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Research Article

Bacterial Community Structure, Diversity, and Fertility of Soil with and without Press Mud in Two Sites in Panay, Philippines

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ABSTRACT

Sugarcane farmers commonly use press mud as organic fertilizer to improve the properties of soil and increase crop production. This study compared the effects of press mud on bacterial community structure, bacterial diversity, and physicochemical parameters of sugar mill soil in two sites, the URC Passi Sugar Central and Passi Sugar Central, Inc. both in Panay, Philippines. DNA and soil analyses were conducted to characterize the soil samples with and without press mud from both sites. The results showed that all nutrient contents increased in both areas after press mud application, except in URC Passi Sugar Central, where no increase in phosphorus and sulfur was observed. Bacterial diversity did not significantly increase six months after press mud application. Community pattern results showed that if soil samples within sites were compared, soil with press mud was significantly higher compared to the soil without press mud in terms of OTU richness (Capiz sugar central: 46.00±1.00, 40.00±1.50, respectively; URC Passi Sugar Central: 48.00±0.50, 45.00±0.00, respectively) and carrying capacity (Capiz sugar central: 169±7.36, 125±9.48, respectively; URC Passi Sugar Central: 181±3.80, 162±0.00, respectively) ($p<0.05$). The same trend was observed in soil samples between sites that were compared. Soil without press mud from URC Passi Sugar Central had a significantly higher compared with soil without press mud of Capiz Sugar Central in terms of OTU richness (45.00±0.00, 40.00±1.50, respectively) and carrying capacity (162±0.00, 125±9.48, respectively) ($p<0.05$). However, no significant difference was observed in soil with press mud between the two sites in OTU richness, carrying capacity, Shannon, and Evenness analyses ($p>0.05$). The phylogenetic tree analysis showed that *Massilia* sp. is closely related to *Burkholderia arboris*, and *Lysobacter* sp. is closely related to both *Massilia* sp. and *B. arboris*. Generally, press mud application helps increase the available nutrients, diversity, and community patterns in soil, making it good organic fertilizer.

Keywords: Bacterial community, Bacterial diversity, Organic fertilizer, Press mud

Introduction

Press mud is a waste product from the clarification of cane juice. It contains organic compounds, calcium, phosphorus, potassium, nitrogen, magnesium, sulfur, cellulose, hemicellulose, lignin, protein, and wax [1]. The composition and properties of press mud vary depending on the cane variety, locality, mill efficiency, type of soil, and nutrients in the soil [2]. Farmers commonly use it to improve soil properties. Different studies cited that the application of press mud as organic fertilizer increased the sugar yield and cane juice quality [3, 4, 5, 6]. Interestingly, though press mud is considered by the Bureau of Agriculture and Fisheries Standards as organic soil amendments, there is no data about the production and utilization of press mud in the Philippines [7].

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Fresh press mud is used as organic fertilizer in organic farming to enhance the quality of the soil. The meta-analysis study conducted by Lori et al. [8], showed that organic farming enhances microbial abundance and activity. The nutrients in organic fertilizer will be available only to the plants after the microbes decompose the materials and obtain the energy. Microorganisms are responsible for controlling the availability of nutrients in the soil by mineralizing or immobilizing the nutrients [9, 10, 11, 12]. The properties of soil like electrical conductivity, pH, temperature, carbon, and nitrogen, significantly correlate to soil microbial diversity [13, 14]. In soil microbial ecology, microorganisms are widely recognized for their significant role in soil fertility [15, 16, 17], in which soil fertility influences plant productivity. Soil fertility is defined as the capacity of soil to provide the essential nutrients needed by plants to make sure optimum plant productivity [18]. Different studies concluded that organic fertilizer enhances microbial diversity [19]. However, limited studies were available that directly explain the mechanism of organic fertilizer in improving the existing microbial diversity in the soil.

Previous studies showed that press mud is a good source of nutrients for microbial growth [20, 21, 22]. Although press mud is widely used in the Philippines as organic fertilizer, especially in sugarcane crops, no study was conducted about the effects of press mud on bacterial communities, bacterial diversity, and soil fertility.

Assessment of soil microbial diversity is essential to understand the relationship between diversity, soil fertility, and productivity in a specific ecosystem [23, 24]. Assessment of microbial diversity can be done in two ways: culture-dependent methods and culture-independent methods [25]. The culture method uses a culture media and is limited only to the general requirements of specific bacteria. For that reason, it causes biases in determining the true diversity of one community [26]. Some bacteria are sensitive and grow slowly, and later are outcompeted by other bacteria in a culture-dependent method [23]. In solving the problem, the culture-independent method is used to assess the diversity of microbial communities. Culture-independent methods can capture those species that are hard to culture using culture-dependent methods [27]. This study compared the effects of press mud on bacterial community structure, bacterial diversity, and physicochemical parameters of sugar mill soil in two sites in Panay, Philippines, using DNA fingerprint and soil analysis.

The results of the study will provide baseline information for future studies that focus on the potential of press mud as organic fertilizer. In addition, it helps to elucidate the mechanism of organic fertilizer in increasing microbial diversity in the soil.

Material and Methods

Soil Analysis

The soil samples were collected from Capiz Sugar Central Inc. located at President Roxas, Capiz, Philippines (11°25'37.84 "N, 122°55'51.32 "E) and URC Passi Sugar Central located at Passi, Iloilo, Philippines (11°05'37.54 "N, 122°39'10.16 "E) in areas covered with press mud and without press mud from the two sites (Figure 1). Due to the unavailability of literature on the decomposition time of press mud in a natural process, the collection of samples was scheduled six (6) months after the last milling operation. This is to allow the press mud to cool down, allowing the colonization of bacteria and ensuring the complete decomposition of press mud. The environmental condition during the decomposition process was not controlled to allow natural decomposition, which is the same condition employed by the farmers when they apply press mud as organic fertilizer. The soil samples were collected randomly as proposed by the Regional Soil Laboratory of the Department of Agriculture. The total cores taken were 25 at 30 cm depth in both sites. The soil sample from each core was pooled and homogenized in a sterilized container. The soil for bacterial analysis was separated on-site and stored at 4°C, and the remaining soil was used for physicochemical analysis. The samples for DNA isolation were processed immediately at the University of the Philippines Visayas (UPV)-National Institute of Molecular Biology and Biotechnology (NIMBB) laboratory on the same day of collection. The samples for physicochemical analysis were air-dried before the analysis and sent to the Department of Agriculture Region VI, Regional Soils Laboratory, and Southeast Asian Fisheries Development Center (SEAFDEC) soil laboratory, Philippines.

Polymerase Chain Reaction (PCR)–Denaturing Gradient Gel Electrophoresis (DGGE) analysis

Genomic DNA extraction was carried out us-

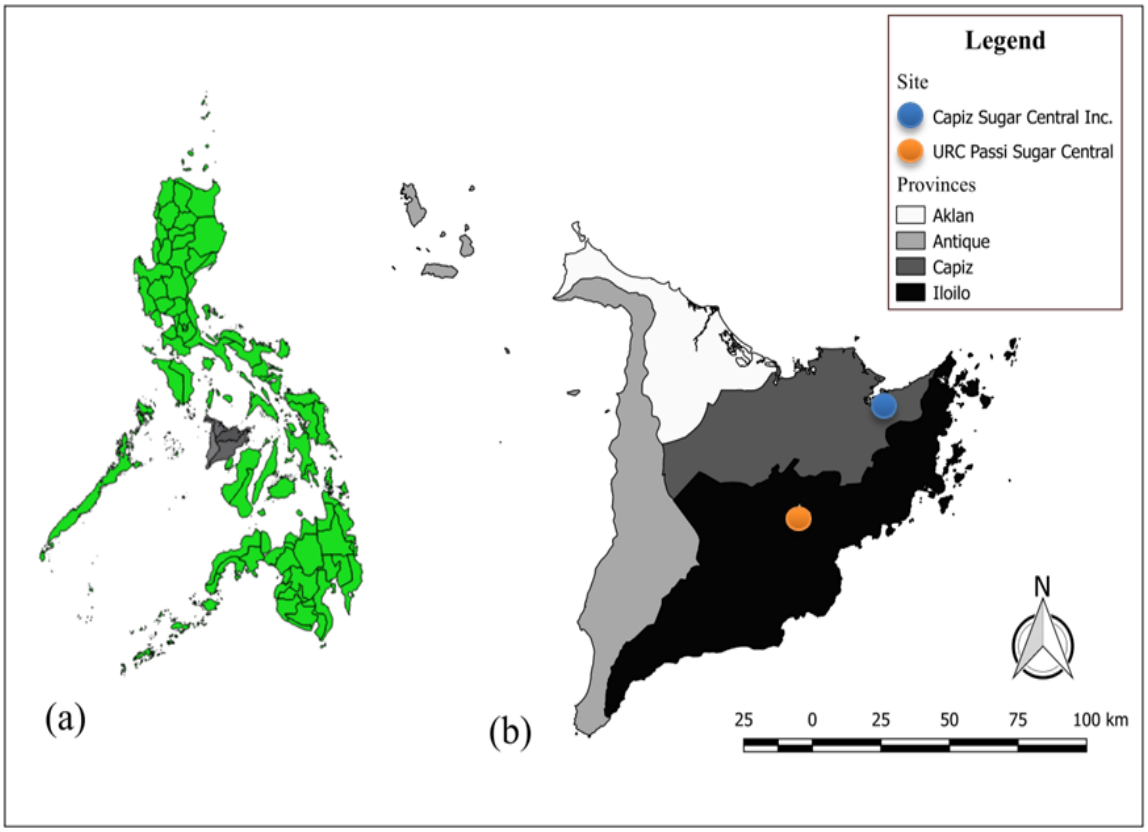

Figure 1. Map of the study site. (a) The map of the Philippines emphasizing Panay; and (b) The map of Panay highlighting Capiz Sugar Central (blue circle) and URC Passi Sugar Central (orange circle).

ing a DNeasy PowerSoil Pro kit (Qiagen, USA) following the protocol provided by the manufacturer. The DNA extracted from the soil was amplified using a conventional polymerase chain reaction (PCR). The universal primers 357F (5' CCTACGGGAGGCAGCAG-3') and 518R (5' ATTACCGCGGCTGCTGG-3') were used to amplify the 16sRNA gene region [28]. The GC clamp was added to the primer 357F for DGGE analysis. The primers' efficiency was evaluated by running an *in silico* PCR on the SILVA database [29]. The PCR reaction mixture consisted of 25 µL of EmeraldAmp®GT PCR Master Mix which is composed of DNA polymerase, optimized reaction buffer, dNTPs, and density reagent (TAKARA BIO INC., Japan), 2 µL (0.4 µM of final concentration) of each primer, and 100 µg/ml of DNA template. The final mixture was adjusted at 50 µL PCR reaction by the addition of RNase-free water. PCR was carried out using BioRad MyCycler™ thermal cycler with an initial denaturation at 94°C for 5 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 45 seconds, and final elongation at 72°C for 7 min.

DGGE analysis was performed using the protocol formulated by Green et al. [30] with some modifications. The following reagents were used in concentrations of 40% Polyacrylamide solution (acrylamide and bisacrylamide in 37.5:1 ratio; Bio-Rad), Urea (Vivantis) Formamide (deionized; Vivantis), 50X TAE buffer (Bio-Rad), and TEMED (Bio-Rad). The polyacrylamide in the gel was 8%, following the protocol of Nieme *et al*. [31]. The denaturing gradients used were 40% to 80%, decided after the optimization was conducted. A total of 300 ng DNA sample was loaded into each well. The buffer was set to 60˚C and run at 35V for 10 min, followed by running at 85V for 16 h [28]. The dominant bands were excised and were sent (20ng/µL) to Macrogen, Korea, for sequencing.

Bioinformatics analysis and phylogenetic tree construction

The sequence alignment of the 16S rRNA gene sequences was carried out with the available sequences present in the public databases of the National Center for Biotechnology Information (NCBI) webserver BLAST [28]. In the construc-

tion of an unrooted phylogenetic tree, sequences of selected known species that are important in nutrient cycling were accessed in Genbank and their relationship with the species obtained from this study was examined. The unrooted phylogenetic tree is focused more on determining the relationships among the taxa rather than on the directionality of evolutionary change [32], hence this was used in this study. Sequences were aligned using the Muscle algorithm, and the phylogenetic tree was constructed using the maximum likelihood [33, 34, 35, 36] using Molecular Evolutionary Genetic Alignment X (MEGA-X) software [33]. The Tamura–Nei was used as a model with uniform rates [37, 38]. The partial deletion option is used as Gaps/Missing Data Treatment, wherein missing data were removed when necessary [33]. The excess bases on both ends were also deleted by editing the alignment manually [39]. Bootstrap analysis was done using the adjusted values of 1,000 replicates to test node stability [33]. A minimum of 70% bootstrap value will be considered as high support [40].

Operational and Taxonomic Units (OTU) diversity and community patterns analysis

The result of the DGGE image was captured and analyzed using the Image LabTM Software. It was used to detect the bands and calculate the relative contribution of each band to the total band intensity in the lane after subtracting a rolling disk background value. The bands occupying the same position or migration distance in the different lanes were recognized through the relative front (Rf) values using the same software. The abundance was calculated using the band intensity in the lane after subtracting a rolling disk background value [41]. The cluster analysis was carried out using the Biodiversity Pro software. The unique and shared OTUs were identified using the Rf or the migration distance of each band. The calculation of the Shannon index and evenness was carried out using the Past 4.0 software [42]. Shannon index was used to measure the alpha diversity because it depends more on species richness and is sensitive to even small diversity changes; thus, it is widely used to assess the actual state of the environment. Evenness analysis was used to measure the equality of abundance in a community [43]. To measure the carrying capacity, the formula of Weighted Richness Index (Rr) was utilized wherein Weighted Richness (Rr) = $N^2 \times Dg$: N, is the over all number of bands in the pattern; and Dg, is the denaturing gradient comprised from the first to last band of the pattern. Statistical analyses of the experimental data of soil analysis, diversity, and community pattern analyses were carried out using analysis of variance (ANOVA) followed by Tukey's test using IBM SPSS statistics 20. The Independent Sample T-test was used for the comparison of nutrient content using the same software.

Results and Discussion

Soil Analysis

For standard comparison, the generated values from the soil with press mud were subtracted from the soil without press mud. The results showed that the increase of available organic matter (OM) was higher in URC Passi Sugar Central (12.55±0.41 %) than in Capiz Sugar Central (7.38±0.36 %) as shown in Figure 2. The same trend was observed in iron (Fe), wherein URC Passi Sugar Central generated a higher increase (12.68±0.56 ppm) compared to Capiz Sugar Central (0.60±0.46 ppm). On the other hand, potassium (K), phosphorus (P), and sulfur (S) were higher in Capiz Sugar Central (96.00±6.00 ppm, 50.31±3.11 ppm, and 27.31±2.40 ppm, respectively) compared to URC Passi Sugar Central (56±7.94 ppm, 0.00±0.00 ppm, and 0.00±0.00 ppm, respectively). The overall results showed that all nutrients increased in both sites after press mud application, except in URC Passi Sugar Central where no increase was detected for phosphorus and sulfur (Figure 3). These suggest that press mud is responsible for increasing the macro and

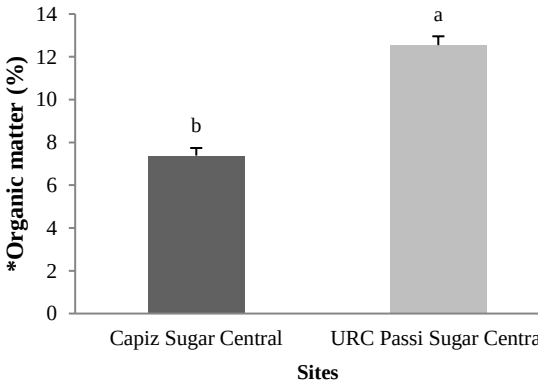

Figure 2. Comparison of increased OM between the two sites. *Values are mean ± SD of three determinations. Values with the same superscript are not significantly different ($p>0.05$).

micronutrients present in the soil with press mud. The results of the standardized comparison between the two sites suggest that Capiz Sugar Central released more K, S, and P to the soil compared to URC Passi Sugar Central, and press mud in URC Passi Sugar Central released more Fe and OM to the soil compared to Capiz Sugar Central after six months (Figures 2 and 3). The increased available nutrients in both sites can be associated with the microbial community. Press mud increased the diversity and OTU richness in Capiz Sugar Central when values with press mud were subtracted from values without press mud (Table 1). As a result of increased alpha diversity and OTU richness, the variety of nutrients at Capiz Sugar Central significantly increased (K, S, and P) after press mud application, while in URC Passi Sugar Central, only Fe and OM increased. The increase in the variety of available nutrients can be attributed to the high microbial diversity.

High diversity community would mean more guilds to mobilize various organic to inorganic nutrients. The same result was observed in the study of Curd et al. [44] that bacterial diversity is positively correlated with soil heterogeneity (e.g., pH, mineral composition, moisture) and nutrients (organic matter in the soil and litter).

Samples from URC Passi Sugar Central with and without press mud did not differ significantly in P and S (Figure 3). Microorganisms are the ones responsible for the release of nutrients from organic matter. Organic S is abundant in sugar cane residues and the release of S from sulfonates is catalyzed via a bacterial multi-component mono-oxygenase system. Sulfonates are the dominant organo-S source in soil. It is possible that the absence of newly transported microorganisms capable of fixing S in URC Passi Sugar Central caused the low S content in the soil samples. According to the study of Gahan and Schmalenberger [44], most aromatic sulfonate mobilizing bacteria belong to Betaproteobacteria and their abundance is positively correlated with pH [46]. Hence, the slightly acidic condition of the soil might have contributed to the low number of the absence of sulfonate mobilizing bacteria and thereby explains the low S content of the samples from this site (Figure 3). In the present study, the absence of beta proteobacteria was indeed observed in samples with press mud from URC Passi Sugar Central.

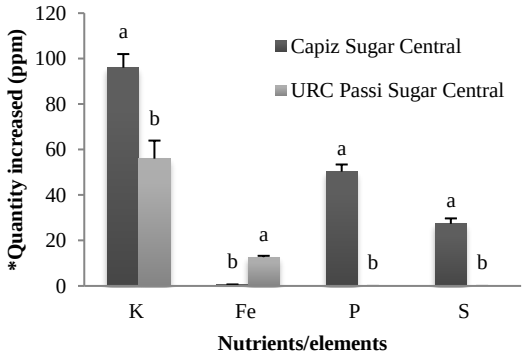

Figure 3. Comparison of increased nutrient contents between the two sites after press mud application. The following initials mean: (K) potassium; (Fe) Iron; (P) Phosphorus; and (S) Sulfur *Values are mean ± SD of three determinations. Values per nutrient with the same superscript are not significantly different ($p>0.05$).

Similar to the results of sulfur, the absences of newly transported microorganisms responsible for fixing P in URC Passi Sugar Central can be a possible cause of no significant results of P from URC Passi Sugar Central. The no significant difference of available P and S in soil with and without press mud in URC Passi Sugar Central also suggests that the absence of microorganisms in control for the assimilation of nutrients will not alleviate the soil fertility even with the presence of organic fertilizer.

Cations influence the acidity and alkalinity of the soil. In the present study, the difference in the pH of soil samples from the two sites could be due to the presence of base-forming cations. High levels of sand in the soil samples can also contribute to its acidity [47], and sandy soil characteristics were observed in samples without press mud from

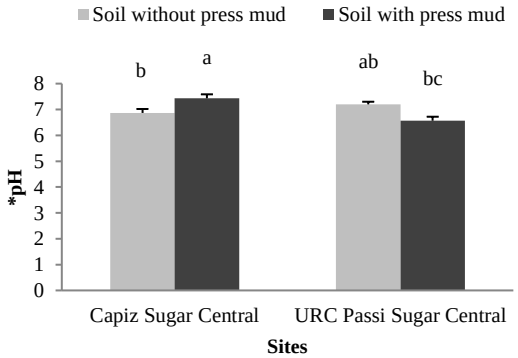

Figure 4. pH analysis of soil. *Values are mean ± SD of three determinations. Values with the same superscript are not significantly different ($p>0.05$).

Capiz Sugar Central and samples with press mud from URC Passi Sugar Central. Despite varied pH in all sites, the pH of the samples is still within the optimal range for most crops, between 5.5 and 7.5 (Figure 4) [48].

Operational Taxonomic Unit (OTU) diversity and community patterns analysis

Microorganisms that are accountable for aiding nutrient cycling can be classified into two autochthonous and allochthonous microorganisms. Some microorganisms are autochthonous or indigenous within a given habitat. They occupy the available environmental niches and can escape predation and compete successfully with the other microbial community members. Other so-called allochthonous microorganisms are grown elsewhere and transported into a given habitat to be transient community members [49]. In this study, the microorganisms in the press mud that are transferred into the soil are considered allochthonous microorganisms.

The nutrients present in the habitat could influence the microbial species richness, abundance, and diversity of a community [50]. The present study showed that soil with press mud from URC Passi Sugar Central had the highest value, followed by soil with press mud from Capiz Sugar Central, soil without press mud from URC Passi Sugar Central, and the lowest value observed in Capiz Sugar Central soil without press mud. These were true in OTU richness (48.00±0.50, 46.00±1.00, 45.00±0.00, and 40.00±1.50, respectively), Shannon (3.53±0.04, 3.52±0.08, 3.51±0.02, and 3.44±0.02, respectively), and carrying capacity (181±3.80, 169±7.36, 162±0.00, and 125±9.48, respectively) (Table 1). If compared within sites, statistical analysis showed a significant difference between soil with press mud and soil without press mud in terms of OTU richness and carrying capacity ($p<0.05$) in both sites. If soil samples were compared between sites, soil without press mud had a significant difference in OTU richness and carrying capacity ($p<0.05$), and no significant difference was observed in soil with press mud in all analyses ($p>0.05$) (Table 1). The high value of OTU richness in samples with press mud could be due to the allochthonous bacteria present in the press mud that was transferred to the soil, and growth was supported by high organic matter. In this habitat, several resources were available that promote high species richness, as shown in the soil analysis results. The OM, P, K, Fe, and S in soil samples with press mud are significantly higher compared with the soil without press mud (Figures 2 and 3). The results of this study show that a higher available nutrient in the soil means that it has a higher carrying capacity and the variety of nutrients available in the soil to support a diverse community. As a result of a higher K, S, and P at Capiz Sugar Central after press mud application (Figure 3), Capiz Sugar Central generated a higher increase of alpha diversity compared to URC Passi Sugar Central. This result is also shown in sequences wherein the dominant bacteria in soil with press mud at Capiz Sugar Central are Betaproteobacteria, Gammaproteobacteria, and Alphaproteobacteria compared to URC Passi Sugar Central that only Alphaproteobacteria are found to dominate the area. These results support the study of Lin et al. [51] that organic fertilizer shapes the composition of microbial communities.

Microbial diversity improves the productivity and stability of the ecosystem [52, 53, 54]. In this study, the soil with press mud had higher alpha diversity than the soil without press mud in both sites after heavy deposition of organic matter (Table 1).

The increase of alpha diversity between soil with and without press mud did not differ significantly ($p>0.05$) as shown in Table 1. The increase in alpha diversity may be due to some allochthonous microorganisms being transported into a new habitat that survived, grew, and executed an active metabolism and later becomes autochthonous microorganisms [48]. Based on the previous studies, fresh press mud naturally had microorganisms that can potentially be transported to the soil [20, 21, 22, 55]. The transportation of new OTUs may have contributed to the increased diversity of the sample. The allochthonous bacteria from organic fertilizer are introduced to the soil through water fluxes. The main reason for no significant difference in alpha diversity between soil with press mud and soil without press mud after six months is although some allochthonous may execute active metabolism, most of the allochthonous bacteria from press mud are quickly eliminated from the soil because they cannot occupy a niche in a given habitat or are described as a transient member of the community [49, 56]. When both allochthonous and autochthonous bacteria are present in the soil, the assimilation of nutrients present in press mud

Table 1. Diversity and community pattern analyses.

	Soil Samples	*OTU richness	*Shannon	*Evenness	*Carrying capacity
Capiz	Without Press Mud	40.00±1.50^{c}	3.44±0.02^{a}	0.79±0.02^{a}	125±9.48^{c}
	With Press Mud	46.00±1.00^{ab}	3.52±0.08^{a}	0.74±0.05^{a}	169±7.36^{ab}
URC Passi	Without Press Mud	45.00±0.00^{b}	3.51±0.02^{a}	0.74±0.02^{a}	162±0.00^{b}
	With Press Mud	48.00±0.50^{a}	3.53±0.04^{a}	0.72±0.02^{a}	181±3.80^{a}

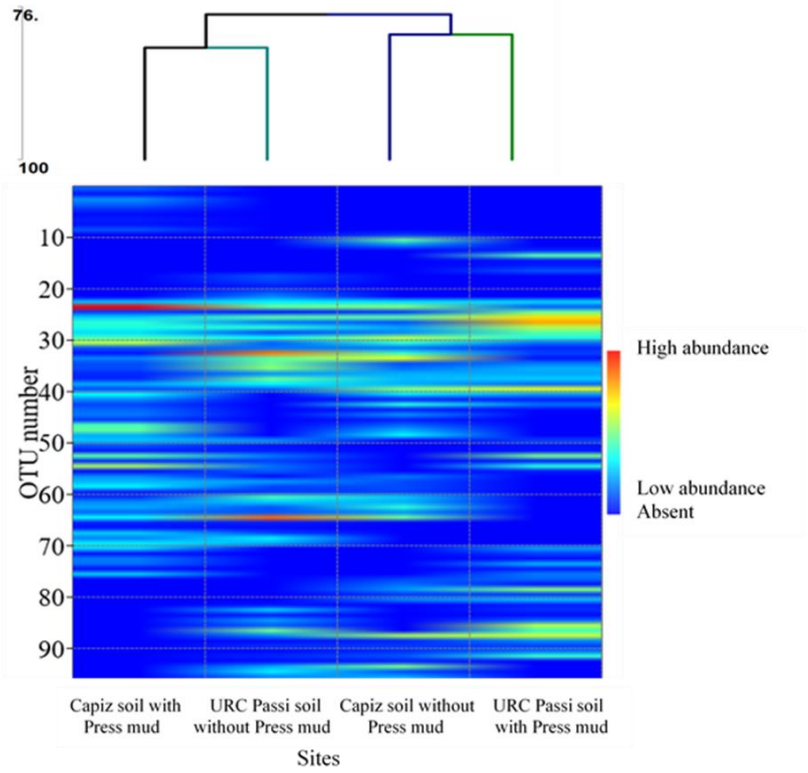

Figure 5. Clustered matrix plot across the sites. The individual relative abundance of operational taxonomic unit (OTU) represented by color. The horizontal direction signifies the relative abundance of each OTU across the site and soil sample. The vertical direction shows the relative abundance of different OTUs in the same site and soil sample.

remains efficient. These trends explain why soil fertility in soil with press mud is significantly higher compared with soil without press mud. Bacterial diversity among all soil samples remains almost the same after six months. The same observations were found in the study of Vinael and Vives [57] and Podmirseg et al. [58] in which indigenous microbiota could out compete the allochthonous microorganisms. All these possibly cause the difference in alpha diversity as supported by the immoderate distribution of abundance in the matrix plot (Figure 5) and reflected in the results of evenness analysis in this study, wherein the soil with press mud from URC Passi Sugar Central (0.72±0.02) generated the lowest value. The soil without press mud from Capiz Sugar Central (0.79±0.02) had the highest value, and the soil without press mud from URC Passi Sugar Central (0.74±0.02) had the same value as Capiz Sugar Central soil with press mud (0.74±0.05). Statistical analysis showed little or no evidence against the null hypothesis among the samples (p=0.07) (Table 1). These results suggest that the reapplication of organic fertilizer is needed. Thus, the increase of diversity in the long-time application of organic fertilizer is the gradual addition of allochthonous microorganisms transported and adapted to the soil environment that can later execute active metabolism and become autochthonous in the given habitat.

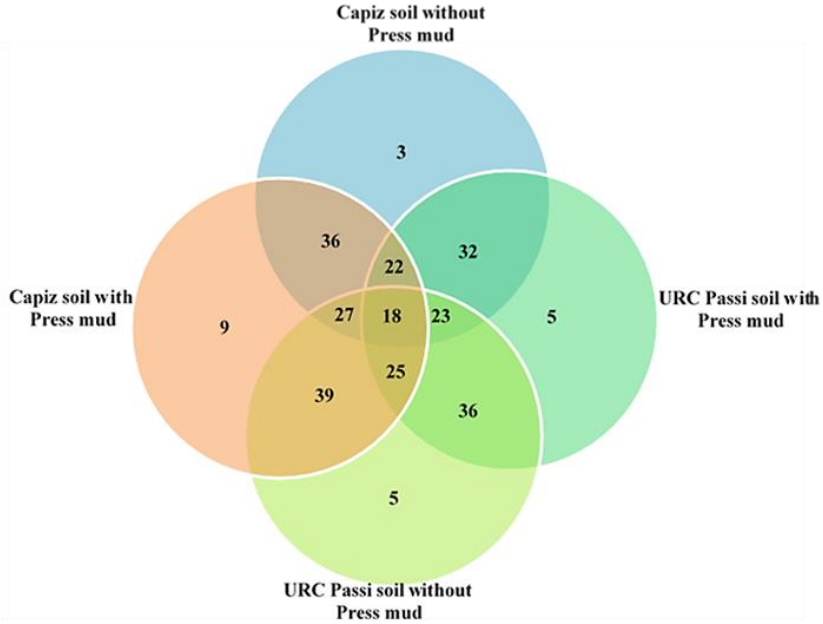

Figure 6. Unique and shared operational taxonomic units (OTUs) within and between the sites. The overlapping represents the set of OTU shared in the counterpart samples, and the single-layer zone represents the number of OTUs uniquely found in the specific sample.

There are two explanations for the elimination or migration of allochthonous bacteria. They migrated or were eliminated because of competition or a lack of tolerance to the new environment. The presence of zymogenous bacteria in soil that proliferates when high energy-containing nutrients are added to the soil [56] could trigger competition. As the highly adapted species, zymogenous bacteria exist in high abundance that encourages competition against other autochthonous or allochthonous bacteria. This result was observed in the matrix plot, where the same OTUs were highly abundant in soil with press mud compared to soil without press mud (Figure 5). This type of habitat generally causes a decrease in species richness [50].

The operational taxonomic unit (OTU) in this study is denoted as a unique band present in each sample. The results of the Venn diagram in Figure 6 indicated that in Capiz Sugar Central, the soil with press mud (9 OTUs) had higher unique OTUs than the soil with press mud (3 OTUs). The results from Capiz Sugar Central showed that soil with press mud and soil without press mud had the same number of unique OTUs (5 OTUs). All sites and soil samples shared a total of 18 OTUs. The unique OTUs in soil with and without press mud in both sites were specialist bacteria, and the OTUs observed that were shared in all sites were generalist bacteria (Figure 6). These OTUs were concluded to be generalist bacteria because they could exist in all sites despite different environmental conditions and nutrient availability. The exact figure showed that the autochthonous bacteria present in all sites and samples were dominated by generalist bacteria.

According to Elsas et al. [59], many bacteria can be categorized as soil generalists because they are often found in high numbers in virtually all soil on Earth. This characteristic of autochthonous bacteria as a generalist can be a significant factor that helps to outcompete allochthonous bacteria. Aside from this, it was proven and known in the literature that autochthonous bacteria used K-selection as their life strategy [56]. The main feature of K-selected species is their highly competitive ability [49].

Sequencing data

The present study showed that the two OTUs matched the genera of *Lysobacter* (96.88%) and *Massilia* (94.5%). There were five (5) *Lysobacter* strains (NR_164964.1, NR_164969.1, NR_043868.1, NR_115948.1, and NR_136845.1) that matched to OTU 2 and nine (9) *Massilia* strains (NR_157751.1, NR_157770.1, NR_157771.1, NR_157772.1, NR_136470.1, NR_137346.1, NR_152009.1, NR_148592.1,

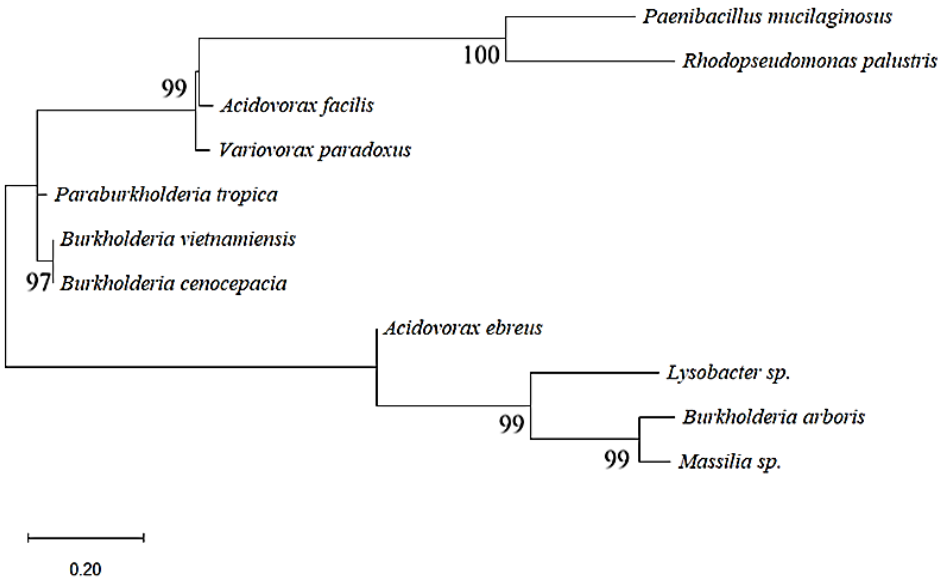

Figure 7. Phylogenetic tree showing the relationship among studied strains and other strains represented by different guilds according to literature (Unrooted). Selected strains are known to be important in nutrient cycling. The scale bar indicates 0.20 substitutions per nucleotide position.

NR_042502.1) that matched to OTU3. The sequencing results are consistent with the literature as these genera were reported to be abundant in soil [60, 61, 62, 63, 64] Other OTUs that did not attain the threshold but with consistent taxonomy, hits were noted. Six (6) OTUs consistently hit alphaproteobacteria. The three (3) dominant OTUs from Capiz Sugar Central soil with press mud belong to beta proteobacteria, gammaproteobacteria, and alphaproteobacteria. On the other hand, the 5 OTUs from URC Passi Sugar Central soil with press mud belong to alphaproteobacteria.

The sequencing data showed that the dominated OTUs came from the phylum Proteobacteria. This result is similar to the study of Spain et al. [65], wherein the results showed that proteobacteria were the dominant phylum in the soil community. Specifically, all OTUs under the threshold but with consistent hits are identified as alphaproteobacteria, *Massilia* is from Betaproteobacteria, and *Lysobacter* is from Gammaproteobacteria [66]. Microorganisms facilitate nutrient cycling in different manners depending on their metabolic preference or activity. Microbial populations that metabolically use and process the same resources are called guilds [50]. In the present study, the dominant bacteria *Massilia* sp. was classified in the guild of phosphate-mobilizing bacteria [67]. These bacteria produce phosphatases that are responsible for the hydrolytic cleavage of phosphate esters. On the other hand, the genus *Lysobacter* can produce bioactive compounds such as chitinase, glucanase, and protease [68]. These bioactive compounds can be essential compounds for the assimilation of nutrients present in press mud. The domination of alphaproteobacteria in URC Passi Sugar Central soil with press mud supported the lesser availability of phosphorus and sulfur than in Capiz Sugar Central dominated by alphaproteobacteria, gammaproteobacteria, and beta proteobacteria.

Phylogenetic tree of selected dominant species

The result of phylogenetic tree analysis of selected dominant species showed that *Massilia* sp. is closely related to *Burkholderia arboris* and *Lysobacter* sp. is closely related to both *Massilia* sp. and *Burkholderia arboris* (Figure 7).

In this study, the results of phylogenetic analysis are supported by their metabolic activity and taxonomic classification. The *Massilia* and *Burkholderia* are from Betaproteobacteria and *Lysobacter* from Gammaproteobacteria [67]. In terms of metabolic activity, *Burkholderia arboris* and *Massilia* sp. are both phosphate-mobilizing bacteria [67, 69]. The dominance of these species can be correlated to their capacity to tolerate pollution stress. In the study of Wang et al. [6], *Massilia*, *Lysobacter*, and *Burkholderia* are described as Gram-negative bacteria that can react

rapidly to pollution stress.

Relationship of soil analysis, diversity, and community patterns during organic fertilizer application

The proposed relationship of soil analysis, diversity, and community patterns during press mud application is summarized in Figure 8. First, when organic fertilizer is applied to the soil, the allochthonous microorganisms are combined with autochthonous microorganisms, which results in the temporary increase of diversity. The temporary increase is because most allochthonous microorganisms get eliminated or migrated from the area [58]. After all, allochthonous microorganisms cannot occupy a specific niche [49, 56, 57]. There is an exemption to this phenomenon because some of the allochthonous, when they survive, have an active metabolism, and coexist, they become autochthonous later on [49]. It was reflected in the present study, wherein the trend of results found in both sites after the press mud application was shown to increase alpha diversity (Table 1). Second, the present study results and supported by literature stating that the increase of diversity promotes a high and variety of available nutrients [70]. In the present study, if compared between sites, Capiz Sugar Central has higher diversity and OTU richness after press mud application (Table 1). As a result of a diverse community, a variety of nutrients at Capiz Sugar Central significantly increased (OM, K, S, and P) after press mud application compared to URC Passi Sugar Central that only Fe and Organic matter increase (Figures 2 and 3). This result happened because a high diversity community means more guilds to mobilize various organic to inorganic nutrients. The same result was observed in the study of Curd et al. [44] that bacterial diversity positively correlated with soil heterogeneity (2). Lastly, a higher available nutrient means it has a higher carrying capacity, and the variety of nutrients available in the soil support the diverse needs of a more diverse community. As a result of a higher increase of OM, K, S, and P at Capiz Sugar Central after press mud application compared to URC Passi Sugar Central, Capiz Sugar Central generated a higher increase of alpha diversity (if you subtract the result in soil with and without press mud) compared to URC Passi Sugar Central. This result is also shown in sequences wherein the dominant bacteria in soil with press mud at Capiz Sugar Central are Betaproteobacteria, Gammaproteobacteria, and Alphaproteobacteria compared to URC Passi Sugar Central that only Alphaproteobacteria are found to dominate the area. This result is the same as the study of Lin et al. [51] that organic fertilizer shapes the composition of microbial communities

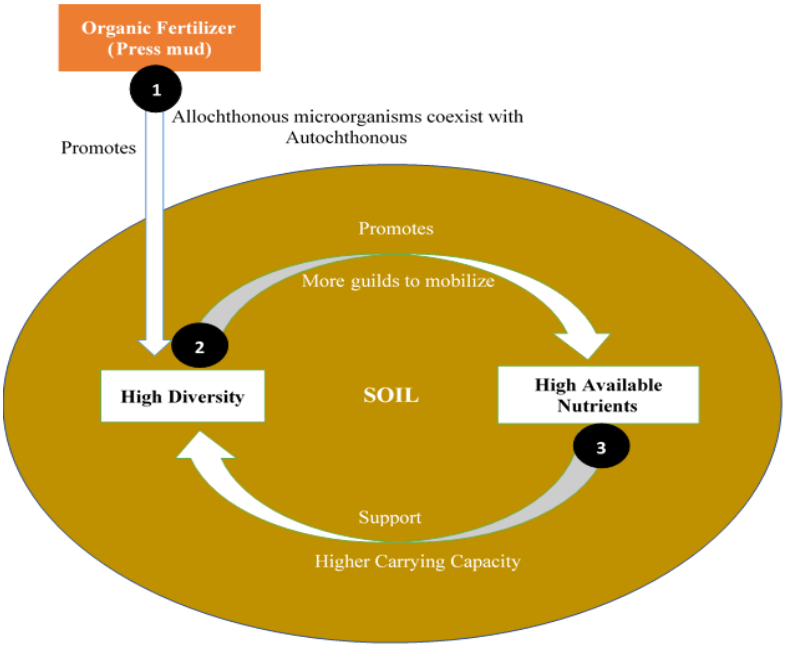

Figure 8. Relationship of soil analysis, diversity, and community pattern during press mud application as an organic fertilizer. The flow of interactions is indicated by arrows and numbers. The brown circle represents the soil, and the orange rectangle is the organic fertilizer.

(3). In summary, the present study showed that the press mud from Capiz Sugar Central generated a higher diversity which helps to increase the number of guilds, OTU richness which helps to mineralize a variety of organic nutrients, and nutrients which help to support the diverse community.

Conclusion

In general, press mud aids to increase the available organic matter (OM), phosphorus (P), potassium (K), iron (Fe), and sulfur (S) in the soil, which makes it a good organic fertilizer. In the present study, the performance and the nutrients available in press mud varied between the two sites and are affected by different factors such as the soil type, location, and microbial community. The increase in bacterial diversity is attributed to allochthonous microorganisms that transported and adapted to the new environment and the allochthonous and autochthonous nutrients support this new community. The result of bacterial diversity inferred that other microorganisms present in the soil and press mud and as a whole had a significant role in nutrient cycling. Furthermore, press mud from Capiz Sugar Central can be considered a more effective organic fertilizer than URC Passi Sugar Central. Further investigation in the actual farming set-up is needed to determine other effects of press mud on microbial diversity and activity and the full potential of press mud in improving soil quality. The proposed mechanism of increasing microbial diversity after organic fertilizer application needs further studies to elucidate more events before, during, and after the application of organic fertilizer to the soil. Furthermore, the use of sophisticated methods and more advanced techniques such as metagenomics is highly preferred to establish the accuracy of observation.

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