dry soil wind weighed as much as 1 gram, then it was put into the erlenmeyer flask and then added 5 ml of K2Cr2O7 and H_2SO_4. The liquid was cooled and diluted with added aquadest up to 200 ml. Then *diphenylamine* solution and titrated with *ammonium ferro sulfate* solution until the suspense turned green. Primary solution (peniter) was recorded and made blank without using soil samples, then it was calculated.

4. N-Total

The clear extract solution of the destructed product was 25 ml each into boiling flask and boiling stone was added, then it was diluted with aquades to 100 ml, plus 20 ml of 30% NaOH and boiling flask was immediately closed. Then boiling flask was connected to a distillation apparatus to distill the removed N and accommodated by an erlenmeyer containing 10 ml of 1% boric acid and three drops of Conway indicator (red). Distillation was carried out until the volume of the 60 ml container solution was

green. The distillation solution was then subjected to H_2SO_4 (0.05 N) until the green color turns pink. A blank solution was then prepared and calculated. (Usman, 2012).

5. Al-exc

Soil samples were weighed 5 gr, then it was put into a 250 ml erlenmeyer flask, added 50 ml 1 N KCl and covered the erlenmeyer flask with plastic and shaken the erlenmeyer flask with a shaking machine ± 15 minutes. After being shaken, filtered soil solution that has been shaken by Whatman sieve was accommodated by plastic bottles. Then 25 ml of the filter was put into a 100 ml erlenmeyer flask and added 5 drops of Fenolphtalein indicator. It was titrated with 0.1 N NaOH solution with 10ml burette to a light pink solution. After that, 1 drop of 0.01 N HCl was added to the erlenmeyer and the erlenmeyer was until shaken the solution was clear white. Then added a solution of 4% NaF was as much as 10 ml, if the solution contains Al then the solution would turn pink again. Then was the solution was re-titrated using 0.01 N HCl until the solution color turned clear white.

The soil reaction (pH), C-Organic, N-total, Al-exc and bacteria population data were analyzed with Anova at $\alpha = 0,05$. If Anova results show a real difference, the data would processed with Tukey's HSD (Honest Significant Difference) test using statistics program 7.0.

RESULTS AND DISCUSSION

Characteristics Soil of Soil Chemistry In Four Land Typologies

Differences of soil chemical properties on various land typologies prior to lime application based on a 5% Tukey's Honest Significant Difference (HSD) test were presented in Table 1:

Table 1. Characteristics soil of soil chemistry in four land typologies

Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
A	$3,87^b$	$51,97^a$	$1,68^a$	$4,49^b$
B	$4,21^a$	$62,56^a$	$1,87^a$	$2,27^a$
C	$4,24^a$	$44,11^a$	$1,48^a$	$2,03^a$
D	$4,11^a$	$59,43^a$	$1,68^a$	$1,77^a$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Based on the significant difference test of Tukey's Honest Significant Difference (HSD) presented in Table 1, it was known that pH and Al-exc on typology A were significantly different from pH and Al-excon typology B, C and D. This is because the soil A pH was lower than soil typology B, C and D. A typology soil was very acidic because it had Al-exc high. Which was known that Aluminum content in soil could be exchanged generally on acid soils. This was supported by the statement by Ewin et al (2015) that the increase of Al-exc content of aluminum saturation soils will also increase the soil pH and also related to the interchangeable aluminum content and aluminum saturation of soil. While C-organic and N-total on typology A, B, C and D showed no significant difference.

Based on Table 1 above it was known that C-organic, N total, pH and Al-exc of the four typologies of tidal lands were not application lime treatment. In the table above the value of C-organic shows the number 44.11 to 62.56, where the amount has shown that the C-organic content in the four typologies of the land were high. It was known that the high amount of C-organic in the soil may decrease the pH of the land. In addition, high C-organic content also becomes a nutrient for microbial life on soils, especially non-symbiotic nitrogen fixing bacteria.

Effect Of Four Land Typologies And Lime Application On The Population Of Indigenous Nitrogen Fixing Bacteria

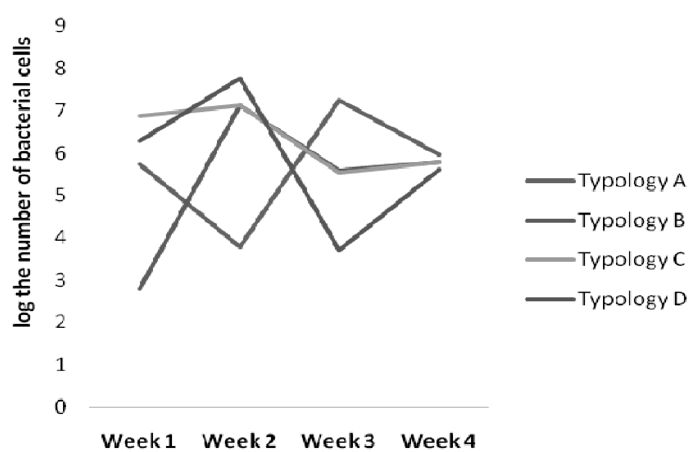


Figure 1. Effect of four land typologies on population of indigenous nitrogen fixing bacteria

Figure 1 above was presented based on the table of the effect of four typologies of land on the number of bacterial populations. According to the figure above the bacterial population at the highest was shown in the second week in the soil typology D, whereas the soil typology A in the second week showed the lowest population. In the fourth week the lowest of bacterial population for all typologies. The figure above showed the distance

between the top of the population of bacteria in typology A and D was quite far away, it was significant difference, but the Anova test results did not show was significant differently. It is caused by the variation of data on the amount of bacteria obtained was very diverse. When viewed as the highest number of populations during the second week in all land typologies, and decreased in the third and the fourth week, it was thought that in the second week is the optimal growth phase in bacteria so that if extended at week three and four many bacteria die, the bacterial population decreased in those weeks.

The lowest bacterial populations obtained in typology B in the first week, when viewed by the amount of nutrients in typology B has very high C-organic and N-total content compared with typology A, C and D (Table 1). High N-total can provide nutrients for bacterial growth, but high N-total results in nitrogen fixing bacteria unable to perform nitrogen fixation, supported by the statement of Imas et al. (1989), nitrogen-fixing bacteria are capable of using nitrogen compounds in the form of ammonium, nitrate, and organic nitrogen compounds. It becomes the most effective nitrogen fixing inhibitor.

Table 2. Effect of lime application on the population of indigenous nitrogen fixing bacteria

Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
	Week 1	Week 2	Week 3	Week 4
ND	$2{,}4x10^5$	$8{,}3x10^6$	$1{,}2x10^{7\ b}$	$1{,}2x10^{7\ b}$
D	$3{,}0x10^5$	$9{,}3x10^5$	$3{,}0x10^{3\ a}$	$3{,}0x10^{5\ a}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Overall, the population of bacteria is more common in unregulated limestone soils. It was thought that limiting certain amounts of lime can inhibit the growth of the indigenous nitrogen fixing bacteria living on the land, according to Havlin et. Al, (1999) the provision of lime to increase soil pH in the tropics often decreases the production due to over liming. Therefore, calcification should be aimed at eliminating the toxic effects of Al^{3+} ions.

Based on Table 2 above, it was found that the population of nitrogen fixing bacteria treated in the third week was a decrease in the bacterial population, while in the fourth week the bacterial population increased and the population was as same as the population in the first week. It was suspected that lime application on over a period of time affects the life of the indigenous nitrogen fixing bacteria in the soil. While the population of nitrogen-fixing bacteria on the soil not application of lime from week to week increased the population of nitrogen-fixing bacteria. It was suspected that the bacteria grew on the soil is an indigenous nitrogen-fixing bacteria that has adapted itself to acid soils.

It was recommended for farmers to plant on soil that has been given a blur so that the plants are not poisoned by pyrite compounds, in the first and second weeks after treatment, because at that time the population of live nitrogen-fixing bacteria still sufficient to supply

free nitrogen through non-symbiotic nitrogen fixation process, it was supported by the statement of Purwaningsih and Saefudin (2012), plant that has high protein content indicates that the plant requires a lot of nutrients, especially nutrients N for growth

Table 3. Interaction effects of four typologies of soil and lime application on the population of indigenous nitrogen fixing bacteria

Soil Typologies	Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
		Week 1	Week 2	Week 3	Week 4
A	ND	$1{,}3x10^{5}$	$1{,}3x10^{7}$ a	$3{,}3x10^{7}$	$1{,}8x10^{7}$
	D	$2{,}0$ $x10^{5}$	$3{,}0$ $x10^{2}$ b	$1{,}3$ $x10^{7}$	$4{,}0$ $x10^{5}$
B	ND	$2{,}0x10^{2}$	$1{,}9x10^{7}$ a	$1{,}1x10^{7}$	$1{,}5x10^{7}$
	D	$2{,}6$ $x10^{3}$	$9{,}0$ $x10^{5}$ ab	$1{,}3$ $x10^{4}$	$2{,}0$ $x10^{5}$
C	ND	$3{,}3x10^{7}$	$2{,}2x10^{7}$ a	$5{,}1x10^{6}$	$1{,}4x10^{7}$
	D	$2{,}0$ $x10^{5}$	$8{,}0$ $x10^{5}$ ab	$2{,}2$ $x10^{4}$	$2{,}0$ $x10^{5}$
D	ND	$5{,}0x10^{5}$	$8{,}8$ $x10^{5}$ ab	$1{,}3$ $x10^{7}$	$8{,}0x10^{5}$
	D	$8{,}1$ $x10^{5}$	$3{,}5$ $x10^{7}$ a	$1{,}9$ $x10^{2}$	$2{,}0$ $x10^{5}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types in tidal land, ND = treatment without dolomite, D = treatment with dolomite)

In the first, third and fourth weeks of the population on the interaction of the four soil typologies and lime treatment and no lime showed no significant difference. The second week showed significant differences in bacterial populations on the soil typology A given lime and soil typology A which was not given lime, but the population of bacteria on calcified soil typology A was not significantly different from the soil typology B, C, D which are lime application or not. In the second week on soil typology A, B, and C bacterial populations were higher in the treatment without lime, whereas in typology D the number of bacterial populations was higher in the treatment of lime.

Effects Of Four Typologies Of Soil And Lime Application On Soil Reactions (pH)

Analysis of Varian (ANOVA) on the effects of four typologies of land to soil reaction (pH) in the first week was obtained $p = 0{,}119964$ ($p > 0{,}05$) at α 5%. It showed that in the first week of soil typology has no significant effect on pH, but in the second week, the third until the fourth of the anova test results showed that the effect of land typology on pH, in which $p = 0{,}00$ ($p < 0{,}05$) at α 5%.

Table 4. The effect of four land typologies on soil reactions (pH)

Soil Typologies	Soil Reactions (pH)			
	Week 1	Week 2	Week 3	Week 4
A	4,33	4,43 [a]	4,50 [ab]	4,42 [a]
B	4,82	5,13 [b]	5,18 [c]	5,12 [a]
C	4,60	4,42 [a]	4,43 [a]	4,50 [a]
D	4,55	4,52 [a]	4,57 [b]	4,53 [b]

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Table 4 above after 5% Tukey's Honest Significant Difference (HSD) test showed that the first week of pH on soil typology A, B, C and D was not significantly different, however in the second, the third and the fourth week pH on typology A, B , C and D showed the significantly different. In the second week the pH of typology B was significantly different from typology A, C and D, but the three typologies were not significantly different from each other. It is in contrast to the third week. The pH of typology A was not significantly different from typology C and D, but it was significantly different from typology B. In contrast, soil typology B was significantly different from soil typology A, C and D. In soil typology C was significantly different from soil typology D.

Based on the data in Table 4, it may be recommended for local farmers to plant on typology A and B fields during the third week after lime treatment, since typology A and B at that time have been a good time for planting with high increase in pH. While for typology C and D, planting by farmer should be done in the first week after lime treatment, because in typology C and D, the highest pH increase reaction occurs in the first week after lime treatment.

Table 5. The effect of lime application on the soil reaction (pH)

Treatment	Soil Reaction (pH)			
	Week 1	Week 2	Week 3	Week 4
ND	4,42 [a]	4,86 [a]	4,45 [a]	4,45 [a]
D	4,73 [b]	4,93 [b]	4,89 [b]	4,83 [b]

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on the 5% Tukey's Honest Significant Difference (HSD) test indicated in Table 5 above that lime application had a significant effect on pH altitude. It was supported by Basuki (2007), one of the goals of lime on acid soil is to increase the soil pH through hydrolysis of $CaCO_3$ so as to affect the dissolution of Al or Fe and prevent poisoning in plants. In the second week the highest pH value was compared to the first, third or fourth week.

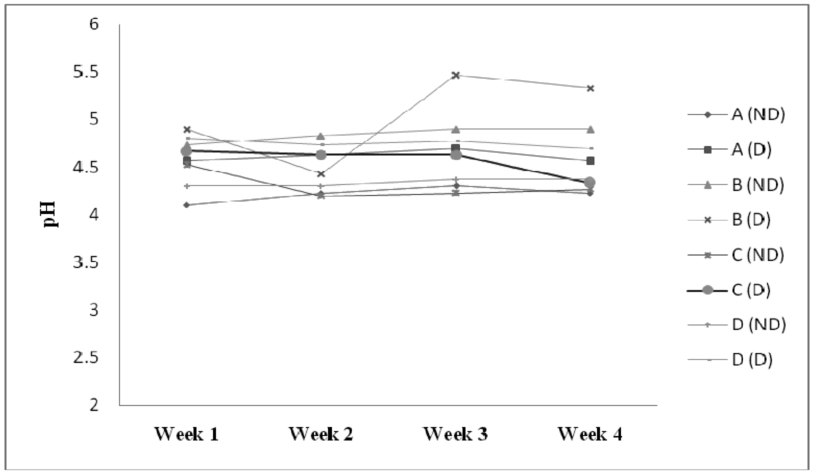


Figure 2. Interaction effects of four land typologies and lime application on soil reactions (pH) (A, B, C and D = tidal soil type, ND = treatment without dolomite, D = treatment with dolomite)

Figure 2 above, based on the Table of effects of four land typologies to pH, in the first week the highest soil pH was found in the soil typology B was treated with lime, soil typology B treated with lime from the first week showed the highest pH ie, range 5, but in the second week was decreased. In typology A, C and D either treated with lime or not, it did not show significant differences in soil pH from the first week to the fourth week.

Soil Chemical Properties in Four Typologies After Soil Treatment

Table 6. Soil chemical properties in four typologies after soil treatment

No.	Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
1.	A	4,42 ^{a}	58,12 ^{b}	3,24 ^{b}	4,43
2.	B	5,12 ^{a}	27,26 ^{a}	1,61 ^{a}	2,55
3.	C	4,50 ^{a}	26,23 ^{a}	1,67 ^{a}	5,21
4.	D	4,53 ^{b}	27,13 ^{a}	1,61 ^{a}	4,11

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

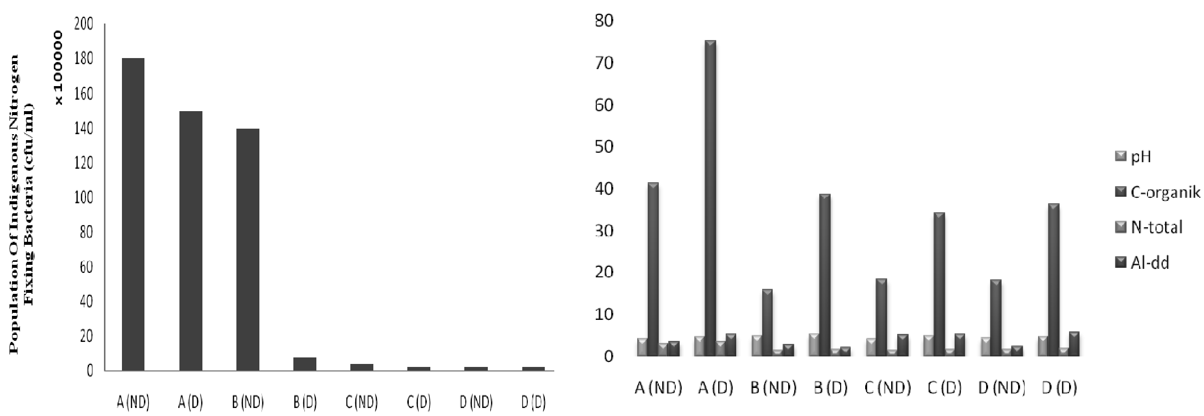
When compared to the initial soil chemical properties measurement of pH values, C-organic and Al-exc on average increased in measurement of soil chemical properties the four typologies after soil treatment. N-total increased significantly only in typology A, compared with typology B, C and D. According to Prasetyo and Suradikarta (2006), high Al saturation value is found in Ultisol soils of sediment and granite (> 60%), and Low value on Ultisol soil from andesitic and volcanic volcanic materials (0%). The ultisole of the tufa material has a low Al saturation in the upper layer (5-8%), but it is high in the bottom layer (37-78%). It seems that Al saturation on Ultisol soils related to soil pH.

Table 7. Soil chemical properties in four typologies after soil treatment at four weeks

Treatment	pH	C-organic (g)	N-total (g)
ND	4,45 [a]	23,35 [a]	1,92
D	4,83 [b]	46,01 [b]	2,22

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on Table 7 according to the 5% Tukey's Honest Significant Difference (HSD) test, it was found that at pH and C-organic significantly affected lime treatment. At lime-treated pH was significantly different from pH which was not given lime. It also occurred in C-organics that was lime application on significantly different from those of not application of lime on C-organic.According to Bot and Benites (2005), the value of C-organic values in soil can show the content of organic matter in the soil which is an important benchmark for management on agricultural soils and C-organic was believed to be the key to resistance to drought and sustainability of food production. In addition, C-organic was also effected by the type of vegetation on soil.



(a) (b)

Figure 3. Interaction effects of four land typologies and lime application on the population of indigenous nitrogen fixing bacteria (a) and Soil chemical properties in four typologies after soil treatment [pH, C-organic, N-total, Al-exc] (b). [A, B, C and D = typologies of tidal land], [ND = treatment without dolomite, D = treatment with dolomite]

Based on Figure 3 it can be seen the number of bacterial population (a) decreased the number of bacteria in the four typologies of soil with lime. At pH (b) there was no significant increase and decrease in the interaction of the four typologies of soil and lime

treatment. In C-organic and N-total increases in the soil typology A given lime. Aldd had no significant effect on the interaction of the four typologies of soil and lime treatment.

Given the initial calculation of soil chemical properties prior to the application of lime amounts of soil C-organic soils in the four high land typologies, it is thought to be one of the causes of soil acidity due to the presence of organic compounds. Lime appication on a certain amount can increase the soil pH but indirectly proportional to the population of growing bacteria. It is suspected that lime application can inhibit the organic acids needed by the living bacteria of the soil. According to Winarso (2005), organic matter in the soil can be defined as the remains of plants and animals in the soil in various weathering, consisting of both living and death. Organic matter in the soil can function or improve on chemical, physical and biological properties of the soil.

CONCLUSION

Based on the research that has been done, then it can be concluded that tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected soil pH. The highest total number of bacteria in the second week on the soil typology D, was 6.0×10^7 CFU / ml and the highest pH of 5.18 on typology B was in the third week. Application of dolomite affected the population of indigenous nitrogen fixing bacteria, where in dolomite application on the soil populations was fewer than untreated dolomite, with the largest bacterial population at 9.3×10^5 CFU / ml in the second week and dolomite increased the soil pH, with the highest soil pH of 4.93 in the second week. And the interaction between land typology and dolomite application on only affected the population of indigenous nitrogen-fixing bacteria with the most populations of 3.5×10^7 CFU / ml on soil typology D in the second week. The highest pH of 5.33 on soil typology B was in the fouth week .

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Effect Of Lime Application On Indigenous Nitrogen-Fixing Bacteria In Tidal Soils Managed For More Than 30 Years

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah, serta menganalisis dampak interaksi tipologi lahan dan pemberian kapur terhadap populasi bakteri penambat nitrogen indigen dan pH tanah. Pengambilan sampel tanah di lahan pasang surut Desa Mulya Sari dan Banyu Urip Kabupaten Banyuasin, Sumatera Selatan. Kemudian sampel diteliti di Laboratorium Mikrobiologi, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sriwijaya, Indralaya, selama bulan Januari-Mei 2017. Berdasarkan hasil Tipologi lahan pasang surut tidak mempengaruhi populasi bakteri penambat nitrogen indigen, tetapi berpengaruh terhadap pH tanah. Jumlah total bakteri terbanyak pada minggu kedua pada tipologi tanah D, yaitu $6,0x10^{7}$ CFU/ml dan pH tertinggi 5,18 pada tipologi B pada minggu ketiga. Pemberian dolomit mempengaruhi populasi bakteri penambat nitrogen indigen, dimana pada tanah yang diberi dolomit populasi bakteri lebih sedikit dibandingkan tanah yang tidak diberi perlakuan dolomit, dengan populasi bakteri terbanyak pada $9,3x10^{5}$ CFU/ml pada minggu kedua dan pemberian dolomit menigkatkan pH tanah, dengan pH tanah tertinggi 4,93 pada minggu kedua. Interaksi antara tipologi lahan dan pemberian dolomit hanya memberi pengaruh terhadap populasi bakteri penambat nitrogen indigen dengan populasi terbanyak $3,5x10^{7}$ CFU/ml pada tipe tanah D pada minggu kedua. pH tertinggi 5,33 pada tipologi tanah B pada minggu keempat.

ABSTRACT

This research purposes to know the effect of soil typology and lime on the population of indigenous nitrogen-fixing bacteria and soil pH, and to analyze the effect of soil typology interaction and lime application on population of indigenous nitrogen-fixing bacteria and soil pH. Sampling of the soil was in tidal land of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. Then the samples be inspected in Microbiology Laboratory at Biology Department of Faculty of Science,Sriwijaya University, Inderalaya, during January-May 2017. Based on the results tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected the soil pH. The highest total number of bacteria at the second week on the soil typology D, ie $6.0x10^{7}$ CFU / ml and the highest pH of 5.18 on typology B in the third week. Application of dolomite affected the population of indigenous nitrogen-fixing bacteria, which in dolomite-treated soil populations were fewer than dolomite un-treated soil, with the largest bacterial population at $9.3x10^{5}$ CFU / ml during the second week and dolomite increased soil pH, with pH the highest ground of 4.93 at the second week. The interaction between land typology and dolomite application only affected the population of indigenous nitrogen-fixing bacteria with the most populations of $3.5x10^{7}$ CFU / ml on soil typology D in the second week. The highest pH on soil typology B of 5.33 was at the fourth week.

Keywords: nitrogen-fixing bacteria, tidal land, lime treatment

INTRODUCTION

Tidal swamps of Mulya sari and Banyu Urip villages, South Sumatra, showed that soil pH was below 5.0 after opening of 30 year old with pH values ranging from 4.0-4.59 (very acid to acid) in all land typologies in combination with high H^+ and Al^{3+} exchangeability. The values of H^+ and Al^{3+} were from 0.65-1.0 cmol $^{(+)}$ / kg and 1.77-4.49 cmol $^{(+)}$ / kg (Budianta et al, 2016).

Villages of Banyu Urip and Mulya Sari, the location of soil sampling with respectively has been guided NPK fertilization for more than 30 years. It is known that the application of chemical fertilizers has side effect compared to natural fertilizers. According to Lokasari (2011), NPK's unfavorable nature is continuously that NPK is not ionizing in soil solution so it is easy to wash, since it can not be absorbed by the soil colloids. To be absorbed by plants had to undergo amonification and nitrification process first. The rapid and slow amide-form changes of the urea to the form of N compounds can be absorbed by the plant depending largely on several factors including population, microorganism activity, soil moisture content, soil temperature and amount of Urea fertilizer provided.

Tidal land of Banyu Urip and Mulya Sari villages were used as the location of soil sampling because the area can represent four typologies of tidal land with low soil pH. According to Budianta et. Al., (2016), soil pH measurements in four soil typologies in Banyu Urip and Mulya Sari villages obtained results, were namely in type A with pH 4.05; Type B with pH 4.59; Type C with pH 4.44; And type D with a pH of 4.55, where the lowest pH in the highest H^+ and Al^{3+} exchanged combinations was found in land typology A after rice growth. It was caused by the nitrification process of nitrogen fertilization causing a decrease in soil pH.

Lime is a source of ameliorant material widely used to improve soil fertility. Lime is an important s ource of Ca nutrients that support plant growth, increase soil pH, reduce soil Al and Mn content (Scott and Fisher, 1989; Mora et al., 2002 ; Koesrini et al., 2015). According to Saputri et al. (2016), the addition of dolomite is needed to regulate the acidity (pH) of growing media, calcium and carbon elements can enrich the mineral content of the media, and function as enzyme activators. According to Winarno (2004) lime also serves as an activator of various types of enzymes that are related in the metabolism of proteins and carbohydrates.

According to Kuswandi (1993), with the increaseof the pH of the soil, the supply of Mg and Ca nutrients can shift the position of H + on the colloid surface to neutralize the soil acidity. Calcification also aims to reduce the risk of aluminium effects, increase the availability of P elements as a result of P extraction from Al-P and Fe-P bonds, increase N fixation and N mineralization to increase CEC, and assist in the completion of the reform with accompanying nutrient release from organic materials and microbial bodies.

MATERIALS AND METHOD

This research was conducted from January to June 2017. Sampling of soil samples was taken in Tidal area of Mulya Sari and Banyu Urip Village of Banyuasin Regency, South Sumatera. The research was conducted in Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya.

Equipment used in the field were sack, soil tester and a shovel. The tools used in the laboratory were autoclave, colony counter, pH meter, erlemeyer ml, hot plate, incubator, ose needle, konduktormeter, bunsen lamp, mixer, mortarporselein, petri dish, 1 ml micropipette, plastic and test tube. Materials needed were LGI medium, aquadest, and dolomite.

This research was used Factorial Complete Random Design, with treatment factor, as follows:

Factor 1: Dolomite Dose (K)

K0 = 0 (without treatment)

K1 = 2 Ton ha^{-1}

Factor 2: Tidal Swamp Land Typology (L)

L1 = Typology A

L2 = Typology B

L3 = Typology C

L4 = Typology D

The number of combinations each repeated 3 times, so it would produce 24 units of experiment.

Soil samples from tidal swamps were types A, B, C and D with aseptic method at 3 soil sampling points and homogenised on each typology. after having homogenized then the samples were inserted into a sterilized sac with different codes according to their respective topological type, then stored in cool box. The soil samples taken from layers of cultivation with a depth of about 0-20 cm in geographical coordinates are located about S 02^0 41'24 ", E $104^0$45'25" for A, S $02^0$39'26 "E $104^0$44'27" for B,S $02^0$39 '44 ", E $104^0$44'027" for C, and S $02^0$38'37 ", E $104^0$43'16" for typology D

Soil with different typology sieved with the sieve size of 0.5 mm hole diameter, then weighed as much as 1000 g, then dried up the ground, after the soil was inserted into polybag, the moisture was measured by using Soil tester. If the soil moisture content is less than 85% (optimum humidity for bacterial growth) first soil has to be given water using 500 ml water bottle poured slowly on the soil, and the humidity increases (Anonymous, 2017). Then dolomite was applicated to each soil in polybags with different dolomite amounts according to the dosage, for 1000 g of soil volume 1.84 g for dose 2 ton ha^{-1} and

on control not application of dolomite. Then it is calculated and observed and measured pH every 1 week for 28 days.

Variables observed were: (1) Total population of bacteria; (2) pH; (3) C-Orgaanic; (4) N-Total; (5) Al-exc

1. Number of Bacterial Populations

The calculation of bacterial cell count was performed by diluting 5 g samples taken from treated soil into 45 mL sterile aquades, made up to a dilution level of 10-6. The result of dilution from 10-4 - 10-6, was taken each 1 mL then put into a sterile petri dish that did not contain the medium of LGI, after inserted 1 ml of dilution, it is the medium of LGI was put wrapped. Then it was incubated at 37^oC for 24 hours. The number of bacteria that grow on counter colonies was Calculated (Modified Munawar, 1999).

2. Soil Reaction (pH)

Soil pH measurements were performed before dolomite given, then it was measured again after dolomite was given once a week for 28 days by means of soil weighed as much as 10 g twice, each was inserted into a shake bottle, and 50 ml of aquades were added to the bottle first (pH H_2O). with a ratio of 1: 5. Then 50 ml KCl 1 M is for second bottle (pH KCl). Then it was shaken with a shaker for 30 minutes. The soil suspension was measured with a calibrated pH meter using a buffer solution of pH 7.0 and pH 4.0. Then the writer recorded the pH value in 2 decimal places. (Balai Penelitaian Tanah, 2005)

3. C-organic

Dry soil wind weighed as much as 1 gram, then it was put into the erlenmeyer flask and then added 5 ml of K2Cr2O7 and H_2SO_4. The liquid was cooled and diluted with added aquadest up to 200 ml. Then *diphenylamine* solution and titrated with *ammonium ferro sulfate* solution until the suspense turned green. Primary solution (peniter) was recorded and made blank without using soil samples, then it was calculated.

4. N-Total

The clear extract solution of the destructed product was 25 ml each into boiling flask and boiling stone was added, then it was diluted with aquades to 100 ml, plus 20 ml of 30% NaOH and boiling flask was immediately closed. Then boiling flask was connected to a distillation apparatus to distill the removed N and accommodated by an erlenmeyer containing 10 ml of 1% boric acid and three drops of Conway indicator (red). Distillation was carried out until the volume of the 60 ml container solution was

green. The distillation solution was then subjected to H2SO4 (0.05 N) until the green color turns pink. A blank solution was then prepared and calculated. (Usman, 2012).

5. Al-exc

Soil samples were weighed 5 gr, then it was put into a 250 ml erlenmeyer flask, added 50 ml 1 N KCl and covered the erlenmeyer flask with plastic and shaken the erlenmeyer flask with a shaking machine ± 15 minutes. After being shaken, filtered soil solution that has been shaken by Whatman sieve was accommodated by plastic bottles. Then 25 ml of the filter was put into a 100 ml erlenmeyer flask and added 5 drops of Fenolphtalein indicator. It was titrated with 0.1 N NaOH solution with 10ml burette to a light pink solution. After that, 1 drop of 0.01 N HCl was added to the erlenmeyer and the erlenmeyer was until shaken the solution was clear white. Then added a solution of 4% NaF was as much as 10 ml, if the solution contains Al then the solution would turn pink again. Then was the solution was re-titrated using 0.01 N HCl until the solution color turned clear white.

The soil reaction (pH), C-Organic, N-total, Al-exc and bacteria population data were analyzed with Anova at α = 0,05. If Anova results show a real difference, the data would processed with Tukey's HSD (Honest Significant Difference) test using statistics program 7.0.

RESULTS AND DISCUSSION

Characteristics Soil of Soil Chemistry In Four Land Typologies

Differences of soil chemical properties on various land typologies prior to lime application based on a 5% Tukey's Honest Significant Difference (HSD) test were presented in Table 1:

Table 1. Characteristics soil of soil chemistry in four land typologies

Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
A	$3,87^{b}$	$51,97^{a}$	$1,68^{a}$	$4,49^{b}$
B	$4,21^{a}$	$62,56^{a}$	$1,87^{a}$	$2,27^{a}$
C	$4,24^{a}$	$44,11^{a}$	$1,48^{a}$	$2,03^{a}$
D	$4,11^{a}$	$59,43^{a}$	$1,68^{a}$	$1,77^{a}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Based on the significant difference test of Tukey's Honest Significant Difference (HSD) presented in Table 1, it was known that pH and Al-exc on typology A were significantly different from pH and Al-excon typology B, C and D. This is because the soil A pH was lower than soil typology B, C and D. A typology soil was very acidic because it had Al-exc high. Which was known that Aluminum content in soil could be exchanged generally on acid soils. This was supported by the statement by Ewin et al (2015) that the increase of Al-exc content of aluminum saturation soils will also increase the soil pH and also related to the interchangeable aluminum content and aluminum saturation of soil. While C-organic and N-total on typology A, B, C and D showed no significant difference.

Based on Table 1 above it was known that C-organic, N total, pH and Al-exc of the four typologies of tidal lands were not application lime treatment. In the table above the value of C-organic shows the number 44.11 to 62.56, where the amount has shown that the C-organic content in the four typologies of the land were high. It was known that the high amount of C-organic in the soil may decrease the pH of the land. In addition, high C-organic content also becomes a nutrient for microbial life on soils, especially non-symbiotic nitrogen fixing bacteria.

Effect Of Four Land Typologies And Lime Application On The Population Of Indigenous Nitrogen Fixing Bacteria

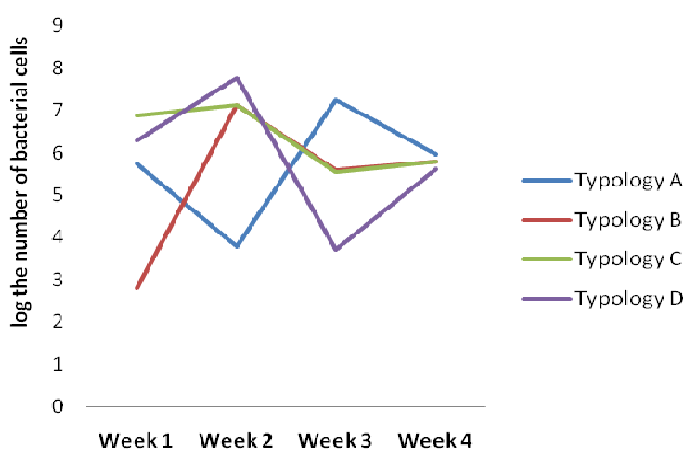


Figure 1. Effect of four land typologies on population of indigenous nitrogen fixing bacteria

Figure 1 above was presented based on the table of the effect of four typologies of land on the number of bacterial populations. According to the figure above the bacterial population at the highest was shown in the second week in the soil typology D, whereas the soil typology A in the second week showed the lowest population. In the fourth week the lowest of bacterial population for all typologies. The figure above showed the distance

between the top of the population of bacteria in typology A and D was quite far away, it was significant difference, but the Anova test results did not show was significant differently. It is caused by the variation of data on the amount of bacteria obtained was very diverse. When viewed as the highest number of populations during the second week in all land typologies, and decreased in the third and the fourth week, it was thought that in the second week is the optimal growth phase in bacteria so that if extended at week three and four many bacteria die, the bacterial population decreased in those weeks.

The lowest bacterial populations obtained in typology B in the first week, when viewed by the amount of nutrients in typology B has very high C-organic and N-total content compared with typology A, C and D (Table 1). High N-total can provide nutrients for bacterial growth, but high N-total results in nitrogen fixing bacteria unable to perform nitrogen fixation, supported by the statement of Imas et al. (1989), nitrogen-fixing bacteria are capable of using nitrogen compounds in the form of ammonium, nitrate, and organic nitrogen compounds. It becomes the most effective nitrogen fixing inhibitor.

Table 2. Effect of lime application on the population of indigenous nitrogen fixing bacteria

Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
	Week 1	Week 2	Week 3	Week 4
ND	$2{,}4x10^5$	$8{,}3x10^6$	$1{,}2x10^{7\ b}$	$1{,}2x10^{7\ b}$
D	$3{,}0x10^5$	$9{,}3x10^5$	$3{,}0x10^{3\ a}$	$3{,}0x10^{5\ a}$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Overall, the population of bacteria is more common in unregulated limestone soils. It was thought that limiting certain amounts of lime can inhibit the growth of the indigenous nitrogen fixing bacteria living on the land, according to Havlin et. Al, (1999) the provision of lime to increase soil pH in the tropics often decreases the production due to over liming. Therefore, calcification should be aimed at eliminating the toxic effects of Al^{3+} ions.

Based on Table 2 above, it was found that the population of nitrogen fixing bacteria treated in the third week was a decrease in the bacterial population, while in the fourth week the bacterial population increased and the population was as same as the population in the first week. It was suspected that lime application on over a period of time affects the life of the indigenous nitrogen fixing bacteria in the soil. While the population of nitrogen-fixing bacteria on the soil not application of lime from week to week increased the population of nitrogen-fixing bacteria. It was suspected that the bacteria grew on the soil is an indigenous nitrogen-fixing bacteria that has adapted itself to acid soils.

It was recommended for farmers to plant on soil that has been given a blur so that the plants are not poisoned by pyrite compounds, in the first and second weeks after treatment, because at that time the population of live nitrogen-fixing bacteria still sufficient to supply

free nitrogen through non-symbiotic nitrogen fixation process, it was supported by the statement of Purwaningsih and Saefudin (2012), plant that has high protein content indicates that the plant requires a lot of nutrients, especially nutrients N for growth

Table 3. Interaction effects of four typologies of soil and lime application on the population of indigenous nitrogen fixing bacteria

Soil Typologies	Treatment	Population Of Indigenous Nitrogen Fixing Bacteria (cfu / ml)			
		Week 1	Week 2	Week 3	Week 4
A	ND	$1,3x10^5$	$1,3x10^{7}$ a	$3,3x10^7$	$1,8x10^7$
	D	$2,0 x10^5$	$3,0 x10^{2}$ b	$1,3 x10^7$	$4,0 x10^5$
B	ND	$2,0x10^2$	$1,9x10^{7}$ a	$1,1x10^7$	$1,5x10^7$
	D	$2,6 x10^3$	$9,0 x10^{5}$ ab	$1,3 x10^4$	$2,0 x10^5$
C	ND	$3,3x10^7$	$2,2x10^{7}$ a	$5,1x10^6$	$1,4x10^7$
	D	$2,0 x10^5$	$8,0 x10^{5}$ ab	$2,2 x10^4$	$2,0 x10^5$
D	ND	$5,0x10^5$	$8,8 x10^{5}$ ab	$1,3 x10^7$	$8,0x10^5$
	D	$8,1 x10^5$	$3,5 x10^{7}$ a	$1,9 x10^2$	$2,0 x10^5$

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types in tidal land, ND = treatment without dolomite, D = treatment with dolomite)

In the first, third and fourth weeks of the population on the interaction of the four soil typologies and lime treatment and no lime showed no significant difference. The second week showed significant differences in bacterial populations on the soil typology A given lime and soil typology A which was not given lime, but the population of bacteria on calcified soil typology A was not significantly different from the soil typology B, C, D which are lime application or not. In the second week on soil typology A, B, and C bacterial populations were higher in the treatment without lime, whereas in typology D the number of bacterial populations was higher in the treatment of lime.

Effects Of Four Typologies Of Soil And Lime Application On Soil Reactions (pH)

Analysis of Varian (ANOVA) on the effects of four typologies of land to soil reaction (pH) in the first week was obtained $p = 0,119964$ ($p > 0,05$) at α 5%. It showed that in the first week of soil typology has no significant effect on pH, but in the second week, the third until the fourth of the anova test results showed that the effect of land typology on pH, in which $p = 0,00$ ($p < 0,05$) at α 5%.

Table 4. The effect of four land typologies on soil reactions (pH)

Soil Typologies	Soil Reactions (pH)			
	Week 1	Week 2	Week 3	Week 4
A	4,33	4,43 ^{a}	4,50 ^{ab}	4,42 ^{a}
B	4,82	5,13 ^{b}	5,18 ^{c}	5,12 ^{a}
C	4,60	4,42 ^{a}	4,43 ^{a}	4,50 ^{a}
D	4,55	4,52 ^{a}	4,57 ^{b}	4,53 ^{b}

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

Table 4 above after 5% Tukey's Honest Significant Difference (HSD) test showed that the first week of pH on soil typology A, B, C and D was not significantly different, however in the second, the third and the fourth week pH on typology A, B , C and D showed the significantly different. In the second week the pH of typology B was significantly different from typology A, C and D, but the three typologies were not significantly different from each other. It is in contrast to the third week. The pH of typology A was not significantly different from typology C and D, but it was significantly different from typology B. In contrast, soil typology B was significantly different from soil typology A, C and D. In soil typology C was significantly different from soil typology D.

Based on the data in Table 4, it may be recommended for local farmers to plant on typology A and B fields during the third week after lime treatment, since typology A and B at that time have been a good time for planting with high increase in pH. While for typology C and D, planting by farmer should be done in the first week after lime treatment, because in typology C and D, the highest pH increase reaction occurs in the first week after lime treatment.

Table 5. The effect of lime application on the soil reaction (pH)

Treatment	Soil Reaction (pH)			
	Week 1	Week 2	Week 3	Week 4
ND	4,42 ^{a}	4,86 ^{a}	4,45 ^{a}	4,45 ^{a}
D	4,73 ^{b}	4,93 ^{b}	4,89 ^{b}	4,83 ^{b}

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on the 5% Tukey's Honest Significant Difference (HSD) test indicated in Table 5 above that lime application had a significant effect on pH altitude. It was supported by Basuki (2007), one of the goals of lime on acid soil is to increase the soil pH through hydrolysis of $CaCO_3$ so as to affect the dissolution of Al or Fe and prevent poisoning in plants. In the second week the highest pH value was compared to the first, third or fourth week.

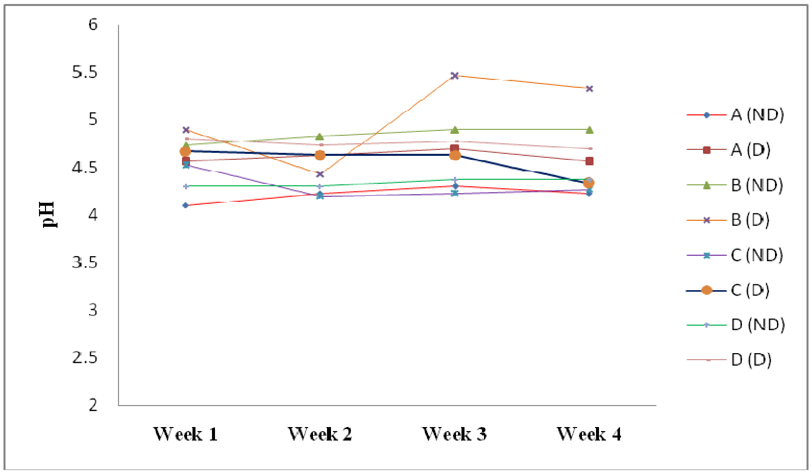


Figure 2. Interaction effects of four land typologies and lime application on soil reactions (pH) (A, B, C and D = tidal soil type, ND = treatment without dolomite, D = treatment with dolomite)

Figure 2 above, based on the Table of effects of four land typologies to pH, in the first week the highest soil pH was found in the soil typology B was treated with lime, soil typology B treated with lime from the first week showed the highest pH ie, range 5, but in the second week was decreased. In typology A, C and D either treated with lime or not, it did not show significant differences in soil pH from the first week to the fourth week.

Soil Chemical Properties in Four Typologies After Soil Treatment

Table 6. Soil chemical properties in four typologies after soil treatment

No.	Soil Typologies	pH	C-organic (g kg^{-1})	N-total (g kg^{-1})	Al-exc (Cmol/ kg^{-1})
1.	A	4,42 ^{a}	58,12 ^{b}	3,24 ^{b}	4,43
2.	B	5,12 ^{a}	27,26 ^{a}	1,61 ^{a}	2,55
3.	C	4,50 ^{a}	26,23 ^{a}	1,67 ^{a}	5,21
4.	D	4,53 ^{b}	27,13 ^{a}	1,61 ^{a}	4,11

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (A, B, C and D = soil types on tidal land)

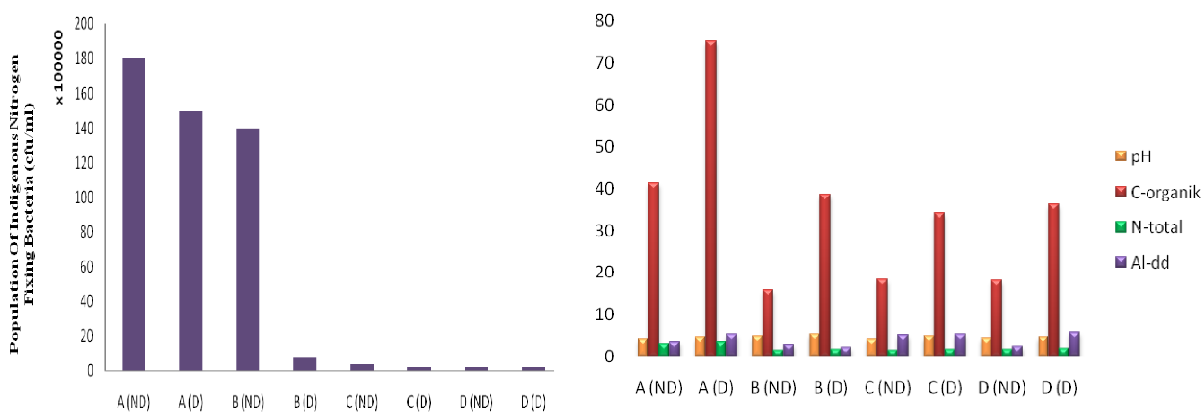
When compared to the initial soil chemical properties measurement of pH values, C-organic and Al-exc on average increased in measurement of soil chemical properties the four typologies after soil treatment. N-total increased significantly only in typology A, compared with typology B, C and D. According to Prasetyo and Suradikarta (2006), high Al saturation value is found in Ultisol soils of sediment and granite (> 60%), and Low value on Ultisol soil from andesitic and volcanic volcanic materials (0%). The ultisole of the tufa material has a low Al saturation in the upper layer (5-8%), but it is high in the bottom layer (37-78%). It seems that Al saturation on Ultisol soils related to soil pH.

Table 7. Soil chemical properties in four typologies after soil treatment at four weeks

Treatment	pH	C-organic (g)	N-total (g)
ND	4,45 [a]	23,35 [a]	1,92
D	4,83 [b]	46,01 [b]	2,22

Ket: The numbers followed by different letters in the same column show significantly different according to Tukey's HSD 5% (ND = treatment without dolomite, D = treatment with dolomite)

Based on Table 7 according to the 5% Tukey's Honest Significant Difference (HSD) test, it was found that at pH and C-organic significantly affected lime treatment. At lime-treated pH was significantly different from pH which was not given lime. It also occurred in C-organics that was lime application on significantly different from those of not application of lime on C-organic.According to Bot and Benites (2005), the value of C-organic values in soil can show the content of organic matter in the soil which is an important benchmark for management on agricultural soils and C-organic was believed to be the key to resistance to drought and sustainability of food production. In addition, C-organic was also effected by the type of vegetation on soil.



(a) (b)

Figure 3. Interaction effects of four land typologies and lime application on the population of indigenous nitrogen fixing bacteria (a) and Soil chemical properties in four typologies after soil treatment [pH, C-organic, N-total, Al-exc] (b). [A, B, C and D = typologies of tidal land], [ND = treatment without dolomite, D = treatment with dolomite]

Based on Figure 3 it can be seen the number of bacterial population (a) decreased the number of bacteria in the four typologies of soil with lime. At pH (b) there was no significant increase and decrease in the interaction of the four typologies of soil and lime

treatment. In C-organic and N-total increases in the soil typology A given lime. Aldd had no significant effect on the interaction of the four typologies of soil and lime treatment.

Given the initial calculation of soil chemical properties prior to the application of lime amounts of soil C-organic soils in the four high land typologies, it is thought to be one of the causes of soil acidity due to the presence of organic compounds. Lime appication on a certain amount can increase the soil pH but indirectly proportional to the population of growing bacteria. It is suspected that lime application can inhibit the organic acids needed by the living bacteria of the soil. According to Winarso (2005), organic matter in the soil can be defined as the remains of plants and animals in the soil in various weathering, consisting of both living and death. Organic matter in the soil can function or improve on chemical, physical and biological properties of the soil.

CONCLUSION

Based on the research that has been done, then it can be concluded that tidal land typology did not affect the population of indigenous nitrogen-fixing bacteria, but it affected soil pH. The highest total number of bacteria in the second week on the soil typology D, was 6.0×10^7 CFU / ml and the highest pH of 5.18 on typology B was in the third week. Application of dolomite affected the population of indigenous nitrogen fixing bacteria, where in dolomite application on the soil populations was fewer than untreated dolomite, with the largest bacterial population at 9.3×10^5 CFU / ml in the second week and dolomite increased the soil pH, with the highest soil pH of 4.93 in the second week. And the interaction between land typology and dolomite application on only affected the population of indigenous nitrogen-fixing bacteria with the most populations of 3.5×10^7 CFU / ml on soil typology D in the second week. The highest pH of 5.33 on soil typology B was in the fouth week .

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