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Research Article

การประเมินจุลินทรีย์และแร่ธาตุบางชนิดบริเวณรอบรากตองกง *(Thysanolaena latifolia)* จากอำเภอแม่สอด จังหวัดตาก ประเทศไทย

Assessing the soil bacterial community and minerals around roots of tiger grass *(Thysanolaena latifolia)* from Mae Sot District, Tak Province, Thailand

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาประชากรจุลินทรีย์และแร่ธาตุในดินรอบรากต้นตองกง (*Thysanolaena latifolia*) จากพื้นที่รกร้างในอำเภอแม่สอด จังหวัดตาก ประเทศไทย จำนวน 3 พื้นที่ ได้แก่ ดินในพื้นที่ 1 เป็นตัวอย่างดินที่เก็บจากพื้นที่ที่เคยปลูกข้าวโพดเลี้ยงสัตว์ ดินบริเวณพื้นที่ 2 เป็นพื้นที่ที่ยังไม่เคยใช้ประโยชน์ในการเกษตร พื้นที่ 3 เป็นตัวอย่างดินจากพื้นที่ที่เคยปลูกกะหล่ำปลี สำหรับการวิเคราะห์กลุ่มประชากรจุลินทรีย์ด้วย HiSeq 2500 System ของ Illumina เพื่อดำเนินการวิเคราะห์ลำดับนิวคลีโอไทด์ของแบคทีเรียที่ตำแหน่ง V3-V4 ของยีน 16S rRNA นอกจากนี้ยังมีการวัดค่า pH ความชื้นในดิน อุณหภูมิ การนำไฟฟ้า อัตราส่วนคาร์บอน (C)/ไนโตรเจน (N) โพแทสเซียม (K) ฟอสฟอรัส (P) กำมะถัน (S) อะลูมิเนียม (Al) แมงกานีส (Mn) นิกเกิล (Ni) ทองแดง (Cu) เหล็ก (Fe) และสังกะสี (Zn) จากผลการทดลองหน่วยอนุกรมวิธานเชิงปฏิบัติการ (OTU) ของจุลินทรีย์ในพื้นที่ที่ 1, 2 และ 3 เท่ากับ 4,226, 3,937 และ 2,958 ตามลำดับ

OTU ที่เหมือนกันทั้ง 3 พื้นที่เท่ากับ 1,684 โดยกลุ่มแบคทีเรียในระดับไฟลัม "*Proteobacteria*" มีอยู่มากในพื้นที่ที่ 2 และ 3 ในทางตรงกันข้าม "*Acidobacteriota*", "*Planctomycetota*" และ "*Verrucomicrobiota*" มีอยู่สูงในพื้นที่ที่ 1 แต่ "*Candidatus* Thermoplasmatota" มีอยู่มากมายในพื้นที่ที่ 2 นอกจากนี้ *Methylobacillus* และ *Pseudarthrobacter* พบมากในพื้นที่ที่ 2 แต่ *Sphingomonas* สูงในพื้นที่ที่ 3 ค่า pH ความชื้นในดิน อัตราส่วน C/N, K, Ni, Cu, Fe และ Zn มีความแตกต่างอย่างเป็นนัยสำคัญทางสถิติระหว่าง 3 กลุ่ม ผลลัพธ์ที่ได้นี้เป็นข้อมูลสำคัญที่จะเพิ่มองค์ความรู้ด้านจุลินทรีย์และแร่ธาตุในดินรอบรากต้นตองกงในพื้นที่รกร้าง กลุ่มแบคทีเรียที่เด่นและแร่ธาตุในดินอาจขึ้นอยู่กับลักษณะของพื้นที่ที่แตกต่างกัน นอกจากนี้ข้อมูลดังกล่าวจะช่วยสนับสนุนการกลับมาฟื้นฟูการใช้ประโยชน์ในอนาคต

คำสำคัญ: ตำแหน่ง V3-4; เทคโนโลยีการหาลำดับเบส; จุลินทรีย์; พื้นที่สูง; ตองกง

Abstract

This study aimed to investigate the soil bacterial community and minerals near the roots of tiger grass (*Thysanolaena latifolia*) from Mae Sot District, Tak Province. Soil samples were collected from three sites. Site 1 was an area that had been cultivated for maize. Site 2 had never been used for agriculture. Site 3 had been cultivated for cabbage. For the microbiota analysis, a HiSeq 2500 system was used to perform Illumina next-generation sequencing to obtain the bacterial sequences targeted to V3-V4 of the 16S rRNA gene. Moreover, pH, soil moisture, temperature, electrical conductivity, the C/N ratio, and the contents of potassium (K), phosphorus (P), sulfur (S), aluminium (Al), manganese (Mn), nickel (Ni), copper (Cu), iron (Fe) and zinc (Zn) were evaluated. The operational taxonomic units (OTUs) of the microbes at sites 1, 2 and 3 were 4,226, 3,937 and 2,958, respectively. The number of OTUs shared by all the samples and sites was 1,684. In phyla, "*Proteobacteria*" was the most abundant at sites 2 and 3. In contrast, *"Acidobacteriota"*, *"Planctomycetota"* and "*Verrucomicrobiota*" were most abundant at site 1, but "*Candidatus Thermoplasmatota"* was predominant at site 2. In terms of genera, *Methylobacillus* and *Pseudarthrobacter* were the most abundant at site 2, but *Sphingomonas* was predominant at site 3. The values for pH, soil moisture, the C/N ratio, and the contents of K, Ni, Cu, Fe and Zn were significantly different among the three sites. These results may provide important data to support an understanding of soil microbes and minerals in waste lands. The results revealed that the core bacterial elements may depend on the characteristics of the soil and environment. In addition, these data may help support the restoration of land in the future.

Keywords: V3-4; NGS; Microbes; Highland; Tiger Grass

Introduction

Tiger grass, *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda (formerly *Thysanolaena maxima*), is a perennial grass that belongs to Family the family Poaceae [1]. This family includes bamboos, goosegrass and cereal grasses [2]. In Southeast Asia, native tiger grasses are widely distributed throughout Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Vietnam and Thailand [3]. In addition, they are utilized

as ornamental plants and for making brooms [4]. The environment, including the climate, mineral content, soil moisture and temperature, is important for the growth of tiger grass [5], as is the diversity of microbes in the rhizosphere [6]. Many studies have investigated root-associated microbes to understand how the roots of different plant species in various environments are colonized by microbial communities [6-8]. The interactions between roots and rhizospheric microbes influence plant growth, plant development and root health [8]. Furthermore, Calvaruso et al. (2006) [9] reported that tree root-associated bacterial diversity increased soil mineral content. Rhizospheric microorganisms in the soil play crucial roles in enhancing and accumulating mineral nutrients [10]. In addition, Choudhary et al. [6] reported the different communities of rhizospheric bacteria in the soil of *T. latifolia* during seasonal changes in Barsua iron ore mines, India. However, the contribution of root-associated microbial diversity to soil mineral content remains unclear for certain plant species. [9]

For decades, the 16S rRNA gene has been used for routine analysis of sequence-based bacteria [11]. Next-generation sequencing is a useful tool to investigate the composition of microbial communities inhabiting soil environments, including plant roots [11]. Bulgarelli et al. [12] reported that bacterial structure and function are associated with the roots of wild and domesticated barley (*Hordeum vulgare*), suggesting host-microbe interactions at the root-soil interface.

In Thailand, tiger grass has spread throughout every region, including the highland areas, such as in western and northern Thailand [3, 13, 14]. In western Thailand, Mae Sot is a district in Tak Province, Thailand that is considered a special economic zone. This district comprises industrial areas, agricultural areas and tourist attractions [15]. Despite the existence of prior studies on *T. latifolia*, knowledge of tiger grass species remains poor. To address this knowledge gap, it is necessary to obtain preliminary data on tiger grass, especially the bacterial community and minerals from the rhizosphere of tiger grass collected from the highlands of Mae Sot District, Tak Province, Thailand.

Objectives

This study sought to assess the soil bacterial communities and mineral content surrounding the roots of tiger grass (*Thysanolaena latifolia*) from the highlands of Mae Sot district, Tak Province, Thailand.

Methods

The soils were sampled in Mae Sot district, Tak Province, in December 2022, from places where soil collection was already permitted by the landowners for academic research (Figure 1a). However, the location coordinates cannot published. Soil was collected from sites with varying soil used. The soil from site 1 was collected from an area that had been cultivated for maize. The soil from site 2 was never used for agriculture. The soil from site 3 was collected from an area that had been cultivated for cabbage. Each sampling site was approximately 5–10 km apart. The soil (2-3 kg.) around the roots of tiger grass (30–60 cm. from the soil surface) was removed and placed into sterile polyethylene bags. Soil was sampled

three times from different tiger grass bunches. The soil samples were stored at 4 °C before being transferred to the laboratory, where they were kept at -4 °C in freezers prior to the experiments.

To analyse the microflora diversity, 1 g of fresh soil was pooled from sites 1, 2 or 3. Three replicates were performed. Total genomic DNA was extracted *via* the Presto™ Soil DNA Extraction Kit according to the manufacturer's instructions. The total genomic DNA of each sample was amplified with the sparQ HiFi PCR Master Mix with universal primers (fw: ACTCCTACGGGAGGCAGCAG and rw: GGACTACHVGGGTWTCTAAT) targeting the 16S rDNA V3-V4 hypervariable region. The PCR conditions were 98 °C for 5 min for predenaturation, followed by 30 rounds at 98 °C for 2 min for denaturation, 57 °C for 40 s for annealing, and 70 °C for 2 min for extension. The last stage was a single cycle of final extension for 10 min. The precise of DNA size was confirmed by gel electrophoresis (1 % of agarose) and then were purified by using a QIAquick Gel Extraction Kit (Qiagen). The PCR products were assessed with the Qubit® dsDNA HS Assay Kit, and a library was constructed with the MetaVX Library Preparation Kit. The sequencing procedure was conducted with the Illumina MiSeq/NovaSeq sequencing platform and yielded operational taxonomic units (OTUs).

The OTUs were assessed using the software USEARCH and Naïve Bayesian Classifier. Taxonomic classifications were created against Greengenes and RDP. The relative abundance of the taxonomic rank and UPGMA (unweighted pair group method with arithmetic mean) tree were determined using R (v3.1.1). A venn diagram was created, and a heatmap was made in R (v3.4.10) and PICRUSt2 v2.3.0-b (V.3.4.10). The nomenclature used for prokaryotes referred to the list of prokaryotic names with standing in nomenclature (LPSN) (https://lpsn.dsmz.de/).

To measure the soil pH, 5 g samples were mixed with 10 ml of deionized water for 30 min. The soil and liquid were allowed to reach equilibrium, and the pH was measured with a portable Hach HQ40D metre. The soil moisture (%), temperature (°C) and electrical conductivity (mS/m) were tested via a Delta-T device WET sensor.

For the elemental analysis of the soil, 1 kg of each sample was dried at 105 °C for 72 hours, then ground and sifted through a 10 mm net. The soil samples were weighed to obtain 2 g and dissolved in hydrofluoric acid, perchloric acid and nitric acid at a ratio of 1:1:1 in a total volume of 20 ml. The samples were extracted and dried at 500 °C with a K-425 SpeedDigester. The residues of the samples were rinsed with 1% nitric acid and filtered through Whatman filter paper. The supernatant was transferred to a volumetric flask, and 1% nitric acid was added to prepare for the analysis of potassium (K), sulfur (S), aluminium (Al), manganese (Mn), nickel (Ni), copper (Cu), iron (Fe) and zinc (Zn). Available phosphorus (P) was analysed via the Bray II method [16] with a spectrophotometer. UV–visible spectrophotometry was used to analyse the percentage of sulfur. Moreover, available carbon and nitrogen in the soil samples were detected with a CHN628 analyser. One-way ANOVA with the Tukey post-hoc test was used to analyse the statistical differences between sites 1, 2 and 3 ($p < 0.05$) in the Statistical Package for the Social Sciences (SPSS) v.18.

Results

In the bacterial analysis, the number of bacterial OTUs at sites 1, 2 and 3 were 4,226, 3,937 and 2,958, respectively. The number of OTUs shared by all the samples and sites was 1,684. At sites 1 and 2, the number of shared OTUs was similar at 669, whereas at sites 1 and 3, the number of shared OTUs was 365. The number of OTUs shared between sites 2 and 3 was 2171 (Figure 1b). The UPGMA tree showed that site 2 was similar to site 3. In contrast, sites 2 and 3 were less similar to site 1 (Figure 1c). The bacterial OTUs from site 2 were more related to those of site 3 than to those of site 1 (Figure 2).

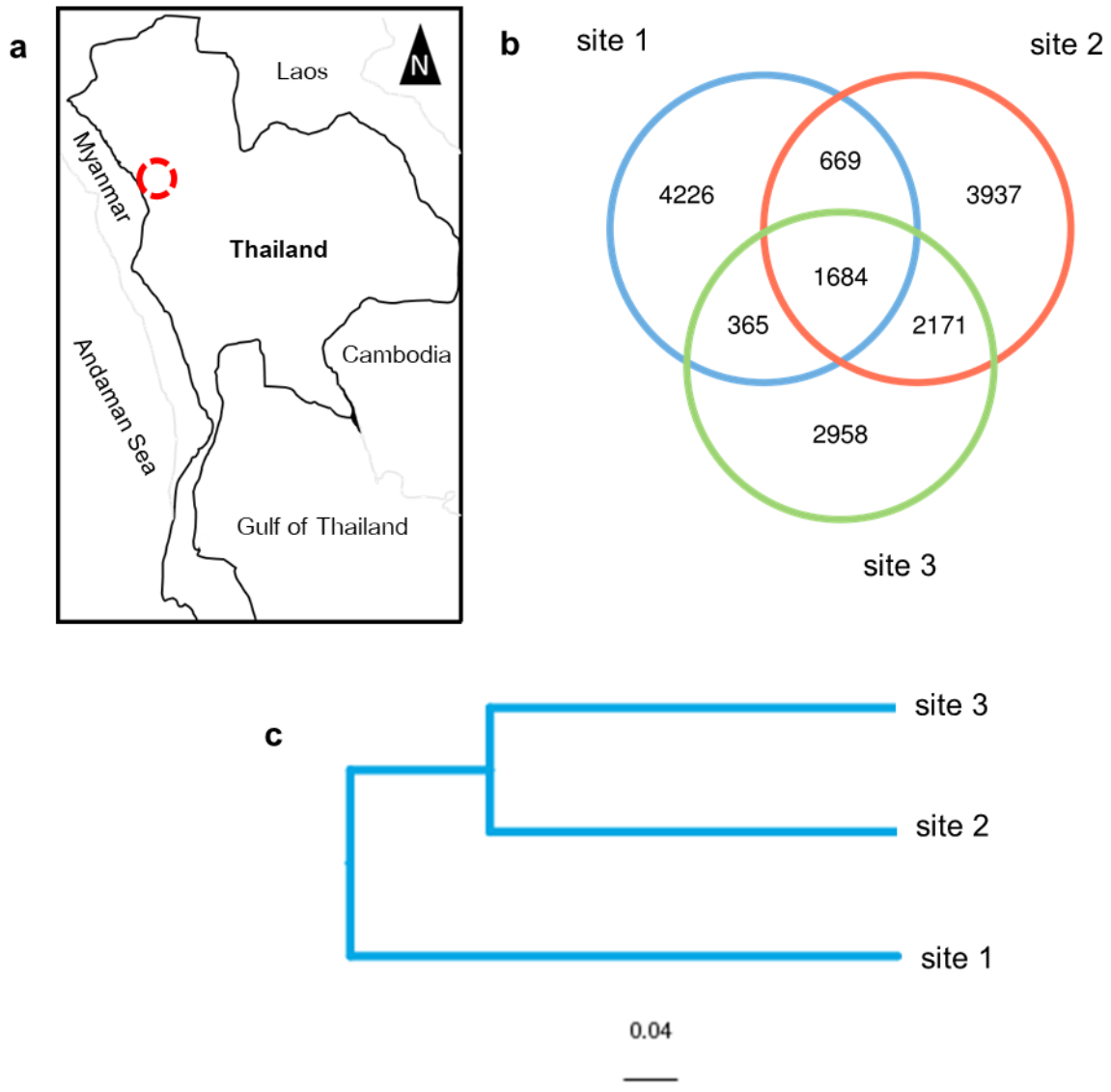

Figure 1 Locations of the sampling sites (red circles) in the highlands of Mae Sot district, Tak Province (a). A Venn diagram (b) and UPGMA tree (c) of the bacterial OTUs from the soil samples near the roots of tiger grass.

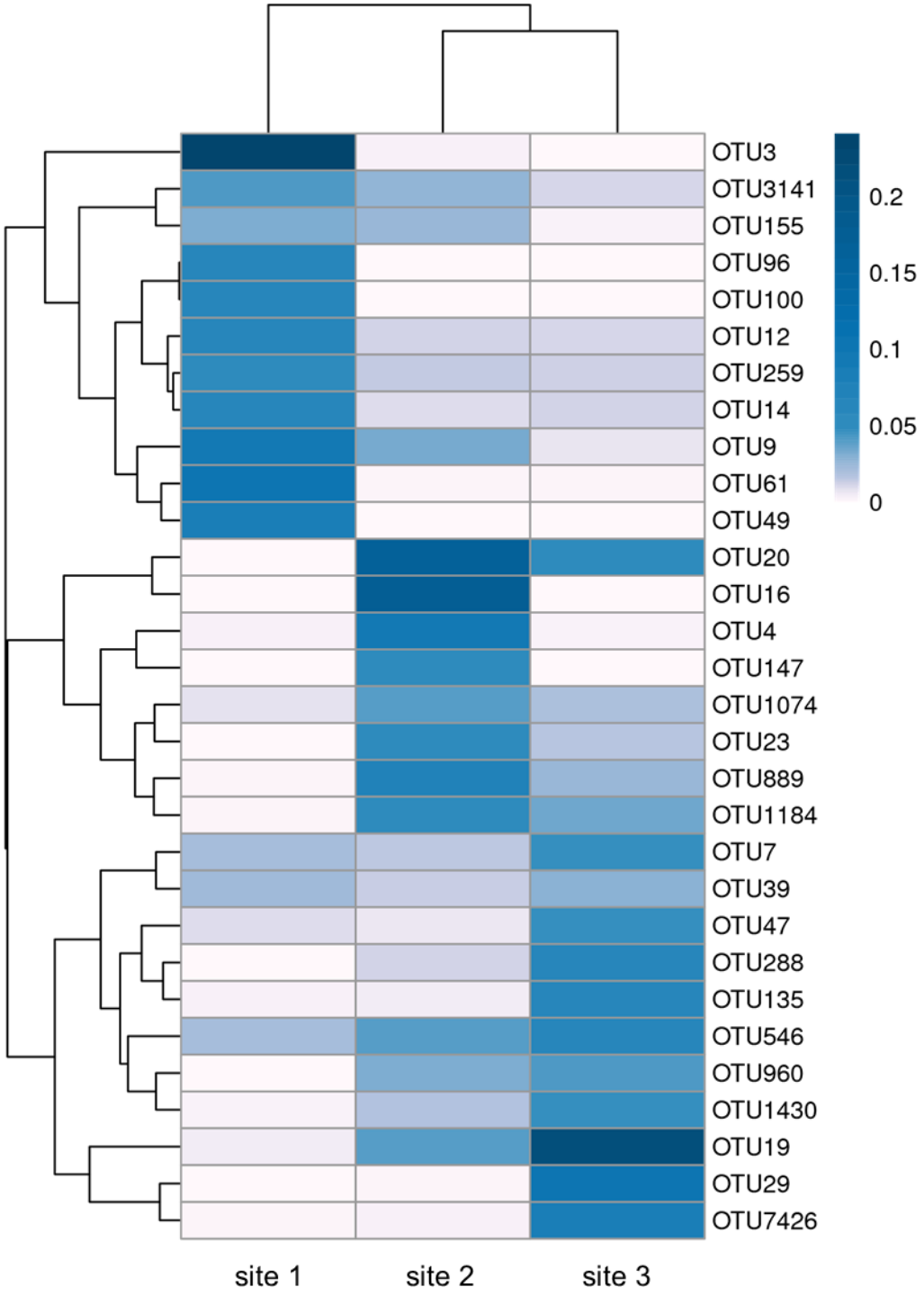

Figure 2 OTU heatmap from soil samples near the roots of tiger grass, which represents the top 30 OTUs with the highest abundance.

Regarding the relative abundance at the phylum level, "*Proteobacteria*" was predominant at sites 2 and 3. In contrast, *"Acidobacteriota"*, *"Planctomycetota"*, "*Candidatus Latescibacterota*" and "*Verrucomicrobiota*" were the most abundant at site 1, but *Candidatus Thermoplasmatota* was the

most abundant at site 2. In addition, *Nitrospirota* was more abundant at sites 2 and 3 than at site 1 (Figure 3). At the genus level, site 2 was dominated by *Methylobacillus* and *Pseudarthrobacter*. However, the abundance of *Sphingomonas* was greatest at site 3. The genus MND1 was more abundant at site 3 than at sites 1 and 2. Moreover, AKYG587 was highly abundant at site 1. The abundance of *Gaiella* was greater at site 3 than at sites 2 and 3 (Figure 4).

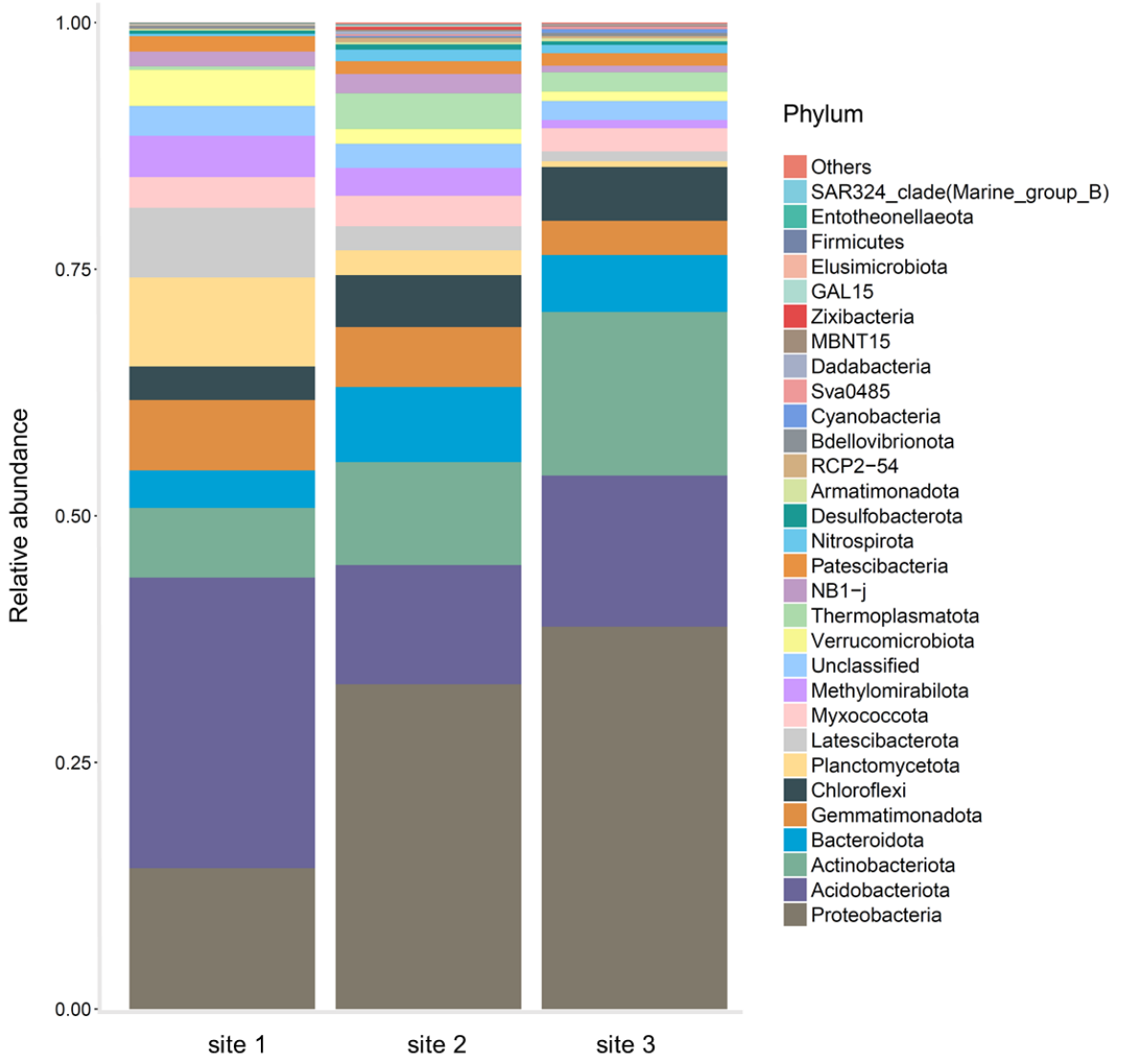

Figure 3 Stacked bar plot of the relative abundance of phylum-level classifications of bacteria in the soil samples surrounding the roots of tiger grass.

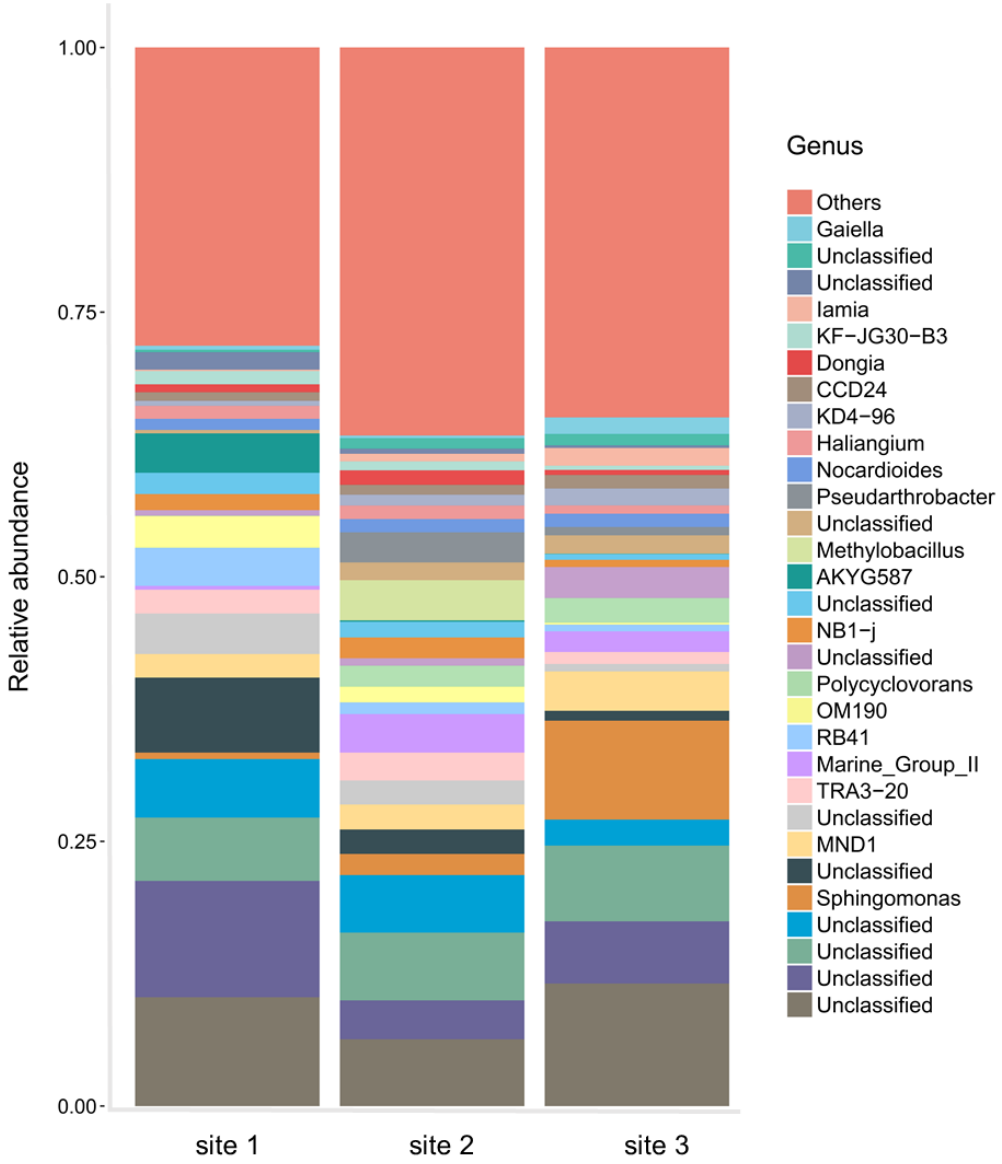

Figure 4 Stacked bar plot of the relative abundance of genus-level classifications of bacteria in the soil samples surrounding the roots of tiger grass.

The pH values (mean ± standard deviation) of sites 1, 2 and 3 were 6.23 ± 0.11, 5.96 ± 0.01 and 6.08 ± 0.06, respectively. The pH of site 1 was greater than that of site 2 ($p < 0.05$). The soil moisture values at sites 1, 2 and 3 were 3.77 ± 1.57, 8.8 ± 1.3 and 8.4 ± 0.55%, respectively. The temperatures at sites 1, 2 and 3 were 33.67 ± 2.4, 34.83 ± 2.6 and 35.23 ± 1.64 °C, respectively. In terms of the electrical conductivity of the soil, the values at sites 1, 2 and 3 were 0.02 ± 0.02, 0.06 ± 0.04 and 0.06 ± 0.03 mS/m, respectively. The moisture and electrical conductivity values were lower at site 1 than at sites 2 and 3 ($p < 0.05$). The C/N values at sites 1, 2 and 3 were 14.12 ± 0.83, 8.87 ± 0.31 and 8.1 ± 0.79, respectively.

The C/N ratio at site 1 was greater than at sites 2 and 3 ($p < 0.05$) (Table 1). In the elemental analysis of the soils, there was no significant difference among the sites in terms of P, S, Al or Mn. In contrast, the contents of K, Ni, Fe and Zn were significantly lower at site 1 than at sites 2 and 3 ($p < 0.05$). In addition, the amount of Cu at site 2 was greater than those at sites 1 and 3 ($p < 0.05$) (Table 1).

Table 1 Soil characteristics around roots of tiger grass from Mae Sot district, Tak province.

	site 1 (mean ± S.D.)	**site 2**	**site 3**
pH	6.23 ± 0.11a	5.96 ± 0.01b	6.08 ± 0.06ab
Soil Moisture (%)	3.77 ± 1.57a	8.8 ± 1.3b	8.4 ± 0.55b
Temperature (°C)	33.67 ± 2.4	34.83 ± 2.6	35.23 ± 1.64
Electrical Conductivity (mS/m)	0.02 ± 0.02	0.06 ± 0.04	0.06 ± 0.03
C/N ratio	14.12 ± 0.83a	8.87 ± 0.31b	8.1 ± 0.79b
K (mg/kg)	205.33 ± 55.18a	1349.67 ± 29.3b	1323.67 ± 100.38b
P (mg/kg)	1.75 ± 2.48	0.11 ± 0.03	0.91 ± 0.34
S (%)	0.1 ± 0.02	0.11 ± 0.01	0.09 ± 0.02
Al (mg/kg)	14160.33 ± 7721.6	19730 ± 5785.13	22050 ± 1732.17
Mn (mg/kg)	513.85 ± 2.81	626.56 ± 20.89	520.29 ± 311.09
Ni (mg/kg)	13.4 ± 11.13a	46.45 ± 7.11b	55.48 ± 7.53b
Cu (mg/kg)	12.03 ± 0.72ab	13.02 ± 0.86a	9.16 ± 2.21b
Fe (mg/kg)	18304.14 ± 964.77a	24523.75 ± 613.36b	23851.22 ± 3183.22b
Zn (mg/kg)	81.69 ± 8.00a	45.67 ± 0.5b	36.33 ± 7.32b

Different letters indicate significant differences ($P < 0.05$).

Conclusions and Discussion

Root-associated microbes can influence plant development, plant health and the accumulation of nutrients in the soil depending on the plant species and environment [6-9]. In this study, we determined the abundances of the different phyla and genera in the soils around the roots of tiger grass. These results reveal the root-associated soil microbiomes of tiger grass collected from Mae Sot district, Tak Province. The finding of the predominant phylum *Proteobacteria* at sites 2 and 3 corresponds to the findings of Spain et al. [17], who also reported that *Proteobacteria* was abundant in the rhizosphere. In terms of genera, *Methylobacillus* and *Pseudarthrobacter* were abundant at site 2, while *Sphingomonas* was abundant at site 3. The genus AKYG587 was highly abundant at site 1. Zhou et al. [18] reported that *Pseudarthrobacter* and *Sphingomonas* were biomarker bacteria for bahiagrass (*Paspalum natatum*). *Methylobacillus* was isolated from the soil of grasslands [19]. Therefore, the bacterial diversity in the soil in our study may indicate that different biomarker bacteria for tiger grass are present in different environments. It has been

shown that AKYG587 is more abundant in dry soil [20]. Similarly, our results suggest that the abundance of AKYG587 is related to the low soil moisture at site 1.

Recently, Kroeksakul et al. [21] reported that the carbon-to-nitrogen ratio of agricultural soils was 31.7-42.0 in Thailand's mountainous region. This finding may suggest that this ratio is greater in agricultural soils than in soils near the roots of tiger grass collected from waste lands. Furthermore, the heavy metal contents decreased in the order of Fe > Mn > Zn > Ni > Cu in the soil samples from the mountainous region [21], which was similar to the results of the present study. *Aquabacterium*, *Massilia* and *Sphingomonas* were the dominant genera in agricultural areas of the study by Kroeksakul et al. [21]. Conversely, *Methylobacillus*, *Pseudarthrobacter* and *Sphingomonas* were the most abundant microbes in the soil surrounding the roots of tiger grass. Notably, *Aquabacterium* is highly abundant in paddy fields [22], and *Massilia* is a major genus of rhizospheric microbes [23]. In addition, the organic carbon content, moisture and pH in natural soils are related to specific communities of bacteria [24]. For the same species, Choudhary et al. [6] described the rhizospheric diversity of bacteria associated with iron ore mines as being composed of "*Proteobacteria*", *"Actinobacteriota"*, *"Chloroflexi"*, "*Gemmatimonadota*", "*Acidobacteriota*", "*Verrucomicrobiota*", "*Planctomycetota*" and "*Chlamydiae*". In this study, the most abundant phyla of bacteria were similar to those identified in Choudhary et al. [6], with the exception of *Chlamydiae*. In terms of genera, *Sphingomonas* was the most abundant in the soil around the roots of tiger grass, which was consistent with the findings of Kroeksakul et al. [21], but neither *Aquabacterium* nor *Massilia* was identified. Therefore, *Sphingomonas* may be a common soil microbe associated with the roots of tiger grass in the highlands of western Thailand.

The results of this study indicated that bacterial diversity and the mineral content of the soil surrounding the roots of tiger grass from the Mae Sot district may depend on the characteristics of the soil environment. The present study has increased the information available regarding the soil-associated microbiota around the roots of perennial grasses, such as tiger grass. Bacterial communities may be indicator for tiger grass diversity and soil characteristics in waste lands of the highlands of Thailand. Furthermore, these results provide information on the diversity of the microbial communities in the soils around tiger grass, which may support future land-use restoration work.

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