VNU Journal of Science: Earth and Environmental Sciences
Journal homepage: https://js.vnu.edu.vn/EES

Original Article

Impacts of Land Uses and Droughts on Dissolved Phosphorus Release and Phosphate Solubilizing Bacteria: A Case Study in Son La, Vietnam

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Received 20th April 2025
Revised 9th May 2025; Accepted 19th May 2025

Abstract: Climate change is causing an increase in extreme weather events, including droughts in many areas, such as forests and agricultural lands. Drought leads to the dessication of soil microbial cells and nutrient lysis caused by osmotic shock, hence releasing dissolved phosphorus (P) into the soil solution. Drought stress for soils of different land uses can shift the soil microbial community significantly. This study compared the biogeochemical impacts of various land uses under drought stress. Soil samples were collected from the natural forest, corn, and coffee plantations in Son La province, Vietnam. Soils were desiccated up to water content about 2-5%, while controls were kept at 60% soil water holding capacity. The experiment was conducted in a climate chamber at 25 ºC for seven weeks in the laboratory. The P release and microbial biomass from natural forest soils, as well as corn and coffee plantation soils were calculated and compared to controls after seven weeks. Soil properties were different among land-use types. Natural forest soil had the highest total P (TP) content, followed by corn and coffee plantation soils, with values of 0.27, 0.27 and 0.09%, respectively. Drought stress increased total dissolved P (TDP) concentrations from 1.0 to 1.17 mg kg^{-1} in natural forest soil, 0.8 to 0.91 mg kg^{-1} in coffee plantation soil, and 0.69 to 0.75 mg kg^{-1} in corn plantation soil. Drought significantly reduced microbial biomass from 1,344 to 526.6 mg kg^{-1} in coffee plantation soil, from 309.2 to 77.2 mg kg^{-1} in corn plantation soil and from 250.6 to 100.9 mg kg^{-1} in natural forest soil. There was a significant difference in carbon mineralization between drought and control conditions. Carbon mineralization in the control samples ranged from 1.04 to 1.08 mg kg^{-1}, while in the drought experiments, it ranged from 0.64 to 0.67 mg kg^{-1}. Drought conditions can lead to the death of phosphate solubilizing bacteria. However, the effects of drought on microbial communities and the recovery of these microorganisms require further study.

Keywords: Carbon mineralization, drought stress, microbial biomass, phosphate solubilizing bacteria, phosphorus release.

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https://doi.org/10.25073/2588-1094/vnuees.5306

1. Introduction

Climate models predict an increase in global surface temperature and a change in precipitation intensity during this century [1]. Abiotic stress like drought, temperature fluctuations raise more and more in both intensity and frequency caused by climate change [2]. Drought is a natural phenomenon that occurs regularly in many soils, especially topsoils a combination of biological and physical processes [3]. Changes in the water content of soil may affect physical processes due to various mechanisms, resulting in different fragment sizes of aggregates. Aggregates may break down by compression of entrapped during wetting (slaking) or by differential swelling of clays [4]. Biological processes include the death of soil microbes due to desiccation and lysis caused by osmotic shock upon rapid rewetting, releasing nutrients into the soil solution. Microorganisms may survive the desiccation by production of cell osmotytes. Many microorganism use simple organics with a good balance of high solubility and limited direct physiological effects [5]. The disruption of soil aggregates after drying-rewetting releases organic matter [6] and nutrients, which are potentially available to plants [7]. Some of the nutrients released during the drying period may be taken up by other soil microbes or might maintain in solution when the soil is rewetted [5]. The decline of soil water content under drought periods reduces soil microbial activity, resulting in higher insoluble carbon compounds and a slow rate of organic matter decomposition; this also affects mineralization processes that release readily available nutrients (e.g., N and P) [8–11]. The C/N ratio also decreases with increasing drought intensity [12]. The effect of drought on soluble nutrients (C, N, as well as P) has been studied [13, 14].

Phosphorus (P) is an essential element for all living organisms as P is active in the energy metabolism, the formation of phospholipids in cell membranes [15, 16]. In soils, P is present as inorganic and organic P. Inorganic P (Pi) usually accounts for 35 to 75% of total P in soil [17] in form of at least 170 different naturally mineral and the most common primary mineral form of P is apatite that was weathered slowly, releasing Pi as orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) [18]. Organic P (Po) comprises 30 to 65 % of the total P in soil, although soil with high organic matter contents can contain up to 90% Po [17]. Soil Po originates from plant residues, animal wastes and the soil microbial biomass [19]. A large proportion of Po in soils is bound in the microbial biomass. Microbial biomass accounts from 2 to 5% of total organic P in cultivated soil, but up to 20% in grassland soils [20] and up to 50% in forest floors [21]. Soil solution P concentrations are relatively low, the quantity of P in the soil solution at a given time is generally on the order of < 1 kg ha^{-1}, or < 1% of the total quantity of P in the soil [22]. Generally, only a small proportion of total stocks of P is in the soil solution, which is the source that is directly used by plant roots [18], and often not sufficient to supply the requirements of plants [23]. In soil solution, a significant proportion of P is represented by dissolved organic P (DOP) [24–26]. The organic P (Po) can be mineralized mediated by soil organisms and plant roots that release phosphatases. Po transformation has a great influence on the overall bioavailability of P in soil [27]. After drought, the total dissolved P in soil increases, and this increase is mainly due to an increase in dissolved organic P (which can increase by up to 50%) by microbial cell lysis [5, 14]. In forest floors, after dried soil at 60 °C, the net dissolved inorganic P (DIP) release increased up to 48-76 mg P kg^{-1} in mineral soil, while the net release of DIP was up to 70 mg P kg^{-1} in organic layer after soil was air-dry at 20 ^oC [28].

Soil drying can kill up to 58% of the total microbial biomass [29–31] and several research groups concluded that the biomass could be an important source of P in soil solution after D/W [32]. Few studies confirmed that the D/W caused change in soil microbial community. The changes were showed in the decrease of microbial biomass [30, 33, 34]; in the change of soil microbial communities structure [35–37]

and bacteria are less affected than fungi [38]. Another study indicated that the different groups of soil microorganisms reacted differently to D/W and P release, with fungi and gram-positive bacteria being less sensitive than gram-positive bacteria [39].

Land-use patterns are an important factor affecting the physical, chemical and biological properties of soil, affecting processes occurring in soil such as organic matter decomposition, erosion, desertification. Total carbon content varies with land-use patterns and this content in natural forest soil is the largest compared to other land-use patterns as demonstrated by many studies [40, 41]. Among different land-use types, the maximum values of pH, cation exchange capacity, total nitrogen content, total P (TP) content, nitrogen content, and available P in soil are not consistent among studies; but the differences among land-use types are concluded by most studies [40–45]. However, information on the response of different land-use patterns to drought through the reslease of P and bacteria is still lacking.

Vietnam is one of the countries to be severely affected by climate change [46]. Hot and sunny weather caused long drought season have occurred dramatically in various region in Vietnam [47, 48]. The global mean air temperature of the regions of Vietnam will increase on average between 1.6 and 3.0 ^{o}C in the basic scenario for the 21st century, specially from 2.5 to 3.7 ^{o}C in some areas [46–48]. The changes in climate like the increase in extreme drought and rainfall events and the variations in rainfall intensity and pattern, strongly influences soil moisture. The response of soil to climate change plays a crucial role in Vietnam. To our knowledge, there are no studies focusing on the effect of drought on the release of dissolved P from different land uses, especially natural forest, corn, and coffee plantations. In this study, we hypothesized that i) The release of P increases with drought; ii) The release of P due to drought is higher in soil of natural forest than in soil of corn and coffee plantations; and iii) Microbial biomass decreases following drought. We conducted a laboratory experiment to test hypotheses using soils from various land use types under both drought and moist conditions.

2. Materials and Methods

2.1. Samplings

Soil samples were collected from the top layer (0-20cm) under different land-use types (coffee plantation (21^{o}18’44’’N, 103^{o}56’40’’E), corn plantation (21^{o}18’38’’N, 103^{o}56’12’’E), natural forest (21^{o}18’40’’N, 103^{o}56’52’’E) in Son La province, Vietnam. These three land-use types have the same soil types (Rhodic Ferralsols). Samples were separately air-dried, gently ground, homogenised and then passed through a 1-mm sieve.

TP, total carbon (TC), total nitrogen (TN), pH, and soil texture were analyzed in all samples. For these analyses, mineral soil will be dried at 105 °C. Total P will be determined after digestion with HNO_3 using an ICP-OES. Total N will be determined by Kjeldahl method. Total carbon will be determined by Walkley and Black (1934) [49]. Soil pH was determined using 1 M KCl (1:2.5, w/v). Soil texture was examined by the pipette method.

2.2. Experimental Design

Moist samples will be arranged in a 2 cm layer in *storage boxes* (12 x 8 x 9 cm) *with lids* in six replicates per soil sample per day. All samples will be adjusted to a water content equivalent to 60% of the maximum water holding capacity (WHC) and will pre incubated for 1 week until processing of drought in order to allow the microbial activity to reach basal rates after changing WHC. At the end of the incubation period, the drought experiment will be started by opening the boxes of the drought treatment to allow the soil to dry. The control boxed will be kept close during the whole experiment and they were controlled WHC by weight daily. The pre-incubation and the experiment will be conducted in a climate

chamber at 25 °C. Soil water potentials will be measured daily until a water content of approximately 2-5% is reached (6 weeks from the opening top). After this point of desication, 3 drought treatment and 3 controls for each of soil sample were destructively harvested to determine total dissolved P (TDP); to determine microbial biomass, to isolate P solubilizing microbes. This experimental design results in 18 solute samples for analysis of TDP (3 different land uses x 2 treatments x 3 replicates), and 18 soil samples for microorganism (3 different land uses x 2 treatments x 3 replicates).

2.3. Analytical Method of Total Dissolved Phosphorus

Soil samples were extracted in deionized water in a soil: water with the ratio of 1:10 by shaking the soil for 140 min on a horizontal shaker, and were filtered through a cellulose membrane acetate filter (0.45 µm, Sartorius AG, Göttingen, Germany) in order to determine TDP using ICP-OES.

2.4. Analytical Method of Microbial Biomass

Microbial biomass carbon (MBC) was measured by the chloroform fumigation-extraction method [30, 50, 51]. After fumigation the soils were extracted with K_2SO_4 0.5 M solution with a soil: solution ratio of 1:4. Total P in the Bray-1 extracts were measured by ICP-OES. Pmic was calculated as difference of inorganic P in the fumigated and non-fumigated soil extracts using a converision factor of 2.5 [52, 53]. Total organic carbon (TOC) was determined using a TOC Analyser.

2.5. Analytical Method of Soil Carbon Mineralization

Soil basal respiration was measured by using alkali trapping methods to determine CO_2 [54,55]. 70 g of soil from treatments and controls were incubated in sealed Mason jars at a constant temperature of 28 ^oC. A small vial containing 10 ml of NaOH 1 N was placed in each Mason jar to absorb CO_2 released by microbial respiration. The vial was replaced after 7 h to measure indirectly the trapped CO_2 by titrating with 0.1 N HCl.

2.6. Isolation of Phosphate-solubilization Bacteria and Evaluation of the Potential of Isolated Phosphate Solubilizing Bacteria in Releasing of Soluble Phosphorus

After treatment, the soil samples were isolated for phosphate solubilizing bac. First, 10 grams of soil from each sample were dissolved in 90 ml of sterilized water. The soil solution was then incubated on a thermostatic shaker at 25 °C for 24 h, with a shaking speed of 100 rpm. The soil solution was then diluted to a concentration of 10^{-4}. To isolate the phosphate-solubilizing bacteria, 0.5 ml of the diluted soil solution was spread evenly over the surface of Pikovskaya agar medium. After incubating in the dark at 25 °C for 7 d, colonies that were capable of solubilizing phosphate were separated and purified on new medium plates.

The ability to solubilize insoluble phosphate was evaluated using Pikovskaya agar. Each strain was inoculated at three different points on the agar plate, and the experiment was repeated 3 replications. After culturing in the dark at 25 °C for 7 d, the diameter of the phosphate solubilization zone was measured. All bacterial isolates showed phosphate solubilizing ability were cultured into Pikovaskaya broth medium at 25 ^oC on a shaker for 7 d, with a shaking speed of 150 rpm. The supernatant was filtered through a cellulose acetate membrane (0.45 µm). The soluble P content in the culture broth was measured spectrophotometrically using the colorimetric method for estimation of ascorbic acid [56]. The bacterial isolate with the strongest phosphate solubilizing ability was identified by sequencing of the 16S rDNA gene region. The bacterial genomes were extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA). PCR amplification of the sequences was performed using the following primer pairs: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-

3'). The PCR products were sequenced using the Sanger technique, and the complete DNA sequences were compared to the GenBank database at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) via BLAST searches.

2.7. Statical Analysis

All statistical analyses were performed in a Windows environment using SPSS software version 27. To analyze the differences in soil properties between various land-use types and the amount of P released, we utilized analysis of variance (ANOVA). This was followed by Tukey's Honestly Significant Difference (HSD) test for post hoc analysis.

3. Results and Discussion

3.1. Soil Properties

Table 1 showed soil properties in different land-use types. The lowest pH value was observed in the soil of coffee plantation (4.8), indicating an acidic soil. The pH value of the soil in the corn plantation and in the natural forest were 5.88 and 5.99 respectively, showing a neutral soil. The observed pH values can be attributed to the depletion of alkaline metal cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), and sodium (Na^{+}) caused by erosion processes in coffee plantations, which have minimal vegetation cover. In comparison, corn plantations possess slightly higher vegetation coverage than coffee plantations, yet still fall short of the coverage found in natural forests. Additionally, both corn and coffee plantation soils were regularly fertilized. The addition of NH_4^+ and NO_3^- ions promotes the oxidation process that converts NH_4^+ into NO_3^-, which increases the concentration of H^+ in the soil solution.

TC, TN and TP concentrations differ significantly among soils under the three land-use types ($p < 0.05$). The TC content in coffee plantation soil was the lowest with 1.1%. In comparison, natural forest soil had a carbon content of 2.54%, while corn plantation soil boasted the highest level at 3.68%. This variation is likely due to differences in vegetation cover. Natural forests have litter layers, contributing organic matter to the soil, while corn plantation areas are covered with by-products from the corn harvest, such as stalks, sheaths, and leaves, which will be decomposed and enrich the soil with organic matter. The TN content was ordered in soil of a corn plantation (0.48%) > natural forest soil (0.34%) > soil of a coffee plantation (0.13%) (Table 1).

Table 1. Soil properties for various land-use types. The values represent means and standard deviations (n = 3).

Land-use type	**pH**	**TC** [%]	**TN** [%]	**TP** [%]	Clay [%]	Limon [%]	Sand [%]
Coffee plantation	4.80 ± 0.03	1.10 ± 0.07	0.13 ± 0.01	0.09 ± 0.01	45.55	43.05	11.40
Corn plantation	5.88 ± 0.03	3.68 ± 0.10	0.48 ± 0.04	0.22 ± 0.03	44.57	44.28	11.15
Natural forest	5.95 ± 0.02	2.54 ± 0.10	0.34 ± 0.01	0.27 ± 0.08	44.79	40.94	14.27

The total P content was highest in natural forest soil, at 0.27%, that was three times higher than the P content found in coffee plantation soil (0.09%). Following that, corn plantation soil had a P content of 0.22%, more than twice that of coffee plantation soil. The carbon/nitrogen (C:N) ratio is commonly used to evaluate the potential rate of decomposition and the quality of soil organic matter [57]. The C:N ratios of the soils were observed to increase in the following order: natural forest soil (7.44), coffee plantation soil (12.22), and corn plantation soil (16.72). All measured C:N ratios were below 20, indicating that the decomposition of organic matter in these areas released sufficient soluble nitrogen (available nitrogen) to support plant growth. The

variation in C:N ratios across different soils may be attributed to the diverse sources of organic matter.

The clay contents ranged from 44.57% to 45.55%, limon contents ranged from 40.94% to 44.28%, and sand contents ranged from 11.15% to 14.27%. There was no significant difference in clay percentage between various land-use types, and this is approximately 45%. Therefore, clay particles are not a major factor for variations in soil nutrient content.

3.2. Release of Total Dissolved Phosphorus

The TDP concentrations in the drought experiment in all land-use types were higher than those in the control experiment. This indicates that the drought process led to a release of TDP in the top layers. The TDP concentration in natural forest soil was consistently higher than that of both coffee and corn plantation soils in both the control and drought experiments. In the control experiment, the TDP concentrations were as follows: natural forest soil with 1.0 mg kg^{-1}, coffee plantation soil at 0.8 mg kg^{-1}, and corn plantation soil at 0.69 mg kg^{-1}. In the drought experiment, the TDP concentrations were ordered natural forest soil (1.17 mg kg^{-1}) > coffee plantation soil (0.91 mg kg^{-1}), and corn plantation soil (0.75 mg kg^{-1}) (Figure 1). Turner (2005) also indicated that the amount of soluble P varies depending on both soil type and land-use, ranging from less than 0.02 mg kg^{-1} of soil to 1.0 mg kg^{-1} of soil [58].

We extracted TDP from soil by distill water after desiccation regime, this can be soil was impacted by drying/rewetting cycle. The change in soil water potential created a strong stress to soil microorganisms [59]. After a drying-rewetting cycle, the microbial biomass is a major source of P release [32, 60]. Additionally, several studies have shown that, following a rewetting drought process, the soluble P released from the soil is greater compared to normal conditions [32, 61].

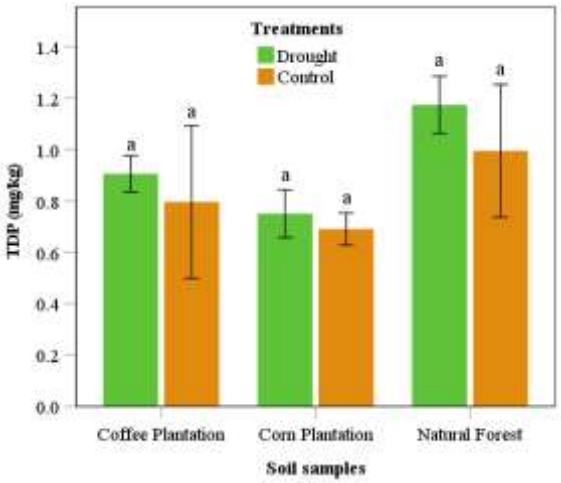

Figure 1. Total dissolved phosphorus (TDP) in different land-use types under the effect of drought. Bars represent the means and standard deviation (n = 3); different letters indicate a significant difference ($p < 0.05$) among soil samples by Tukey's HSD test.

3.3. Response of Microbial Biomass Carbon to Drought

MBC in the control experiments was consistently 2.48 to 4.01 times higher than in the drought experiments ($p < 0.05$). The drought conditions reduced MBC by up to 4.01 times in corn plantation soil, 2.55 times in coffee plantation soil, and 2.48 times in natural forest soil (Figure 2). Specifically, drought reduced microbial biomass by more than 60% in coffee plantation soil, 75% in corn plantation soil, and 60% in natural forest soil compared to the control. This result supports the initial research hypothesis that microbial biomass carbon decreases following drought. This finding is similar to a study by Hueso et al., (2011) [62], who showed that drought conditions can reduce microbial biomass by 48 to 63%, and the soil microflora remained intact and was not destroyed up to two months. The most substantial decrease in microbial biomass occurred in corn plantation soil, which aligns with the high amount of organic matter present in that soil. The decrease of microbial biomass seem likely the main source of P release [32, 60].

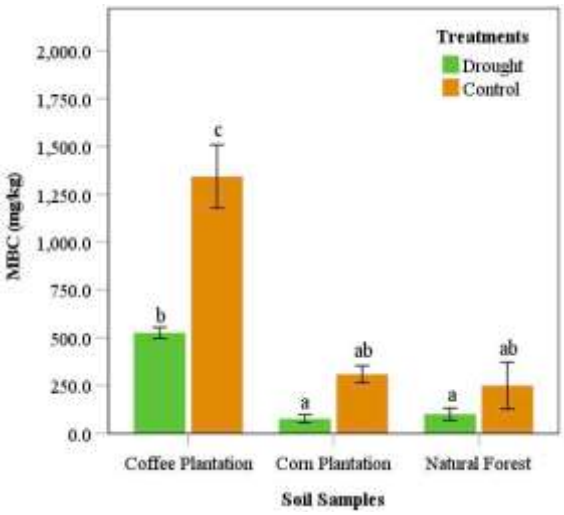

Figure 2. Microbial biomass carbon (MBC) in different land-use types under the effect of drought. Bars represent the means and standard deviation (n = 3); different letters indicate a significant difference ($p < 0.05$) among soil samples by Tukey's HSD test.

3.4. Response of Carbon Mineralization to Drought

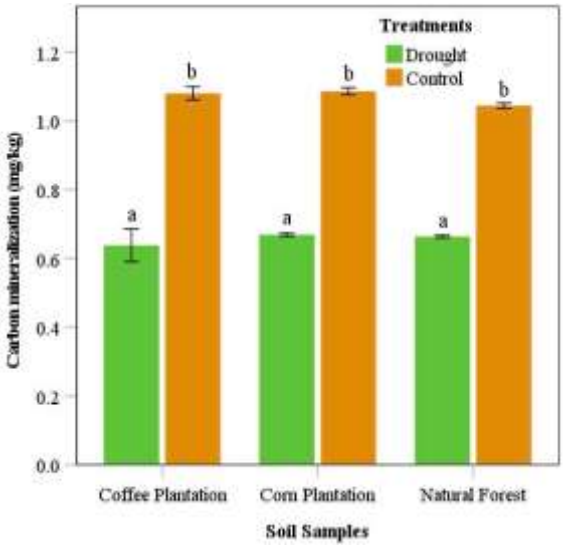

Figure 3. Carbon mineralization in different land-use types under the effect of drought. Bars represent the means and standard deviation (n = 3); different letters indicate a significant difference ($p < 0.05$) among soil samples by Tukey's HSD test.

The response of different land-use types to drought in terms of carbon mineralization to drought did not differ. However, there were a significant difference in terms of carbon mineralization between drought and contronl ($p < 0.05$). Carbon mineralization in control experiments ranged from 1.04 to 1.08 mg kg^{-1}, while those content were from 0.64 to 0.67 mg kg^{-1} in drought experiments (Figure 3). Drought stress on soil microorganisms can cause them to enter a dormant state or result in cell disruption [59]. This phenomenon explains why MBC decreased by 2.48 to 4.01 times under drought conditions compared to the control.

3.5. Response of Phosphorus Solubilizing Bacteria to Drought

Under the same experimental conditions, phosphate-solubilizing bacteria were unable to survive in drought conditions across all land-use types. Furthermore, no bacterial isolates exhibiting phosphate-solubilizing activities were found in the coffee plantation soil (Table 2). The choice of isolation medium can impact the results when isolating P-solubilizing bacteria [63]. Some bacteria did not exhibit a clear zone on agar plates, yet they can solubilize inorganic phosphates in a liquid medium [63, 64]. Hence, Bashan et al., 2013 [65] suggested that P-solubilizing bacteria should be selected using both agar and liquid media.

In the control experiments conducted under moist conditions, three isolates (VK01, VK02, VK03) were obtained from the corn plantation soil. Additionally, six isolates (VK04, VK05, VK06, VK07, VK08, VK09 were collected from the soil of natural forest. These isolates demonstrated the ability to solubilize insoluble phosphate, with the diameter of phosphate solubilization ranging from 2.33 to 9.33 mm on Pikovskaya agar medium. Furthermore, the soluble P content in the culture solutions varied, measuring between 11.55 and 129.71 P_2O_5 mg/100 ml. Although isolate VK09 was not the best P solubilizer in the agar medium, the isolate VK09 demonstrated the highest level of

phosphate solubilization with 129.71 P_2O_5 mg/100 ml after 7 d of incubation. The bacterial isolate (VK09) that exhibited the highest capability for phosphate solubilization was identified at the species level by sequencing the 16S rDNA gene region. The sequencing results were compared with the GenBank database using the BLAST search tool. The isolate was confirmed to be *Burkholderia cenocepacia*, showing a 100% identity match.

Burkholderia cenocepacia CR318 can also be isolated from the roots of starch corn. This isolate exhibited strong activity in Pi and K solubilization. Additionally, it plays a role in promoting plant growth under greenhouse conditions [66]. Another study showed that *Burkholderia cenocepacia* CEIB S5-2 isolated from angricultural soil can degrade fastly glyphosate and metabolite aminomethylphosphonic acid in just 8 h [67].

Table 2. Isolation and phosphate-solubilizing activities of bacteria obtained from various land-use types under drought and moist conditions. The values represent means and standard deviations (n = 3)

Land-use type	**Treatment**	**Isolates**	**Diameter of phosphate solubilization** (mm)	**Soluble phosphorus content** (mg P_2O_5/100 ml)
Coffee plantation	Drought	No phosphate solubilizing bacteria		
	Control	No phosphate solubilizing bacteria		
Corn lantation	Drought	No phosphate solubilizing bacteria		
	Control	VK01	2.33 ± 0.44	22.13 ± 0.19
		VK02	6.50 ± 0.29	26.71 ± 0.96
		VK03	8.00 ± 0.29	29.78 ± 4.01
Natural forest	Drought	No phosphate solubilizing bacteria		
	Control	VK04	9.33 ± 0.93	21.39 ± 3.53
		VK05	9.17 ± 0.88	11.55 ± 3.87
		VK06	5.67 ± 0.17	67.39 ± 2.74
		VK07	2.50 ± 0.29	48.72 ± 1.75
		VK08	6.83 ± 0.44	93.74 ± 6.85
		VK09*	**6.00 ± 0.58**	**129.71 ± 7.19**

* The bacterial isolate showed the stronghest phosphate solubilizing activity

4. Conclusions

This study found that soil under different land-use types respond differently to drought and the release of TDP, with the highest P release observed in the soil of natural forests. Additionally, microbial biomass decreased due to drying conditions, which led to the complete elimination of all phosphate-solubilizing bacteria in the soil. Nine isolates of phosphate solubilizing bacteria were obtained from the soils of the corn plantation and natural forest under moist conditions. Among these, the *Burkholderia cenocepacia* (VK09) isolate demonstrated the highest phosphate solubilizing efficiency. Notably, there were no phosphate solubilizing bacteria found in the soil of coffee plantation in either drought or moist conditions.

Acknowledgements

We would like to thank Do Quang Trung, Tran Thi Hang, and Nguyen Manh Ha for their assistance in the laboratory.

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