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NITRIFICATION OF A TROPICAL PEAT SOIL CULTIVATED WITH OIL PALM (Elaeis guineensis Jacq.)

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By

MOHD RIZAL BIN ARIFFIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

January 2017

DEDICATION

I’d dedicate each pages of this research to:

My wife for her constant encouragement and prayers

My lovely daugthers who motivate me to achieve my future endeavour

My brother who takes the role as a father after my parents passed away

My deceased mother and father whose dream I am fulfilling

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

NITRIFICATION OF A TROPICAL PEAT SOIL CULTIVATED WITH OIL PALM (*Elaeis guineensis* Jacq.)

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Chairman: Ahmad Husni Mohd. Hanif, PhD
Faculty : Agriculture

As knowledge on nitrogen (N) cycle is evolving rapidly, soil nitrification has become the centre of research interest because it is the single most important process in the N-cycle that leads to N losses in broad range of environments. With further expansion of oil palm on tropical peat lands, N cycling is predicted to be modified. However, very few current literatures reported nitrification in tropical peat soils cultivated with oil palm. Therefore, there is a need to understand the nitrification process in cultivated tropical peat soils with oil palm.

Soil sampling were carried out up to 60 cm depth at weeded circle (WC), harvesting path (HP) and frond heap (FH). General chemical analyses were carried out in the laboratory. The results showed that WC contained the highest ammonium (8.48 µg g^{-1} soil), nitrate (6.22 µg g^{-1} soil) and inorganic N (14.70 µg g^{-1} soil) contents compared with FH and HP in 0-10 cm soil depth. PNR indicated, most of the nitrification activity happened at 0-10 cm in WC area. In 20-30 cm WC area, 10-20 cm, 20-30 cm of FH area and 10-20 cm HP area indicated negative value of PNR. This suggests that, nitrification were restricted at these depths and the NO_3^- availability in these areas were predicted to originate from the topsoil through vertical down movement. Laboratory incubation study also indicated, nitrification were affected not only by the availability of substrate (NH_4^+), but also by the type of N fertilizers. Nitrification was inhibited by addition of AS, and higher input of AS led to greater inhibition of nitrification. Net nitrification in urea reached its highest value in 2 kg N $palm^{-1}$ treatment, which is higher (21.33 µg g^{-1} soil day^{-1}) than in AS (3.41 µg g^{-1} soil day^{-1}). In the next laboratory incubation study, the dynamics of NO_3^- did not show significant change with increased soil moisture without addition of urea. This results indicated, nitrification in peat soil needed reactive N supply (urea) regardless of soil moisture conditions. However, increasing soil moisture resulted in lower NO_3^- and PNR which reflect that soil moisture controlled the magnitude of PNR when urea was added.

Aside from laboratory incubation experiments, field soil sampling were also collected in dry and wet season from research plots consisted of ground cover treatments with three N rates. Data from the study suggested that installation of legume cover crops (LCC) in combination with N fertilizers resulted in higher mineralization and nitrification in wet season compared to natural ground cover. Nitrification rate was stimulated by the availability of substrates (NH_4^+) regardless of the moisture condition. LCC can be the contributing factors in higher availabity of NH_4^+ in the wet season that could be loss to the environments. Therefore, LCC may be unnecessary in peat soil where N fertilizers were applied.

Finally, selected soil samples from the study mentioned above were used to determine the microbial composition and to identify potential nitrifiers existed in tropical peat soil cultivated with oil palm using 16S metagenomics. The results indicated that conventional ammonium and/or ammonia oxidizers (*Nitrosomonas* and *Nitrosococcus*) were almost non-existent in all the soil samples. This study observed that soil samples from the surface area of WC, FH and HP area were abundant with *Bacilli* and *Pseudomonales* suggesting heterotrophic pathways of ammonia oxidation in this area. However, pronounced shift in ammonium oxidizing archaea (AOA) number was observed (*Thaumarchaeota)* with inclusion of conventional legume sytems compared with natural ground cover. *Planctomycetaceae* order from the phylum *Planctomycetes* (anammox bacteria) were also abundant in number suggesting anaerobic ammonium oxidation is possible in tropical peat soil cultivated with oil palm.

In conclusion, nitrification was prominent in tropical peat soil cultivated with oil palm disregarding low pH condition and high moisture content. The nitrification rate varies among the operational zones and depth and the process was influenced by N fertilizer addition and also regulated by moisture condition. However, the major pathways of ammonia oxidation (heterotrophic bacteria or by AOA) and the extention of anaerobic ammonium oxidation still remained elusive and require further studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Doktor Falsafah

NITRIFIKASI TANAH GAMBUT TROPIKA YANG DITANAM KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

MOHD RIZAL BIN ARIFFIN

Januari 2017

Pengerusi: Ahmad Husni Mohd. Hanif, PhD
Fakulti : Pertanian

Dengan perkembangan pesat ilmu berkenaan kitaran N, nitrifikasai N dalam tanah menjadi tumpuan penyelidikan memandangkan ia merupakan proses paling utama dalam kitaran N yang menyebabkan N hilang didalam pelbagai persekitaran. Dengan pengembangan tanaman kelapa sawit di tanah gambut tropika, kitaran N dijangka akan berubah. Tetapi hanya terdapat beberapa sahaja kajian yang menunjukkan nitrifikasi di tanah gambut yang ditanam kelapa sawit. Justeru itu, terdapat keperluan untuk kajian nitrifikasi dalam tanah gambut tropika yang ditanam kelapa sawit.

Sampel tanah di ambil sehingga kedalaman 60 cm pada weeded circle (WC), harvesting path (HP) dan juga frond heap (FH). Analisis kimia umum dijalankan di dalam makmal. Keputusan menunjukkan WC mengandungi ammonium (8.48 µg g^{-1} tanah), nitrat (6.22 µg g^{-1} tanah) dan N bukan organik (14.70 µg g^{-1} tanah) tertinggi berbanding FH dan HP pada kedalaman 0-10 cm. PNR menunjukkan kebanyakan nitrifikasi berlaku pada kedalaman 0-10 cm pada kawasan WC. Pada 20-30 cm WC, 10-20 cm dan 20-30 cm FH beserta 10-20 cm HP, PNR menunjukkan nilai negatif. Hal ini mengimplikasikan bahawa nitrifikasi adalah terhad di kedalaman tersebut diatas. Oleh itu, kandungan NO_3^- yang terdapat dikawasan ini dipercayai berasal daripada tanah permukaan dan bergerak ke bawah.

Kajian inkubasi makmal juga menunjukkan nitrifikasi bukan hanya dipengaruhi dengan kehadiran substrat (NH_4^+), tetapi juga oleh jenis baja N. Nitrifikasi juga terhalang dengan penambahan AS (ammonium sulphate) dan input AS yang lebih tinggi akan meningkatkan lagi kadar halangan nitrifikasi. Kadar nitrifikasi bersih mencapai tahap tertinggi pada urea 2 kg N/pokok (21.33 µg g^{-1} soil day^{-1}) lebih tinggi daripada AS 2 kg N/pokok (3.41 µg g^{-1} soil day^{-1}). Dalam kajian inkubasi makmal seterusnya, kadar dinamik NO_3^- tidak menunjukkan kesan perubahan signifikan dengan peningkatan kelembapan tanpa penambahan urea. Hasil kajian ini menunjukkan nitrifikasi pada tanah gambut memerlukan bekalan N yang reaktif (urea) tanpa mengira keadaan kelembapan untuk nitrifikasi terjadi. Walaubagaimanapun, peningkatan kelembapan menyebabkan NO_3^- dan PNR lebih rendah yang menunjukkan kelembapan tanah mengawal magnitud PNR apabila urea dibekalkan.

Selain daripada eksperimen inkubasi makmal, sampel tanah juga di ambil pada musim hujan dan musim kering daripada plot kajian yang mengandungi rawatan perlindungan tanah bersama tiga kadar N. Data daripada kajian ini menunjukkan penambahan LCC dengan kombinasi baja N menyebabkan kadar mineralisasi dan nitrifikasi yang lebih tinggi pada musim hujan berbanding keadaan tanah semulajadi. Kadar nitrifikasi dipengaruhi oleh kedapatan substrate (NH_4^+) tanpa mengira keadaan kelembapan. LCC boleh menjadi faktor penyebab kandungan tinggi NH_4^+ pada musim hujan yang berpontensi hilang ke persekitaran. Oleh itu, penambahan LCC pada tanah gambut yang diberi baja N mungkin tidak diperlukan.

Akhir sekali, sampel tanah terpilih daripada kajian yang disebut diatas digunakan untuk menentukan komposisi mikrob dan juga untuk mengenalpasti mikrob nitrifikasi yang terdapat didalam tanah gambut yang ditanam kelapa sawit menggunakan teknik 16S metagenomics. Keputusan menunjukkan bahawa bakteria konvesional pengoksida ammonium dan/atau ammonia (*Nitrosomonas* and *Nitrosococcus*) hampir tidak wujud dalam kesemua sampel. Pemerhatian menunjukkan sampel daripada kawasan permukaan WC, FH dan HP terdapat banyak mikrob berasal daripada kumpulan *Bacilli* dan *Pseudomonales*. Hal ini menyarankan bahawa terdapat laluan pengoksidaan ammonia secara heterotropik di kawasan ini. Walaubagaimanapun, terdapat perubahan jumlah AOA diperhatikan dengan kemasukan sistem kekacang konvensional berbanding permukaan tanah semulajadi. Kajian juga mendapati terdapat banyak *Planctomycetaceae* order daripada phylum *Planctomycetes* (bakteria anammox) dan keadaan ini menyarankan terdapat proses pengoksidaan ammonium secara anaerobic didalam tanah gambut tropika yang ditanam kelapa sawit.

Pada kesimpulannya, proses nitrifikasi boleh berlaku didalam tanah gambut tropika dalam keadaan pH rendah dan juga kelembapan tinggi. Kadar nitrifikasi adalah berbeza disetiap zon operasi dan kedalaman. Proses nitrifikasi ini dipengaruhi oleh penambahan baja dan dikawal oleh oleh keadaan kelembapan. Perubahan pengurusan tanah seperti penambahan tanaman kekacang dan baja cenderung untuk meningkatkan bilangan bakteria nitrifikasi. Walaubagaimanapun, jalan utama proses pengoksidaan ammonia (samaada heterotropik atau AOA) masih kabur. Kadar sumbangan nitrifikasi oleh bakteria pengoksida ammonia anaerobik juga masih memerlukan kajian di masa hadapan.

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I certify that a Thesis Examination Committee has met on 17 January 2017 to conduct the final examination of Mohd Rizal bin Ariffin on his thesis entitled "Nitrification of a Tropical Peat Soil Cultivated with Oil Palm (*Elaeis guineensis* Jacq.)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment for the degree of Doctor of Philosophy. The members of the Supervisory committee are as follows:

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AMO	ammonia monooxygenase genes
amoA	ammonia monooxygenase archaea genes
AOA	ammonia oxidizing archaea
AOB	ammonia-oxidizing bacteria
AS	Ammonium sulphate
C	Carbon
CO_2	Carbon dioxide
c-PCR	competitive polymerase chain reaction
DCD	diacyandiamide
FH	Frond heap
HAO	hydroxylamine oxidoreductase
HP	harvesting path
LCC	leguminous cover crops
MPN	most probable number
MPOB	Malaysia Palm Oil Board
N_2	dinitrogen
N_2O	Nitrous oxide
NH_2OH	hydroxylamine
NH_3	Ammonia
NH_4^+	Ammonium
N_i	Inorganic N
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOB	nitrite oxidizing bacteria
O_2	oxygen
O_3	ozone
OH	hydroxyl
OM	Organic matter
PNR	potential nitrification rate
q-PCR	quantitative polymerase chain reaction
TC	Total carbon
TN	Total nitrogen
WC	Weeded circle
WHC	Water holding capacity

CHAPTER 1

INTRODUCTION

The microbial mediated conversion of ammonium and ammonia (NH_4^+ and NH_3) to nitrite –N (NO_2^-) is the first step in the nitrification process. Afterwards, NO_2^- is further oxidized to nitrate (NO_3^-). Although NO_3^- production through nitrification is important for plant nutrient uptake, NO_3^- is also susceptible to leaching into water bodies. At the same time nitrification can also produce nitrous oxide (N_2O) as byproduct under aerobic condition which can escape to the atmosphere and affecting the ozone layer (Cameron et al., 2013; O'Sullivan et al., 2011). Therefore, nitrification is an important mechanism affecting nitrogen (N) loss and mobility in soils, a process that lowers N-use efficiency in agriculture systems. In agriculture systems, it is desirable to control the conversion of N fertilizer (applied in stable and reduced form of NH_4^+ or urea) to more mobile oxidized form (NO_3^-) as this allows for longer access to N by the plant roots (Chen et al., 2008).

Higher nitrification rate is assumed to occur in tropical soils because of the hot climate and abundant rainfall which subsequently stimulate nitrification in soils. However, very few studies can be found emphasized on soil nitrification (Breuer et al., 2000; Chao et al., 1993; Pett-Ridge et al., 2013). In tropical peat soils, it is generally believed that nitrification can be neglected because of the extremely acidic condition (pH_{water} <4.5). However, it has become widely accepted that nitrification can occur in a wide range of acid soils (<5.5) (De Boer & Kowalchuk, 2001). Even the ultra-acidic soils (pH_{water} ~3) have been reported to support nitrification. Moreover, natural tropical peat soils are in water saturated condition which suppressed aerobic microbial activity (Furukawa et al., 2005). However, in cultivated peat soils especially with oil palm, water table is normally lowered to about 60 cm to allow oil palm roots well aerated (Mutert et al., 1999). Through this, nitrification can become prominent as aerobic conditions are enhanced (Regina et al., 1996). This is also evident through production of high N_2O gas upon cultivating peat soils with oil palm (Melling et al., 2007; Sakata et al., 2015). Many studies have been focused on N_2O release in oil palm cultivated on peat soils (Arai et al., 2014; Jauhiainen et al., 2012; MÜller et al., 2016; Melling et al., 2007), but only few studies emphasized on nitrification in tropical peat soils and oil palm cultivated peat soils (Nurulita et al., 2016; Pett-Ridge et al., 2013).

In addition, cultivated peat land (mainly oil palm in Malaysia) received of N fertilizer (140-150 kg N/ha) for yield optimization (Anuar et al., 2008; Schroth et al., 2000; Tung et al., 2009). Addition of N fertilizer generally stimulate nitrification as it served as available substrates for the nitrifiers community (He et al., 2007; Shen et al., 2014). For the past decade, it was assumed that the process of ammonia oxidation, the rate limiting step of nitrification, was restricted to a small range of autotrophic bacteria belonging to β-subclasses of *Proteobacteria* (Bothe et al., 2000). Collectively known as the ammonia-oxidizing bacteria (AOB), these microorganisms have been extensively studied in terms of their biochemistry, physiology, contribution and factors affecting their activity towards nitrification (Bowatte et al., 2006; Chu et al., 2007). However, the recent discovery of ammonia oxidizing archaea (AOA) from the *Crenarchaeota* lineage challenges the assumption that nitrification in soil is chiefly dominated by AOB (Treusch et al., 2005; Venter et al., 2004). It has been suggested that the AOA are favored over AOB at lower soil pH (Nicol et al., 2008) and lower NH_4^+ availability (Martens-Habbena et al., 2009). In addition, AOA generally dominates fertilized agricultural soils especially with ammonia fertilizers (Hayatsu et al., 2008; Leininger et al., 2006) mainly due to the use of urea, including other N fertilizers in agriculture have been shown to be used through hydrolysis for autotrophic nitrification (Burton and Prosser., 2001). Therefore, added N source could be one of the contributing factors of nitrification in tropical peat soils cultivated with oil palm as it affects nitrifiers diversity. However, up to date, very few literature can be found on nitrifiers population, diversity and abundance in tropical peat soils used for oil palm cultivation.

Various physical, environmental and chemical factors affect soil nitrification. Specifically, nitrification process is affected by NH_4^+ availability, oxygen (O_2) supply and temperature. These factors affect the nitrifying population (type, density and diversity) and also regulate NH_4^+ concentration and availability (Krave et al., 2002). Typical microbes are sensitive to disturbance in pH. Acidic condition will decrease their growth and activity because they are unable to regulate their intracellular pH value when there is environmental changes in pH (Rousk et al., 2010). However, nitrifiers possess survival mechanisms that permit them to remain alive and well distributed in different terrestrial ecosystems. This is through their autotrophic nature which enables them to create biomass through biosynthetic pathways (Allison & Prosser, 1991). At the same time, nitrifiers cell also can be maintained under starvation condition through low level of cytoplasmic respiration and lower anabolic processes to undetectable levels (Hagopian & Riley, 1998). Nonetheless, information on nitrification rate in oil palm under tropical peat soils and the major factors influencing it such as soil moisture, substrate availability and land use remained limited and not well characterized. It is hypothesized that nitrification is prominent in in oil palm cultivated peat soils and the process rate is influenced by substrate availability, soil moisture and availability of acid-tolerant microorganisms to perform nitrification.

1.1 Objectives of the study

Generally, there is a need to elucidate the nitrification status in oil palm under tropical peat soils. Furthermore, there is lack of information on the microbial part of nitrification as well. This study aimed a) to determine the nitrification rate in tropical peat soil in relation to the major factors influencing it. The study also b) identified and characterized the microorganism involved in nitrification including their abundance. Further understanding in this process is expected to contribute to knowledge of the N cycling and losses which affect nutrient use efficiency. A better understanding of nitrification rates and their regulation helps research effort towards increasing N-use efficiency and maintaining environmental quality especially in situations where the loss of applied N following nitrification is high. It will also provide strong basis for decision making that relates to fertilizer management in peat soils.

The present study was carried out with the following specific objectives:

I. to determine the influence of operational zones, harvesting path (HP), weeded circle (WC) and frond heap (FH) and their depths on potential nitrification rate and inorganic N (N_i) availability related to soil physicochemical parameters,
II. to determine the dynamic of NH_4^+, NO_3^-, N_i and potential nitrification rate (PNR) in relation to different N source (Urea and Ammonium sulphate) and rate,
III. to determine NH_4^+, NO_3^-, N_i and PNR under different moisture levels and fluctuating moisture condition with and without the addition of urea,
IV. to compare NH_4^+, NO_3^-, N_{in} and PNR in oil palm under peat soils with different rates of urea applied and in combination with different leguminous cover crops (LCC) (agronomic practices),
V. to determine the interactions between agronomic practices and seasonal difference in NH_4^+, NO_3^-, N_{in} and PNR,
VI. to identify and quantify the type of nitrifiers available in peat soil cultivated with oil palm and understand their distribution at different operational zones, depth, agronomic practices and season

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