

Analysis of The Role of Biotic Components in Ecosystem Equilibrium Through Aquaponic Modeling Using Covariate Analysis

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ABSTRACT

Overusing pesticides has caused numerous problems in the agricultural sector and led to mutagenic effects in fish. Additionally, land conversion across Indonesia is a major challenge for farmers. Farmers have adopted an aquaponic system by combining catfish and hydroponic rice cultivation to address this. This method has been widely used in Indonesia; however, it still lacks a scientific benchmark. This research aims to observe changes in biotic component behavior after degradation of bacterial strains, namely *Lactobacillus*, *Nitrobacter*, and *Photosynthetic Bacteria*. Through this, we will analyze the interaction between catfish and paddy using a parametric ANCOVA assay. The results show that the use of bacterial strains significantly affects the catfish growth in terms of length and weight. However, the correlation between the growth of catfish and paddy is not significant. While the well-being of catfish contributes to better paddy growth, the absence of catfish does not negatively impact paddy growth, as catfish are not the primary source of nutrients required by the paddy.

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1. INTRODUCTION

Biotic components are living organisms in natural and artificial ecosystems [1]. Based on their role in the ecosystem, biotic components are divided into producers, consumers, and decomposers. A producer is an organism that produces their food. The consumer is an organism that consumes the producers. Lastly, a decomposer is an organism that decomposes metabolic waste and other organisms' corpses [2]. The role of biotic components is important in maintaining ecosystem stability, due to the close interaction of one component with another. For this reason, research on biotic components is required, especially before designing an artificial ecosystem [1].

According to The Conversation News from 2023, Indonesia is the third highest pesticide user in the world. The huge amount of pesticide use can cause water pollution later on. Fish, as one of the aquatic biota, will experience structural and functional changes, along with its histological conditions, when it's contaminated by the chemical substances from pesticides for a long time. This problem will also affect humans, as consumers of the fish themselves. Furthermore, according to CNBC Indonesia Research in 2023, the land conversion throughout Indonesia reached 100,000 hectares per year.

To face those problems, farmers had come up with a solution. An aquaponic system is a form of artificial ecosystem that integrates an aquaculture system (fish farming) with hydroponics (water-mediated agriculture). This system works by converting the debris from the metabolic waste of fish and the excess feed into a source of nutrients for plants. The aquaculture method adopts Indonesia's conventional agricultural system called mina padi. This method needs to be developed because it's feasible as a solution for agricultural problems, due to excessive pesticide use and limited agricultural land.

The main objective of this research is to dissect the role of each biotic component on ecosystem equilibrium, from the producer (paddy), consumer (catfish), and decomposer (degrading bacteria). These objectives were

divided into three. Firstly, observation of the shifting of the biotic component behavior after the strain of degradation bacteria, such as *Lactobacillus*, *Nitrobacter*, and *Photosynthetic Bacteria* (PSB) had been added. Secondly, analyzing the significance of ecosystem behavior change after each strain of bacteria is added. Thirdly, measure the interaction between the catfish and the paddy. The test was conducted by the parametric ANCOVA assay.

Both parametric and non-parametric assays were used in this experiment. The parametric testing was used when the data was well distributed ($p\text{-value} > 0.05$). The first parametric assay uses two-way ANOVA, a statistical assay to assess the effect of multi-variant variables, such as their interaction. The second assay, ANCOVA, was a statistical assay used to compare two or more variables. The second variable was considered to influence or control the third variable. The non-parametric assay was an alternative test if the data obtained was not well distributed ($p\text{-value} < 0.05$). The non-parametric assay method, which was considered capable of representing a two-way ANOVA assay, was the Friedman test. The Friedman assay was conducted to determine differences in multi-variant groups which interrelated.

This experiment also used a complete random design to minimize bias and improve the reliability of results.

2. LITERATURE REVIEW

2.1 *The Effectiveness of Using Nitrosomonas and Nitrobacter to Improve Water Quality of Tilapia Cultivation Media (Oreochromis niloticus) (10pt)*

This research results that *Nitrosomonas* and *Nitrobacter* in Nila fish cultivation increased the water quality of the media growth, as shown by the low percentage of ammonia present at the end of the experiment. The *Nitrobacter* possesses nitrification by converting ammonia into a simpler form of nitrogen that can be absorbed by the plants as a

nutritional source. Ammonia is a very toxic substance both for the catfish that lives inside there and the paddy that lives above the water. First, ammonia will be turned into nitrite, then nitrate, and by then it can be absorbed by the plants as nutrients.

2.2 The Effect of Probiotic Bacteria (*Lactobacillus plantarum*) on Feed for The Growth of Nila Fish (*Oreochromis niloticus*)

This research resulted in the conclusion that the use of *Lactobacillus plantarum* as food for Nila fish directly impacted the growth in weight of the Nila fish. However, the research also shows that the use of *Lactobacillus plantarum* probiotic doesn't have a significant impact on the growth of length of the Nila fish. *Lactobacillus* mainly affected the growth in weight of the Nila Fish, this is because the ability of *Lactobacillus* itself to provide digestive enzymes that improve the digestive mechanism for the plant to digest and absorb nutrients from the environment, such as protein, fat, and carbohydrate, better.

2.3 The Effect of Photosynthetic Bacteria (PSB) on the Growth and Yield Quality of the Pakcoi Plant (*Brassica rapa subsp Chinensis* L.)

This research resulted in the conclusion that the use of *Photosynthetic Bacteria* directly impacted the number of leaves grown in the Pakcoi Plant. The function of the *Photosynthetic Bacteria* is to help plants undergo the photosynthetic process during the night when sunlight is not available. *Photosynthetic Bacteria* contain the bacterial chlorophyll compound that enables photosynthesis. It's required to synthesize organic

nitrogen that's needed by the plants to grow.

2.4 The Contribution of Nitrogen from Endophytic Bacteria to Rice Plants

This research resulted in the conclusion that the growth of rice plants was independent of external bacterial treatment. This is due to the presence of endophytic bacteria located on the roots of the rice plants. It can excrete growth hormones, such as indole acetic acid, that impact the growth of primary, secondary, and hair roots of a plant. So, this hormone has a very significant role in a plant's growth, and it could bind N₂, which leads to the conclusion that the waste of catfish is not the main source of nitrogen that can be obtained by the plant. The degradation bacteria support the supply of nitrogen needed by the paddy by helping the nitrification process during the conversion of ammonia into nitrogen. However, even without it, the plant can still survive and grow well.

3. METHODS (11 PT)

3.1 Time and Place of Research

Time: June 1st – December 5th 2024

Place: Chemistry Lab, Little Sun Senior High School

3.2 Tools and Materials

Tools: Aquaculture Instruments – maintenance container: 16-20 liters, wire mesh, pump, aerator, TDS meter, heater, gallon, Hydroponic instruments – pipe, plastic glass (net pot) 250 ml

Material: Catfish fingerlings size 5-6 cm, paddy seeds, bacteria culture, Nitrobacter, photosynthetic bacteria, lactobacillus, coarse salt, growing media (sugarcane pulp and banana stem), liquid organic fertilizer.

3.3 Design Experiment

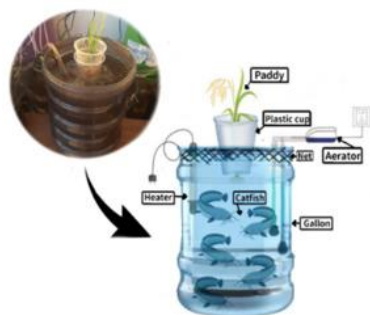


Figure 1. Experimental design

The photo above illustrates the design of the aquaponics used in this study. Each gallon consisted of 10 catfish, providing a controlled environment for observing their growth. The gallons were equipped with an aeration system to maintain adequate oxygen levels, supporting the optimal development of the catfish in this research. A plastic cup containing paddy was placed on top of each gallon, allowing researchers to examine the symbiotic relationship between the growth of catfish and paddy. The placement of the paddy cups ensured that the roots had access to the water, which facilitates nutrient exchange between the catfish waste and the paddy. This design aimed to show aquaponic ecosystems, where aquatic organisms and plants coexist and benefit from each other.

In total, 12 gallons of samples were used, which were divided into 4 treatments. Each of the three samples was added with *Lactobacillus*, *Sp*, *Nitrobacter*, and PSB, while the remaining samples were treated without any bacteria as a control. These control groups provided a baseline comparison to determine the effectiveness of the bacterial treatments. To assess the effects of these treatments, data collection was conducted weekly, every Friday, for 8 weeks to observe the growth of 10 catfish in each sample. The measurement of catfish was performed in a small container filled with water. The weight of the catfish was measured using digital scales, while their length was measured with a ruler to ensure precise growth tracking. Additionally, paddy growth was evaluated for 4 weeks following the seedling period, with height

measurements taken from the base of the stem.

Repetition	Treatment			
	Negative Control	Lactobacillus	Nitrobacter	PSB
	C1	L1	N1	P1
	C2	L2	N2	P2
	C3	L3	N3	P3

Figure 2CRD of the experiment

This study also used a Completely Randomized Design (CRD) as an experimental framework. A Completely Randomized Design is an experimental design in which subjects or experimental units are randomly assigned to different treatments. It ensured that each unit had an equal chance of receiving any treatment, which minimized bias, improved the reliability of results, and ensured that external variables did not influence the outcomes of the study. In this study, CRD was used to test the effects of different bacterial culture treatments on catfish and paddy growth. The experimental design consists of four treatments, which are Negative Control, *Lactobacillus*, *Nitrobacter*, and PSB. Each of the treatments was replicated three times, and a total of 12 experimental units were used, labeled as C1-C3 (Control), L1-L3 (*Lactobacillus*), N1-N3 (*Nitrobacter*), and P1-P3 (PSB). The random assignment of treatment in CRD helps to ensure the accuracy and validity of the experimental findings.

3.4 Parametric Assay – ANOVA Two-Ways

ANOVA Two-Ways is a statistical assay used to test the effect of two independent variables on a dependent variable, as well as their interaction. In this research, the ANOVA Two-Ways assay was used to examine the impact of bacterial treatments on the growth of catfish and paddy. The basic formula of ANOVA Two-Ways is as follows:

$$Y = \alpha + \beta X + \varepsilon$$

Information:

Y = Dependent variable (paddy growth)

X = Independent variable (catfish growth)

α = Constant

β = Regression coefficient of the independent variable x

ε = Residual

The ANOVA Two-Ways assay is carried out by considering several factors. The

sample population must be normally distributed, the sample must be independent, the sample variation must be the same based on the homogeneity test, and the sample size must be the same. However, if the normal distribution test requirements are not achieved, the test will be carried out using non-parametric assays, such as the Friedman assay. The expected hypothesis from this assay is:

- Ho : The presence of degrading bacteria doesn't affect the growth of catfish (length and weight) and rice growth (height)
- Hi : The presence of degrading bacteria doesn't affect the growth of catfish (length and weight) and rice growth (height)
- Ho : The character of degrading bacteria doesn't affect the growth of catfish (length and weight) and rice growth (height).
- Hi : The character of degrading bacteria affects the growth of catfish (length and weight) and rice growth (height)

If the ANOVA Two-Ways value has a significant value of $p < 0.05$, then H_0 will be rejected, and the variability between each variable is considered not to have a significant effect.

3.5 Parametric Assay – ANCOVA

ANCOVA is a statistical assay used to compare two or more variables, where the second variable is considered to have an influence or control over the third variable. In this research, ANCOVA assay is used to analyze the symbiotic relationship between each of the biotic components. The basic formula for ANCOVA is as follows:

$$Y = \alpha + \beta X + \gamma Z + \varepsilon$$

Information:

Y = Dependent variable (paddy growth)

X = Independent variable (catfish growth)

Z = Covariate variable

α = Constant

β = Regression coefficient of the independent variable X

γ = Regression coefficient of covariate variable Z

ε = Residual

The ANCOVA assay is carried out by considering several factors. The sample population must be normally distributed, homogeneity of data variance, a linear relationship between covariate variables and independent variables, homoscedasticity of data from covariate variables to independent variables, and the absence of interaction between covariate variables. If the data is not normally distributed, then the test will be carried out using a non-parametric assay without a further correlation assay. The hypothesis test of the ANCOVA assay is carried out twice based on the Sun Square Linearity Test (SS) type III:

Ho : There is no correlation between catfish growth (length and weight) and paddy growth (height).

Hi : There is no correlation between catfish growth (length and weight) and paddy growth (height).

If the ANCOVA value has a significant value of $p < 0.05$, then H_0 will be rejected, and the variability between each variable is considered to have a significant influence.

3.6 Non-Parametric Assay – Friedman

Non-parametric assay is an alternative assay that needs to be done if the data obtained doesn't pass the normal distribution test ($p\text{-value} < 0.05$). The non-parametric assay is considered capable of representing the ANOVA Two-Ways, Friedman Assay. Friedman's Assay is carried out to determine the differences in more than 2 sample groups interrelated. This research was used to examine whether each bacterial culture treatment results in a significant difference in its ecosystem, with the interpretation results presented in the form of rankings. The basic formula for the Friedman assay is as follows:

$$X_r^2 = \frac{12}{nk(k+1)} \sum_{j=1}^K R_j^2 - 3n(k+1)$$

Information:

X_r^2 = Friedman value

n = Amount of data for each variable

k = Amount of paired variables

R_j = Number of rankings for each variable

4. RESULTS AND DISCUSSION

Based on observations made for 1 month, it was found that increasing the amount of flocculant had a significant impact on catfish welfare.

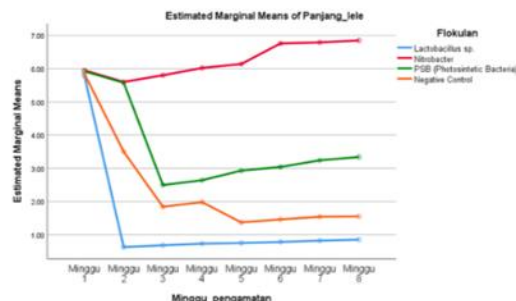


Figure 3. The Graph of Catfish Growth in Length (Sample 1)

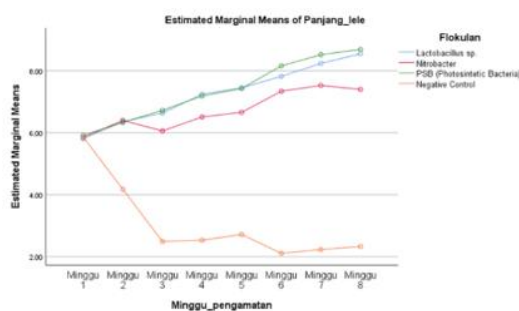


Figure 4. The Graph of Catfish Growth in Length (Sample 2)

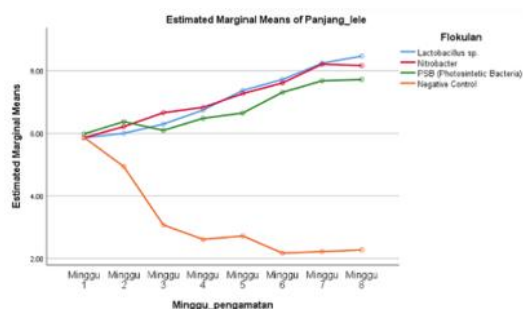


Figure 5. The Graph of Catfish Growth in Length (Sample 3)

The selection of degrading bacteria was based on a demand for commercial bacterial cultures, which are *Lactobacillus* sp., *Nitrobacter*, and *Photosynthetic Bacteria* strains. The three strains of bacteria have different characteristics, which will directly lead to different ecosystem behavior. However, since the data was not distributed normally ($p = 0.00 < 0.05$), the Friedman test was conducted

to measure the significance of each bacterial strain.

Table 1. Friedman Rank of Estimated Marginal Means of Catfish Growth

	Lactobacillus	Nitrobacter	PSB	Control
Catfish Length	3.31	3.31	2.25	1.13
Catfish Weight	3.50	2.83	2.67	1.00

Sig. value of length: $0.01 < 0.05$ (Reject H_0)
Sig. value of weight: $0.07 > 0.05$ (Accept H_0)

Based on the Friedman Rank, we can conclude that *Lactobacillus* and *Nitrobacter* occupied the highest rank in the data of catfish length. This result is linear to the characteristics of both *Lactobacillus* sp. and *Nitrobacter*. *Lactobacillus* sp. plays a role in improving the digestive system of catfish, which directly impacts its growth in length. In the same rank, we have *Nitrobacter*, whose role is to help in the nitrification process of converting toxic ammonia into nitrogen to maintain optimum ecosystem health. Nitrogen is an important compound required to support the growth of a plant. Nitrogen ensures energy is available anytime when plants need it. Other than that, nitrogen also helps regulate water and nutrient uptake in the roots. So, a sufficient amount of nitrogen in the environment is important for the plants' growth.

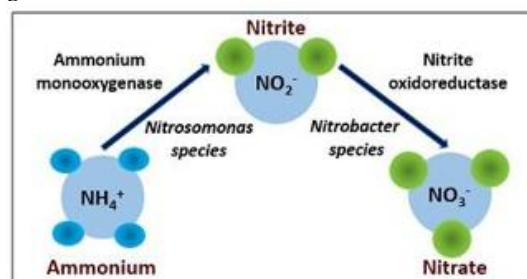


Figure 6. Overall Nitrification Process

This result is supported by the statement of Hayatun (2022) [4]. In his research on the effectiveness of using *Nitrosomonas* and *Nitrobacter* to Improve the Water Quality of Tilapia Cultivation Media (*Oreochromis niloticus*), which resulted in the conclusion, that the lowest ammonia content was obtained in the sample with *Nitrosomonas* and *Nitrobacter*, this is because there is presence of autotroph bacteria in the cultivation media that helps in optimizing water quality by doing oxidation upon fishes' waste located at the bottom of the cultivation

media. This proves that *Nitrobacter* can reduce ammonia and turn it into a simpler compound (nitrogen), which is used by other organisms, in this case, paddy.

However, the significant value of the length is 0.01, which is ($p < 0.05$). So, H_0 is rejected, which shows that different characteristics of bacteria don't affect catfish length. In contrast, it will affect fish weight, which will be discussed below.

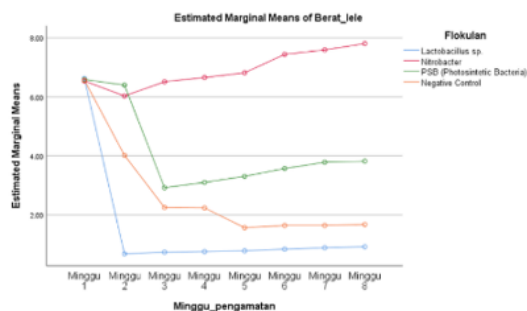


Figure 7. The Graph of Catfish Growth in Weight (Sample 1)

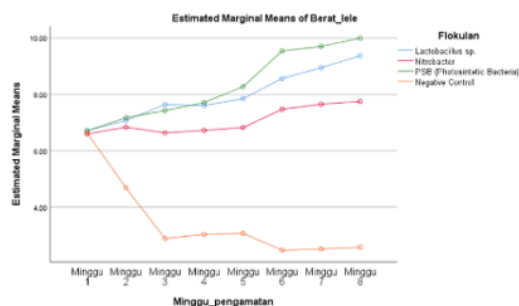


Figure 8. The Graph of Catfish Growth in Weight (Sample 2)

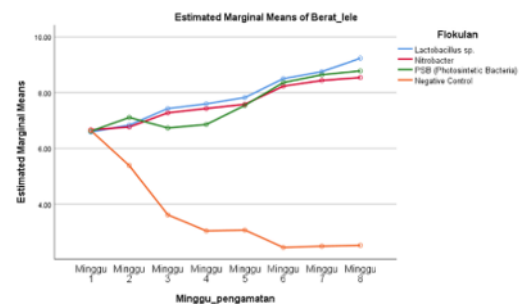


Figure 9. The Graph of Catfish Growth in Weight (Sample 3)

Based on Table 1, we can conclude that the growth in weight of fish is mostly affected by the presence of *Lactobacillus* sp. This is supported by the experiment conducted by Sartika, dkk (2022) [7]. Their experiment shows that *Lactobacillus* sp. results in better growth of fish. *Lactobacillus* sp. provides enzymes that support the

digestive mechanism to digest and absorb nutrients such as protein, fat, and carbohydrates. The significant value is 0.07, which is ($p > 0.05$). This shows that the presence of *Lactobacillus* sp. in the environment directly affects the weight growth of the organism living in it.

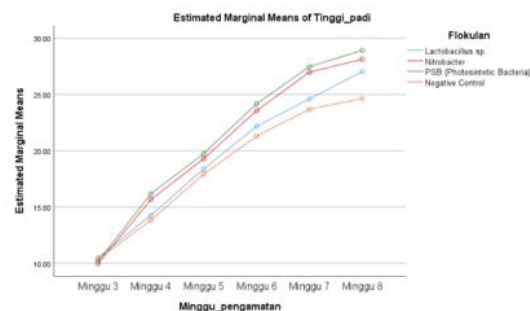


Figure 10. The Graph of Paddy Growth in Height (Sample 1)

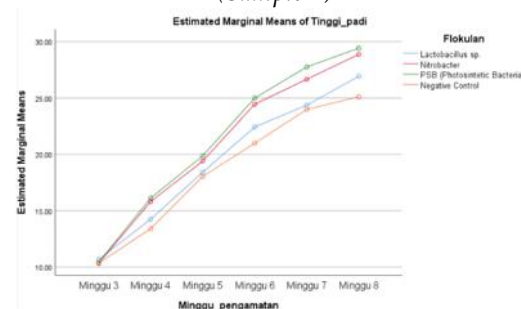


Figure 11. The Graph of Paddy Growth in Height (Sample 2)

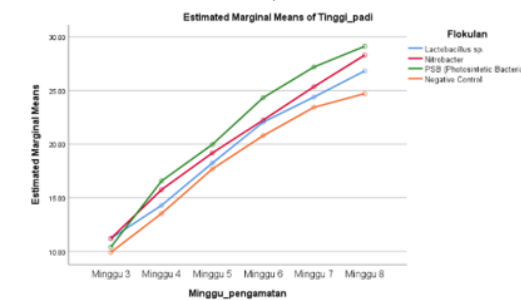


Figure 12: The Graph of Paddy Growth in Height (Sample 3)

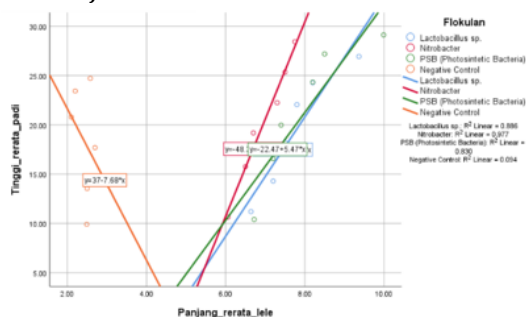
All 3 graphs show increasing trends for each bacteria treatment are linear with the control variable. It shows that the presence of degrading bacteria influences paddy growth. Table 2. ANOVA 2-Ways of the Estimated Marginal Means of Paddy Height

	Control	Lactobacillus	Nitrobacter	PSB
Paddy Height	18.65 ^a	19.51 ^b	20.92 ^c	21.43 ^d

The results of the 2-way ANOVA test showed that each variant of degrading bacteria had a different effect on rice growth

(p -value > 0.05). PSB bacteria are stated as the best strain for supporting the accretion of paddy because it is consistent with the research conducted by Miftakhul Rizah (2024) [5]. This research shows that the use of PSB (Photosynthetic Bacteria) can improve soil structure, strengthen the ability of soil to absorb water, and help it expand nutrient availability. All of these have major contributions to improving plant growth.

Sig. value (Type III Sum of Square) = 0.00 < 0.05 (Reject H0)



Sig. value (Type III Sum of Square) = 0.02 < 0.05 (Reject H0)

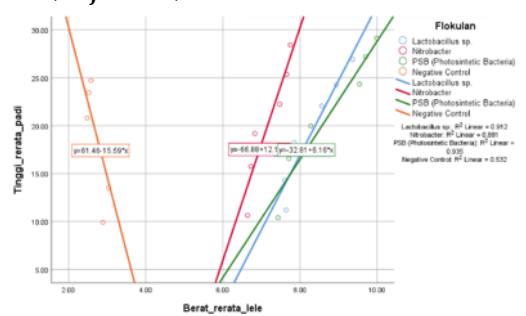


Figure 13. Comparing the Estimated Marginal Growth of Catfish with The Estimated Marginal Height of Paddy for Each Type of Bacteria Treatment

The denser bacteria and the role of catfish did not control the rice growth due to the high nutrients available to rice ($p > 0.05$). It can also be proved by the Pearson value ($r = 0.388$), which is less than 0.515 (R_{crt}). This result is supported by research from Nur Maulidya Zain, dkk, 2018 [8]. Her manuscripts show that even without external bacterial treatment, paddy can grow just fine because the presence of endophyte bacteria that live in the root and stem tissues increases iron substance in the soil, as well as phosphorus and nitrogen for the plants. Some diazotroph endophytic bacteria can also bind N_2 , as well as excrete indole-3-acetic acid, a

growth hormone that directly impacts the growth of primary root, secondary root, and root hairs.

The result shows that the well-being of catfish will lead to the well-being of paddies. However, if the catfish are dead, then there is no significant impact on the paddy. The nutrients needed by paddy are not the only ones supplied by catfish; they still survive even without the presence of catfish.

5. CONCLUSION

This research aims to dissect the role of each biotic component on ecosystem equilibrium. The conclusion stated that the decomposer component plays a crucial role in maintaining ecosystem equilibrium, as the *Nitrobacter* helps to reduce ammonia, which is toxic for the environment through the nitrification process, and *Lactobacillus. Sp.* helped in improving the digestive system of the catfish, which led to a better growth in weight of the catfish, and Photosynthetic Bacteria helped in the photosynthetic process of paddy, which led to better growth of paddy, in terms of leaf length. Secondly, the existence of catfish does not influence paddy growth, although catfish are a contributor to nitrogen sources. Paddy, just like any other plant, has endophyte bacteria living on its root; these bacteria play a very significant role in producing growth hormone that directly impacts the growth of the paddy. As mentioned previously, nitrogen is one of the most important nutrients required by a plant to maintain its life. In this case, nitrogen from the waste of catfish is also a source of nitrogen for the paddy, however, the paddy is not completely dependent on it as a nitrogen supply. That's why when the catfish was living well, the paddy would also be impacted, however, when the catfish died, there was no significant impact upon the paddy. Thirdly, rice proves its independence as a producer, it also has less attachment to other components.

As a reference for future research that wants to research more deeply about a similar topic, researchers suggest that this mathematical research is ready to be developed to break down the benchmark

details for each component, such as the population ratio. Because now, especially farmers in Indonesia, they haven't used a scientific benchmark in their application of degradation bacteria. This can lead to inefficiency in terms of capital and resources. It can also be implemented for complex agricultural systems, like the Long Yam farming model. Long Yam farming model is a type of farming that operates above a pond.

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Researchers hope that this study will contribute valuable insights to the scientific community and serve as the foundation for further exploration into sustainable agriculture and aquaculture practices, promoting environmentally friendly solutions.

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BIOGRAPHIES OF AUTHORS (10 PT)

The recommended number of authors is at least 2. One of them is a corresponding author.

Please attach a clear photo (3x4 cm) and a vitae. Example of biographies of authors (9 pt):

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