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The Abundance of N-Transforming Bacteria in Ungrazing and Grazing Area Under Oil Palm- Cattle Integration Management in South Kalimantan

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ABSTRACT
Systematic management of oil palm-cattle integration considers as one of the best alternative solutions to meet the need of increasing local beef and dairy. The change in oil palm plantation management systems can influence the soil microbial community. Microbes mediate many key processes in soils and are essential in driving ecosystem stability and sustainability of oil palm production. The research aimed to investigate the influence of grazing in an oil palm plantation on the bacteria involved in the soil nitrogen cycle. We evaluated the abundance of diazotrophic, nitrifying, and denitrifying bacteria in the grazing and ungrazing area of oil palm plantations. Grazing area subject to 11 and 10 times cattle grazing rotation with the two-month interval between rotation were studied. The enumeration of bacteria was evaluated using the Most Probable Number (MPN) technique. Generally, diazotrophic bacteria, nitrifier, and denitrifier were higher in the grazing area compare to ungrazing area. However, the difference between that two grazing-plantation management is not significant ($P>0.05$). In summary, our results show that an addition of organic materials from the dung of cattle in the grazing area of oil palm plantation influences the soil microbial community including N-transforming bacteria

Keywords: *denitrifier, diazotrophic, grazing, nitrifier, ungrazing*

1. INTRODUCTION

Oil palm cultivation in Indonesia is rapidly expanding nowadays. In 2008, Indonesia has reached about 7.33 million hectares of oil palm plantations area and increased become approximately 12.8 million hectares in 2018 [1]. The offensive expansion of oil palm plantations should take into account since this monocropping land utilization will influence ecosystem and economic risk. One of the alternative approaches to minimize the risk and simultaneously utilize the plantation area towards resource maximization is through integrated systematic management of beef cattle and oil palm plantations in the same area. Integration of the beef cattle management under the oil palm plantation consider as one of the best green solutions to increase the production of local beef and dairy to meet the demand. Systematic management of oil palm-cattle integration (OPCI) is commonly known as SISKA (Sistem Integrasi Sapi-Kelapa Sawit) in Indonesia [2]. Ayob and Kabul [2] define stematic management of cattle integration in oil palm plantation as management of cattle that integrated with oil palm. The OPCI system aims are to maximize the land used through the optimal utilization of resources and also to control the weeds through biological control agent. Integration of cattle and oil palm plantations has a great positive influence on the improvement of farm operation management effectively. Studying the correlation between soil characteristics, soil microbial community dynamic, and functional traits with changing environmental factors has proven to be an informative approach to study the N cycle process in the soil as ecosystem responses [3].

Microorganisms are important for soil fertility, stability, and plant productivity because they intercede many key processes in soils [4,5]. The cycling of nutrients by microbial activity influences many ecological processes of the soil ecosystem including plant growth, plant productivity, soil carbon (C) sequestration, soil microbial community function, rate of decomposition, N mineralization, the abundance and activity of various functional microbe i.e. nitrifying, denitrifying, phosphate solubilizing and others microorganisms and greenhouse gas (GHG) emissions [6,7].

It has been identified by many researchers that one of the key nutrient cycles that drive nutrient structure in various ecosystems is the nitrogen cycle. In the nitrogen cycle, microorganisms act as a key element that plays an important role in the biological process for the availability of N to plant growth and avoiding nitrogen leakage from the soil ecosystems [4, 6,8,9, 10, 11]. Nitrogen (N) is a

component of proteins and nucleic acids in a living organism that makes it act as a vital element [9]. The four nitrogen cycle is a series of four microbiological processes included fixation and mineralization of nitrogen, nitrification, and denitrification [8,12]. Furthermore, Hayatsu et al. [12] explained that denitrifying bacteria and fungi, nitrifying bacteria and archaea, anammox bacteria, and heterotrophic nitrifying microorganisms are the key players of microbiological processes of nitrogen transforming in the various ecosystem.

Some studies have described that the grazing activity in different livestock management practices influences the community structure of microbial nitrification and denitrification in soil [13]. It was identified that the grazing activity in many ecosystems influences the aboveground productivity and nutrient cycle which is limited by nitrogen availability in soil [14]. The abundance of N2-fixing, nitrifier, and denitrifier bacterial communities in this study was evaluated in the grazing and ungrazing area of oil palm plantation since they are very important for nitrogen content stability in soil ecosystems [6]. Nitrogen availability has been reported often the limiting factor in various ecosystem productivity. Accordingly, the impact of grazing on the abundance of N-transforming bacteria and soil characteristics has also been studied because it will impact the regulation of C and N dynamics and soil fertility. Furthermore, it will feedback on plant productivity since soil nitrogen pools are direct reservoirs of nutrients to plant.

Approximately 97% of the natural N input in terrestrial ecosystems comes from biological N2 fixation that is performed by Bacteria and Archaea [15,16]. Land use management, soil nutrient content and availability (N, C, P, and micronutrients), soil texture, soil pH, clay content, season, and different vegetation type alter the nitrogen fixation rate of the free-living diazotrophic. This will directly impact the diversity and community structure of the free-living diazotrophic in the soil [17]. Biological N2 fixation relays the activity of a phylogenetically diverse list of bacteria, archaea, and symbioses [8]

The key microbiological activity that conveys to nitrous oxide emission from soil is nitrification. Nitrification is the microbial oxidation of ammonia to NO2- and NO-. The microbe that active in nitrification in most soil ecosystems are chemolithoautotrophic ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) [12, 18]. The rate of nitrification in the most ecosystem depends on the ammonia oxidation process which is act as the global nitrogen cycle process. Ammonia oxidation is an oxidation process of ammonia to nitrate via nitrite [19]. The AOB all are known to belong to a monophyletic group within the subclass of *Proteobacteria*, which has two genera, *Nitrosospira* and *Nitrosomonas* [20].

Denitrification is the biological process that contributes to the nitrogen cycle by release NO, N_2O, and N_2 gases from NO_3^- under anoxic conditions. This process involves the activities of denitrifiers genes such as *narGHIJ*, *napAB*, *nirKS*, *norBC*, and *nosZ* genes [21]. Denitrifying microorganisms in soil are approximately accounted between 0.5 and 5% of the bacterial community in soil that includes a wide range of heterotrophic bacteria such as *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Paracoccus denitrificans* as well as autotrophic bacteria such as *Thiobacillus denitrificans* [6].

2. MATERIALS AND METHODS

2.1. Soil Sampling and Analysis

Soil samples were obtained from grazing and ungrazing area of mature oil palm plantations of PT Buana Karya Bhakti, South Kalimantan in June-July 2019. Grazing area with 10 and 11 times grazing rotation with around 200 cows and ungrazing within 30 ha of mature oil palm plantation area was sampled. Cattle grazing rotation in the oil palm plantation area of PT. Buana Karya Bhakti was a two-month interval between rotations. Soil samples within the plantation area were obtained from the harvesting path zone, circle zone and front stack zone. *Jalur angkong* is the harvesting path zone, an area where the labor or mechanical engine commonly used for fertilizing, harvesting, or other agronomic activity for oil palm trees. *Piringan* is the circle zone, the area for spreading fertilizer in each plant with 1 m width around the oil palm trees. *Jalur mati* is the front stack zone, the path for pile-up pruned branches and leaves of oil palm trees. Soil sampling in every zone was repeated 3 times. The samples were acquired from 0-10 cm of soil depth in each site.

Soil pH was recorded using a pH electrode with a soil-to-water ratio of 1:5, w/v, and 1 M KCl (1:5, w/v). Soil moisture was measured gravimetrically. The soil chemical properties: C-organic, total N, C/N ratio, total P, P_2O_5, K_2O, Ca-exch, Mg-exch, K-exch, Na-exch, Cation Exchange Capacity (CEC), Base saturation, Al_3^+, H^+ were recorded using a method as defined by Eviati and Sulaiman [22].

2.2. Enumeration of diazotrophic

The population density of the cultivated diazotrophic bacterial community was determined using the Most Probable Number (MPN) technique described by Cappucino and Sherman [23]. A series of 3 sequential MPN tubes were prepared. The MPN was set up as a 3-tube 5-dilution MPN (for each sample) that representing 10, 1, 0.1, 0.01, and 0.001 g of sample. Soil samples (1 g) from each sampling area (front stack zone, circle zone, and harvesting path zone) from each grazing treatments were submitted to successive serial dilutions (10-1 to 10-5) in a salt solution (0.85 % NaCl), and 0.5 mL aliquots of the diluted

suspensions were inoculated onto semi-solid culture media of Nfb containing (g/L): malic acid 5.0 g, K2HPO4 0.5 g, MgSO4.7H2O 0.2 g, NaCl 0.1 g, CaCl2.2H2O 0.02 g. Micronutrient solution: 2 ml (gL-1 : CuSO4.5H2O 0.4 g, ZnSO4.7H2O 0.12 g, H3BO3 1,13 g, Na2MoO4 1,0 g, MnSO4.H2O 1.5 g), bromthymol blue solution 0.5% in 2 N KOH 2 ml, FeEDTA 1.64% 4 ml, vitamin 1 ml (Biotin 10 mg dan Pyriodoxol-HCl 20 mg per 100 ml) and bacterial agar 1.75 g. pH was adjusted to 6.8 with KOH. After two weeks of incubation at 30oC, the tube showed the forming growth-characteristic film on semisolid NFb medium was considered diazotrophic positive. The Most Probable Number (MPN) of bacteria per gram of sample was determined using the MPN table from the US Food and Drug Administration's Bacterial Analytical Manual (BAM) for MPN 3 tubes.

2.3. Enumeration of Denitrifying Bacteria

Enumeration of denitrifying bacteria was performed using the medium as described by Trolldenier [24] as follows: 8g of nutrient broth, containing of 5 g peptone, 3 g meat extract, 1.5 g of potassium nitrate were dissolved in 1000 ml of distilled water and pH was adjusted to 7.0. The broth medium then dispensed 9-ml aliquots in culture tubes that filled with upturned Durham tubes according to the MPN 3 tubes technique. The air stuck in the Durham tubes was removed by gentle shaking, the tubes were caped loosely, and then were autoclaved for 15 min at 121ºC. After autoclaved, remove the gas bubbles in Durham tubes by gently shaking again. Each sample was inoculated according to MPN dilutions 3 tubes. After 2 weeks of incubation at 30ºC, the tube with bubble formation on Durham tubes was counted as a positives tube.

2.4. Enumeration of Nitrifying Bacteria Analysis

The nitrifying bacteria was determined using the media as described by Nakos and Wolcott (1979). The medium formulated as follow (g/L): 0.3 $(NH_4)_2SO_4$; 0.136 $CaCl_2$; 0.175 $MgSO_4 \cdot 7\ H_2O$; 0.5 $NaHCO_3$; 13.5 Na_2HPO_4; 0.7 KH_2PO_4; 0.005 $FeSO_4 \cdot 7\ H_2O$ and 0.00375 NaMoO4. The medium was made to pH 8.2 and sterilized at 121°C for 20 min. Each sample was inoculated on MPN dilutions as described above for diazotrophic. All test was done in triplicate and all tubes were incubated for 2–4 weeks at 30°C. The positive samples were evaluated visually. The test tubes were scored at two-day intervals for the change in the color after the indicator was added. The color change displayed the growth of ammonium and nitrite oxidizers. A Most Probable Number (MPN) table 3 tubes were used to observed the enumeration of nitrifying bacteria.

2.5. Data Analysis

All data were subjected to statistical analysis using Agricolae package from R (R Core Team). Analysis of variance (ANOVA) was determined to analysed the mean difference comparison between the treatments and then by Tuckey's HSD test function (α=0.05).

3. RESULTS AND DISCUSSION

3.1. Soil Characteristics

There is no significant difference between ungrazing and grazing area on soil pH, total phosphorus (P_2O_5), P_2O_5 potential, C/N ratio, and Na+. This result is not in line with several studies which determined that grazing increases the pH and phosphorus concentrations of soil. A similar result has been reported by Xie et al. [13], who observed that grazing does not influence the soil pH, phosphorus, and soil water content in Tibetan alpine meadows. This study resulted that the total N, C-organic, K_20, CEC, and K^+ concentration in soil increased significantly at grazing rotation 11 times. This finding could be explained by the deposition of cattle dung and urine in the grazing area. Some studies reported that grazing affects the soil properties in different ways according to ecosystem types and grazing intensity [13]. Furthermore, Rui et al. [25] and Xie et al. [13] explained that grazing treatments increased the soil nitrate concentration under alpine meadows ecosystems. We observed that total N, P_20_5, and K_20 potential increased with grazing intensity.

3.2. Diazotrophic

Our study observed that no significant differences of number diazotrophic bacteria were found between grazing and ungrazing area (Figure 1). Our study observed that no significant differences of number diazotrophic bacteria were found between grazing and ungrazing area (Figure 1). The number of diazotrophic calculated higher in grazing area compared to ungrazing. This result correlated with the total phosphorus measured in soil. Batterman et. al. [26] explain that nitrogen fixation will take place as a result of a response to soil nitrogen availability, the fixation will be up-regulated when soil nitrogen content is low and down-regulated when soil nitrogen is abundant and this results in high demand for phosphorus, consequently fixation may only happen if the phosphorus content in the soil is adequate. On the other hand, Houlton et al. [27] explained that N_2 fixation in tropical forests is activated in phosphorus-poor soil because fixation would allow the fixers to accumulate supplementary soil phosphorus through the reserves of fixed nitrogen in extracellular phosphatases in the soil. Moreover, Levy-Booth and Winder [6] reported that the *nifH* gene, nitrogenase genes which code for the Fe-protein of the nitrogenase enzyme that catalyzes N_2 fixation, was associated with organic C content in soil because the N fixation can determine interlinked N- and C-cycling processes.

Table 1 Soil characteristic in the different grazing treatments. For each variable,

Variable	Ungrazing	Grazing 10 times	Grazing 11 times
pH (H_20)	5.23a	5.23a	5.13a
pH (KCl)	3.94a	3.92a	3.85b
C-Organic (%)	1.762a	1.583b	1.896a
N total (%)	0.136ab	0.129b	0.143a
C/N ratio	13.06a	12.56a	13.00a
P_20_5 total (ppm)	1.68a	1.80a	2.44a
P_20_5 potential (mg/100g)	14.27a	14.97a	14.03a
K_20 potential (mg/100g)	20.64b	23.64a	25.53a
K^+ (cmol(+)/kg)	0.065b	0.072ab	0.09a
Na^+ (cmol(+)/kg)	0.000a	0.006a	0.001a
Ca^+ (cmol(+)/kg)	0.67b	0.94a	0.76ab
Mg^+ (cmol(+)/kg)	0.24b	0.39a	0.36a
CEC	7.49b	8.34b	9.29a
Base saturation (%)	12.06b	17.37a	12.76b
Al_3^+ (cmol(+)/kg)	2.84b	3.28a	3.70a
H^+ (cmol(+)/kg)	0.53b	0.77a	0.88a

Values within the same row not followed by the same letter differ significantly (P <0.05).

The number of diazotrophic bacteria was significantly different between sampling locations in the plantation area, where the highest number of diazotrophic bacteria were observed in *Jalur Mati* (Table 3). This can be explained that *Jalur Mati* is huge in organic matter from the branch and leaves of oil palm that promote the microbial community. It was more likely that the diazotrophic community in this study is altered by soil characteristic, diversity of above-ground vegetation, phenological stage, agronomic activity, and by the niche of colonization than by the activity of cattle on the tree and ecosystem during grazing. Those variables are factors that influenced the diazotrophic community in degraded pastures in Brazil as reported by Oliveira et al. [28].

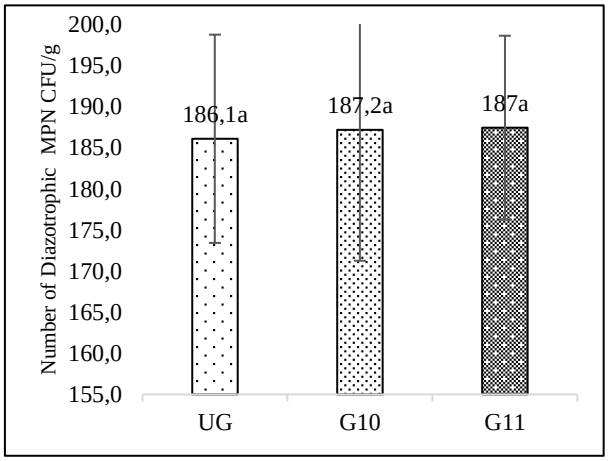

Figure 1 Number of diazotrophic under grazing treatments, UG=ungrazing, G10= grazing 10 times rotation and G11= grazing 11 times rotation.

3.3. Denitrifiers

The abundance of denitrifiers tended in correlation with the total N concentration measured in soil samples at grazing treatments. The MPN value of denitrifying bacteria did not change with grazing activity at 10 and 11 times rotation however, the number of denitrifiers at grazing 11 times rotation was highest. It can be implying that the increasing number of denitrifying bacteria in line with the increase of grazing intensity. This result in accordance with the result conducted by Lindsay et al. [29] and Xie et al. [13] who determined that the grazing intensity has a positive correlation with increased abundance of nirK, a gene encoding nitrite reductase of nitrite-reducers, significantly. The number of denitrifiers at *Piringan* soil sample were statistically not different (P<0.005) from those that enumerated from the soil samples of *Jalur Mati* and *Jalur Angkong* (Table 2 and Table 3), however in *Jalur Angkong* the number of denitrifiers was lowest. This result can be explained that the labor and machine activity on *Jalur Angkong* may contribute to disturbing the niche microbial colonization in the soil. Levy-Boot et al. [6] concluded that the quantity of nirS and nirK, genes encoding the denitrification pathway are influenced by different aspects, comprising soil water content and temperature, total N, $NH4^+$, and $NO3^-$ concentration in soil, available phosphorus (P), soil organic matter, dissolved organic carbon and pH. In contrast, Järvan and Edesi [4] reported that the abundance of denitrifying bacteria in sandy loam Albeluvisol soil of Central-Estonia during the growing season did not affect by the farming treatments and weather conditions.

Table 2 Number of N-transforming bacteria enumerated from different grazing treatments

Bacteria (MPN CFU/g)	**Ungrazing**	**Grazing 10 times**	**Grazing 11 times**
Diazotrophic	186.07a	187.16a	187.43a
Denitrifiers	61.42a	60.41a	97.76a
Nitrifiers	0.15b	22.04a	29.68a

Values within the same row followed by the same letter do not differ significantly (P <0.05).

Table 3 Number of N-transforming bacteria enumerated from different soil sampling location in the oil palm plantation area

Bacteria (MPN CFU/g)	**Piringan**	**Jalur Mati**	**Jalur Angkong**
Diazotrophic	182.89ab	216.96a	160.20b
Denitrifiers	76.86a	82.74.a	51.16.76a
Nitrifiers	10.18a	14.38.a	14.45.a

Values within the same row followed by the same letter do not differ significantly (P <0.05).

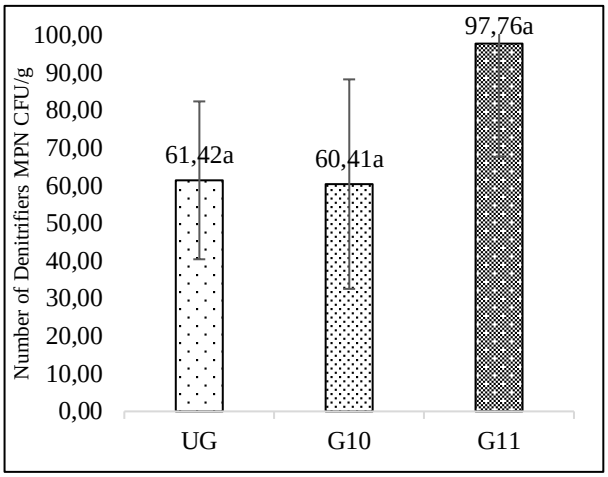

Figure 2 Number of denitrifiers under grazing treatments, UG=ungrazing, G10= grazing 10 times rotation and G11= grazing 11 times rotation.

3.4. Nitrifiers

The abundance of nitrifiers significantly increased in response to grazing intensity (Table 2). This result is correlated with the result of Xie et al. [13], that the abundances of the AOA and the AOB were aligned responded to grazing intensity and soil NO3- concentration, total nitrogen, C/N ratio, and available phosphorous. The number of nitrifiers population in the ungrazing area enumerated very low, suggesting that nitrification process is impaired in ungrazing area. This result could be explained by the input of dung and urine in the grazing area, but it should be taken into account that the activity of the microbial community at the sampling site may change from time to time differently.

Furthermore, we also have to consider the possibility of potential bias in MPN analysis.

We did not identify a major soil characteristic variable that driving the impacts of grazing on nitrifiers abundance. Szukics et.al [30] and Jarvan and Edesi [5] reported the changing environmental condition such as precipitation regime, soil temperature, soil treatment, year, season growth, and their interaction even in the short-term change altered activities and the abundance of soil nitrifying and denitrifying bacteria.

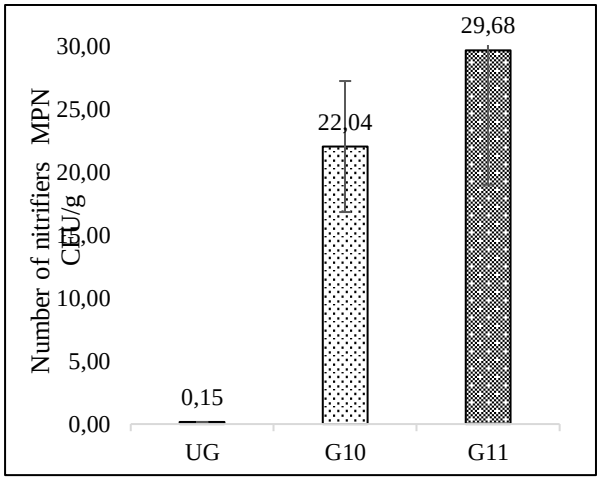

Figure 3 Number of nitrifiers under grazing treatments, UG=ungrazing, G10= grazing 10 times rotation and G11= grazing 11 times rotation

4. CONCLUSION

The abundance of diazothropic, denitrifiers and nitrifiers at the study area tended to increase in response to grazing intensity. These results could be explained by the addition of organic materials from dung and urine from cattle in the grazing area of oil palm plantation influences the soil microbial community including N-transforming bacteria. The result also indicates that soil N microbial cycle may be sustained even under grazing management.

In summary, our study describes the analysis of the abundance of N-transforming bacteria of the soils from oil palm plantation under grazing treatments based on enumeration of N-transforming bacterial data using MPN method. We identified the most abundant N-transforming bacteria were represented based on the result of enumeration of their population by MPN method. The result of this analysis suggesting the active microbial involved in nitrogen cycling are generally consistent with soil characteristics, suggesting N-transforming microbial communities could provide nitrogen nutrients for oil palm and above ground plants in the oil palm plantation area.

To have better information on the abundance of N-transforming bacteria and their activity under grazing treatments in oil palm plantation areas, a molecular technique, such as 16S rRNA sequencing that integrates molecular-level dynamics into ecosystem-level processes should be applied. MPN technique used in this study is an estimate based on probabilistic tables that introduce additional errors, therefore the causes and consequences of cattle integration in oil palm plantation management alterations may not clearly be understood.

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REFERENCES

[1] R. Hirschmann, 'Total area of oil palm plantations Indonesia 2008-2018', Accessed: May 27, 2020. [Online]. Available: https://www.statista.com/statistics/971424/total-area-of-oil-palm-plantations-indonesia/

[2] M.A. Ayob, and M.A. Hj Kabul, 'Cattle Integration in Oil Palm Plantation through Systematic Management.' *The 1st International Seminar on Animal Industry*, Bogor, pp. 23-24, 2009 .

[3] M. Espenberg, M.Truu, Ü. Mander, K. Kasak, H. Nõlvak, T. Ligi, K. Oopkaup, M. Maddison, and J Truu, 'Differences in microbial community structure and nitrogen cycling in natural and drained tropical peatland soils', *Scientific Reports*, vol. 8, pp 47- 42, 2018, doi:10.1038/s41598-018-23032-y

[4] M. Järvan, and L. Edesi, 'Nitrogen cycle bacteria in soils of organically and conventionally managed crop rotations', *Zemdirbyste-Agriculture*, vol. 102, No. 1, pp. 15−22, 2015, doi: 10.13080/z-a.2015.102.002

[5] H. Tan , M. Barret, O. Rice, D.N. Dowling, J. Burke J, J.P. Morrissey, and F. O'Gara F, 'Long-term grichemical use leads to alterations in bacterial community diversity'. *Plant, Soil and Environment*, vol. 58 no.10, 452–458, 2012.

[6] D. J. Levy-Booth, C. E. Prescott, and S. J. Grayston, 'Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems', *Soil Biology & Biochemistry*, vol. 75, pp. 11-25, 2014.

[7] I.A. Janssens, W. Dieleman, S. Luyssaert , J.A. Subke, M. Reichstein, R. Ceulemans, P. Ciais, A.J. Dolman, J. Grace, G. Matteucci, D. Papale, S.L. Piao, E.D. Schulze, J. Tang, and B. E. Law, 'Reduction of forest soil respiration in response to nitrogen deposition', *Nature Geoscience*, vol. 3, pp. 315-322, 2010.

[8] M. E. Ritchie, and R. Raina, 'Effects of herbivores on nitrogen fixation by grass endophytes, legume symbionts and free-living soil surface bacteria in the Serengeti', *Pedobiologia* vol. 59, pp. 233–241, 2016.

[9] P.M. Vitousek, and R.W. Howarth, 'Nitrogen limitation on land and in the sea how can it occur', Biogeochemistry, vol. 13, pp. 87-115, 1991.

[10] B. Thamdrup, 'New Pathways and Processes in the Global Nitrogen Cycle', *Annu. Rev. Ecol. Evol. Syst., vol.* 43, pp. 407–428, 2012.

[11] H. Yao, D. Bowman, and W. Shi, 'Seasonal variations of soil microbial biomass and activity in warm- and cool-season turfgrass systems', *Soil. Biol. Biochem*.,vol. 43, pp. 153-1543, 2011.

[12] M. Hayatsu, K. Tago, and M. Saito, 'Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification', *Soil Science and Plant Nutrition*, vol. 54, no. 1, pp. 33–45, 2008.

[13] Z. Xie *et.al.*, 'Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil

environmental drivers in Tibetan alpine meadows', *Soil Biology & Biochemistry*, vol. 77, pp. 89-99, 2014.

[14] T. Liu, Z. Nan, and F. Hou, 'Grazing intensity effects on soil nitrogen mineralization in semi-arid grassland on the Loess Plateau of northern China', *Nutrient Cycl. Agroecosyst*., vol. 91, pp. 67-75, 2011.

[15] J.N. Galloway *et al*., 'Transformation of the nitrogen cycle: recent trends, questions, and potential solutions', *Science*, vol. 320, pp. 889–892, 2008.

[16] R.R. Eady, 'The dinitrogen-fixing bacteria, in: A. Balows A, H.G. Tr¨uper, M. Dworkin, W. Harder, K.H. Schleifer (Eds.), The prokaryotes.A handbook on the biology of bacteria: ecophysiology, isolation, identification, application', *Springer-Verlag*, New York, 1992.

[17] B.S. Mirza, C. Potisap, K. Nüsslein, B. J. M. Bohannan, and J. L. M. Rodriguesa, 'Response of Free-Living Nitrogen-Fixing Microorganisms to Land Use Change in the Amazon Rainforest', *Appl. Environ. Microbiol*, vol. 80, no. 1, pp. 281- 288., 2014.

[18] Pajares, and B.J.M. Bohannan, 'Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils', *Front. Microbiol*, vol. **7**, pp. 1–20, 2016.

[19] G.A. Kowalchuk, and J. R. Stephen, 'Ammonia-oxidizing bacteria: a model for molecular microbial ecology', *Annu. Rev. Microbiol.*, vol. 55: pp. 485–529, 2001.

[20] J.R. Stephen, A. E. McCaig, Z. Smith, J. I. Prosser, and T. M. Embley, 'Molecular diversity of soil and marine 16S rRNA gene sequences related to subgroup ammonia-oxidizing bacteria', *Appl. Environ. Microbiol*., vol. 62, pp. 4147- 4154, 1996.

[21] M. Ren, *et al.*, 'Diversity and Contributions to Nitrogen Cycling and Carbon Fixation of Soil Salinity Shaped Microbial Communities in Tarim Basin', *Front. Microbiol*, vol9: 431 (2018).

[22] Sulaiman and Eviati, 'Soil chemical, plant, water and fertilizer analysis procedure', 2nd Edition, *Balitbang Pertanian*, Bogor, 2009.

[23] G. J. Cappucino, and N. Sherman, Microbiology: A Laboratory Manual, Ed-7, *Addison-Wesley Publishing Company Inc*, California, 2007.

[24] T. Han, J.P. Katoen, and D. Berteun, 'Counterexample generation in probabilistic model checking', *IEEE Trans. Softw. Eng.*, vol. 35, no. 2, pp. 241–257, 2009.

[25] G. Trolldenier, 'Influence of some environmental factors on nitrogen fixation in the rhizosphere of rice', *Plant Soi,l* vol. 47, pp. 203-217, 1977.

[26] S.A. Batterman, N. Wurzburger, and L. O. Hedin, 'Nitrogen and phosphorus interact to control tropical symbiotic N2 fixation: a test in Inga punctata', *J. Ecol*., vol. 101, pp. 1400-1408, 2013.

[27] B.Z. Houlton, Y.P. Wang, P.M. Vitousek, and C.B. Field, 'A unifying framework for di-nitrogen fixation in the terrestrial biosphere', *Nature*, vol. 454, pp. 327–330, 2008.

[28] J.T.C. Oliveira, *et.al*., 'Diazotrophic Bacterial Community of Degraded Pastures. *Appl. Environ. Soi. Sci*., vol. 1, pp. 1 - 10, 2017, doi: 10.1155/2017/2561428

[29] E.A. Lindsay, M.J. Colloff, N.L. Gibb, and S.A. Wakelin, 'The abundance of microbial functional genes in grassy woodlands is influenced more by soil nutrient enrichment than by recent weed invasion or livestock exclusion', *Appl. Environ. Microbiol.*, vol. 76, pp. 5547-5555, 2010.

[30] U. Szukics, G.C.J. Abel, V HödB. Mitter, A. Sessitsch, E. Hack, and S. Zehmeister-Boltenstern, 'Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil', *FEMS Microbiol. Ecol*', vol. 72, pp. 395–406., 2010.