INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
23–0265/2023/30–4–291–300
DOI: 10.17957/IJAB/15.2087
http://www.fspublishers.org

Full Length Article

Relationship Between Nitrogen Fixing Bacteria and Nitrogen Element in Soil After Burning in Sugarcane Field of Kanchanaburi Province, Thailand

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Received 19 July 2023; Accepted 24 August 2023; Published 11 September 2023

Abstract

Kanchanaburi is the largest sugarcane plantation in the central region of Thailand. Nitrogen is the main element for plantation and postharvest quality of sugarcane. Burning sugarcane scraps in fields take a long time to fire extinguish as well as to drop the ambient temperature until normal. This research was aimed at to examine relationship between the number of nitrogen-fixing bacteria via 16S rRNA Next Generation Sequencing and the amount of nitrogen in soil for biological map reconstruction on Kanchanaburi map. The biological map was created with QGIS 3.26 for focusing a change of nitrogen fixing bacteria and nitrogen element before and after burning in four periods at five sugarcane fields in Mueang and Bo Phloi Districts of Kanchanaburi province. In the beginning of study, the results of all fields showed that gram-positive nitrogen fixing bacteria were the most abundant in the phylum Firmicutes, namely *Bacillus subtillis* and *B. pumilus*, followed by gram-negative bacteria in the phylum Proteobacteria, namely *Azospirillum brasilense*. Before burning, the soil from Nong Pak Bung Village indicated the highest number of bacteria (0.52%), corresponding with the highest nitrogen content (0.16%), while these bacteria were not present in the sugarcane soil from Thung Masang Village (NB3) and the nitrogen content was minimal (0.09%). It is possible that this nitrogen remained in the soil from the chemical fertilizers. However, moisture and nitrogen in soil decreased to half within 1–2 days after burning and increased slightly in two months' duration after incineration. In the same way, the number of nitrogen-fixing bacteria increased slowly. Therefore, if farmers stop burning and change sugarcane scraps to fertilizer, sugarcane production will be sustainable and reduce cost of fertilizer in production as well.

Keywords: Sugarcane scraps; Sugarcane plantation; Nitrogen fixers; Nitrogen content

Introduction

Sugarcane (*Saccharum officinarum* L.) is an important economic crop in Thailand, which drives the economy in the farmer level and the processing industries level. In 2021, Thailand was the 3rd sugar exporter of the world, followed by India and Brazil. The main planting areas were the central and the west region of the country. The 1,123.56 million square meters of sugarcane planting area in Kanchanaburi province was the largest area in the west region (Office of the Cane and Sugar Board 2021).

In Thailand, the sugarcane stem is commonly harvested manually even though few of sugarcane field are cut by machine. Therefore, sugarcane harvesting is still a limitation. Preharvest, 70% of whole sugarcanes is burnt to get rid of sharp foliage and reduce injury of labors but increases economic returns (Phukongchai and Kaewpradit 2018). After harvest, dried sugarcane scraps are burned to prevent fire in the field and prepare the field for the next crop. Now a day, this action has effects on environmental pollution and is viewed seriously around the world. Moreover, unsuitable harvesting managements are significantly correlated with a reduction of the quality of product, such as yield, commercial cane sugar (CCS), brix and Pol content.

Severity and recurrence of burning is linked to the soil ecosystem and have effects on above-ground and below-ground soil properties. Soil microorganism community is one of key factors of soil quality. They drive 80–90% of the soil

To cite this paper: Hadthamard N, M Nakkuntod, S Arksri, S Poodkuntod (2023). Relationship between nitrogen fixing bacteria and nitrogen element in soil after burning in sugarcane field of kanchanaburi province, Thailand. *Intl J Agric Biol* 30:291–300

fertility improvement, which depends on temperature, moisture, and physical and chemical properties of soil as well (Pompranee 2009). The impact of fire on the soil after burning was reported to decrease of fungal and bacterial population (Dooley and Treseder 2012). The rest of microorganisms survive on nutrients from the dead microorganisms to grow, while this situation requires long recovery times for months or years. However, high recurrence burning areas presented negative effects of fire on soil microorganisms and cycling bacteria for 5–10 years after burning. Unfortunately, soil recovery might not take place (Pereg *et al.* 2018; Rodríguez *et al.* 2018; Barreiro and Díaz-Raviña 2021). Furthermore, burning may affect not only soil quality but also sugarcane product quality in the future.

The growth of sugarcane requires sufficient nitrogen to get good yields and a high sugar content (Osotsapar 2016). Generally, nutrients in the soil occur from the activity of soil microorganisms such as *Azotobacter*, *Beijerinckia* and *Azospirillium*, with nitrogen-fixing properties at the soil surface and in the soil around the plant roots to promote plant growth (Lopes *et al.* 2013). The addition of fertilizer containing macroelements may have a direct effect on high production costs and physical quality loss of soil in the long term (Sriwarakhan *et al.* 2012).

The previous study found that the morphological characterization of soil microorganisms in sugarcane field could be classified on carboxy methyl cellulose agar and stained with congo red, which it took more than 2 weeks (Pompranee 2009). Conventional microbial community study by PCR technique is applied to decrease time, but it still begins from culture cell and sequences on short region of nucleotides only. Therefore, it is expensive for examination at the 16S rRNA region, which is up to 1,500 base pairs long. Recently, metagenomic is used to study community microorganisms directly in natural environment. It can identify species of microorganisms and determine their functions and behaviors using the target region of genome (Chiu and Miller 2019). The principle is processing nucleotide sequences with high throughput at the same time. The detection region, 16S rRNA for bacteria and archaea and 18S rRNA for eukaryotes, is faster and cheaper than the common method (Lim *et al.* 2010).

Chemical and biological properties of the soil altered to different soil and crop management. Microbial community in sugarcane soils were analyzed to determine soil health by metagenomics target sequencing of 16S rRNA at V3–V4 region to obtain populations of bacteria and archaea in the soil samples (Trivedi *et al.* 2016; Bigott *et al.* 2019; Liu *et al.* 2021). Each burning of sugarcane leaves takes a long term until the fire is extinguished. While on fire, the accumulated heat in the soil makes the temperature climb up to 90ºC. This event had a direct disadvantage on humidity and ecosystem. Comparison of total organic carbon content of soil in sugarcane field, the unburned soil was better than burned soil (Souza *et al.* 2012). However, timing for microorganism recovery after incineration is a challenging topic because it depends on the number of microorganism's survival, environment, and management in field.

For Thailand, the research about the quantity and change in bacterial population in burned sugarcane field is not clear. Therefore, the researchers purposed to study the relationship and comparison the amounts of nitrogen-fixing bacteria and nitrogen elements in the soil before and after burning of sugarcane fields using Mueang and Bo Phloi Districts of Kanchanaburi province as the models. These fields were the major sugarcane cultivation, but they were burned frequently. So, the impact on the plantation will affect overall sugarcane yield. This research focused the effect of burning sugarcane fields on the type and abundance of nitrogen-fixing bacteria because nitrogen is a macronutrient in the soil and a key component of amino acids, and chlorophyll in the growth of stems and leaves. This information will be useful for management in the fields including planning to apply fertilizer in the right amount and time for awareness of the disadvantages of burning sugarcane leaves on soil quality.

Materials and Methods

Preparation of soil samples

Five fields with fully mature sugarcane crop were selected for this case study in Mueang and Bo Phloi Districts of Kanchanaburi Province, Thailand. For two selected fields in Mueang District are located at Nong Klang Phong Village, Wang Yen Subdistrict (WM) and Nong Phak Bung Village, Kaeng Sian Subdistrict (KM) and all three fields in Bo Phloi Districts are located at Thung Masang Village, Nong Kum Subdistrict (NB1, NB2 and NB3). Soil samples were collected from each field in four durations: (1) no more than five days before the fire, (2) after burning 1–2 days, (3) after burning a month and (4) after burning 2 months, respectively (Table 1).

The soil samples of each field were randomly taken from 12 points during 11:00 a.m. to 1:00 p.m. All points covered the burning area and around the sugarcane's root. The soil collection was followed as these steps. First, the soil surface was cleaned without humus or charcoal and excavated in deep to 20–30 cm to collect 1000 g of the soil sample (Bigott *et al.* 2019). All the soil samples were divided into two bags. One bag was stored in an ice box with dry ice to determine the nitrogen-fixing bacteria, and another bag was stored in an airtight plastic box and sent to the Product Research and Development Unit in Kanchanaburi Rajabhat University to analyze soil moisture and nitrogen content. Each sample collection was recorded for the geographic coordinates of sampling point, temperature in air and moisture content of soil.

DNA extraction

Genomic DNA of soil organisms were extracted using

commercial DNeasy PowerSoil Pro Kit (Qiagen, Germany). A 250–300 mg of fine soil sample was removed to a PowerBead Pro tube and 800 µL of solution CD1 was added. The mixture was incubated at room temperature for 10 min, and centrifuged at 13,000 rpm for 1 min. The supernatant was transferred to a new 2 mL microcentrifuge tube and 800 μL of solution CD2 was added, mixed using vortex, and centrifuged at 13,000 rpm for 1 min. Next, the new supernatant was mixed with 600 μL of solution CD3, replaced it in the MB Spin Column tube and centrifuged at 13,000 rpm for 1 min. The MB Spin Column was shifted to a new collection tube. Then, 500 μL of solution EA was added, and centrifuged at 13,000 rpm for 1 min. After that, 500 μL of solution C5 was added in MB Spin Column and centrifuged again. Next, the MB Spin Column was taken in a new Collection tube, centrifuged at 13,000 rpm for 2 min. Then, the MB Spin Column was moved on 1.5 mL microcentrifuge tube, added 40 μL of solution C6, set it at room temperature for 2 min and centrifuged for 1 min to keep DNA solution. Finally, all DNA samples were stored in a refrigerator before use.

DNA quality determination

Quantity and quality of DNA was checked for breakage, purity, and concentration. For the DNA breakage study, 5 μL of DNA sample was mixed with 1 μL of loading dye and 4 μL of distilled water. The DNA mixture was loaded into the additional wells of the 1% agarose gel, run the gel in 1xTAE buffer using electrophoresis at 100 volts for 30 min and replaced gel to stain with ethidium bromide solution for 10 min. The fragment of DNA was visually analyzed under UV light by UV transilluminator.

For the purity and concentration studies, DNA sample was determined by a NanodropTM Spectrophotometer (T042, Thermo Scientific, USA) using solution C6 as a blank to measure the absorbance at wavelengths 260 and 280 nm. Then the ratio of the A260/A280 was calculated. The ratio was found at 1.8–2.0. This means that the purity and concentration were sufficient for usability.

Study of the diversity of nitrogen fixing bacteria

The soil microbial communities from four fields were determined by metagenomics target sequencing of 16S rRNA at the V3-V4 region that is specific for bacteria and archaea. DNA samples were delivered to the sequencing service company (Macrogen Inc., South Korea) for nucleotide sequencing. Then, 16S metagenomic sequencing data were analyzed for taxonomic profile by following the pipeline of Qiime2 (Bolyen *et al.* 2019).

Spatial diagram of nitrogen-fixing bacteria of sugarcane soil

Data on the diversity of nitrogen-fixing bacteria and the nitrogen content of each field in difference term were taken from the geographical map of Kanchanaburi Province using QGIS 3.26 (free download at https://www.qgis.org/en/site/) together with the map of Thailand, (tha_adm_rtsd_itos_20210121_SHP.zipSHP(358.4M)) using the central database from https://data.humdata.org/dataset/cod-ab-tha? The administrative area was divided into provinces, districts and subdistricts, which were last updated on January 27, 2022, to create a spatial diagram showing the types and populations of nitrogen-fixing bacteria.

Results

Characteristics of the soil samples

All fields were grown with the same sugarcane variety, namely Khon Kaen 3, machine was used to harvest. After that, the farmers burned sugarcane residues to cultivate a new crop. About a week after harvest, farmers started to manure. Fertilizer application was frequent within the first 1–2 months, depending on the height of the sugarcane. During plantation, the soil management of WM, KM, NB2 and NB3 were added with chemical fertilizer, manure and green manure. NB1 was applied with fermented sugarcane residues combined with other fertilizers. The irrigation system at KM, NB1, NB2, and NB3 used rainwater and dripping. In WM applied the pouring water conjugated with both. Postharvest burning negatively affected the moisture and nutrients contents in soil, especially total nitrogen content, a major indicator of sugarcane plantation. Soil was blackened (Fig. 1), it was the first appearance that represented impacts.

Physico-chemical characteristics in soil

The moisture and nitrogen (N) contents in soil decreased within 1–2 days after burning and increased slightly after two months of incineration, except the moisture content of WM, because of raining after harvest. Reduction of moisture content in soil at NB1, NB2, KM and NB3 were 44.35, 28.11, 27.70 and 11.98%, respectively. Comparison of the N contents during, before and after burning 1–2 days showed that the total N contents decreasing in soil after burning 1–2 days of KM, WM, NB3, NB2 and NB1 were 75, 70, 50, 33.33 and 12.38%, respectively (Fig. 2).

Changing of nitrogen fixing bacteria communities in soil of sugarcane field

The extracted DNA solution presented the complete band of genomic DNA and disappeared fracture. Their concentration and purity values were 3.4–98.5 ng/μL and 1.86–2.37, respectively. Whole the metagenome sequencing of 16S rRNA at V3-V4 region from 20 soil samples found that total length of nucleotides 16.6–28.2 million base pairs. Final total reads were considered to conserve bacterial genomes about 47,768–120,374 reads and percentages of GC were 56.18–58.46 (Table 2).

Table 1: Samples of soil in five sugarcane fields

Samples	Location	Longitude and Latitude
WM-B, WM-A, WM-A1M, WM-A2M	Nong Klang Phong Village, Wang Yen Subdistrict, Mueang District (WM)	99.3968, 13.94098
KM-B, KM-A, KM-A1M, KM-A2M	Nong Phak Bung Village, Kaeng Sian Subdistrict, Mueang District (KM)	99.4744, 14.06711
NB1-B, NB1-A, NB1-A1M, NB1-A2M	Thung Masang Village, Nong Kum Subdistrict, Bo Phloi District (NB1)	99.47136, 14.2004
NB2-B, NB2-A, NB2-A1M, NB2-A2M	Thung Masang Village, Nong Kum Subdistrict, Bo Phloi District (NB2)	99.47168, 14.1890
NB3-B, NB3-A, NB3-A1M, NB3-A2M	Thung Masang Village, Nong Kum Subdistrict, Bo Phloi District (NB3)	99.4717, 14.2066

Note: B = before burning, A = 1-2 days after burning, A1M = a month after burning, and A2M = 2 months after burning

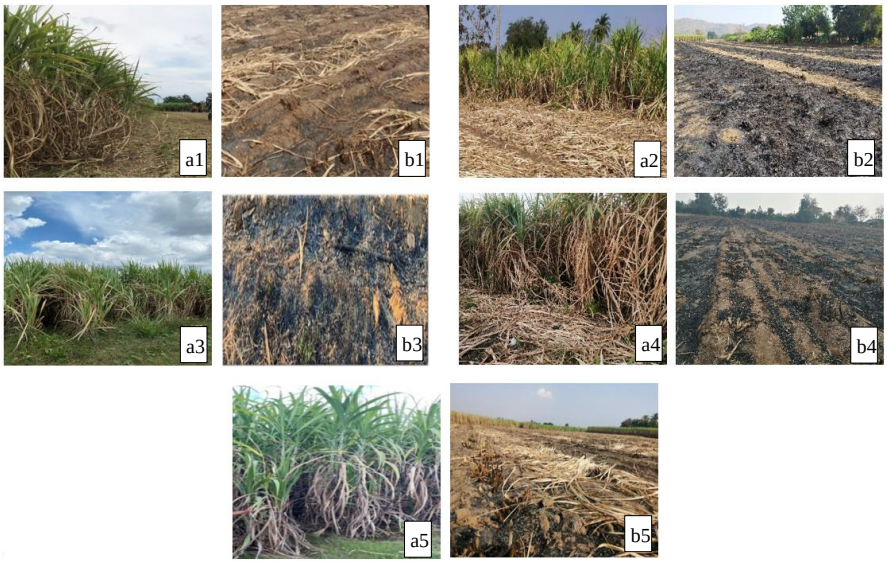

Fig. 1: Unburned and burned (1–2 days) of sugarcane fields in Mueang and Bo Phloi Districts of Kanchanaburi province, Thailand
a1-a5 = unburned sugarcane fields at WM, KM, NB1, NB2, and NB3, respectively; b1-b5 = 1–2 days burned sugarcane fields at WM, KM, NB1, NB2, and NB3, respectively

Table 2: Characteristics of 16S rRNA at V3-V4 region of bacteria in soil

sample	Total bases (million bp)	Total reads	GC (%)
WM-B	21.5	99,198	57.73
WM-A	16.6	76,920	57.51
WM-A1M	19.8	91,670	57.94
WM-A2M	17.7	81,612	57.28
KM-B	27.5	60,557	57.92
KM-A	22.5	49,567	57.71
KM-A1M	21.7	47,768	57.61
KM-A2M	27.9	61,601	57.87
NB1-B	25.9	56,921	57.9
NB1-A	26.1	57,584	58.19
NB1-A1M	23.6	51,998	58.38
NB1-A2M	23.0	50,749	58.01
NB2-B	23.4	51,438	58.42
NB2-A	21.8	48,263	56.18
NB2-A1M	23.9	52,560	58.33
NB2-A2M	28.2	61,989	58.46
NB3-B	18.53	85,931	57.92
NB3-A	26.2	120,374	57.33
NB3-A1M	18.8	87,716	57.58
NB3-A2M	18.2	83,728	57.95

The extracted DNA genome was obtained from 727 species of bacteria from 16 phyla, namely Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Chlamydiae, Chloroflexi, Cyanobacteria, Dictyoglomi, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, Proteobacteria, Thermodesulfobacteria, Verrucomicrobia and Synergistetes. Soil microbial communities before burning time from all fields are presented by phylum following as Actinobacteria (the most abundant), Firmicutes, Proteobacteria and Acidobacteria, respectively. Considering by area, soil from NB1, NB2 and NB3 field had higher bacterial abundance in the phylum Actinobacteria than KM and WM, but less in the phylum Acidobacteria. However, bacteria in the phylum Chloroflexi, Gemmatimonadetes, Nitrospirae, Planctomycetes, Armatimonadetes, Chlamydiae, Cyanobacteria, Dictyoglomi, Synergistetes, and Thermodesulfobacteria were found to be less than 5% (Fig. 3).

All of the identified bacteria belonged to three species of nitrogen fixing bacteria: particularly *Bacillus subtilis*, *B. pumilus* and *Azospirillum brasilense*. For *B. subtilis* and *B. pumilus* were gram-positive bacteria belonging to the phylum Firmicutes while *A. brasilense* was gram-negative bacteria in the phylum Proteobacteria.

After burning for 1–2 days, the nitrogen fixing bacteria communities in all fields were changed. *B. subtilis* increased in abundance in all four sugarcane field soils, except the WM, which had a slight drop. In particular, the sugarcane soil of NB2 had a 70-fold (0.70%) increase in *B. subtilis* from 0.01%

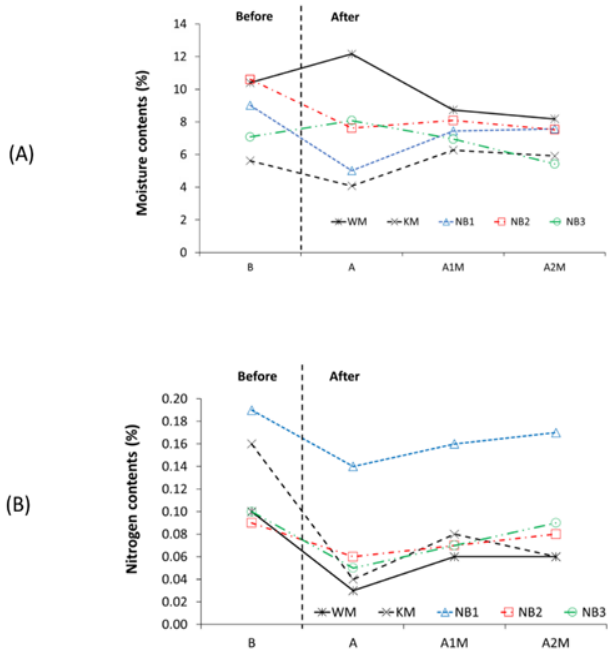

Fig. 2: Moisture (A) and nitrogen (B) contents of five sugarcane fields before and after burning sugarcane residues

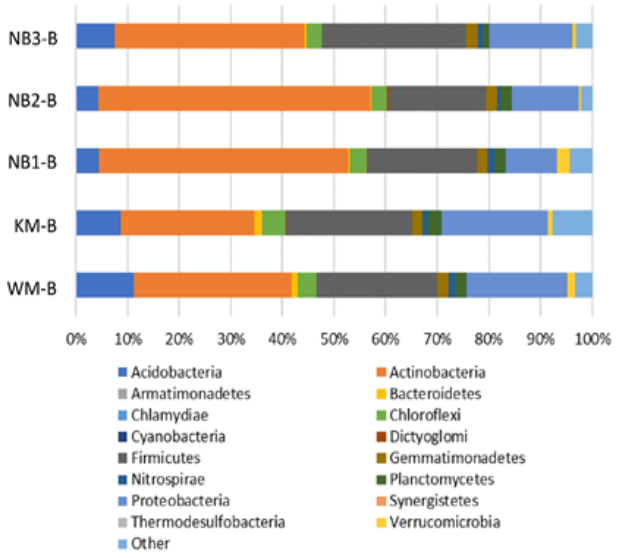

Fig. 3: Soil microbial communities before burning of the five sugarcane fields

in pre-burned soil, while there was a slight increase in the NB1, NB3 and KM fields. *B. pumilus* was found only in 2 fields (KM and NB2). KM abundance raised from 0.03 to 0.15%, while NB2 field abundance stood at 0.05% from not being found before burning. *A. brasilense* was found in KM, NB1 and NB2 sugarcane soils. NB1 and NB2 abundances increased from 0.01 to 0.02% and 0.01 to 0.30%, respectively, while KM was constant. In soils of WM and NB3, it decreased from 0.02 and 0.01% until it was

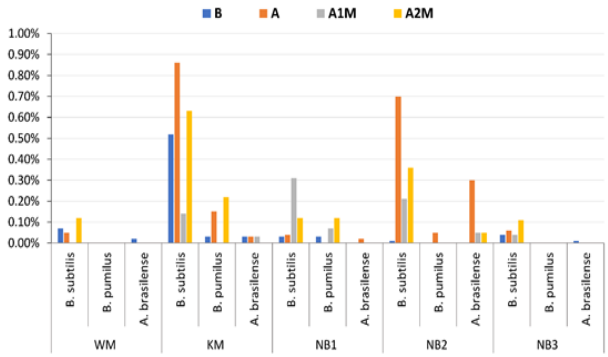

Fig. 4: Abundance of nitrogen fixing bacteria in five sugarcane fields before and after burning

undetectable.

A month after burning in the sugarcane field, the soil nitrogen fixing bacterial community changed differently at each site. For WM field, the above three species of nitrogen fixing bacteria disappeared while *B. subtilis* and *B. pumilus* were found to decrease in KM soils, but *A. brasilense* remained constant as the same with before burning. On the other hand, the population of *B. subtilis* and *B. pumilus* was increased only in NB1 from 0.04 to 0.31% and from undetectable to 0.07%, respectively but bacteria in NB2 and NB3 decreased after burning for a month, especially *B. subtilis* and *A. brasilense* abundance in NB2 field after burning decreased sharply.

Two months after burning sugarcane residues in fields, nitrogen-fixing bacterial population changed again. *B. subtilis* increased in all areas except NB1, which decreased more than half. *B. pumilus* was not detected in sugarcane WM and NB3 fields, while it increased in KM and NB1 fields. However, *A. brasilense* was undetectable after one month in all fields.

Nitrogen content and nitrogen fixing bacteria

Even sugarcane burning may not directly affect nitrogen-fixing bacteria. However, the high heat of the land from burning for a long time affected the soil quality and the balance of the soil bacteria. It can be seen from the significant decrease in nitrogen contents of all fields, as well as the loss of structural properties and moisture contents in soil.

According to the spatial diagram showing the amounts of nitrogen fixing bacteria and nitrogen contents in the five sugarcane fields, their patterns had the same tend (Fig. 5–8). Before the sugarcane plantation was burned, the soil from KM had the highest nitrogen fixing bacteria (0.52%), corresponding to the high nitrogen content (0.16%). On the other hand, when the surrounding air contained more nitrogen gas. N-fixing bacteria grew rapidly to fix nitrogen. At that moment, population of *A. brasilense* was not significantly different (Fig. 5 and 8), but the population of *B. subtilis* and *B. pumilis* increased after burning for 1–2 days (Fig. 6–7).

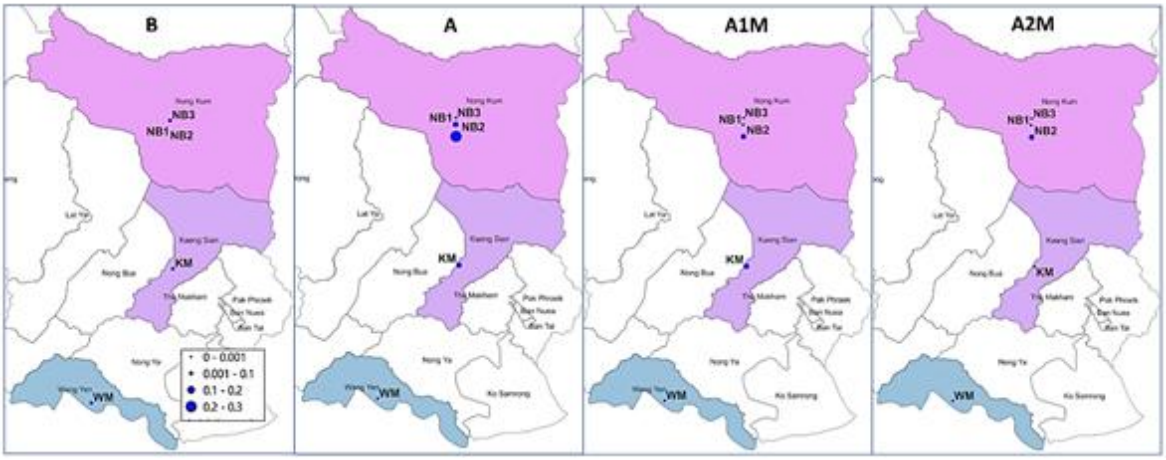

Fig. 5: *Azospirillum brasilense* in five sugarcane fields with four phases (B = before burning, A = 1–2 days after burning, A1M = 1 month after burning, and A2M = 2 months after burning)

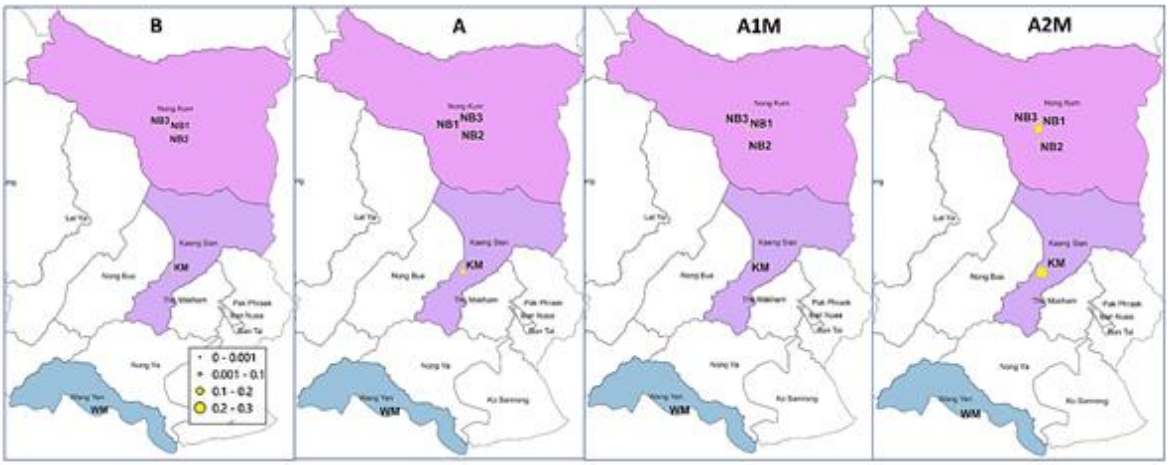

Fig. 6: *Bacillus pumilus* in five sugarcane fields with four phases (B = before burning, A = 1–2 days after burning, A1M = 1 month after burning, and A2M = 2 months after burning)

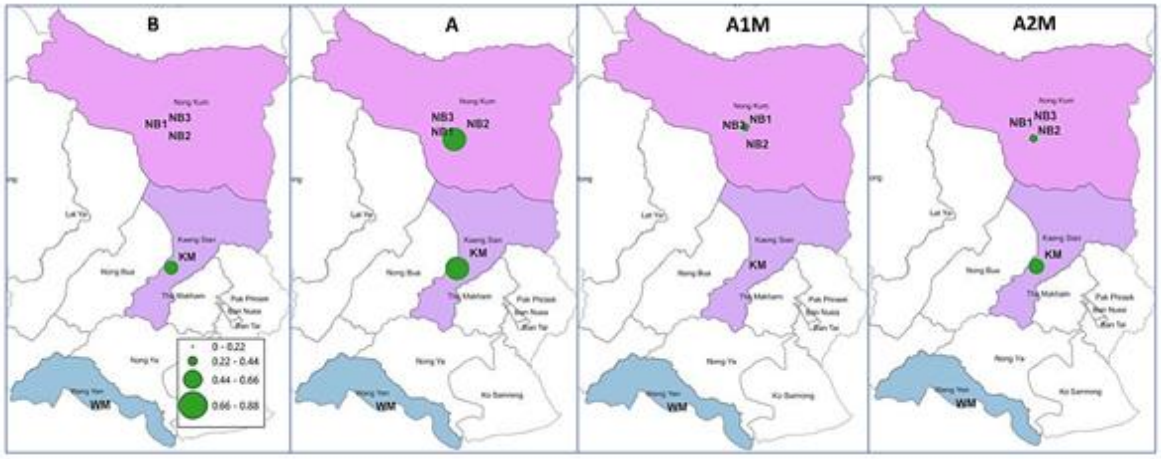

Fig. 7: *Bacillus subtilis* in five sugarcane fields with four phases (B = before burning, A = 1–2 days after burning, A1M = 1 month after burning and A2M = 2 months after burning)

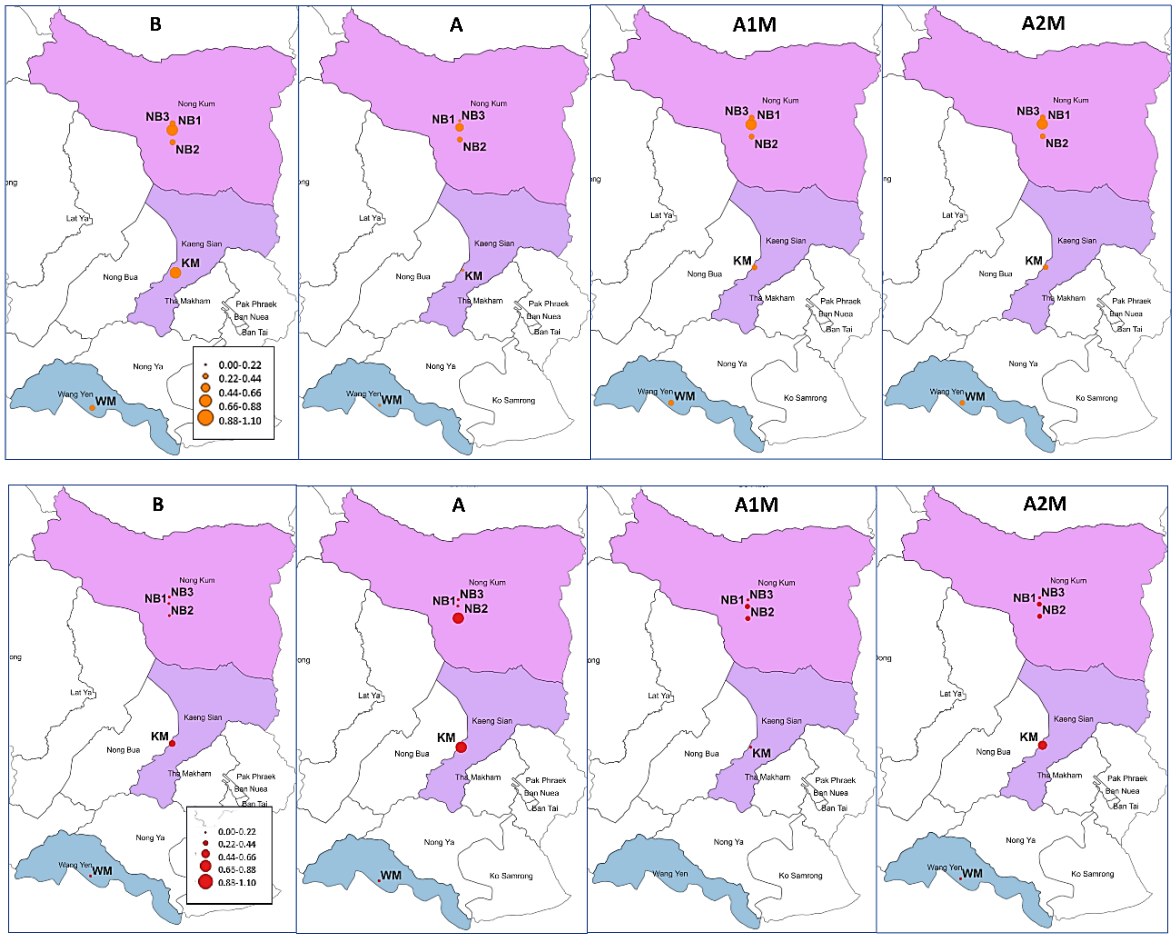

Fig. 8: Nitrogen contents (orange) and nitrogen-fixations bacteria (red) in five sugarcane fields with four phases (B = before burning, A = 1–2 days after burning, A1M = 1 month after burning, and A2M = 2 months after burning)

Discussion

Even the farmers had irrigation system for two months, but the moisture level of the burnt field decreased gradually, due to loss of water repellency capacity in the soil (Badía *et al.* 2017). Besides that, total weight of sugarcane biomass after burning decreased, due to which the moisture in soil evaporated rapidly (Dengia and Lantinga 2018). Badía *et al.* (2017) reported that burning depleted 25–50% of the organic carbon content in the wet and dry soil, especially the first centimeter of soil depth lost the total N-content about 1/3 of the original value. Comparison of the N and moisture contents showed that the rate of nitrogen loss was higher. This may be due to damage level of the physical, chemical, and microbiological qualities in soil due to burning (Moitinho *et al.* 2021).

The 16S rRNA sequence showed that communities of Acidobacteria, Actinobacteria, Firmicutes, and Proteobacteria in all fields were similar. Before burning, Actinobacteria was the most abundant. After 1–2-day burning, the bacterial abundances in the soils of WM, KM, and NB2 decreased completely. Fire severity (temperature and time) directly affected soil warming microbial communities. In low and moderately severe fires, microbial communities are resilient (Moya *et al.* 2022). This phenomenon was similar to change in microbial population after forest fire. The intense fire led to reduction of microbial biomass, organic carbon and nitrogen content and pH increasing in the upper layer of soil (Villadas *et al.* 2019). Damage of microbial biomass may be due to malfunction of their cellular components and enzymes from high temperature (>120ºC) shock (Köster *et al.* 2021). In addition, fire generally reduced microbial communities more at the soil surface (0–5 cm in depth) than in deep soil (5–10 cm). Thus, the sugarcane plantation of NB1 and NB3 were not affected by post-fire. However, the remaining bacteria in each field increased slightly and sharply during 1–2 months, especially Actinobacteria and Firmicutes. Actinobacteria is related to a heat resistance gene that could improve survival after fire (Nelson *et al.* 2022). Firmicutes-producing endospores take advantage of the high temperature (Villadas *et al.* 2019).

In this study, three species of diazotrophs spread closer to the sugarcane roots, which acted as the plant growth promoting rhizobacteria (Que *et al.* 2012; Singh *et al.* 2020). Determination of microbial abundance revealed that only KM field represented these nitrogen fixing bacteria completely, while *B. subtilis* was the highest. Mainly the *B. pumilus* was detected in soil of KM and NB1 with equal number (0.03% of the total bacteria), while *A. brasilense* was found in soil of WM, KM and NB3. The N-fixing bacterial abundance before and after burning is presented in Fig. 4. *B. subtilis* and *B. pumilus* could associate with other microbial genera in soil of corn cultivar. Thus, Bacillus bacteria were detected in every field with the highest abundance. However, moisture content may be a major condition for the growth of *B. subtilis*, *B. pumilus* and *A. brasilense* (Jabir *et al*. 2018). Jiang and De-Ti (2009) found that bacteria in phylum Proteobacteria grew up to 63–84% in semi-arid soil, especially orders Sphingomonadales and Rhizobiales in class Alphaproteobacteria. Besides, Shu *et al.* (2012) reported that 27.6% of class Alphaproteobacteria were in wetlands rice soils. Accordingly, soil from KM entailed the greatest number of *A. brasilense*, despite of least moisture content (5.62%). The NB1 and NB2 soils had the highest moisture content (9.02 and 10.60%), therefore there was no *A. brasilense*.

Nitrogen fixing bacteria play an important role in inorganic compound degradation and nitrogen fixation (Eilers *et al.* 2010). However, the sugarcane soil from NB2 had the least bacterial abundance (0.01%) and the nitrogen content was 0.06–0.09% (Fig. 1). It might be that the measured N-content was caused by chemical fertilizers added to the soil. And there are still nitrogen nutrient components left over from the evaporation of ammonia, as well as the experiments of Sun *et al.* (2020a). These bacteria gradually grew up for 2 months. Here, the fixed nitrogen changed to ammonia for backup in soil. The soil in WM field was detected that nitrogen fixing bacteria decreased within 1–2 days after burning since this land might have suffered heat more than 45ºC thereby affecting to the population of the surviving microorganism and microbial recovery (Dosta *et al.* 2008; Fernandes *et al.* 2018). This is due to the reason that temperature and moisture properties are main factors affecting the growth of microorganisms (Ali and Okabe 2015; Ma *et al.* 2016). *A. brasilense* is amongst the nitrogen fixing bacteria, which are often found in semi-arid soils (Jiang and De-Ti 2009). This is consistent with the pre-burned sugarcane soil of KM, which had the lowest moisture content (5.62%). But this field had the highest of *A. brasilense* (0.03%) and the highest nitrogen content (0.16%). The genus Bacillus was found generally in all fields due to their ability to coexist well with other microorganisms (Jabir *et al.* 2018). However, a temperature of 35–40ºC from incineration caused a decrease in oxygen and caused release of ammonia from soil. Thus, after 1–2 days of burning in the sugarcane field, the nitrogen content in the soil decreased by 75% (from 0.16 to 0.04%).

Sugarcane plantation in Mueang and Bo Phloi Districts of Kanchanaburi Province, farmers will add at least three types of fertilizers, for examples chemical fertilizer, manure, and green manure (plowing). The first time for adding fertilizer was started within a week after harvest. However, chemical fertilization alone lost nitrogen up to 60% through ammonia evaporation. This loss could be reduced to 5% if *B. subtilis* is used due to the expression of narG, nirK, nirS and nosZ gene in denitrification process of *B. subtilis* increased (Sun *et al.* 2020b). As the plant and animal residues are being decomposed, fertilizer is added periodically to provide sufficient nutrients for the sugarcane growth. However, the soil management in NB1 differed from other fields when it was added with the fermented sugarcane residues too. Negative ion in organic matter played buffer capacity of soil to delay nitrogen loss (Sun *et al.* 2019). Thus, reduction in nitrogen from NB1 field was the least. KM field had the most surviving *B. subtilis* abundant (more than 0.5%), and these areas showed the high nitrogen content too.

Conclusion

Postharvest burning of the sugarcane residues destroys the physical quality of soil and disturbs the nitrogen fixing bacteria community in the soil. This situation has a negative effect to moisture and nitrogen content in soil. When nitrogen content is low, sugarcane plantation faces a low yield problem because nitrogen is necessary for sugarcane's growth in vegetative part such as stem and leaf. The selected sugarcane fields in Mueang and Bo Phloi Districts of Kanchanaburi province presented three species of nitrogen fixing bacteria: *B. subtilis*, *B. pumilis*, and *A. brasilense* from the 727 species of bacteria in sugarcane soil. The recovery of soil microorganism depended on the severity and recurrence of burning, and damage level of soil. Solving the problem by adding chemical fertilizers could maintain the soil quality only a short term.

Acknowledgements

The authors would like to thank National Research Council of Thailand (NRCT) for financial support in fiscal year 2021 and the sugarcane farmers in Mueang and Bo Phloi Districts of Kanchanaburi province.

Author Contributions

SP collected samples from the fields. NH and MN planned the research, determined chemical and biological properties of soil, and interpreted the results. SA produced biological mapping and illustrations.

Conflict of Interest

All authors agree with the contents of this manuscript and have no conflicts of interest to declare.

Data Availability

Specific locations of each field cannot be made publicly available due to privacy of research participants.

Ethics Approval

Protocol identification was exemption considered by ethics committee of Kanchanaburi Rajabhat University.

Funding Source

This research was supported by National Research Council of Thailand (NRCT).

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