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Abstract: Tropical peat swamp forest beds that have been reclaimed for agricultural use are generally an active source of nitrous oxide (N_2O) efflux, however, the mechanism by which reclaimed tropical peat soils promote the emergence of N_2O emistion potential and its correlation with mineral nitrogen contents in reclaimed soils. Using a culture-based N_2O emission assay, the N_2O emission potentials of soil at various depths (0-450 cm) were investigated in two oil palm plantations in Sarawak, Malaysia, which had elapsed times of two years (E2Y) and 10 years (E10Y) after deforestation, respectively. On the basis of the relationship between the vertical profiles of N_2O emission potentials and the contents of mineralized nitrogen in the peat soils at various depths, the impact of land management on soil microbial communities was discussed. The peat soil at plantation site E2Y showed a trend of high N_2O production in deep layers (200-400 cm), whereas the older plantation site E10Y showed considerably more active N_2O emission potentials that were inversely proportional to the contents of NO_3^- . This contrasting vertical correlation between N_2O emission potentials and mineralized nitrogen contents in bulk soils suggests that active N_2O emission in deep layer (C/N) ratio of the peat soil, whereas at E10Y, such a regulatory system has been lost due to advanced soil degradation, leading to dynamic changes in the nitrogen cycle in shallow soil.

Key words: N₂O emitters, tropical peat soil, vertical N₂O emission potential, C/N ratio, oil palm plantation.

1. Introduction

Nitrous oxide (N_2O) is a greenhouse gas responsible for about 7% of global warming [1] and for depletion of the stratospheric ozone layer [2-4]. Globally, the total anthropogenic N₂O emission in 1994 was estimated at 17.7 Tg·N/yr [5], with a minimum estimate of 58% being a byproduct of agriculture and 38% specifically from farm soil [1, 6]. Tropical peat swamp forests, particularly reclaimed for agricultural use, are prominent N_2O hot spots [7-10]. The closed chamber method, which can measure gas flux across soil surface, is the most common technique for monitoring trace gas [11, 12]; however, this approach only covers an area at the bottom of the chamber and is used to estimate total N_2O emission from deep to shallow top soils. Although some methods for vertical distribution of gas efflux potentials have been established for CO_2

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[13, 14] and N₂O using a lysimeter [15], much simple ways of examining N₂O productivity at different depths of the bulk soil has not yet been established. To discuss more on the correlation between vertical distribution of N₂O emission potentials at different depths and NO₃⁻ and NH₄⁺ contents of the oil palm plantation soils should be determined.

In this study, the vertical distribution of N_2O -emitting potentials in oil palm (*Elaeis guineensis*) plantation soils was investigated using a culture-base N_2O emission assay for soil microbial communities. Two reclaimed peatlands that had been used for oil palm production for two and 10 years were compared for vertical distribution patterns of their N_2O emission potentials and mineral nitrogen contents.

2. Materials and Methods

2.1 Site Description

Soils were sampled from field experimental plots on two oil palm plantation sites near Sibu, Sarawak, Malaysia, where permission was granted by a privately-owned oil palm plantation to Tropical Peat Research Laboratory. At one oil palm plantation (02°08'N, 111°54'E), the palms had been planted for

10 years prior to sampling (elapsed times of 10 years (E10Y) site), while at the other site $(02^{\circ}11'N,$ 111°50'E), the oil palms had been planted two years prior to sampling (elapsed times of two years (E2Y) site). Depths of the peat layer at E10Y and E2Y sites were 370 cm and more than 450 cm, respectively. Annual water table depths were monitored at the sites with a pressure-type water gauge connected to a data logger DL/N70 (Koshin, Tokyo, Japan) (Fig. 1). The study area was originally a secondary logged-over peat swamp forest consisting primarily of evergreens such as Shorea spp., showing a similar property of the peat soils. The E10Y site was previously an Alan swamp forest, which is generally a flooded swamp with associations of Shorea albida-Gonstylus-Stenonurus (locally known as Alan batu) or S. albida-Stenonurus consociation (locally known as Alan bunga) [16]. While, the E2Y site was located in a mixed peat swamp forest, which is characterized by associations of Gonystylus-Dactylocladus-Copaifera without S. albida. Both forests were opened for oil palm cultivation following clear-cutting, water drainage, soil compaction with heavy machinery, and chemical



Fig. 1 Water table depths in the sampling sites during 2009 and 2010.

The water table depth at the E2Y site was deeper than the E10Y site by June 2010. At the sampling time, January 2010, soil at E2Y was obtained before mitigation of water management. Bars \pm SD (standard deviation) (n = 3).

fertilization, and are now regularly fertilized and sprayed with herbicides and pesticides.

2.2 Soil Sampling

Vertical soil samples were collected during the rainy season in late January of 2010. Soil samples were collected at depth ranges of 0-5, 5-10, 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400 and 400-450 cm using a peat auger sampler. For data plotting on a dispersion diagram for correlation analyses between soil depth and other parameters, the depth of each soil sample was shown by each margin (e.g., in 150-200 cm, a margin of 175 cm). Soil samples (approximately 300 g each) were placed in clean polyethylene bags equipped with fasteners for ejection of air inside the bag and transported to the laboratory.

2.3 Soil Analysis

Soil samples were dried and analyzed for pH, NO₃, NH_4^+ , carbon-nitrogen (C/N) ratio, total C, total N, loss-on-ignition (LOI) and pyrophosphate solubility index (PSI). Soil pH was determined using a suspension of 10 g of soil in 25 mL of H₂O. The organic matter content was determined as LOI at 400 °C [17]. Total N and total C were determined using a CNS2000 carbon, nitrogen, sulfur analyzer (Leco, St. Joseph, MI, USA). The PSI was determined through pyrophosphate solution extraction followed by spectrophotometric analysis at 550 nm [18]. Soil NH_4^+ and NO_3^- were extracted in a soil:water (1:5) mixture [19] and analyzed using ion chromatography (Metrohm 761, Herisau, Switzerland). For the determination of NH₄⁺, a Metrosep C2-250 cationic column (Metrohm, Herisau, Switzerland) and a Metrosep C2 guard column (Metrohm) were used, and the eluent consisted of 4 mmol/L tartaric acid and 0.75 mmol/L dipicolonic acid (flow rate: 1 mL/min). Soil NO₃⁻ was determined using a Metrosep A SUPP 5 250 anionic column equipped with a Metrosep A SUPP 5 guard column (Metrohm). The eluent (flow rate: 0.7 mL/min) consisted of 1 mmol/L NaHCO₃ and 3.2 mmol/L Na₂CO₃. Total N and C were shown as percentage of air-dried soil (w/w), and from these N and C contents, the C/N value of each soil sample was calculated. The total N content of each wet soil sample was determined based upon the soil moisture content.

2.4 Culture-Base N₂O Emission Assay

The medium for the culture-base N_2O emission assay contained no sugar, but 5 mM KNO₃ (0.52 g/L) was added as a substrate for N_2O production. Winogradsky's mineral mixture served as the medium for the N_2O emission assay [20]. The medium was autoclaved, adjusted to a pH of 4.0 with 2 M H₂SO₄, and then solidified to a soft gel with 0.5% gellan gum (Wako Pure Chemical Industries Ltd., Osaka, Japan). After cooling, 10.0 mL aliquots of medium were poured into 30 mL gas chromatography vials equipped with butyl-rubber plugs (Nichiden-Rika Glass Co., Kobe, Japan). The headspace volume was kept at 22.6 mL [21].

Three portions (approximately 10 mg) of each soil sample were added to 10 mL of sterile water, vortexed for 30 s and allowed to stand for 10 min. A 100 μ L aliquot of each suspension supernatant was inoculated onto the soft gel medium contained in a gas chromatography vial, leaving a 22.5 mL headspace volume. The vials were vortexed thoroughly and incubated at 25 °C in the dark for 7 d. Headspace gas (50 µL) was sampled from each vial using a Hamilton gas-tight syringe and injected into a gas chromatograph equipped with a 1 m Porapak N column (Waters, Milford, MA, USA) maintained at 60 °C and an electron-capture detector (Shimadzu GC-14B, Kyoto, Japan) maintained at 340 °C. The carrier gas consisted of Ar and 5% CH₄. The N₂O emission data were not corrected for the time lag associated with analysis (max. 1 h) due to the comparatively long duration of the incubation period (168 h). Production of N₂O is expressed as either µg/mL or ng/mL in the headspace gas, during the 7-day incubation.

3. Results

3.1 Characteristics of Sarawak Peat Soils at E10Y and E2Y Sites

The chemical characteristics of the soil samples collected from the oil palm plantations in Sarawak, Malaysia, are presented in Tables 1 and 2. At the 2-year-old oil palm plantation (E2Y) site, the soil pH was less than 4 (pH 3.14-3.45) at all the depths (0-450 cm), indicating of acidic conditions in the soil ecosystem. The peat soil had an extraordinarily high LOI (approximately 97%-99%, determined using a

furnace), indicating that the peat soil contained 53%-87% w/w of water and 10%-45% of organic material. The average of duplicate PSI values for the soil samples ranged between optical density at 550 nm (OD₅₅₀) 22.1 and 56.6. Soils from the E2Y site consisted of 25%-57% total carbon (determined using oven-dried soil) and 0.8%-1.5% total nitrogen, resulting in a C/N ratio of 26-55.

Conversely, the E10Y site exhibited progressed peat soil degradation, which was shown particularly in the shallow soil layers by relatively low total carbon (15%-50%) and high soil pH (pH 5.32 at the topsoil, *cf*.

 Table 1
 Physicochemical properties of soils collected at different depths from a 10-year-old oil palm plantation (site E10Y).

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Depth (cm)	Soil pH^{\dagger} (H ₂ O)	Loss of ignition [†] (%)	PO ₄ ⁻ (mg/kg)	NO ₃ ⁻ (mg/kg)	NH4 ⁺ (mg/kg)	Total C	Total N	C/N			
0-5	5.0 ± 0.2	90.3 ± 0.0	1.42	41.8	15.28	29.0	1.1	25.5			
5-10	4.8 ± 0.1	92.7 ± 0.0	2.03	30.1	3.93	20.5	0.7	27.5			
10-20	4.4 ± 0.1	93.4 ± 0.0	1.94	42.6	0.17	34.5	1.2	29.8			
20-30	4.0 ± 0.2	94.3 ± 0.1	4.68	18.5	14.43	35.3	1.1	32.7			
30-50	3.9 ± 0.1	91.2 ± 0.1	1.29	7.9	16.37	34.4	0.9	36.9			
50-100	3.9 ± 0.0	94.6 ± 0.2	0.07	4.6	7.80	48.0	1.2	41.0			
100-150	3.8 ± 0.0	94.3 ± 0.3	0.00*	0.6	0.01	48.4	1.3	37.7			
150-200	3.8 ± 0.1	96.7 ± 0.1	0.38	4.8	0.04	34.6	0.8	45.4			
200-250	3.8 ± 0.0	96.9 ± 0.1	0.09	2.4	0.01	46.1	1.0	48.2			
250-300	3.7 ± 0.1	97.0 ± 0.4	0.00*	4.1	0.01	49.7	1.0	50.6			
300-350	3.6 ± 0.0	97.5 ± 0.1	0.00*	4.2	0.00*	14.7	0.4	35.9			
350-400	4.1 ± 0.1	24.7 ± 0.7	0.00*	1.3	0.02	0.3	0.3	0.8			

[†]Values show the mean \pm SD, n = 3, *less than the detection limit.

 Table 2
 Physicochemical properties of soils collected at different depths from a 2-year-old oil palm plantation (site E2Y).

Depth	Soil pH [†]	Loss of ignition [†]	PO ₄ ⁻	NO ₃ -	$\mathrm{NH_4}^+$	Total C	Total N	C/N
(cm)	(H_2O)	(%)	(mg/kg)	(mg/kg)	(mg/kg)	Total C	I Otal IN	
0-5	3.4 ± 0.0	96.9 ± 0.2	1.14	45.2	0.11	35.0	1.4	25.9
5-10	3.3 ± 0.0	97.9 ± 0.2	0.45	53.2	0.03	31.9	1.2	27.6
10-20	3.3 ± 0.0	98.0 ± 0.1	0.25	95.7	0.03	44.4	1.5	29.8
20-30	3.2 ± 0.1	97.8 ± 0.1	7.11	146.0	7.68	45.7	1.5	30.1
30-50	3.2 ± 0.1	97.6 ± 0.2	7.29	150.8	0.04	25.1	0.9	29.5
50-100	3.1 ± 0.1	97.5 ± 0.3	6.30	214.0	32.36	38.3	1.1	34.3
100-150	3.4 ± 0.1	98.3 ± 0.1	4.41	8.9	13.47	41.9	1.0	41.3
150-200	3.5 ± 0.1	97.6 ± 0.3	0.00*	2.7	4.19	40.8	1.0	38.0
200-250	3.4 ± 0.0	98.2 ± 0.1	0.00*	6.6	9.23	56.8	1.4	40.6
250-300	3.3 ± 0.0	98.8 ± 0.2	0.00*	1.1	0.33	40.3	0.8	49.3
300-350	3.4 ± 0.1	98.4 ± 0.1	0.01	2.5	0.55	48.8	0.9	55.2
350-400	3.3 ± 0.0	98.5 ± 0.0	0.00*	7.6	0.71	52.4	1.0	51.7
400-450	3.2 ± 0.1	97.6 ± 0.1	0.00*	5.1	1.16	49.1	1.0	47.2

[†]Values show the mean \pm SD, n = 3, *less than the detection limit.

pH 3.62 was the most acidic layer at a depth of 300-350 cm). The soil degradation resulted in compaction and subsidence of the soil at the E10Y site, while E10Y soil obtained from depth of 350-400 cm was obviously mineral soil. At the depth of 350-400 cm, the mineral soil was highly affected by the upper peat layers, showing a total carbon value of 0.3% (Table 1).

The water table depths at the sampling sites showed a clear difference (Fig. 1). In 2009, the E2Y site was relatively dry due to excessive water drainage. The different moisture conditions of each site may have affected N₂O emissions associated with effective nitrification and anaerobic denitrification in the bulk soils. For appropriate water and land managements at the E2Y site, the water drainage was adjusted to mitigate water table in June 2010.

3.2 N₂O Emission by Soil Suspension Cultures in KNO₃-Containing Medium

The level of N₂O present in the headspace gas of vials inoculated with medium used to wash soil samples showed a clearly different pattern between the two sites. As shown in Fig. 2, the shallow soils (0-100 cm) at E10Y showed highly active N₂O-emitting potentials. The most active N₂O-emitting potential shown by the culture-base N₂O emission assay was found in soils collected at depths of 0-5 cm, and all the soil layers up to 50 cm

were active. Conversely, deeper soils (100-450 cm) at the E10Y site showed relatively low levels of N_2O emission, except for the peat soil at 350-400 cm that was highly affected by mineral soils at the base (C/N ratio, 0.8) (Table 1).

In contrast to the soils at E10Y site, suspensions of shallow soils (0-100 cm) from the E2Y site showed no significant emission of N₂O. Deeper peat soils from E2Y were, however, more active than the shallow soils; the highest rate of N₂O emission (0.70 \pm 0.13 µg/mL) was observed in the soil collected at 350-400 cm, of which total C content was 52.4% (Table 2).

3.3 Correlation of N₂O Emission Potentials with Mineral Nitrogen Contents in Soil

N₂O emission potentials observed in the soils at different depths showed a significant correlation with contents of NO₃⁻ in the tropical peat soils at both sites, E10Y and E2Y (Fig. 3). At E10Y, where oil palms had been planted for 10 years, a positive correlation ($y = 0.02 x^{0.91}, R^2 = 0.25$) was found between the vertical gradient of NO₃⁻ concentration in dry soil and the N₂O production potentials of soil bacterial communities shown by the N₂O emission culturing assay. Also, a high positive correlation was also observed between N₂O emission and the concentration of NH₄⁺ in dry soil ($y = 0.000016 x^{1.66}, R^2 = 0.72$) at the site E10Y (Fig. 3a).



Fig. 2 Vertical distribution of N₂O emission potentials at different soil depths from the two oil palm plantation sites. Soil samples from E10Y (a) and E2Y (b) were kept at 4 °C until they were used for inoculation. Bar = \pm SD. At E10Y, the deep soil layer at 350-400 cm was highly affected by the mineral soil. Hence, this soil layer should be distinguished from other peat soils.

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Fig. 3 Correlation between N_2O emission potential and NH_4^+ and NO_3^- concentrations in soil of 10-year and 2-year cultivated oil palm plantation soils.

Correlation coefficients are given as R (n = 13 for E2Y and n = 11 for E10Y). Concentrations of NH₄⁺ (\blacktriangle) and NO₃⁻ (\bullet) in dry soils of E10Y (a) and E2Y (b). At E10Y (a), NH₄⁺ content of the deep soil at 300-350 cm was less than the detection limit. Hence, this soil was excluded from the plots. Both of the *x*- and *y*-axes are shown as logarithmic scales.

Conversely, N₂O emission potential was in inverse proportion to NO₃⁻ content in the soil at the E2Y site $(y = 190.2 x^{-0.62}, R^2 = 0.23)$, while no correlation was showed between NH₄⁺ contents and virtual N₂O emission ($R^2 = 0.05$) (Fig. 3b). In contrast to the shallow soils at E10Y site, the deep E2Y soils (at depth of 100-450 cm) with relatively high N₂O emitting potentials contained low levels of NO₃⁻ (1-9 mg/kg dry soil, Table 2). In the E2Y site, soils at the shallow layers rather contained higher NO₃⁻ than those of E10Y site (45-214 µg/kg dry soil). As the shallow soils at E2Y site, which did not show any N₂O emission potentials, the topsoil at this site resulted in accumulation of NO₃⁻ but almost no conversion of NO₃⁻ into N₂O gas.

3.4 Relationship between C/N Ratio of Peat Soil Layers and N_2O Emission Potentials

We determined that the C/N ratio and N₂O emission

of the soil collected from each depth at the E10Y site were in inverse proportion (Fig. 4, shown by the green repression line). A high potential of N₂O emission in shallow soils of E10Y, in which soil degradation had accumulated a relatively high concentration of NO₃⁻, showed convergence of the soil C/N ratio within a range from 25.5 to 36.9. The equation of $y = 59.0 x^{-0.094}$ in E10Y soils described the inverse proportion to the C/N ratio and N₂O emission potentials.

Conversely, E2Y soils showed a direct proportion between the two measures (Fig. 4, shown by the red regression line). A relatively high potential of N₂O emission in the deep soils of E2Y, in which soil degradation had progressed less, showed increased remediation activity throughout due to high denitrification potentials. This was determined by elevated N₂O emission potentials and clear convergence of the C/N values at around 50 or more with an equation of $y = 27.2 x^{0.101}$.



Fig. 4 Correlation between soil C/N ratio and N_2O emission potential by a culture assay.

Correlations between C/N ratios of more than 20 (for peat soil or peat soil affected by mineral soils) and N₂O emissions in cultures of the soils, are plotted on the diagram. Soils collected from E10Y ($\mathbf{\nabla}$, n = 11, after removal of peat soil highly affected by mineral soil at 350-400 cm) and E2Y ($\mathbf{\Phi}$, n = 13) are respectively shown by their primary regression equation with mathematical schemes on the diagram. Both of the *x*- and *y*-axes are shown as logarithmic scales.

4. Discussion

4.1 Relation of Land Management and Progressive Soil Degradation with N₂O-Emitting Potentials in Tropical Peat Soil

In the soils from E10Y and E2Y sites, correlations between N₂O emission potentials and NO₃⁻ content were obviously incompatible with each other. A report by McCarty et al. for nitrogen deposition and denitrification activity of riparian wetland soils in Maryland, US, suggests that natural wetland accumulates more NO₃⁻ at shallow layers of the soils due to less active denitrification in soils [22]. At E2Y site of oil palm plantation, active accumulation of NO₃⁻ observed at shallow soil layers is proximate to the natural wetland ecosystems. Although a high accumulation of NO₃⁻ in the shallow soils of E2Y is partly due to nitrification, the anthropogenic water drainage and chemical fertilization, low N₂O emitting potentials at the shallow soils of E2Y indicate that N_2O emitters rarely existed in the tropical peat swamp forest bed and 2-year-cultivated oil palm plantation soils. Instead, it is more natural to consider that the N_2O emitters inhabiting deeper soils (200-400 cm) are native soil microorganisms. It is suggested that, at the E2Y site, the deep soils do not contribute to N_2O emission from the young oil palm plantation since the deep soils contains almost zero levels of NO_3^- , and N_2O in deep soils may be quenched by some other denitrifiers similar to that reported in a paddock soil [23].

Conversely, the direct proportion between N_2O -emitting potentials of the soil suspension cultures and the NO_3^- in the E10Y soil suggests that constant provision of NO_3^- in the shallow soils (8-43 mg NO_3^-/kg of dry soil, Table 1). Since shallow soils (0-50 cm) at the E10Y site accumulated NO_3^- due to nitrification and frequent chemical fertilization for oil palm management, it was an active efflux source of N_2O . Rain squall probably interrupts oxygen supply to the topsoil, leading to a temporal anaerobic soil condition.

Our culture-base N_2O emission assay inoculated with peat soil suspensions from the shallow layers at the E10Y site showed active N_2O emission only when NO_3^- was supplied to the culture medium. Therefore, it was suggested that, at the E10Y site with highly advanced soil degradation, supplemental NO_3^- activate mainly denitrifiers in the shallow soil layers to produce N_2O (Fig. 3a). Indeed, NH_4^+ supplied to the culture medium instead of NO_3^- never resulted in N_2O emission into the headspace.

4.2 Roles of N₂O Emission in Soil Ecosystems, and Its Link to the Soil C/N Ratio

The C/N ratio inversely related with N_2O emission potentials in the E10Y soils suggests that nitrogen-rich peat soils with low C/N ration allow for the emergence of denitrifiers in the soil microbial communities. These denitrifiers serve the purpose of

increasing the C/N ratio. Conversely, the positive correlation between C/N values and N₂O emission in E2Y soil suggests that denitrification, including N₂O emission, does not regulate C/N values of the peat soil, probably due to secondary forest trees most contributing to adjust soil C/N value within a range of 38.0 to 55.2. It is seemed that young oil palms with their poor root system rarely affect soil C/N values within 2-year cultivation.

It is well known that the soil [24], bacteria (*Erwinia* sp.) [25], plants (*Arabidopsis*) [26], and even the forest ecosystem itself [27] control nitrogen balance by themselves. Denitrifying bacteria play a particularly important role in regulating the concentration of mineral nitrogen in peat soil ecosystems [28]. As detected in our culturing assay, active N₂O emission by denitrifying bacteria in the degrading soil itself could play a role in adjusting the nitrogen content of the anthropogenic ecosystem of the oil palm plantation.

4.3 Contribution of Soil Conditions to Denitrification and N₂O Emission

In pristine tropical peat swamp forest beds, relatively high redox potentials (> 200 cm to 400 cm deep) are often observed as similar vertical profiles, and this is partly due to the presence of below-ground water flow [29]. It is still a mystery why farming soils of the 10- and 2-year-old oil palm plantations show such drastic differences in the vertical distribution of N₂O-emitting potentials shown in Fig. 1. Experiments with tracers indicate that 61%-92% of N₂O efflux in temperate agricultural soils is derived from denitrification [30]. Our studies suggest that the relatively high level of N2O emission observed in deeper E2Y soils containing only trace levels of NO₃⁻ can be attributed to denitrifying bacteria. Indeed, anaerobic denitrifiers are often found as survived community in deep, nitrate-free, anaerobic sediments lying beneath fresh water [31].

4.4 Contribution of Human Activity to N_2O Efflux in Tropical Peat Soils

Using the chamber method, Melling et al. [32] found that N₂O efflux from the soil of an oil palm plantation on reclaimed peat land in Sarawak, Malaysia, was lower than that from peat soil used for the cultivation of sago. The oil palm plantations produced 0.9-58.4 μ g N₂O/m²·h, of which the highest observed rate was only 33% of that observed on unfertilized sago plantation farmland (1.0-176.3 μ g N₂O/m²·h).

Chemical fertilization with minerals and nitrogen, and application of herbicides and pesticides, are particular land management strategies used on oil palm plantations. Chemical fertilization activates microorganisms residing in the surface horizon, accelerating decomposition of peat and efflux of CO₂ from shallow soil layers [9, 33, 34].

5. Conclusions

On an average, it has been calculated that relatively deep forest peat layers in Sarawak accumulating at a rate of 1 m took 200-400 years [35]. Hence, it is natural to think that difference of the tree species in the secondary forests before the reclamation between the two sites less affected original community structures of soil microorganisms, mainly eubacteria and archaea. Our current study suggests that the emergence of active N₂O-emitting microorganisms in land reclaimed from tropical peat swamp forests was accelerated in the oxidatively decomposed layers of soil near the surface (at a depth of 5-100 cm) from the E10Y site, while soil collected at a depth of 100-350 cm did not display this property (Fig. 2a). In contrast, the absence of N₂O production in the more shallow layers of the soil, and the high N₂O production in soil over depths of 100 cm on E2Y, represents a pristine state of soil microbial communities or a transient state of soil degradation (Fig. 2b). Further investigation of N₂O emittable bacteria in the soil microbial community for these soils is warranted.

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