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Impact of Paraquat Herbicide Application on Soil Microbial (Bacterial and Fungal) Populations and Some Physico-Chemical Properties of Soils under Plantain (*Musa paradisiaca*) Plantation

Ikponmwosa Ogbemudia[*)], Edosa Valerie Ifeyinwa, Abamba Esther Ejiro, Ogboghodo Ikponmwosa Abraham

Department of Soil Science and Land Management, University of Benin. Benin City, Nigeria
P.M.B. 1154, Ugbowo Lagos Road, Benin City, Edo State, Nigeria.
[*)]Corresponding author: ikponmwosa.ogbemudia@uniben.edu

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Abstract

Paraquat, a widely used non-selective herbicide, is commonly applied in plantain (*Musa paradisiaca*) plantations to suppress weeds. However, its repeated application raises concern about adverse effects on soil microbial communities and key soil properties that determine fertility and sustainability. This study evaluated the impact of paraquat application on soil microbial (bacterial and fungal) populations and some selected physical and chemical properties of soil under a plantain plantation to understand its influence on soil health. Soils were collected from a plantain plantation in Benin City, Nigeria and treated with paraquat at control (0), recommended rate (RR), and twice the recommended rate (2RR). The experiment was laid out in a Completely Randomized Design (CRD) with three replicates per treatment. Microbial populations and soil properties were monitored at 0, 7, and 14 days after application using standard microbiological and physico-chemical analyses. The RR paraquat application stimulated bacterial and fungal populations over time, suggesting microbial tolerance at moderate concentrations. In contrast, the 2RR application suppressed microbial populations, reduced total nitrogen and organic carbon, and altered nutrient dynamics. Available phosphorus and exchangeable cations (K, Ca, Mg, Na) increased with paraquat rate, while exchangeable acidity (H^+, Al^{3+}) decreased. Soil pH and texture influenced paraquat's bioavailability and effects, with the sandy, moderately acidic soil enhancing its mobility and toxicity at higher doses. Moderate paraquat use can enhance microbial activity and maintain soil fertility, but excessive application disrupts microbial communities and nutrient balance. These findings reveal the need for judicious paraquat application to safeguard soil health and support sustainable weed management.

INTRODUCTION

The intensification of agricultural practices has led to widespread use of herbicides as a means of controlling weeds and improving crop productivity (Cobb, 2022 and Parven et al., 2025). Among these, paraquat, a non-selective, fast-acting contact herbicide (Dennis et al., 2018) has been extensively used in tropical and subtropical regions due to its effectiveness and affordability (Janaki et al., 2015). In plantain (*Musa paradisiaca*) plantations, which are economically and nutritionally important in many developing countries, paraquat is commonly applied to suppress weed growth and reduce competition for water and nutrients (Sumekar et al., 2024). However, the repeated and indiscriminate use of paraquat has raised concerns about its potential adverse effects on soil health, particularly the soil microbial communities and key soil

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properties that determine long-term soil fertility and ecosystem functioning.

Soil microbes, including bacteria and fungi, play critical roles in nutrient cycling, organic matter decomposition, soil structure stabilization, and plant health (Ogbemudia et al., 2022). Changes in the abundance, diversity, and activity of these microorganisms due to herbicide residues may compromise soil quality and sustainability (Janaki et al., 2015). Similarly, paraquat may alter soil physical and chemical properties, such as pH, organic matter content, and porosity, which influence both microbial habitats (Singh et al., 2020) and plant growth. Previous studies have reported variable effects of paraquat on soil microorganisms, ranging from transient suppression of microbial populations to shifts in community composition, depending on factors such as soil type, climate, and application rate (Teke et al., 2024). For example, some reports (Stanley et al., 2013; Singh et al., 2020; Rathod et al., 2021 and Gandhi et al., 2021) indicated that paraquat binds strongly to soil particles and reduces its bioavailability, while others suggest it can inhibit microbial enzymes and reduce microbial biomass, particularly with frequent application (Meena et al., 2020). Despite these findings, there is still limited and inconsistent information on how paraquat affects microbial communities and soil quality, specifically under perennial cropping systems like plantain plantations, where herbicides are applied repeatedly over long periods.

Given the importance of maintaining soil health for the sustainability of plantain production, it is crucial to better understand the extent to which paraquat influences soil microbial dynamics and some soil properties. The present study addresses the research gap by evaluating the impact of paraquat application on soil bacterial and fungal populations as well as selected physical and chemical properties of soils under a plantain plantation to provide a more comprehensive assessment of paraquat's effects on soil health, thereby informing more sustainable weed management practices under a plantain plantation.

MATERIALS AND METHODS

Soil Samples Collection Preparations

This study was conducted using soils collected from a *Musa paradisiaca* (plantain) plantation located within the University of Benin, Benin City, Nigeria. The plantation is located at 6°23'58.512"N to 6°28'58.734"N and 5°37'46.416"E to 5°37'47.502"E. The area falls within the humid tropical rainforest zone of southern Nigeria, characterized by high annual rainfall, warm temperatures, and deeply weathered, lateritic soils typical of the region. The soils are generally sandy-loam to loamy in texture, moderately acidic, and support a diversity of crops under rain-fed conditions.

Soil samples were collected randomly from the plantation using a soil auger at a depth of 0–15 cm, which represents the active root zone and the layer most influenced by agricultural management practices, including herbicide application. Sampling was done at multiple observation points across the plantation to capture spatial variability. The individual samples were thoroughly mixed to form a composite sample representative of the site. The composite soil was then air-dried under shade to preserve microbial integrity while reducing moisture content, and gently crushed to pass through a 2 mm sieve to remove stones, roots, and other debris.

Three (3) kg of the prepared soil was weighed into well-labelled, perforated plastic containers to allow for drainage and aeration. Three treatments were applied, including (i) control (no paraquat application), (ii) recommended rate of paraquat application, and (iii) twice the recommended rate of paraquat application to assess both standard usage and potential over-application effects. Each treatment was replicated three times, making a total of nine experimental units arranged in a Completely Randomized Design (CRD). This setup enabled the evaluation of paraquat's impact on soil microbial (bacterial and fungal) populations and selected physical and chemical properties of soils under controlled conditions while reflecting field-relevant situations.

Herbicide Application Procedure

The herbicide used in this study was paraquat, chemically known as methyl viologen (1,1-dimethyl-4,4'-bipyridinium dichloride), a widely used non-selective contact herbicide. Commercial-grade paraquat was procured from a registered agricultural input supplier located at Ring Road, Benin City, Edo State, Nigeria. The application rates were based on the manufacturer's recommendation for field use, specified as 1.5 liters of paraquat diluted in 50 liters of water per hectare.

To simulate field-relevant exposure levels in the

controlled laboratory experiment, the recommended rate was converted to the equivalent amount per kilogram of soil in the experimental containers. Specifically, 0.004125 mL of paraquat was diluted in 0.15 mL of water to achieve a recommended dose of 0.154125 mL of paraquat per 3 kg of soil (i.e., 0.051375 mL kg^{-1}). Similarly, for the twice recommended rate treatment, 0.00825 mL of paraquat was diluted in 0.3 mL of water to achieve a dose of 0.30825 mL of paraquat per 3 kg of soil (i.e., 0.10275 mL kg^{-1}). Meanwhile, the control treatment received no herbicide application.

The paraquat solutions were thoroughly mixed before application to ensure homogeneity. Application to the soil was performed uniformly using a precision micro-pipette to deliver the calculated volume evenly across the surface of each soil-filled container. Care was taken to avoid spillage, ensure even distribution, and maintain sterile conditions to prevent external contamination.

Following application, the soils were kept under ambient laboratory conditions, and the containers were monitored throughout the experimental period. To assess the short-term effects of paraquat on soil microbial populations and physico-chemical properties, samples were collected and analyzed at three time intervals: 60 minutes after herbicide application (0 days), 7 days and 14 days after application (DAA). This sampling schedule allowed for observation of the soil system's response to paraquat exposure over time.

Enumeration of Microbial (Bacterial and Fungal) Population

The serial dilution method was used to prepare soil for microbial population determination: representative soil samples were collected from the study site using a sterile soil auger. The samples were carefully placed into sterile plastic containers, sealed, and transported to the laboratory for microbial analysis while minimizing contamination.

In the laboratory, one gram (1 g) of the soil sample was accurately weighed and transferred into a sterile flask containing 9 mL of sterile distilled water or physiological saline. The mixture was shaken vigorously to detach bacteria from the soil particles and to produce a homogeneous soil suspension. Thereafter, a set of sterile test tubes, each containing 9 mL of sterile diluent, was prepared. From the initial soil suspension, 1 mL was aseptically transferred into the first tube and mixed thoroughly, yielding a 10^{-1} dilution. Subsequent tenfold serial dilutions were then prepared by transferring 1 mL from one tube to the next and mixing at each step until the desired dilution levels were achieved. From the 10^{-5} dilution, an aliquot of 0.1 mL was aseptically pipetted onto the surface of prepared agar plates: Nutrient Agar (NA) for bacterial enumeration and Potato Dextrose Agar (PDA) for fungal enumeration. The inoculum was evenly spread over the surface of the agar using a sterile glass spreader to ensure uniform distribution.

The inoculated plates for bacteria were incubated at a temperature of 25–30°C for approximately 24–48 hours until visible colonies developed. For fungi, the plates were incubated at the same temperature for a longer period, typically 24–72 hours, to allow fungal colonies to grow sufficiently. After incubation, plates exhibiting 30 – 300 colonies were selected for counting to ensure statistical accuracy and reliability of the results. The number of colony-forming units (CFU) per gram of soil was then calculated using the following standard formula:

$$\text{CFU/g soil} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume plated (mL)}}$$

(Cappuccino, and Sherman, 2014)

Physical and Chemical Analysis of the Soil Samples

The pH of the soil samples was determined (at 1:1) in water and in 1N potassium chloride (KCl) using glass electrode pH meter. Mechanical analyses of the soil was done using the Bouyoucos method as modified by Mwendwa (2022) to determine the sand, silt and clay fractions of the soil. Total nitrogen was determined using the Micro-Kjeldahl digestion method. Organic carbon was determined using wet oxidation method based on Walkley and Black modified by de Sousa et al. (2024). Exchangeable acidity was determined using 1 N extraction and titrated with 0.05 N NaOH solution to determine the extractable Al3+ and H+. Available phosphorus was determined using the Bray 1 method. The exchangeable bases (Na, K, Ca, Mg) were extracted from the soils with 1 N neutral ammonium acetate solution. The Na and K concentrations of the extract were determined using flame photometer, while Ca and Mg concentrations were determined using the Atomic Absorption Spectrometer (AAS).

Data Analysis

The statistical analysis was performed using the GenStat statistical software package (2018), and the means were separated using Duncan Multiple Range Test (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

Effects of Paraquat on Average Bacterial Population

On day 0 (one hour after paraquat application), the bacterial population slightly increased with the increasing paraquat rate (Fig. 1). The soils treated with paraquat at the recommended rate (RR) and twice the recommended rate (2RR) recorded bacterial populations of 9.18×10^5 and 9.40×10^5 CFU g^{-1}, respectively, compared to 7.05×10^5 CFU g^{-1} in the control. Although the differences were not statistically significant ($p > 0.05$), this early stimulation of bacterial growth is consistent with Maheswari and Ramesh (2019), who noted that herbicides can initially stimulate microbial activity by supplying a novel carbon and nitrogen source or by disrupting competing microbial communities.

By day 7, the bacterial population in soils treated with the recommended rate of paraquat rose significantly ($p < 0.05$) to 12.37×10^5 CFU g^{-1}, higher than in both control (7.88×10^5 CFU g^{-1}) and 2RR treatment (8.83×10^5 CFU g^{-1}). On day 14, the population peaked at 29.3×10^5 CFU g^{-1} under the RR treatment, followed by 15×10^5 CFU g^{-1} in the control and only 5.53×10^5 CFU g^{-1} in the 2RR treatment. This pattern suggests that while moderate paraquat application may promote bacterial growth, excessive concentrations (2RR) inhibit it, aligning with findings by Med et al. (2021), demonstrating dosage dependent herbicide toxicity on soil microbes.

The elements responsible for these effects are primarily the bipyridinium cations in paraquat (Dennis et al., 2018), which are redox-active and highly reactive. At moderate levels, certain bacterial species can metabolize the carbon and nitrogen within paraquat molecules or use them indirectly, boosting their growth (Saleh et al., 2022). However, at higher concentrations, the strong oxidative properties of paraquat disrupt cell membranes, inhibit enzymatic processes (Zaller et al., 2017), and generate reactive oxygen species, leading to reduced bacterial viability (Farago, 2022).

The soil's physical and chemical properties likely had impact on these results. The slightly acidic pH (6.0) and moderate organic carbon content (20.5 g kg^{-1}) can support microbial activity and reduce the toxicity of herbicides. This report corroborates the findings of Takeshita et al. (2018), who reported that

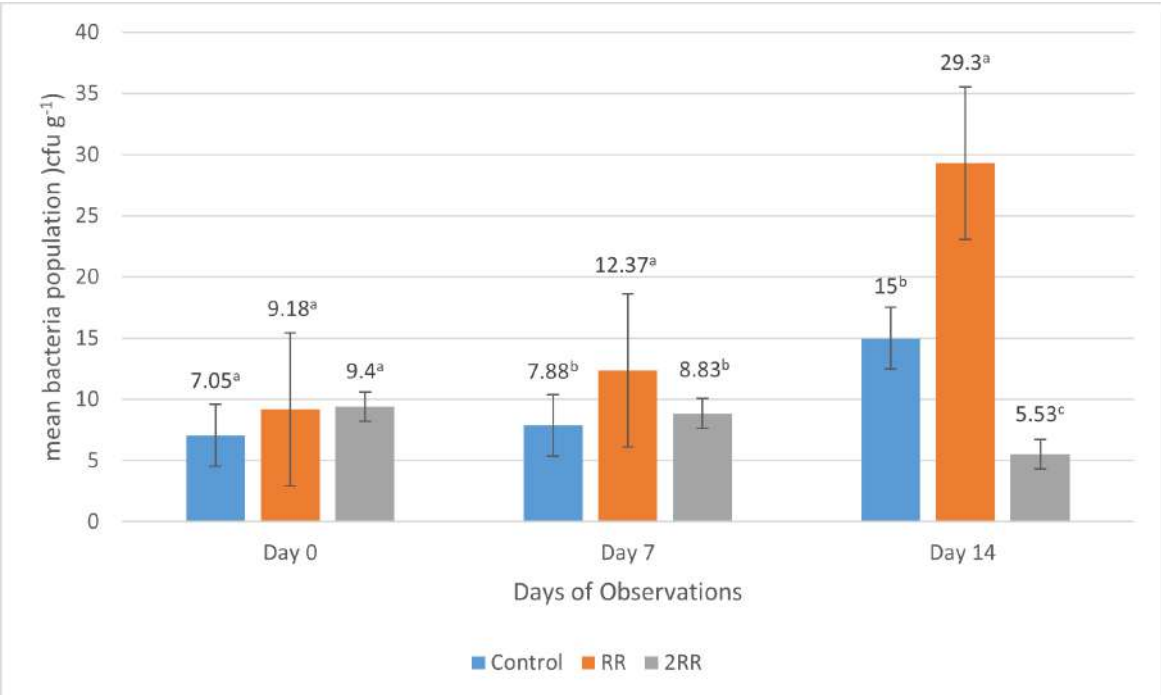

Figure 1. Average number of bacteria (x 10^5 $cfug^{-1}$) of soils at day 0, 7 and 14

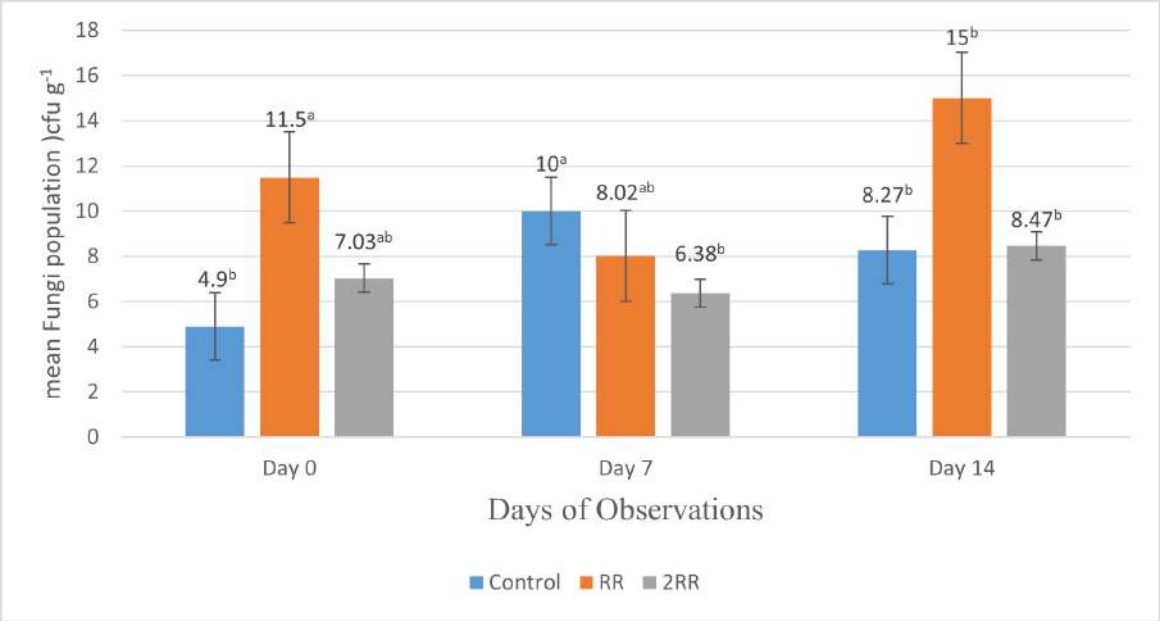

Figure 2. Average Number of Fungi (x 10^5 $cfug^{-1}$) of soil samples at day 0, 7 and 14

organic matter (OM) in the soil can decrease the mobility of herbicides in the soil profile. The sandy texture (86% sand) provides good aeration but may lead to lower adsorption of paraquat compared to clay-rich soils, increasing its bioavailability and toxicity at higher doses. Additionally, the low exchangeable aluminum (0.006 cmol kg^{-1}) and moderate base cations reduce stress on microbes, allowing the community to withstand paraquat inputs at recommended concentration.

Effects of Paraquat on Average Fungal Population

Figure 2 shows the average fungal population in soils subjected to different paraquat treatments over time. On day 0 (one hour after application), the fungal population ranged from 4.99 × 10^5 CFU g^{-1} in the control to 11.5 × 10^5 CFU g^{-1} in soils treated with the recommended rate (RR) of paraquat. The RR treatment showed a significantly ($p < 0.05$) higher fungal population than the control, suggesting that some fungi were able to utilize paraquat or its decomposition products as a substrate, at least transiently (Maheswari and Ramesh (2019).

By day 7, the highest fungal population (10 × 10^5 CFU g^{-1}) was observed in the control soil, while the lowest (6.38 × 10^5 CFU g^{-1}) was recorded in the soil treated with twice the recommended rate (2RR). The fungal population under the 2RR treatment was greatly repressed in comparison to the control, even though there was no significant difference between the RR and control treatments at the 5% significance level. This is because soil organisms do not survive excessive concentration of paraquat in soil (Meena et al., 2020). On day 14, the fungal population peaked at 15 × 10^5 CFU g^{-1} in the RR treatment, significantly higher than in both control (8.27 × 10^5 CFU g^{-1}) and 2RR treatment (8.47 × 10^5 CFU g^{-1}). This result is similar to the result obtained for bacteria, and it further suggests that moderate levels of paraquat can stimulate fungal activity, while excessive levels inhibit it. These findings are consistent with Singh et al. (2020), who noted that while recommended rates of some herbicides have limited effects on microbial activity, exceeding those rates can lead to significant suppression of microbial communities.

The observed responses can be linked to the chemical nature of paraquat as well as the physical and chemical properties of the soil. Paraquat is a bipyridinium herbicide that contains reactive nitrogen and carbon elements, which at low to moderate levels may serve as substrates for some fungi, enhancing their metabolism and growth. However, at high concentrations, paraquat's strong oxidative properties produce reactive oxygen species (ROS), which damage fungal cell membranes, enzymes, and DNA leading to growth inhibition (Moustaka, 2015; Gill et al., 2018).

However, the moderately acidic pH (6.0) of the soil, coupled with its sandy texture (86% sand), and

moderate organic carbon content (20.5 g kg^{-1}) could influence the extent of paraquat's effect. The acidic pH and low clay content reduce the soil's cation exchange capacity (CEC), limiting the adsorption of paraquat onto soil particles and making it more bioavailable, and thus, more toxic, especially at higher doses. The sandy texture also promotes rapid drainage and less retention of herbicide residues, which could partly explain why the inhibitory effects are transient and less pronounced at lower doses.

Effects of Paraquat on Soil Chemical Properties

The effects of paraquat on texture and some chemical properties of soil on day 0, 7 and 14 are presented in Table 1. Day 0, was examined an hour after Paraquat was applied to the soil to assess its immediate effects. However, texture did not change as it is understood to be a fixed property of soil. There was a significant difference ($p < 0.05$) between the pH of the control and treated soils. The pH value ranged from 5.91 – 6.37, and the highest value (6.34 – 6.37) was obtained when paraquat was applied at twice the recommended rate. This may be due to the facts that, some chemicals can cause a temporary shift towards higher pH levels, depending on how they interact with the soil's base or acidic components (Frimpong et al., 2018).

Paraquat application significantly influenced total nitrogen (TN), total organic carbon (TOC), and available phosphorus (Av. P) compared to the control on day 0, 7, and 14 (Table 1). Total nitrogen was consistently the highest in soils treated with the recommended rate (RR) of paraquat (1.15, 1.17, and 1.20 g kg^{-1}) and the lowest in soils treated with twice the recommended rate (2RR: 0.65, 0.75, and 0.89 g kg^{-1}). At higher concentrations, paraquat's bipyridinium cations, which are redox active and highly oxidative, are likely to have disrupted the microbial community responsible for nitrogen mineralization and reduced soil buffering capacity, resulting in lower TN in 2RR (Ng et al., 2022).

Similarly, TOC was reduced in the 2RR treatment (12.03–19.18 g kg^{-1}) compared to both control (18.90, 18.07, and 16.77 g kg^{-1}) and RR treatment. Excess paraquat at 2RR probably inhibited microbial decomposition of plant residues and organic matter (Singh et al., 2020), thereby slowing the recycling of carbon into the soil. This is consistent with the observations of Meena et al. (2020), who reported suppressed organic carbon turnover under high herbicide loads.

Interestingly, available phosphorus increased progressively with paraquat application rates, which was the lowest in the control (25.23, 23.67, and 21.50 mg kg^{-1}), higher in RR (31.00, 32.40, and 32.73

Table 1. Effects of Paraquat on Physical and Chemical Properties of Soil

	pH	T. N	TOC	Av.P	K	Ca	Mg	Na	H^+	AL^{3+}	Sand	Silt	Clay
		g kg^{-1}	g kg^{-1}	mgkg^{-1}			cmolkg^{-1}					g kg^{-1}	
						DAY 0							
Control	6.04^b	0.96^b	18.90^b	25.23^c	0.19^c	0.81^c	0.19^c	0.12^b	0.08^b	0.05^a	854.00^a	82.67^a	63.33^a
RR	6.30^a	1.15^a	23.03^a	31.00^b	0.24^b	1.02^b	0.23^b	0.13^b	0.04^a	0.02^a	855.00^a	81.33^a	63.67^a
2RR	6.36^a	0.65^c	12.03^c	34.17^a	0.28^a	1.10^a	0.25^a	0.16^a	0.03^a	0.04^a	860.67^b	78.67^a	60.67^a
						DAY 7							
Control	6.08^b	0.90^b	18.07^b	23.67^c	0.24^b	0.76^c	0.21^b	0.12^b	0.07^a	0.05^a	865.70^a	80.33^a	54.00^a
RR	6.28^a	1.17^a	23.37^a	32.40^b	0.25^b	1.06^b	0.25^a	0.15^a	0.05^b	0.03^a	860.00^{ab}	78.67^a	61.33^a
2RR	6.34^a	0.74^c	12.03^c	35.07^a	0.31^a	1.09^a	0.26^a	0.15^a	0.03^b	0.04^a	863.30^b	79.67^a	57.00^a
						Control							
Control	5.91^b	0.68^{ab}	16.77^c	21.50^c	0.24^c	0.73^c	0.21^c	0.60^a	0.07^a	0.08^a	865.00^b	79.70^a	55.30^a
RR	6.31^a	1.20^a	23.77^a	32.73^b	0.32^b	1.26^b	0.29^b	0.16^b	0.05^b	0.03^a	871.00^{ab}	74.00^{ab}	55.00^a
2RR	6.37^a	0.89^b	19.18^b	34.39^a	0.34^a	1.32^a	0.31^a	0.15^b	0.03^b	0.05^b	874.30^a	70.30^b	55.30^a

Means with similar alphabet within a column are not significantly different at $P > 0.05$
KEY: RR = Recommended rate, 2RR = Double Recommended rate

mg kg^{-1}), and the highest in 2RR (34.17, 35.07, and 34.39 mg kg^{-1}) on day 0, 7, and 14 respectively. This trend can be explained by the fact that paraquat's strong adsorption to soil particles displaces phosphate ions from exchange sites, thereby increasing phosphorus availability in the soil solution (Rathod et al., 2021). Secondly, paraquat's oxidative stress on microbes can lead to decreased phosphorus immobilization by microbial biomass, making more phosphorus available to plants.

Other exchangeable cations (K, Ca, Mg, and Na) also increased with the increasing paraquat levels, although the measured K and Mg remained below the critical thresholds required for optimal plant growth (Guimaraes et al., 2024). These increases may reflect the displacement of these cations from soil colloids by paraquat's positively charged molecules.

Conversely, exchangeable acidity (H^+ and Al^{3+}) showed a decreasing trend with paraquat application. Hydrogen ions were the highest in the control (0.08 cmol kg^{-1}) and the lowest in 2RR treatment, while Al^{3+} was the lowest in RR treatment and the highest in the control, although these differences were not statistically significant ($p > 0.05$). The reduction in acidity may also result from paraquat's cationic nature competing with H^+ and Al^{3+} for exchange sites, thus reducing their activity in the soil.

CONCLUSIONS

This study highlights the complex and dosage dependent effects of paraquat herbicide on soil biological and chemical properties in a plantain plantation. Moderate paraquat application at the recommended rate enhanced microbial populations, particularly bacteria and fungi, and maintained favorable soil fertility indicators, suggesting that some soil microorganisms can tolerate or even metabolize paraquat at these levels. However, excessive application at twice the recommended rate suppressed microbial communities, reduced total nitrogen and organic carbon, and increased the bioavailability of phosphorus and exchangeable bases, likely due to paraquat's strong cationic and oxidative interactions with soil colloids and organic matter.

The findings underscore that the bipyridinium cations in paraquat not only exert toxic effects at high concentrations but also alter nutrient dynamics by displacing essential and toxic ions from exchange sites and disrupting microbial mediated nutrient cycling. The response of the soil microbial community and nutrient availability was also modulated by the soil's inherent properties, including its moderate acidity, sandy texture, and organic matter content.

Finally, this study emphasizes the need for judicious use of paraquat at recommended rates to preserve soil microbial diversity and maintain soil fertility, while avoiding the deleterious effects observed at higher doses. These insights contribute to a better understanding of herbicide–soil interactions and support the development of sustainable weed management strategies that minimize negative impacts on soil health and ecosystem functioning.

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