Microbial activity of potential and actual acid sulphate soil from Kalimantan Island

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Abstract. Drainage on acid sulphate land causes a change in the type of acid sulfate (AS) soil, from the potential to actual due to pyrite oxidation. The alteration might cause changes in microbial composition and activity. The objective of the study was to compare the microbial activity of two types of acid sulphate (AS) soil, potential AS soil from South Kalimantan and actual AS soil from Central Kalimantan. The microbial activity of both soils was determined by measuring respiration, dehydrogenase, and nitrogenase activity. The presence of culturable soil rhizospheric microorganisms was determined by Total Plate Count methods and their nitrogen fixation function was determined by the ability of culturable rhizospheric soil bacteria to grow in semisolid nitrogen-free medium, as well as, nifH detection. The result of the research revealed that the respiration, dehydrogenase, and nitrogenase activity soil from potential (1.34 mg CO$_2$.kg$^{-1}$.hour$^{-1}$ and 1.75 nmol C$_2$H$_4$.g$^{-1}$.hour$^{-1}$) and actual AS soil (1.23 mg CO$_2$.kg$^{-1}$.hour$^{-1}$ and 2.00 nmol C$_2$H$_4$.g$^{-1}$.day$^{-1}$) were relatively not different. However, the dehydrogenase activity of actual AS soil (6.29 µg TPF.g$^{-1}$.hour$^{-1}$) was higher than that of potential AS soils (3.32 µg TPF.g$^{-1}$.hour$^{-1}$). Similarly, the microbial abundance of both soils was not different and the abundance of bacteria was higher than that of fungi. The soils contained nifH genes. In both soils have been detected the presence of microbial abundance and functional microorganisms as a nitrogen fixer, a culturable phosphate solubilizer, or phytohormone producer. However, limiting factors of both soils, i.e., very acidic soil, low nutrient availability, and Al toxicity brought about the low microbial activity.

Keywords: Acid sulphate soil, respiration, dehydrogenase, nitrogenase, nifH gene

1. Introduction

Acid sulphate soils (ASS) are soils or sediments with a very low pH (referred to as real ASS) due to the oxidation of iron sulphides, and/or soils containing iron sulphides or other sulfidic components that have not been exposed to air and oxidized (referred to as potential ASS). Actual and potential ASS are frequently discovered in the same soil profile, which can be flooded by tidal water during both wet and dry seasons. The land surface is still lower than the average tidal elevation [1]. Sulphate acid soil covers around 6.7 million hectares in Indonesia, while it covers roughly 3.5 million hectares in Kalimantan [2]. Sulphate acid soil is a marginal soil that is a target of the Indonesian government, for increasing food production.

Acid sulphate and peat soil are included in swampland which is soil always saturated with water throughout the year or waterlogging [3]. Based on tidal overflow topography and drainage, swampland
is grouped as A, B, C, and D. The swampland of class A hydrological topography is a swampland that can be flooded by tidal water, both in the rainy season and in the dry season. The land surface is generally still lower when compared to the average tidal elevation. The class B hydrological topography is a swampland area that only can be overflowed with tidal water in the rainy season. The land surface is generally still above the high tidal elevation average in the dry season, but still below the high-water level average in the rainy season. The swampland with class C is a swampland that cannot be overflowed with tidal water all the time or occasionally. The land surface is generally relatively high than of class A or B so that tidal water only affects the water table with a depth of less than 50 cm from the land surface. Class D swampland, a swampland that is high enough so that it is completely inaccessible to the rising tides (more like dry land). The groundwater table is generally deeper than 50 cm from the land surface [4].

Pyrite (Fe$_2$S) layer presence is an inherent characteristic of acid sulphate soil which limits its usefulness for agriculture [5]. In potential acid sulphate soil, the pyrite is not oxidizable due to waterlogging conditions whilst in actual acid soil, it is oxidizable due to natural or anthropogenic drainage which forms sulfuric acid. The soil becomes acidic (pH < 3.5), exchangeable Al$^{3+}$ and Fe$^{3+}$ increase, as well as, the acidic pH link to the low of nutrients available [6]. This study used potential (L1) and actual (L2) acid sulphate soil taken from Tanjung Harapan Village, Alalak, Barito Kuala and Tamban Baru Tengah Village, Kecamatan Tamban Catur, Central Kalimantan, respectively. The L1 type acid sulphate soil has B class while L2 has C class hydrological topography.

Alteration in acid sulphate soil type would change the soil microbial activity and function. The database of microbial activity and function of acid sulphate soil is still few whereas it can describe the soil fertility and health ([7][8][9]. Microbial activity in the soil can be determined by respiration, dehydrogenase, and nitrogenase activity measurement. Soil respiration measurements are widely used to quantify carbon fluxes and ascertain soil biological properties related to soil microbial ecology and soil health [7]. Soil dehydrogenase activity (DHA) is considered a measure of overall soil microbial activity due to dehydrogenases occurring intracellularly in all living microbial cells and do not accumulate extracellularly in the soil. As part of the respiration pathways of soil microorganisms, dehydrogenases oxidize SOM by transferring protons and electrons from substrates to acceptors; hence, DHA may indicate soil potential to support biochemical processes necessary for soil fertility preservation [9]. Biological nitrogen fixation (BNF), a key reaction of the nitrogen cycle, is catalyzed by the enzyme nitrogenase. BNF, which is only catalysed by bacteria and archaea, is a key nitrogen source for agricultural ecosystems, contributing 40–100 Tg N per year to the terrestrial ecosystem [10]. Biological nitrogen (N$_2$) fixation as a source of new N input into the soil by free-living diazotrophs is important for achieving sustainable rice agriculture [11, 2020].

Since lack study which examine responses of microbial activity such as respiration, dehydrogenase, and nitrogenase to alteration in the potential to actual acid sulphate soil. The objective of the study was to investigate microbial activity with a focus on soil respiration, dehydrogenase, and nitrogenase activity of two types of acid sulphate soil, potential and actual. To find out the relationship between the physical-chemical properties of soil and microbial activity, several physical and chemical parameters of soil are analyzed. Soil microbial activity describes soil microbial community structure and function. Phosphate solubilization and IAA production test of the isolated microbe was conducted for assessing soil potential and capacity.

2. Materials and Methods

The research steps conducted including soil sampling, soil microbial activity analysis, and determination of the abundance and functional characteristic of culturable microbial rhizosphere soil.

2.1. Soil sampling and physico-chemical analysis

Potential and actual acid sulphate soil, namely Desa Tanjung Harapan, Alalak, Barito Kuala and DesaTamban Baru Tengah, KecamatanTamban Catur, respectively. The soil sample was taken from
nine locations for each acid sulphate soil type. Each location of the soil sample was from composite soil of ten points.

The procedures described in Buku Petunjuk Analisa Tanah halaman 211 [12] were used for physicochemical soil analysis. With an Agilent Technologies 3200 M Multifunction Analyzer pH meter, the pH of the soil was evaluated using a 1:5 soil to demineralization water ratio. The Walkley & Black and Kjeldahl methods were used to determine soil organic carbon and total nitrogen (Gerhardt distiller, VAP45S, Cologne, Germany). The soil's total P and K levels were determined using 25 percent HCl extraction procedures and measurement of the extracted element by A Cary Series UV-Visible Spectrophotometer (Agilent Technologies, USA). The cation exchange capacity (CEC) of 1 M ammonium acetate was measured (pH 7.0). Cation exchange capacity (CEC) was determined through 1 M ammonium acetate (pH 7.0) extraction and measurement of extracted soil leachates for exchangeable Ca, Mg, K, and sodium Na using an atomic absorption spectrometer (AAS). Exchangeable Al$^{3+}$ and H$^+$ were determined using a 1 N KCN extraction technique and AAS measurement. HNO$_3$ extractant technique was used to determine total Fe and S, which was then quantified by AAS (Agilent Technologies, 200 Series AA, 240F5 AA). The pipette method was used to determine the physical parameters of the soil.

2.2. Microbial activity of acid sulphate soil analysis

2.2.1. Soil respiration

Soil respiration was estimated by the alkali absorption method as described by Alef [13]. The estimation of the respiration based on CO$_2$ measurement resulted in the incubation period of the soil sample with 0.2 M NaOH solution. The absorbed CO$_2$ in NaOH was titrated with 0.2 M HCl. Respiration rate was calculated with the equation as follows: CO$_2$(µg g$^{-1}$ h$^{-1}$) = [(V0 – V1) x 4.4]/Ms.dwt where V0 and V1 are the volume of HCl consumed in control and the tested sample (ml), respectively; Ms is the mass of soil sample (g); dwt is the dry mass fraction of soil sample; and 4.4 is the conversion factor (1 ml of 0.2 molar HCl corresponds to 4.4 of CO$_2$).

2.2.2. Soil dehydrogenase activity

Dehydrogenase activity was measured by the method of Moeskop et al. [14] with slight modification. Five grams of soil sample was mixed with a milliliter of 3% triphenyl tetrazolium chloride (TTC) as substrate and two milliliters of tris-HCl buffer (pH 7.3) in 20 ml tube with screw cap. For soil sample mixed with four milliliters of tris-HCl as control. All mixtures were incubated in incubator shaking at 37°C for 24 hours in dark condition. Triphenyl formazan (TPF) extraction used methanol and TPF was detected using spectrophotometer at 485 nm wavelength. The dehydrogenase activity was expressed as µg TPF g$^{-1}$ soil h$^{-1}$.

2.2.3. Acetylene reduction measurement

The activity of soil nitrogenase in soil was evaluated using an acetylene reduction assay, as described by Bellenger et al. [15]. Glass vials were sealed with silicon and 10 mL air was taken out of the headspace of 100-mL vials using a gas-tight hypodermic syringe. Following that, the soils were treated with 10 mL C$_2$H$_2$ to ensure a 10% C$_2$H$_2$ enrichment. Finally, C$_2$H$_4$ concentrations were evaluated by extracting 1 mL of gas from C$_2$H$_2$-enriched soil samples using gas chromatography (GC-2014C; Shimadzu, Kyoto, Japan) at 1 hours. The amount of ethylene produced was estimated by measuring the concentration of C$_2$H$_4$ in the sample, which was obtained by comparing the sample's peak height to that of a C$_2$H$_4$ reference standard. The nitrogenase activity was expressed as nmol ethylene g$^{-1}$ soil h$^{-1}$.

2.2.4. nifH gene detection of rhizospheric soil

Rhizosphere microbiome DNA was extracted and purified from rhizospheric soil sample using ZymoBIOMICSTM DNA Microprep Kit. A NanoDrop spectrophotometer was used to determine the concentration of isolated DNA at wavelengths of 260 and 280 nm, and the extracts were kept at 20 °C for subsequent analysis. Detection of nifH gene by amplification using primer nifHr
(5‘CCATCGTGATCGGGTGGGATG‘3), nifHf (5‘GGCAAGGGGCGGTATCGGCAAGTC‘3). A total of 2 µL of DNA was added to the PCR cocktail consisting of 12.5 µL of GoTaq master mix, 1 µL of each primer nifHr, nifHf, and 8.5 µL of nuclease-free water. The amplification process was carried out at initial denaturation temperature of 94 for 3 minutes with 1 cycle, denaturation of 94℃ for 30 seconds, annealing of 55℃ for 30 seconds, and extension of 72℃ for 30 seconds with 29 cycles, final extension 72℃ for 5 minutes with 1 cycle [16].

2.3. Determination of the abundance and functional characteristic of culturable microbes

2.3.1. Rhizospheric microbial abundance

The total plate count method was used to determine the number of rhizobacteria and rhizospheric fungus. Rhizobacteria were isolated from rhizosphere samples by dilution with sterile phosphate-buffered saline to make a 1:10 dilution in a 500-mL Erlenmeyer flask sealed with aluminum foil, and then successive dilutions up to 10^-6. Trypticase soy agar/broth (trypticase or tryptone 17.0 g, Soytone 3.0 g, NaCl 5.0 g, K2HPO4 2.5 g, glucose 2.5 g, Agar 18 g, dH2O 1000 ml) was used for rhizobacteria, and Rose Bengal+Chloramphenicol Agar glucose 10.0 g, peptone 5.0 g, KH2PO4 1.0 g, MgSO4.7H2O 0.5 g, rose Bengal 0.025 g, chloramphenicol 0.1 g, agar 15.0 g, dH2O 1000.0 ml).

2.3.2. Phosphate Solubilization

Bacterial isolates were tested by plate assay using Pikovskaya medium (Pikovskaya, 1948) containing per liter: 10 g glucose, 0.5 g (NH4)2SO4, 0.1 g MgSO4.7H2O, 0.5 g yeast extract, 0.2 g KCl, 0.2 g NaCl, 0.002 g FeSO4.7H2O, 0.002 g MnSO4.H2O, 5 g Ca3(PO4)2 and 1000 mL distilled water (pH 7). Phosphate-solubilizing bacterial colonies are recognized by clear halos after 5 days of incubation at 28°C. The activity was indexed as the diameter (cm) of the colony and halo divided by the diameter of the colony.

Ca3(PO4)2, AlPO4, FePO4 or phosphate rock were used as phosphate sources.

2.3.3. IAA production

Gordon and Weber’s reagent was used to colorimetrically detect IAA in the supernatants of bacterial cultures (Gordon and Weber, 1951). The isolates were cultured overnight in modified nutritional broth M 26 before being inoculated in a 10 mL minimal salt medium containing 5 mM tryptophan (Frankenberger and Poth, 1988). Following a 44-hour incubation period, IAA was measured using the following steps: bacterial cells were centrifuged out of the culture medium, and 2 mL of Gordon & Weber’s reagent [17] (1 mL of 0.5 M FeCl3 dissolved in 50 mL of 35 % HClO4 in a dark bottle) was mixed with 1 mL of supernatant. After incubation at 28 °C for 25 minutes, the produced color was assessed using a spectrophotometer at 535 nm. The amount of IAA in each culture medium was calculated by comparing with a standard curve made up of recognized IAA concentrations.

3. Results and Discussion

3.1. Soil physico-chemical properties.

The analysis result of soil physico-chemical parameters of potential and actual acid sulphate soil is presented in Table 1.

According to the Physico-chemical examination of soil parameters, the potential acid sulfate soil from South Kalimantan and the actual acid sulfate soil from central Kalimantan have silty clay and clay-textured soil, respectively. Both soils had a very acidic soil reaction (pH 3.97 and 3.80), as well as a high exchangeable Al³⁺ concentration (8.23 and 7.75 cmol(+)/kg-1) that indicated a strong phosphorus fixation potential of both soils. Because the total P2O5 is high (43 mg.100⁻¹ in L1 and very high in L2 (62 mg.100⁻¹), the P may be unavailable to the plant because it is in the Al-fixed form. Furthermore, the level of Al³⁺ in the soil could be hazardous to plants and soil microbes.
Table 1. Physico-chemical properties of acid sulfate soil type.

| Soil Parameters                        | Potential | Actual  | Category*  |
|----------------------------------------|-----------|---------|------------|
| **Textural Grade (pipet), %**          |           |         |            |
| **Sand**                               | 11        | 34      |            |
| **Silt**                               | 43        | 29      |            |
| **Clay**                               | 46        | 37      |            |
| **pH (1:5, H\(_2\)O**                  | 3.97      | 3.80    | Very Acid  |
| **Organic matter**                     |           |         |            |
| **C, %**                               | 6.80      | 13.45   | Very High  |
| **N, %**                               | 0.37      | 0.59    | Medium     |
| **C/N**                                | 18        | 23      | Medium     |
| **Extractant (HCl 25%)**               |           |         |            |
| **P\(_2\)O\(_5\) (mg.100 g\(^{-1}\))**| 43        | 62      | High; Very High |
| **K\(_2\)O (mg.100 g\(^{-1}\))**      | 15        | 14      | Low        |
| **Cation Exchangeable value (NH\(_4\)-Acetate 1N, pH 7) (cmol\(_{(+)}\) kg\(^{-1}\))** | | | |
| **Ca**                                 | 0.85      | 0.83    | Very Low   |
| **Mg**                                 | 1.40      | 2.13    | Medium; High |
| **K**                                  | 0.16      | 0.18    | Low        |
| **Na**                                 | 0.70      | 0.69    | Medium     |
| **Total**                              | 3.11      | 3.83    |            |
| **CEC**                                | 27.66     | 35.03   | High       |
| **Base saturation (%)**                | 11        | 11      | Low        |
| **Exchangeable (KCl 1 M) (cmol\(_{(+)}\)kg\(^{-1}\))** | | | |
| **Al\(^{3+}\)**                        | 8.23      | 7.75    | High       |
| **H\(^{+}\)**                         | 0.31      | 0.45    | Low        |
| **Total HNO\(_3\)**                   | 2.18      | 2.31    | Low        |
| **Fe (ppm)**                           | 0.05      | 0.43    | Low        |
| **S (%)**                              |           |         |            |

* Guide book of soil analysis

The medium nitrogen content of L1 (0.37 percent) and L2 (0.59 percent) as a source of nutrition for plants and energy for microbial growth and activity, as well as the high organic C content of the potential (6.8%) and real ASS (13.45 percent). Furthermore, organic carbon served as a CEC source, with L2 having a higher organic carbon than L1. As a result, L2 soil had a greater CEC (35.05 cmol\(_{(+)}\) kg\(^{-1}\)) than L1 soil while having less clay content. The negative charge of soil and organic colloid acts as a storage facility for key cation elements required for plant growth. Despite having a high CEC, the potential and actual ASS had low base saturation (both 11%). It's probably that Al\(^{3+}\) occupied the majority of the soil particle and organic carbon exchangeable sites. Qu and co-workers [18]0 found that plant density, total biomass (TB), soil water content (SW), and C:N ratio (C/N) were the key driving forces contributing to the carbon source utilization structure of the soil microbial communities, among other edaphic features and plant attributes. In grasslands, parameters such as belowground SW, C/N, and aboveground plant density and TB influenced soil microbial metabolic activity the most.

3.2. Microbial activity.
Soil microorganisms play important roles in nutrient cycling, ecosystem functioning, and ecosystem health. Because of their significant involvement in soil nutrient cycling, particularly in regulating soil organic matter (SOM) turnover, soil microbe is commonly used to mediate soil fertility. Soil microbial activity may be responsible for the build-up and breakdown of soil organic carbon (SOC) during SOM turnover [19].
Table 2. Soil microbial activity of acid sulfate soil type.

| Acid Sulphate Soil Type | Respiration (mg CO₂ kg⁻¹ h⁻¹) | Dehydrogenase (µg TPF g⁻¹ h⁻¹) | Nitrogenase (nmol C₂H₄ g⁻¹ h⁻¹) |
|-------------------------|-------------------------------|-------------------------------|-------------------------------|
| Potential (L1)          | 1.34                          | 3.32                          | 1.75                          |
| Actual (L2)             | 1.23                          | 6.29                          | 2.00                          |

Soil respiration of soil sample from acid sulfate soil was relatively the same between potential and actual about 1.34 mg CO₂ g⁻¹ dry soil h⁻¹ for potential acid sulfate soil and 1.23 mg CO₂ kg⁻¹ dry soil h⁻¹ for actual acid sulfate soil. There was no increase in soil respiration due to the shift of potential to actual type soil. Compared to other studies (Table 2), these soil respiration values were lower than values resulting in seasonally dry tropical in China about 2.69 - 3.09 mg CO₂ kg⁻¹ dry soil h⁻¹ (Yu et al., 2018), as well as, in sub-humid moist and semi-arid dry agroecology in India, namely 2.45 and 2.00 mg CO₂ kg⁻¹ dry soil h⁻¹ [20]. Given the high organic carbon and medium organic nitrogen contents of both acid sulphate soil types, however low soil respiration is the properties of acid sulphate soil which caused by soil limiting-factors, such as high soil acidity and its consequence. Decomposition is impeded in flooded and saturated soils so that primary production in wetlands will often exceed decomposition, leading to a net accumulation of soil organic matter. Furthermore, as reported by De Marco and co-workers [21], species vegetation where soil originated-litter determines microbial activity in soil. Black locust and blackberry litter have a marked inhibitory effect on decomposer microorganisms, which, in turn, reduce organic matter mineralization with possible ecosystem consequences by increasing C sequestration in mineral soil.

Result analysis of dehydrogenase activity indicated that actual acid soil had higher activity than that of potential acid sulphate soil, i.e 6.29 and 3.32 µg TPF, g⁻¹ h⁻¹ respectively. Conventional (1.04 - 2.08 µg TPF, g⁻¹ h⁻¹), organic agriculture (5.83 - 12.5 µg TPF, g⁻¹ h⁻¹), organic-2 year (9.70 - 10.42 µg TPF, g⁻¹ h⁻¹) upland agriculture ecosystem, and secondary forest (18.75) in Indonesia [14]. High management practices were found to significantly increase DHA (2.15 µg TPF g⁻¹) compared to low management (1.78 µg TPF g⁻¹), whilst no significant differences were found in DHA between irrigated and rainfed systems [20]. The higher DH activity of actual acid sulphate soil than those of potential acid sulphate soil than of potential ASS which may due to the higher organic C content and C/N ratio.

Similar to soil respiration, nitrogenase activity of both soils seemed relative not different, namely 1.75 for potential acid sulphate soil and 2.00 nmol g⁻¹ h⁻¹. However, these values were lower than data obtained by [11] in China with values of about 35 nmol C₂H₄ g⁻¹ h⁻¹ (tropical), 95 nmol C₂H₄ g⁻¹ h⁻¹ (subtropical) 245 nmol C₂H₄ g⁻¹ h⁻¹ (warm temperate) 117 (mid-temperate). In the upland farmland ecosystem at paddy soil ecosystem, the nitrogenase activity was found about 0.04 -0.72 nmol C₂H₄ g⁻¹ h⁻¹ [25]. In mangrove forests, Shiau et al. [24] was found that at downstream mangrove forests, the value was higher (13.2 – 15.6 nmol C₂H₄ g⁻¹ h⁻¹) than the value at upstream mangrove forest (0.2 – 1.4 nmol C₂H₄ g⁻¹ h⁻¹) which receives more N (i.e., 3.13 mg N-NH₃ L⁻¹) from the upland watershed than does the downstream forest (Bali) (i.e., 1.06 mg N-NH₃ L⁻¹). Because breaking the triple link between the two N atoms demands a lot of energy, microbial N₂ fixation occurs only in N-limited settings [26] thereof the nitrogenase activity of the two soils to be low considering that the total N of both soils was moderate. Besides that, it is also due to the high acidity factor of both soils (Table 1). The availability of Mo micronutrient as a component of nitrogenase is low in acidic soil so that may limit the ability of soil to acquired N from N₂ fixation [27]. Also, toxicity of Al in potential and actual acid sulphate soil in this study which is due to very low soil pH may constraint the ability of both soils to receive N from N₂ fixation. Moreover, the amount and type of organic C factor determine the amount of fixed N₂ by N₂ fixer. The addition of glucose (13 nmol g⁻¹ h⁻¹) and sucrose (15 nmol g⁻¹ h⁻¹) to the soil enhanced nitrogenase activity by more than 20 times, and the addition of mannitol (10 nmol g⁻¹ h⁻¹) raised it by 15 times. While the addition of lactate and acetate to the soil from tidal mangrove ecosystem had no influence on nitrogenase activity. The nitrogenase activity measurement method factor used did not
cover all bacterial or archaeal groups such as sulphate-reducing bacteria, photosynthetic, or anaerobic bacteria [24] which might exist in acid sulphate soil.

Table 3. Soil microbial activity of various ecosystems from other studies.

| Soil microbial activity | Soil Ecosystem | Value* | Author |
|------------------------|----------------|--------|--------|
| Respiration            | Agroecology    | mg CO₂ kg⁻¹ h⁻¹ | [20] |
|                        | Sub-humid moist| 2.45   |        |
|                        | Semi-arid dry  | 2.00   |        |
|                        | Arid           | 1.62   |        |
|                        | a seasonally dry tropical forest | 2.69 - 3.09 | [22] |
|                        | Semi-arid forest | 0.45 – 2.62 | [23] |
| Dehydrogenase          | Agricultural (Up land) | µg TPF. g⁻¹. h⁻¹ | [14] |
|                        | Conventional   | 1.04 - 2.08 |        |
|                        | Organic Agricultural | 5.83 -12.5 |        |
|                        | Organic-2 years | 9.70 - 10.42 |        |
|                        | Secondary forest | 18.75  |        |
|                        | Semi-arid forest | 0.11 - 16.47 | [23] 2020 |
| Nitrogenase            | Mangrove forest | nmol C₅H₄.g⁻¹.h⁻¹ | [24] |
|                        | Downstream mangrove forests | 13.2–15.6 |        |
|                        | Upstream mangrove forests | 0.2–1.4 |        |
|                        | Farmland (upland) | 0.04 - 9.72 | [25] |
|                        | Paddy Soil     |        |        |
|                        | Tropical        | 35     |        |
|                        | Subtropical     | 95     |        |
|                        | Warm-temperate  | 245    |        |
|                        | Mid-temperate   | 117    |        |

Figure 1. Amplicons of PCR result, nifH gene ~ 360 bp from rhizospheric DNA of indigenous plant growing in potential (number in red) and actual (number in balck) acid sulfate soil.
The nitrogenase enzyme, whose many subunits are encoded by the genes \( nifH \), \( nifD \), and \( nifK \), is responsible for nitrogen fixation. The most sequenced of the three, \( nifH \) (encoding the nitrogenase reductase subunit), has become the go-to marker gene for researchers looking into the phylogeny, variety, and abundance of nitrogen-fixing microbes. As a result, various PCR primers targeting the \( nifH \) gene have been created to amplify this gene sequence from environmental materials [28]. In this study, we have succeeded to detect the \( nifH \) gene presence from the rhizosphere of various indigenous plants such as karamunting (\( Rhodomyrtus tomentosa \)), kalakai or fern (\( Stenochlaena palutris \)), purun tikus (\( Eleocharis dulcis \)), etc as presented in Figure 1. However, we had not succeeded to quantify the gene copy number using RT-PCR due to technical problems. With this result, at least we have information that the rhizosphere of indigenous plants in acid sulphate soil was harboured by functional rhizobacteria, an \( N_2 \) fixer that are contributors of \( N \) input in acid sulphate soil.

3.3. The Abundance of soil heterotrophic fungi and rhizobacteria and their function as \( P \) solubilizer and IAA producer.

Differences in culturable fungal and bacterial abundance and relative microbial activity could result in large effects on subsequent soil biogeochemical cycling [29]. The total culturable heterotrophic rhizobacteria and fungi of both soils seemed the same where total rhizobacteria were higher than total fungi. This observation was the same as the result of Shamshuddin et al. [30] in the rhizosphere from acid sulfate soil in Peninsular Malaysia that the total bacterial and actinomycete population was higher than the fungal population.

| Acid Sulfate Soil | Microbe    | Microbial Number (Log CFU/g fresh soil sample) | Phosphate Solubilization Index | IAA (ppm) |
|-------------------|------------|-----------------------------------------------|--------------------------------|-----------|
|                   |            |                                               | \( Ca_3(PO_4)_2 \) | \( AlPO_4 \) | \( FePO_4 \) | \( P \) Rock |          |
| Potential (L1)    | Rhizobacteria | 6.38                                      | 1.11 - 1.25 | - | - | - | 6.80 – 22.39 |
|                   | Fungi       | 5.20                                      | 1.04 - 2.00 | - | - | - | NT* |
| Actual (L2)       | Rhizobacteria | 6.30                                      | 1.04 - 1.20 | - | - | - | 6.93 – 27.66 |
|                   | Fungi       | 5.15                                      | 1.06 - 2.97 | - | - | - | NT* |

*NT = have not been analyzed

The ability of the microbe to solubilize P is a good predictor of inorganic P's mineralization potential of soil where the microbes were taken. Isolated rhizobacteria from the abundance count were shown to be able to solubilize insoluble P as \( Ca_3(PO_4)_2 \), with a solubilization index of 1.11 to 1.25 for potential acid sulphate soil and 1.04 to 1.20 for actual acid sulphate soil rhizobacteria. Phosphate solubilization ability was also found in the isolated fungus group, with index solubilization against \( Ca_3(PO_4)_2 \) ranging from 1.04 to 2.00 for potential acid sulphate soil and 1.06 to 2.97 for actual acid sulphate soil. Fungi were found to have a better ability to solubilize \( Ca_3(PO_4)_2 \) than rhizobacteria (Table 4, Figure 2). However, the P solubilization index of the rhizobacteria and rhizosphere fungi from this study was lower than that of Noya et al. [31], i.e., heterotrophic rhizobacteria from the rhizosphere of pioneer plants growing in a copper mined-out area, which ranged from 2 to 5. In addition, both rhizobacteria and fungi were unable to solubilize insoluble P in the form of \( AlPO_4 \) or \( FePO_4 \) or phosphate rock, but ASS was plagued by a lack of accessible P due to Al or Fe-fixed P. This information suggests that inoculation of microbe with effective P solubilization of insoluble P (fixed to Al, Fe, or as P rock) and adaptive with ASS are needed for friendly and sustainable soil productivity.
Figure 2. The fungi group indicated P solubilization on $\text{Ca}_3(\text{PO}_4)_2$ as insoluble P in media.

Microorganisms in soil could produce phytohormone which has the function for plant growth, fitness, and their survival to abiotic and biotic stress (Egamberdieva et al. 2017). Rhizobacteria isolated from potential and actual ASS were shown to be capable of producing IAA in the range of 6.89 to 22.39 for potential ASS and 6.93 to 27.66 for actual AAS (Table 3).

4. Conclusion
Microbial activity of potential and actual acid sulphate in terms of respiration, dehydrogenase, and nitrogenase activity was not different. Several soil factors affected the low value of the microbial activity in acid sulphate soil.

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