Research Article

Correlation of Soil Physiochemical Properties, Microorganism Numbers, and Bacterial Communities Following Unburned and Burned Sugarcane Harvest

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The effects on soil properties were studied following sugarcane burning during harvesting based on the analysis of soil properties and the number of microorganisms. The soil bacterial community structure was observed using metagenomics. It was found that burned sugarcane harvesting reduced the soil moisture and total nitrogen contents and decreased the numbers of bacterial fungi and actinomycetes. Furthermore, there were decreased numbers of nitrogen-fixing and phosphate-solubilizing bacteria beneficial to plants. The Firmicutes phylum (46.79%) was found abundantly in the soil after burned sugarcane harvest. Paenibacillus (34.20%) and Bacillus (9.19%) were dominant at the genus level. On the other hand, in the soil after unburned sugarcane harvest, the diversity index was higher than that after burned sugarcane harvest. Actinobacteria (25.92%) dominated at the phylum level, and Candidatus koribacter, Gaiella, Pseudolabrys, and Sphingomonas dominated at the genus level in the unburned plots. Changing the bacterial community resulted in a change in correlation with soil properties. Therefore, the impacts from burned sugarcane harvesting should be realized, specifically that soil physiochemical and biological properties are degraded (except for some groups of bacteria) along with their functions in the soil.

1. Introduction

The burning of agricultural residues is a universal phenomenon and can cause global air quality decline[1, 2] and contribute to greenhouse gas emissions [3]. Air pollution and greenhouse gases are caused by carbon dioxide, methane, nitrous oxide, and fluorinated gases [4]. Furthermore, the particulate matter generated from biomass burning has negative effects on both the environment and human health [5, 6]. Burning agricultural residues are a serious threat to soil security and food chain sustainability. For example, the effect of burning wheat residues on soil quality has caused nutrient losses in both developing and developed countries [7]. Sugarcane burning practices negatively affect soil quality, human health, and harvested sugarcane moisture loss [8]. During the agricultural harvesting season in Thailand (November to February), burning on cultivated land is a major problem, especially the pre-harvest burning of sugarcane.

Sugarcane is a crucial source of sugar and ethanol production in Thailand, with 1.74 million ha planted with sugarcane producing 2.00 million in the production year 2020/21 [9]. Annually, sugar factories purchase sugarcane from farmers as either fresh sugarcane (73.6%; 4.90 million t) or burned sugarcane (26.4%; 1.76 million t). Although government measures have become more stringent to limit the practice of burning during harvesting, farmers still burn sugarcane. Substantial losses of carbon, nitrogen, and organic matter (OM) due to sugarcane residue burning have been reported and have resulted in the deterioration of soil...
quality [8, 10]. Increases in soil temperature during pre-
harvest burning affect soil microorganisms and water
content [11, 12]. Rachid et al. [13] reported that under
different management regimes (preharrow burn and me-
chanical, unburnt harvest, or green sugarcane), there were
significant changes in the total structure, ammonia-
oxidizing, and denitrifying bacterial communities. In ad-
dition, soil microorganisms play critical roles in soil OM
decomposition, nutrient availability, and cycling [14, 15].
Thus, the soil microbiome must be managed to ensure re-
liable agricultural production and effective control of plant
diseases [16]. It is known that burning affects soil bacteria
and soil properties; therefore, this work extends the in-
vestigation of the relationship between soil bacterial com-
nunities and soil chemical properties as a changing
relationship or not and how it affects soil properties. The
purpose of the current research was to compare and study
correlations in the effects of unburned and burned sugarcane
harvesting on the soil properties and the population
structure of soil microorganisms to better understand the
ecological impact of burning sugarcane.

2. Materials and Methods

2.1. Soil Sample Collection and Physicochemical Analysis be-
fore Planting and after Harvesting. Composite samples in a
zig-zag pattern were collected from a homogeneous plot of
land (8–10 ha), consisting of a mixture of 10–20 subsamples
[17]. Samples were collected at a depth of 0–30 cm in a
Kamphaeng Saen soil series in Bo Suphan subdistrict, Song
Phi Nong district, Suphan Buri province (14.165747°N,
99.800659°E), Thailand. Some of the soil properties before
planting sugarcane were analyzed. The potential of hydrogen
(pH) was measured using a pH meter. Soil samples were
processed using H₂SO₄-Na₂SO₄-Se mixture digestion as
specified to analyze the total nitrogen content based on the
Kjeldahl method for analyzing soil OM [18]. The phosphorus
content was analyzed according to Bray and Kurtz [19],
kilograms of nitrogen per kilogram of soil. Soil samples were
treated for soil analysis, microbial counts, and deoxy-
ribonucleic acid (DNA) extraction. The samples were kept
on ice before transporting to a laboratory for microbial
analysis.

2.3. Microbial Counts. A soil solution dilution method was
used in this experiment. The soil solution was diluted to a
range of 10⁻¹ - 10⁻⁶ with 3 replications. Then, 0.1 ml of the
appropriate dilution solution was pipetted into a Petri dish.
Different types of microorganism-specific media were used:
nutrient agar (NA) for bacteria, actinomycetes isolation agar
(AA) for actinomycetes, potato dextrose agar (PDA) for
fungi (Himedia), Pikovskaya (PVK) medium for phosphate-soluble bacteria [23], and N-free medium (NF)
for nitrogen-fixing bacteria [24]. Microorganisms were
counted after incubation at 30 °C for 24 h for bacteria and for
7 days for actinomycetes and fungi. The numbers of mi-
croorganisms were compared using the logarithm of colony-
forming units (log CFU) per 1 g of dry soil weight (Dw).

2.2. Sugarcane Planting and Harvesting. Khon Kaen 3,
a sugarcane cultivar in the first ratoon cane, was used in the
current research. Two different treatments were carried out
for sugarcane harvest (unburned and burned sugarcane
fields) with 3 replications. The plot size was 5 rows
planted ×12 m (1.4–1.8 m between planted rows). Fertilizer
was applied following recommendations according to the
soil analysis for sugarcane by the Department of Agriculture,
Thailand (N: P₂O₅: K₂O at the rate of 2.88: 0.96: 2.88 kg ha⁻¹,
respectively). The composite soil samples were collected
from 10–20 points in each plot at a depth of 0–30 cm. The
soil from fields growing sugarcane aged 12 months was
collected following unburned and burned sugarcane har-
vesting for soil analysis, microbial counts, and deoxy-
ribonucleic acid (DNA) extraction. The samples were kept
on ice before transporting to a laboratory for microbial
analysis.

2.4. DNA Preparation and Metagenomics. DNA was
extracted from 0.25 g of soil using a NucleoSpin soil DNA
Purification Kit (Macherey-Nagel, Germany) following the
protocol supplied by the manufacturer. DNA samples were
quality-checked based on 1% agarose gel electrophoresis and
quantified using a Nanodrop spectrophotometer (Maestro,
Taiwan). A sample of DNA (20–30 ng) was used to generate
amplicons. The library preparation and sequencing were
used for the V3 and V4 hypervariable regions of the pro-
karyotic 16S ribosomal RNA (rRNA) gene. The library was
quantified to 10 nM; PE250/FE300 paired-end sequencing
was performed according to the Illumina MiSeq/NovaSeq
(Illumina, USA) instrument manual. Processing of the 16S
rRNA libraries was performed using the quantitative in-
sights into microbial ecology (QIIME) software, version
1.9.1 [25]. The resulting sequence for the operational tax-
ononomic unit (OTU) clustering used the VSEARCH clus-
tering software, version 1.9.6 [26]. Nonmetric multidimensional scaling (NMDS) was used to display beta
diversity visualization, based on the distance between using
the Bray–Curtis metric. The OTU analysis was used with a
random sampling of the sample sequences to calculate the
indices Ace, Chao1, Shannon, Simpson alpha diversity and
Good’s coverage, and community species sequence (se-
quence similarity was set to 97%). The diversity indexes were
calculated using the following equation: Ace index [27]:
\[ R = S_{\text{obs}} + a \cdot S_{\text{DI}} \]
where \( a \) is the number of taxa observed at least once in a
sample and \( a \) is the unknown number of species present
in the community but not observed. Chao’s index [28, 29]:
\[ S_{\text{Chao}} = S_{\text{obs}} + (a^2 + b^2) \]
where \( a \) is the maximum no. of species, \( b \) is the number of species observed in different
samples, and \( S_{\text{DI}} \) is the number of species represented by one individual each, and \( b \) is the
doubleton number of species represented by two individuals each. Shannon’s index [30]:
\[ H' = \sum_{i=1}^{S_{\text{obs}}} P_i \ln P_i \]
and Simpson’s index [31]:
\[ D = 1 - \sum_{i=1}^{S_{\text{obs}}} P_i^2 \]
where \( P_i \) is the fraction of the entire population made up of
species $i$, and $R$: numbers of species encountered. Good's coverage index [32]: $1 - F1/N$ where $F1$: the number of singleton OTUs, and $N$: the total number of individuals or the sum of abundances for all OTUs.

2.5. Statistical Analysis and Correlation. Analysis of variance was carried out using the R software, version 2.15.3 [33] with difference comparisons between mean values using Duncan’s new multiple range test. The difference between treatments was tested as either significant or highly significant ($P < 0.05$ and $P < 0.01$, respectively). The correlation coefficient was displayed in a heat map of the relationships between quantitative variables using the Minitab, version 16.2.0 [34] software.

3. Results

3.1. Soil Properties before Planting. The soil properties of the Kamphaeng Saen soil series before sugarcane planting based on sampling at a soil depth of 0–30 cm were as follows: pH 6.22; OM, total nitrogen, and exchangeable potassium at low levels of 1.43%, 0.05 mg kg$^{-1}$, and 44.21 mg kg$^{-1}$, respectively; available phosphorus high at 49.83 mg kg$^{-1}$; sand, silt, and clay at 58.74, 21.11, and 20.15%, respectively; and the texture was a sandy clay loam. These results were used to apply the relevant recommendations for the use of fertilizers for sugarcane planting [35].

3.2. Changes in Soil Physiochemical Properties after Harvesting Unburned and Burned Sugarcane. The soil physiochemical properties at a soil depth of 0–30 cm were analyzed after unburned and burned sugarcane harvesting (Table 1). There were no changes in the OM, available phosphorus, and exchangeable potassium contents or in the CEC of the soil. The most obvious changes in soil properties for the burned sugarcane harvesting compared to the unburned were in the reduced levels for the pH (reduced from 5.54 to 4.60) and the soil moisture (1.42%) and total nitrogen (0.01%) contents.

3.3. Changes in Microbial Numbers after Harvesting Unburned and Burned Sugarcane. Soil microorganisms were analyzed for the two different sugarcane harvesting systems (unburned and burned sugarcane harvesting). The numbers of bacteria, actinomycetes, and fungi were significantly different between unburned and burned sugarcane harvest (Figure 1(a)). The numbers of bacteria, actinomycetes, and fungi were 5.68, 4.76, and 4.73 log CFU g Dw$^{-1}$, respectively, in unburned sugarcane harvest compared to 4.40, 3.34, and 3.65 log CFU g Dw$^{-1}$, respectively, in burned sugarcane. The number of soil microorganisms was less for burned sugarcane harvest. The numbers of phosphate-solubilizing (3.67 log CFU g Dw$^{-1}$) and nitrogen-fixing (3.95 log CFU g Dw$^{-1}$) microorganisms following burned sugarcane harvest were less than those for unburned sugarcane harvest (Figure 1(b)).

3.4. Changes in Bacterial Communities after Harvesting Unburned and Burned Sugarcane. The soil samples after unburned and burned sugarcane harvest were used to generate V3-V4 16S rRNA gene profiles. Bacterial diversity and abundance were evaluated. In total, 1,295,634 reads were achieved with an average count of 215,939 per batch of samples, and in total, 367 OTUs were generated, of which 318 were shared by unburned and burned sugarcane harvesting. The NMDS ordination (Figure 2) showed the structural differences and separation of soil bacteria communities of the unburned and burned sugarcane harvest. The relative abundance at the phylum level (Figure 3(a)) was dominated by Actinobacteria (25.92%), Proteobacteria (24.19%), Acidobacteria (22.72%), and Firmicutes (10.68%) in unburned sugarcane harvest, while in the burned sugarcane harvest, the dominant phyla were Firmicutes (46.79%), Actinobacteria (15.33%), Acidobacteria (13.59%), and Chloroflexi (10.84%). Microbial composition abundance (Figure 3(b)) for the top-three at the genus level for unburned sugarcane harvest were unclassified bacteria (16.27%), Bacillus (5.67%), and Sphingomonas (4.84%). In contrast, in the burned sugarcane harvest, the top-three were Paenibacillus (34.20%), unclassified bacteria (10.24%), and Bacillus (9.19%). Community richness estimates and diversity indices for differences in the soil after unburned and burned sugarcane harvest are shown in Table 2. The values for Ace, Chao 1, Shannon, and Simpson indices were high for both abundance and evenness of the species present in a community for unburned sugarcane harvest compared with burned sugarcane harvest. Good coverage was found at 1.0 for unburned and burned sugarcane harvest.

3.5. Correlations of Soil Properties, Microbial Counts, and Bacterial Structure. The correlation matrix heatmaps of soil properties, microbial counts, and bacterial structure are shown in Figure 4. Unburned sugarcane harvest, OM, and available phosphorus were positively correlated with exchangeable potassium. The pH, total nitrogen, bacteria, and actinomycetes were positively correlated with nitrogen-fixing bacteria. The OM and exchangeable potassium were positively correlated with Paracoccus, Dongia, and Burkholderia. The CEC was positively correlated with Byobacter and Occallatibacter. The soil moisture content was positively correlated with Gaiella, phosphate-solubilizing bacteria, and

### Table 1: Soil physiochemical properties after unburned and burned sugarcane harvesting at soil depth 0–30 cm.

<table>
<thead>
<tr>
<th>Average</th>
<th>Unburned</th>
<th>Burned</th>
<th>$F_{\text{test}}$</th>
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<tbody>
<tr>
<td>Organic matter (%)</td>
<td>1.58 ± 0.12</td>
<td>1.40 ± 0.43</td>
<td>ns</td>
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<td>Hydrogen potential</td>
<td>5.54 ± 0.24</td>
<td>4.60 ± 0.21</td>
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<tr>
<td>Total nitrogen (%)</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.004</td>
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<tr>
<td>Available phosphorus (mg kg$^{-1}$)</td>
<td>33.19 ± 6.57</td>
<td>28.48 ± 7.08</td>
<td>ns</td>
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<td>Exchangeable potassium (mg kg$^{-1}$)</td>
<td>61.04 ± 7.24</td>
<td>50.93 ± 0.41</td>
<td>ns</td>
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<tr>
<td>Cation exchange capacity (cmol·kg$^{-1}$)</td>
<td>13.50 ± 2.14</td>
<td>10.85 ± 0.002</td>
<td>ns</td>
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<tr>
<td>Moisture content (%)</td>
<td>6.71 ± 0.46</td>
<td>1.42 ± 0.82</td>
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</table>

Data presented as mean ± standard deviation, $n$: 3, *$P < 0.05$ and **$P < 0.01$, and ns: not significant.
C. Koribacter. On the other hand, the OM and exchangeable potassium were negatively correlated with soil moisture content and the phosphate-solubilizing bacteria Gaiella, Conexibacter, and C. Koribacter. Phosphate-solubilizing bacteria were positively correlated with Conexibacter and Dongia. Bacteria had a positive correlation with actinomycetes, Burkholderia, Sphingomonas, Tumebacillus, and Bradyrhizobium, while fungi had a positive correlation with Paenibacillus, Bacillus, Streptomyces, Pseudomonas, and Jatrophihabitans. The CEC, total nitrogen, and nitrogen-fixing bacteria were negatively correlated with Amnoniphilus. The pH had a negative correlation with the fungi, Paenibacillus, Bacillus, Streptomyces, while the available phosphorus had a negative correlation with the CEC, Nitrospira, Candidatus Solibacter, Gemmatimonas, Byobacter, and Occallatibacter.

With burned sugarcane harvest, the CEC and phosphate-solubilizing bacteria were positively correlated with Gaiella, Conexibacter, and Nitrospira. The OM, available phosphorus, and exchangeable potassium were positively correlated with Streptomyces, C. Solibacter, Byobacter, and Bradyrhizobium. Fungi and nitrogen-fixing bacteria were positively correlated with Paenibacillus. Conversely, the OM, available phosphorus, and exchangeable potassium were negatively correlated with soil moisture content, fungi, nitrogen-fixing bacteria, Paenibacillus, and Paracoccus. The pH was negatively correlated with actinomycoses. The CEC was negatively correlated with bacteria, nitrogen-fixing bacteria, and Paracoccus.

4. Discussion

The results clearly showed that burning the sugarcane affected the measured soil properties. The pH was more acidic, the moisture loss was about 20%, and the total nitrogen was reduced compared to not burning (Table 1). These results were consistent with those of Flores-Jiménez et al. [36], who reported volatilized nitrogen nutrients and water loss from the soil during sugarcane burning. Arocena and Opio [37] reported that a pH decrease was generally caused by the loss of base cations during burning. The opposite effect was reported by the authors of reference [38], who compared to the control (unburned) sites for pine forest and oak forest, the soil pH levels of burnt pine and oak forests were higher by 0.41 and 0.78 units, respectively. In the current study, the numbers of bacteria, actinomycetes, and fungi decreased. In addition, phosphate-solubilizing and nitrogen-fixing bacteria numbers were reduced (Figure 1). The results were consistent with the results of Mills and Alley [39] who reported heterotrophic bacteria decreasing to 76% at a depth of 0–3 cm and up to 90% at a depth of 5–10 cm after burning of
stubble, with *Azotobacter* (nitrogen-fixing bacteria) and nitrifying bacteria destroyed in the topsoil. Significant reductions in nitrogen-related bacteria in the rhizosphere soil and sulfur cycle functions accounted for the continuous effective reduction of the total nitrogen and sulfur contents in the sugarcane yield and sugar content [40]. However, the authors of reference [38] found that the bacterial population tended to increase after the fire because of more available carbon sources. In the current study, the bacterial structure in the soil changed after burning during sugarcane harvest (Table 2 and Figure 3). Notably, a large proportion of *Paenibacillus* was found in the soil after burned sugarcane harvest (Figure 3). These endospore-forming bacteria are heat-tolerant [41] and can survive in a burned crop. Several known species of *Paenibacillus* sp. are known to colonize the plant rhizosphere, promoting the growth and productivity of crops, including rice, maize, pumpkin, poplar, and switchgrass [42]. *Candidatus Koribacter*, *Gaiella*, *Pseudomonas*, and *Sphingomonas* in the soil after unburned sugarcane harvesting had greater relative abundance than in soil after burned sugarcane harvesting. These bacteria are beneficial for sugarcane growth, with *C. Koribacter* in the Acidobacteria related to nutrient mineralization [43], while *Sphingomonas* and *Gaiella* decompose lignocellulose and contribute to nutrient cycling [44]. *S. paucimobilis* ZJSH1 promoted plant growth through nitrogen fixation and various phytohormones, including salicylic acid, indole-3-acetic acid, zeatin, and abscisic acid [45]. The diversity of *Pseudomonas* spp. was associated with nitrogen fixation and indole-3-acetic acid production in sugarcane in Guangxi, China [46], and the effect of *Pseudomonas fluorescens* with other inocula reduced the dose of phosphate fertilizer and phosphorus accumulation in sugarcanes at the end of the cycle [47]. These results were related to the work of Kirkby

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**Figure 3**: Relative abundance of major bacteria by (a) phylum and (b) genus from soil samples of unburned and burned sugarcane plots.

**Table 2**: Soil bacterial diversity indices and richness estimates of unburned and burned sugarcane harvest determined using Illumina MiSeq sequencing analysis.

<table>
<thead>
<tr>
<th>Diversity index</th>
<th>Unburned</th>
<th>Burned</th>
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<tbody>
<tr>
<td>Ace</td>
<td>335.23 ± 4.33</td>
<td>226.80 ± 5.20</td>
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<td>Chao I</td>
<td>336.21 ± 6.94</td>
<td>226.88 ± 7.32</td>
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<tr>
<td>Shannon</td>
<td>7.04 ± 0.06</td>
<td>4.53 ± 0.08</td>
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<tr>
<td>Simpson</td>
<td>0.986 ± 0.002</td>
<td>0.664 ± 0.003</td>
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<tr>
<td>Good’s coverage</td>
<td>1.000 ± 0.001</td>
<td>0.999 ± 0.001</td>
<td>ns</td>
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Data presented as mean ± standard deviation, n: 3, **P < 0.01, and ns: not significant.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bac</th>
<th>Actino</th>
<th>Fungi</th>
<th>PSB</th>
<th>NF</th>
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<td>Organic matter (OM)</td>
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<td>Potential of hydrogen (pH)</td>
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<td>Total nitrogen (TotalN)</td>
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<td>Available phosphate (AvaiP)</td>
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<td>Exchangeable potassium (ExchK)</td>
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<td>Cation exchange capacity (CEC)</td>
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<td>Moisture</td>
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<td>Bacteria (Bac)</td>
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<td>Actinomycetes (Actino)</td>
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<td>Fungi</td>
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<td>Phosphate-solubilizing bacteria (PSB)</td>
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<td>Nitrogen fixing bacteria (NF)</td>
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<td>Paenibacillus</td>
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<td>Bacillus</td>
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<td>Streptomyces</td>
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<td>Candidatus solibacter</td>
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<td>Anmoniphilus</td>
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<td>Gaiella</td>
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<td>Pseudolabrys</td>
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<td>Bryobacter</td>
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<td>Paracoccus</td>
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<td>Conexibacter</td>
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<td>Dongia</td>
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<td>Bradyrhizobium</td>
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<td>Candidatus koribacter</td>
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<td>Occallatibacter</td>
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<td>Nitrospira</td>
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<td>Burkholderia</td>
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<td>Jatrophihabitans</td>
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<td>Tumebacillus</td>
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<td>Gemmatimonas</td>
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**Figure 4: Continued.**
and Fattore [48], who reported that the stubble retained in soils had higher microbial biomass and microbial activity than in stubble-burned soils. Turning the harvest from burning to green (unburned) reportedly positively affected many soil properties, such as carbon stock, microbial biomass, soil enzymatic activity, and soil aggregation [49, 50]. Crop residues from sugarcane harvesting provide ecosystem services, nutrient recycling, soil biodiversity, water storage, carbon accumulation, and soil erosion control and restrict weed infestation [51]. Therefore, soil management using unburned sugarcane harvest and allowing the waste products to decompose in the field should be a better option than burned sugarcane harvest.

With burned sugarcane harvest, the available phosphorus and exchangeable potassium had a positive correlation with the dominant bacterial community (Figure 4). The reason for the increase in nutrients was the carbon dioxide released from burning [52], combined with water to produce carbonic acid that affected the soil pH; thus, the pH controls the soil biology and soluble nutrients [53]. Another reason for the dominance of the Bacillus genus in the current research was its reported effectiveness in dissolving phosphorus and potassium [54, 55]. However, the moisture, actinomycetes, bacteria, and fungi had a high negative correlation with the dominant bacterial community in the current research; with burning, some groups of bacteria decreased, while others increased. The cellulolytic and amylolytic populations slightly decreased, but the ammonium oxidizers were positively affected by fire [56]. A similar situation was evident in the current research, where the phyla Acidobacteria and the genera C. solibacter and C. koribacter, Streptomyces spp., and Bradyrhizobium, which produce cellulolytic or amylolytic enzymes decreased, while an ammonia-oxidizing bacteria Paenibacillus increased. The moisture also affects bacterial numbers in the soil [57]. However, further research is required because different soil series and the soil management methods associated with postharvest burning may change the bacterial community structure and its relationship to soil properties.

5. Conclusion

Burning during sugarcane harvest resulted in reductions in soil properties (pH and the total moisture and nitrogen contents) and the number of microorganisms. The correlation between soil properties and soil bacteria population structure changed patterns in unburned and burned sugarcane harvest, with the bacterial structure in the burned
sugarcane harvest decreasing. These bacteria are important for soil fertility and quality that affect plant growth. However, spore-forming bacteria survived the sugarcane burning, of which *Bacillus* was correlated with phosphorus and potassium solubilization.

**Data Availability**

The metagenomics data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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