COMPARATIVE STUDY OF MICROORGANISMS IN SOIL OF TRUNKING AND NON-TRUNKING SAGO PALMS PLANTATION

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<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
</tr>
<tr>
<td>Acknowledgements</td>
</tr>
<tr>
<td>Table of Contents</td>
</tr>
<tr>
<td>List of Tables</td>
</tr>
<tr>
<td>List of Figures</td>
</tr>
<tr>
<td>List of Plates</td>
</tr>
<tr>
<td>List of Abbreviations</td>
</tr>
<tr>
<td>Abstract</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
</tr>
<tr>
<td>1.1. Introduction</td>
</tr>
<tr>
<td>1.2. Literature review</td>
</tr>
<tr>
<td>1.2.1. Growth pattern of sago palm</td>
</tr>
<tr>
<td>1.2.2. Soil microbial community structure</td>
</tr>
<tr>
<td>1.2.3. Soil physicochemical characteristics</td>
</tr>
<tr>
<td>1.2.4. Soil CO₂ emission</td>
</tr>
<tr>
<td>1.2.5. Soil microbiota</td>
</tr>
<tr>
<td>1.2.6. Mycorrhiza characteristics</td>
</tr>
<tr>
<td>1.2.7. N₂ fixation</td>
</tr>
<tr>
<td>1.3. Objectives of the study</td>
</tr>
<tr>
<td>1.4. Scope of study</td>
</tr>
<tr>
<td>1.5. Strategy of study</td>
</tr>
</tbody>
</table>
Chapter 2: Materials and Methods

2.1. Locality 20

2.2. Sampling 20

2.3. Soil physical characteristics 21

2.3.1. Dry weight 21

2.3.2. Soil pH 22

2.4. Soil CO$_2$ detection by soda lime absorption 26

2.5. Total recoverable viable aerobic bacteria via plate count method using Tryptic Soy Agar 27

2.6. Quantification of degree of mycorhizal-fungus infection 28

2.7. Total recoverable viable N$_2$ fixers via plate count method using Winogradsky Nitrogen-Free Minimal Agar 29

2.8. Microbiological media and chemical reagents 29

2.9. Aseptic technique 30

2.10. Statistical analysis 31

2.10.1. Statistical significance 31

2.10.2. Shapiro-Wilk Normality Test 31

2.10.3. Descriptive statistics 32

2.10.4. Comparison of means 33

2.10.5. Regression analysis 34

2.10.6. Partial Correlation Analysis 34

Chapter 3: Results and Discussion 36

3.1. Sampling 36

3.1.1. Site description 36
LIST OF TABLES

Table 1.1: Scope of study 18
Table 3.1: Descriptive details of each sample 37
Table 3.2: Results of partial correlation analysis between trunk volume and other parameter while controlling the effects from other microbiological parameters 63
Table 3.3: Standardized coefficients 63
LIST OF FIGURES

Figure 2.1: Map showing the location of Dalat Sago Plantation 23
Figure 2.2: Map showing the layout of Dalat Sago Plantation 24
Figure 2.3: Map showing the site of study 24
Figure 2.4: Map showing the sampling points 25
Figure 3.1: Bar chart showing the dry weight of the soil samples 41
Figure 3.2: Bar chart showing the pH of the reconstituted dry soil 41
Figure 3.3: Map showing the soil pH in relation to trunking and non-trunking block 42
Figure 3.4: Scatter plot with regression line showing correlation between sago trunk volume and soil pH 43
Figure 3.5: Bar chart showing soil respiration of the soil samples as CO₂ emission 46
Figure 3.6: Map showing the rate of soil respiration in relation to trunking and non-trunking block 47
Figure 3.7: Bar chart showing the total recoverable viable aerobic bacteria count of the soil samples 51
Figure 3.8: Scatter plot with regression line showing correlation between TRVAB count and soil pH 51
Figure 3.9: Map showing the count of TRVAB population within the study area 52
Figure 3.10: Bar chart showing total aerobic nitrogen fixers count of the soil samples 57
Figure 3.11: Scatter plot with regression line showing correlation between TANF and TRVAB

Figure 3.12: Scatter plot with regression line showing correlation between soil respiration and total aerobic N₂ fixers

Figure 3.13: Map showing the count of aerobic nitrogen fixer population within the study area
LIST OF PLATES

Plate 2.1: Field sampling
Plate 2.2: Group photograph
Plate 2.3: Soil respiration
Plate 2.4: Decimal serial dilution of soil sample in physiological saline
Plate 2.5: Tryptic soy agar
Plate 2.6: Root sample preparation
Plate 2.7: Winogradsky Nitrogen-Free Minimal Agar
Plate 2.8: Anaerobic incubation of WNFMA using anaerobic jar

Plate 3.1: Photograph showing a panoramic view of one of the sampling site in DSP
            Phase 1A Block 1
Plate 3.2: Photograph showing a panoramic view of one of the sampling site in DSP
            Phase 1A Block 7
Plate 3.3: Block 1
Plate 3.4: Block 7
Plate 3.5: Plate count of TRVAB on TSA
Plate 3.6: Stained mycorrhizae at various magnification
Plate 3.7: Plate count of aerobic nitrogen fixers on WNFMA
LIST OF ABBREVIATIONS

cfu colony forming unit
DBH diameter at breast height
DSP Dalat Sago Plantation
GPS Global Positioning Satellite
LCDA Land Custody and Development Authority of Sarawak
MPN most probable number
PGPR plant growth-promoting rhizobacteria
SR soil respiration
TANF total aerobic N₂ fixers
TAnNF total anaerobic N₂ fixers
TRVAB total recoverable viable aerobic bacteria
TSA Tryptic Soy Agar
TV trunk volume
UV-sterilized ultraviolet-sterilized
WNFMA Winogradsky Nitrogen-Free Minimal Agar
ABSTRACT

Sarawak is the principal exporter of sago starch to the world market, exporting about 25 000 – 30 000 tonnes of sago starch annually. Commercialization of the sago industry and plantation could ensure the production of sago starch in large quantity and of homogeneous quality. Hence LCDA (Land Custody and Development Authority of Sarawak) developed the world’s first commercial deep peat sago plantation in Mukah, Sarawak in the year 1987, with minimal management intervention. However, most of those sago palms planted are not showing any sign of trunking after 10 years old. Moreover, they suffered stunted growth and high mortality rate. This study is a part of an umbrella study looking into factors influencing the growth patterns of sago palms under minimally managed peat swamp cultivation in Dalat and Mukah. This study applies a comparative-inductive study design to compare selected soil physicochemical and microbiological parameters, such as soil pH, soil CO₂ emission and soil microbiota, focusing on mycorrhizae and N₂ fixers, of soil samples collected from trunking and non-trunking sago palms. Two sampling plots were identified and subjected to further study, they are Block 1 (as trunking block) and Block 7 (as non-trunking block) of Dalat Sago Plantation (DSP) Phase 1A. Sago trunk volume was used as the index of trunking. It was found that DSP was completely water-logged during the duration of the study (September, 2003 – January, 2004) and that its peat soil was very acidic (dry soil pH, 2.82 – 3.13). Moreover, the soil pH for trunking block (2.98) was marginally higher than non-trunking block (2.90) \((p = 0.0667)\). Using a field method of CO₂ detection by soda lime absorption, soil CO₂ emission for trunking block (3.5 g CO₂/ m²/ day) was also...
found to be significantly higher than non-trunking block (2.1 g CO₂/m²/day) (p = 0.0127). Total recoverable viable aerobic bacteria (TRVAB) count has been enumerated using standard plate count method and this study is 80.46% confident (only marginally significant) that TRVAB count for trunking block (9.8 x 10⁵ cfu/g dry soil) was higher than non-trunking block (7.2 x 10⁵ cfu/g dry soil). Attempts at quantifying degree of mycorrhizal infection using gridline-intersect method were unsuccessful due to the inability of the technique to distinguish between living and non-living roots. Enumeration of total aerobic N₂ fixers (TANF) using standard plate count method did not reveal any significant difference between the TANF count for trunking (9.1 x 10⁵ cfu/g dry soil) and non-trunking block (8.2 x 10⁵ cfu/g dry soil). This suggested that most of the soil bacteria quantified were aerobic N₂ fixers which were probably inactivated by the water-logging condition. Only one colony of anaerobic N₂ fixers was isolated. Regression and Partial Correlation Analysis have proven that all the soil microbiological parameters were related to the trunk volume of the sago palm (r² = 1.000, p = 0.008). TRVAB was found to be the most influential parameter. However, it was undermined by the high level of soil acidity. Therefore, under existing natural field condition, soil pH seemed most important in affecting trunk volume. In light of these results, two soil amendment strategies are suggested, i.e. the improvement of soil aeration by lowering the water table and increasing the soil pH by liming. However, soil microbiological interactions are so complex that the actual consequences of these amendments though very influential and significant, are quite unknown without further in-depth study into the subject. Therefore it is high time that the effects of soil microorganisms on the growth of sago palms be subjected to intensive research since proper understanding of soil microbiology would benefit
the plantation as well as the natural environment because microbiological amendments on the soil are as a rule, environmentally-friendlier and less capital-intensive.
KAJIAN PERBANDINGAN MIKROORGANISMA TANAH ANTARA LADANG SAGU YANG MEMBENTUK DAN TIDAK MEMBENTUK BATANG POHON

Sarawak merupakan pengeksport sagu yang utama di dunia, dengan jumlah eksport tahunan sebanyak 25 000 – 30 000 tonne sagu. Pengkormersilan industri dan ladang sagu akan mampu mengeluarkan sagu dalam kuantiti yang banyak dan pada kualiti yang sekata. Oleh itu, LCDA telah membangunkan ladang komersil sagu yang pertama di dunia di Mukah, Sarawak pada tahun 1987, yang memerlukan pengawasan yang minimum. Namun begitu, pohon sagu yang ditanam masih tidak membentuk batang pohon selepas 10 tahun ditanam. Tambahan pula, pohon-pohon sagu ini mengalami pertumbuhan yang terbantut dan kadar kematian yang tinggi.

Kajian ini merupakan sebahagian daripada satu kajian menyeluruh yang mengkaji faktor yang mempengaruhi pertumbuhan pohon sagu yang ditanam berasaskan konsep pengawasan minimum di Dalat dan Mukah. Kajian ini menggunakan teknik kajian perbandingan-induktif untuk membandingkan ciri-ciri fizikal-kimia dan mikrobiologi tanah, seperti pH tanah, penghasilan CO₂ dari tanah dan mikrobiota tanah terutama mikoriza dan pengikat N₂, bagi sampel tanah yang dikumpul dari blok pohon sagu yang membentuk batang pohon dan yang tidak membentuk batang pohon. Dua plot sampel telah dikenalpasti untuk dikaji, iaitu Blok 1 (sebagai blok yang membentuk batang pohon) dan Blok 7 (sebagai blok yang tidak membentuk batang pohon) di DSP Fasa 1A. Isipadu batang sagu telah digunakan sebagai indeks pembentukan batang pohon. Kajian mendapati bahawa DSP adalah dibanjiri air
sepanjang masa kajian ini dijalankan (September, 2003 – Januari, 2004) dan tanah gambutnya adalah sangat berasid (pH tanah kering, 2.82 – 3.13). Di samping itu, pH tanah untuk blok yang membentuk batang pohon (2.98) didapati lebih tinggi secara marginal berbanding blok yang tidak membentuk batang pohon (2.90) (p = 0.0667). Dengan menggunakan satu teknik lapangan untuk pengesanan CO₂ iaitu dengan cara penyerapan oleh kapur soda, penghasilan CO₂ tanah untuk blok yang membentuk batang pohon (3.5 g CO₂/ m²/ hari) didapati nyata lebih tinggi daripada blok yang tidak membentuk batang pohon (2.1 g CO₂/ m²/ hari) (p = 0.0127). TRVAB juga telah dijumlahkan dengan menggunakan teknik piawai kiraan plat dan kajian ini adalah 80.46 % yakin (secara marginal) bahawa kiraan TRVAB bagi blok yang membentuk batang pohon (9.8 × 10⁴ cfu/ g tanah kering) adalah lebih tinggi daripada blok yang tidak membentuk batang pohon (7.2 × 10⁴ cfu/ g tanah kering). Percubaan untuk menganggarkan darjah jangkitan mikoriza dengan menggunakan teknik persilangan-garis-grid tidak berjaya kerana teknik yang digunakan tidak mampu membezakan akar yang hidup daripada yang sudah mati. TANF yang dijumlahkan dengan teknik piawai kiraan plat tidak menunjukkan sebarang perbezaan yang ketara di antara kiraan TANF bagi blok yang membentuk batang pohon (9.1 × 10⁵ cfu/ g tanah kering) dengan blok yang tidak membentuk batang pohon (8.2 × 10⁵ cfu/ g tanah kering). Ini mencadangkan bahawa kebanyakan bakteria tanah yang dikulturkan adalah pengikat N₂ yang aerobik yang kemungkinan besar telah dinyahaktifkan oleh keadaan tanah yang dibanjiri air. Hanya satu koloni pengikat N₂ yang anaerobik yang berjaya diasingkan. Analisis Regresi dan Korrelasi Separa telah membuktikan bahawa semua ciri-ciri mikrobiologi tanah adalah berkait rapat dengan isipadu batang pohon sagu (r² = 1.000, p = 0.008). Adalah didapati bahawa TRVAB merupakan parameter
1.1. Introduction

Starch from stems of palms is a product of local importance throughout the mainland and islands of Southeast Asia, in parts of Melanesia, and certain islands of Micronesia, where it is obtained from the stems of some, mostly native, palm species (Ruddle et al., 1978). Among these palm species, sago palm (*Metroxylon* spp.) ranks top. Ruddle et al. has presented a very good review of the importance of sago to these producing regions in terms of historical, cultural and economical point of view.

Sago has always been the crops recommended for plantation in peat soil (Teng, 2002) with low-cost and minimal management as it can tolerate water-logging condition (Jong, 1995). Therefore, Sarawak naturally becomes the largest sago producer in Malaysia with its 1.7 Mha of peatland (Teng, 1994). Sarawak peatland is largely made up of basin peat swamps under natural water-logged conditions (Teng, 2002). Tie et al. (1991) estimated that the total sago area in Sarawak was at 19 720 ha and nearly 75 % of this occurred in Oya-Dalat, Mukah, Igan and Balingian areas, whereby 62 % of the sago was grown in peat soil.

Currently, Sarawak is the principal exporter of sago starch to the world market, exporting about 25 – 30 Gg of sago starch annually. Sago has been
one of the primary revenue earners for over 50 years, ranking at the fifth highest agricultural revenue earner after pepper, rubber, oil palm and cocoa (Zulpilip et al., 1991). Zulpilip et al. (1991) has also reported that price of refined and superior sago starch was always increasing and this trend is expected to continue into the future. However, concerted measures on assuring the quality of sago starch are lacking because small-holders, who are the main sago producers, have limited resources to do this properly. If sago starch of homogeneous quality can be supplied in large quantities, it can become more important than corn, potato, tapioca and sweet potato. This could only be achieved through commercialization of the sago industry and plantation.

In light of these potentials from sago, Land Custody and Development Authority of Sarawak (LCDA) developed the world’s first commercial deep peat sago plantation in Mukah, Sarawak, in the year 1987. It was thought that sago palm would grow on deep peat swamps, such as the one in Mukah, with minimal management intervention. If the plantation were successful, it would mean the possible revival of the vast peat swamp areas in Sarawak into productive agricultural lands.

However, Melling (2000) found that the plantation is experiencing some problems that were never anticipated. The sago palms grown both in Dalat and Mukah Sago Plantation suffered stunted growth and high mortality rate. After more than 10 years old, these palms are not showing any sign of trunking. Generally they are unhealthy as shown by the formation of small crowns, low number of fronds and premature frond desiccation. LCDA is
CHAPTER 1 INTRODUCTION

depedly concerned as they are the owner of these sago plantations and this is a mega development project, therefore it is important to study the factors limiting the healthy growth of sago palms in the plantation, in which soil microbiological property is one of these factors.

1.2. Literature review

1.2.1. Growth pattern of sago palm

A review of sago ecology has been reproduced from Jong (1995) as follow. Sago palm occurs between 10° N and 10° S with the best yield coming from palms grown below 400 m above sea level. The optimum temperature is 25 – 30 °C while an evenly distributed annual rainfall of 2000 – 4000 mm is desirable. Precipitation must exceed evaporation so as to maintain the water-logging condition. Full sunshine and a soil pH of 4 or higher are very suitable for the growth of sago palms. Nevertheless, it can tolerate low pH and grow on peat or mineral soils but growth is better on mineral soils. This can be seen from its maturation age of 8 – 10 years on mineral soil whereas it takes 15 – 17 years to mature on peat soil. Sago palm may remain trunkless for the first 3 – 6 years. Mature trunk has a diameter of about 35 – 60 cm and a length that measures 7 – 15 m. Sago trunk should be harvested before its flowering stage as starch content in the trunk diminishes after the flowering stage. Locals have their own system of
classifying the growth stage of sago palm. This system is mainly based on the height of the sago trunk which represents the harvestable volume. Therefore, to be more accurate in determining the harvestable volume, the trunk volume as calculated from the DBH (diameter at breast height) and the height of trunk (measured from the base to the sheath base of the oldest living frond) will be used as an index to represent the harvestable trunk.

1.2.2. Soil microbial community structure

Generally, microorganisms are found to prefer well-aerated soil with high moisture content and high organic matter concentration. Hence, soil microbial population and its related activities are most active at the soil surface and decreased with increasing soil depth (Ekelund et al., 2001; Fisk et al., 2003; Mergel et al., 2001; Stout, 1971).

Soil microbial community structure and distribution are highly dynamic under natural condition. Fungi generally dominated soil microbial population in terms of biomass while bacteria are the most abundant in number. However, as the soil improves, the microbial community structure began to shift and would be dominated by bacteria (Grayston et al., 2001). Moreover, even when soil moisture was not limiting, precipitation would still cause a sudden but short-lived upsurge of bacterial biomass (Clarholm & Rosswall, 1980).
However, the effect of precipitation in waterlogged soil such as peat remains unknown. Nevertheless, Reichardt et al. (2001) had observed that fungi, N-remobilizing, proteolytic bacteria and ammonium oxidizing nitrifiers population were very low under flooding condition in contrast to anaerobes and denitrifiers populations that were high.

Van Beelen & Fleuren-Kemilä (1989) reported that peat soil contained $3 \times 10^{10}$ bacteria per gram dry soil through direct counts. Apun et al. (2003) also reported that the peat soil in a sago plantation in Mukah contained a total bacterial count in the range of $10^8$ cell / g soil. On the other hand, it has always been acknowledged that total soil bacteria count was always underestimated, as only 15 – 30 % of the total bacteria were active even under favourable growth conditions (Clarholm & Rosswall, 1980). Though the dynamism of soil microbial population has always been a paradigm in soil microbiology, a contrasting paper stated that soil rich in organic carbon supported uniform distribution of soil microbial community regardless of soil water content and depth. Spatial isolation has been quoted as the reason for the observed uniform distribution and high diversity of soil microbial community (Zhou et al., 2002). In support of it, Boehm et al. (2003) stated that level of peat decomposition neither affected the dominancy of any species of rhizosphere bacteria nor the bacterial species diversity. However, level of peat decomposition did influence the composition of bacterial species in the rhizosphere.
1.2.3. Soil physicochemical characteristics

Soil microbial population and its activities are always subjected to various environmental stresses. Among the most important physicochemical characteristics is soil pH (Jones & Bangs, 1985; Kahindi et al., 1997; Mårtensson & Witter, 1990). Soil pH influences the physiology of most microorganisms especially the N2-fixers.

Other often-cited factor is water logging condition which decreases the soil CO2 emission (Aerts & Ludwig, 2003; Best & Jacobs, 1997) and affected the dynamism of N-cycle related microorganisms (Reichardt et al., 2001). Soil fertility also influences the soil CO2 emission in which increases in soil fertility corresponds to increases in soil CO2 emission (Aerts & Ludwig, 2003; Bridgham & Richardson, 1992; Grayston et al., 2001). Soil depth (Apun et al., 2003; Ekelund et al., 2001; Stout, 1971) and aeration (Herbert, 1975) tends to affect soil microbial community structure similarly – microorganisms are the most abundant in the surface soil where aeration is good and vice versa. As with all living processes, temperature affects the rate of physiological processes and thereby limiting the soil microbial population (Egamberdiyeva & Höflich, 2003; Jones & Bangs, 1985). Other factors like level of peat decomposition (Boehm et al., 2003), precipitation (Clarholm & Rosswall, 1980) and soil types (Egamberdiyeva & Höflich, 2003) cause changes in the microhabitat and consequently its microbial
community structure. Availability of C (Jones & Bangs, 1985; Stout, 1971; Zhou et al., 2002) and presence of heavy metal (Kahindi et al., 1997; Mårtensson & Witter, 1990) is important to the growth of soil microorganisms as their growth is usually substrate-induced as well as subjected to substrate-poisoning. Protozoa grazing and salinity (Kahindi et al., 1997) control soil microbial community through predation and physiological limitations. Most of these factors are inter-related, for example aeration and water logging condition or soil depth; soil fertility and level of peat decomposition, available C or soil types; and precipitation and water logging condition.

1.2.4. Soil CO₂ emission

Peat plays a significant role in the global emission of CO₂. Sustaining agriculture into the future might very much rely on proper understanding and management of nature’s nutrient cycle, of which C cycle ranked among the tops. The extent to which complex interrelationships between microorganisms and soil organic matter influenced plants’ growth is very critical to our understanding and application of sustainable agriculture.

This study would use similar approach whereby soil respiration and soil CO₂ emission will be used interchangeably as in most literature. Soil CO₂ emission is mostly attributed to its microbial population. Hence, most of the environmental factors discussed
previously are applicable here. Nevertheless, there are a few major factors that necessitate further explanation. By far, water logging condition (Aerts & Ludwig, 2003; Best & Jacobs, 1997) and soil fertility (Aerts & Ludwig, 2003; Bridgham & Richardson, 1992) are the most important factors. Under waterlogged condition, soil environment became anaerobic thus reducing the emission of CO$_2$, an aerobic respiration product. In addition, low level of nutrients also diminished soil microbial population and its respiration. Therefore, it was proven as expected that soil base respiration, microbial biomass and carbon content were highly correlated (Witter et al., 1993). This was further established by the fact that soil respiration has been successfully used to gauge biomass and activity of peat soil microorganisms (Brake et al., 1999).

Apart from these, Haraguchi et al. (2002), also showed that soil CO$_2$ emission was a good indicator of level of decomposition of organic matter in peat soil. It was observed in an earlier study that microbial oxidation of peat declines with increasing age of the carbon (Stout, 1971).

1.2.5. Soil microbiota

By virtue of their physiological adaptability and metabolic versatility, bacteria in plant root zones are a key agent of change in soil agroecosystems. Interactions between plant root systems and