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Concentration-dependent effects of atrazine on culturable soil microbial groups and biodegradation in Gerif soil, Sudan

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Atrazine is widely used for weed control, but its persistence and effects on soil microorganisms remain poorly understood in semi-arid Sudanese soils. This study evaluated the concentration-dependent effects of atrazine on culturable soil microbial groups and its biodegradation in Gerif silty clay loam soil collected from the Blue Nile Bank, east of Khartoum, Sudan. Soil samples were treated with four atrazine concentrations (0.678, 1.69, 3.39, and 5.08 mg g^{-1} soil) and incubated at 28 ± 1 °C for 150 days. Microbial populations were enumerated at 0, 15, 30, 60, 90, 120, and 150 days using selective culture media, while atrazine residues were quantified by gas chromatography. Atrazine significantly affected culturable microbial groups in a concentration- and time-dependent manner. Organic nitrogen-utilizing bacteria showed inhibition up to 73.6% at the highest concentration after 90 days, while fungal populations were inhibited by up to 70.0% at 3.39 mg g^{-1} soil after 15 days. Mineral nitrogen-utilizing bacteria were strongly suppressed during the early incubation period, with inhibition reaching 80.7% at 1.69 mg g^{-1} soil after 30 days, followed by partial recovery and stimulation at later stages. Microorganisms growing on nitrate agar were dominated by Mycobacterium spp., suggesting selective enrichment of atrazine-tolerant culturable taxa. Atrazine degradation began within 15 days and increased progressively during incubation; after 150 days, degradation reached 75.0% at 0.678 mg g^{-1} soil but only 54.2% at 5.08 mg g^{-1} soil. Kinetic analysis further showed that atrazine half-life in Gerif soil ranged from 69 to 125 days, with the longest half-life recorded at the highest concentration. These findings indicate that atrazine imposes selective pressure on culturable soil microorganisms and persists longer at higher concentrations in low-organic-matter Gerif soil. The results support optimized herbicide application rates, improved soil organic matter management, and microbial-based remediation strategies in semi-arid agroecosystems.

KEYWORDS
atrazine, biodegradation, culturable microbial groups, half-life, herbicide persistence, microbial adaptation, semi-arid soils

1 Introduction

The rapid growth of the global population continues to increase the demand for food production, driving the widespread use of agricultural technologies aimed at improving crop yields. Among these technologies, agrochemicals, particularly herbicides, insecticides, and fungicides, remain important components of modern agricultural systems (1–3). Herbicides play a critical role in controlling weeds that compete with crops for nutrients, water, and light. However, their extensive and sometimes poorly regulated use has raised increasing environmental concerns, particularly regarding soil health, biodiversity, microbial functioning, and ecosystem sustainability (4–6).

Pesticide residues are frequently detected in soil and aquatic environments, where they may persist, transform into potentially toxic metabolites, move through soil profiles, and affect non-target organisms and ecological processes (7–10). In soil ecosystems, these residues are of particular concern because microorganisms regulate nutrient cycling, organic matter decomposition, enzymatic activity, and soil fertility. Disruption of microbial processes can therefore reduce soil quality and alter the ecological functions that support sustainable crop production.

Atrazine is among the most widely used herbicides worldwide, particularly in maize, sugarcane, and sorghum cultivation, because of its effectiveness in controlling broadleaf and grassy weeds (11). Despite its agronomic value, atrazine is considered environmentally persistent and mobile, with potential to leach into groundwater. Its ecotoxicological effects include disruption of soil microbial activity, inhibition of enzymatic processes, and adverse impacts on non-target organisms (12). For example, atrazine exposure has been associated with tissue damage in freshwater mussels (13), while phytotoxic effects have been reported in non-target crops such as cassava (14).

In Sudan, herbicide use has historically been important in major irrigated agricultural schemes such as Gezira, New Halfa, and El Rahad. Earlier national data indicated that atrazine represented an important component of herbicide imports, ranking after 2,4-D (15). However, agrochemical-use patterns have changed substantially in recent years, with current research emphasizing integrated weed management, safer application practices, and improved monitoring of herbicide residues in soil and water (7, 8, 16). Therefore, evaluating atrazine behavior under Sudanese soil conditions remains important for sustainable herbicide management and environmental risk reduction.

Soil is the primary sink for applied herbicides, and herbicide fate is governed by interacting physicochemical and biological processes. Recent studies confirm that atrazine sorption, desorption, mobility, and degradation are strongly influenced by soil organic carbon, clay content, mineral composition, pH, temperature, moisture, and organic or inorganic amendments (17–23). These factors regulate atrazine bioavailability to microorganisms and determine whether the herbicide is degraded, retained in soil, or transported to water bodies.

Gerif soils, which occur in Sudanese agricultural landscapes, are typically characterized by low organic matter content and limited nutrient status. These properties may reduce microbial biomass and enzymatic activity, thereby limiting the capacity of soil microorganisms to degrade herbicides efficiently. Consequently, herbicides such as atrazine may persist longer in these soils and pose a greater risk to soil biological functioning and water quality (15, 20, 24). This concern is particularly relevant in semi-arid environments, where high temperature variability and low organic matter can influence microbial resilience and herbicide degradation dynamics (25).

Several remediation strategies have been explored to reduce atrazine persistence in soil. Organic amendments, composts, and mineral-based adsorbents can enhance microbial activity, modify herbicide bioavailability, and promote degradation or immobilization processes. For example, vermicompost has been shown to increase atrazine degradation while stimulating specialized degrading microorganisms (12), and other organic or mineral amendments may reduce herbicide mobility and support microbial-mediated remediation (26).

Despite these advances, there remains a critical knowledge gap regarding the response of soil microorganisms to atrazine and its degradation dynamics in Sudanese Gerif soils. Most existing studies have been conducted outside semi-arid Sudanese conditions, limiting their applicability to soils with distinct physicochemical properties and microbial constraints. The novelty of this study lies in its focus on atrazine effects and degradation dynamics in Gerif soil, a low-organic-matter semi-arid Sudanese soil for which limited experimental information is available. Unlike many previous studies conducted under temperate or higher-organic-matter soil conditions, this work integrates concentration-dependent responses of culturable microbial groups, atrazine residue dissipation, and degradation kinetic parameters under controlled laboratory conditions. Therefore, this study aimed to (i) evaluate the effects of different atrazine concentrations on major culturable soil microbial groups and (ii) assess the biodegradation dynamics and persistence of atrazine in Gerif soil under controlled laboratory conditions. The findings provide useful insights into atrazine behavior in semi-arid soils and support the development of sustainable herbicide management and soil remediation strategies in Sudan and similar agroecosystems.

2 Materials and methods

2.1 Soil sampling and preparation

Surface soil samples (0–15 cm depth) were collected from Gerif soil located along the Blue Nile Bank, east of Khartoum, Sudan. Composite soil samples were obtained by randomly collecting multiple subsamples using a soil auger across the study area and combining them to ensure representativeness.

The samples were transported to the laboratory in clean polyethylene bags. In the laboratory, soil samples were air-dried at room temperature (25 ± 2 °C), gently crushed to break aggregates, and sieved through a 2 mm mesh to obtain a homogeneous sample. The processed soil was stored under dry conditions prior to analysis and experimental use.

Selected physicochemical properties of the soil were determined following the standard procedures described by Rayan et al. (27), and the results are presented in Table 1. Soil pH was measured in a soil-water suspension using a glass electrode pH meter, while electrical conductivity was determined using an EC meter. Particle-size distribution was determined using the hydrometer method, and the soil textural class was assigned according to the USDA textural classification system. Organic matter was determined using the Walkley-Black oxidation method, total nitrogen by the Kjeldahl method, available phosphorus colorimetrically, and potassium by flame photometry. The soil was non-saline, non-sodic, and characterized by low nutrient status, particularly nitrogen and phosphorus. Based on particle size distribution (31.7% clay, 53.6% silt, and 14.7% sand), the soil was classified as silty clay loam according to the USDA textural classification system. According to USDA Soil Taxonomy, the soil belongs to the Typic Haplustepts (fine, mixed, iso-hyperthermic) group.

2.2 Experimental design and atrazine treatment

Technical-grade atrazine (500 g L^{-1}) was obtained from the Sudanese Agricultural Business Company Ltd., Khartoum, Sudan. The experiment was conducted under controlled laboratory conditions using a completely randomized design.

Soil samples were divided into five treatments, including one untreated control and four atrazine-amended treatments. Atrazine was applied at concentrations of 0.678, 1.69, 3.39, and 5.08 mg g^{-1} soil, representing a gradient from low to relatively high contamination levels.

For each treatment, soil (on a dry weight basis) was placed in incubation containers and thoroughly mixed to ensure uniform distribution of atrazine. Soil moisture content was adjusted to approximately 60% of field capacity and maintained throughout the incubation period by periodic addition of distilled water, based on weight monitoring.

All treatments were prepared in triplicate (n = 3). The samples were incubated in the dark at 28 ± 1 °C for 150 days. Subsamples were collected destructively at 0, 15, 30, 60, 90, 120, and 150 days for microbiological and chemical analyses.

TABLE 1 Chemical and physical properties of Gerif soil.

Parameter	Value
pH	7.3
Electrical conductivity (dS m^{-1})	0.58
Clay (%)	31.7
Silt (%)	53.6
Sand (%)	14.7
Textural class	Silty clay loam
Total nitrogen (%)	0.11
P_2O_5 (%)	0.43
K_2O (%)	0.13
Organic matter (%)	0.31

2.3 Microbial enumeration

The effects of atrazine on soil microbial populations were assessed using culture-dependent enumeration methods. Microbial populations were quantified using selective culture media prepared according to Tepper et al. (28), including Czapek's Dox Agar (CZA) for fungi, Meat Peptone Agar (MPA) for organic nitrogen-utilizing bacteria, Starch Ammonium Agar (SAA) for inorganic nitrogen-utilizing bacteria and actinobacteria, and Nitrate Agar (NA) for microorganisms capable of growing on nutrient-poor media. Each medium was freshly prepared, autoclaved at 121 °C under 15 lb in^{-2} pressure for 20 min, cooled, and poured into sterilized Petri dishes before use.

For viable count determination, 1 g of atrazine-treated soil was suspended in 9 mL of sterilized distilled water and shaken thoroughly to obtain the first dilution (10^{-1}). Serial dilutions were then prepared up to 10^{-9}. One milliliter of the appropriate dilution was transferred onto the surface of the corresponding agar medium and spread using sterilized glass spreaders. Plates were incubated at 28 °C. Incubation periods were 3 days for MPA and CZA plates, 7 days for SAA plates, and 10 days for NA plates. At the end of the incubation period, colonies were counted and expressed as colony-forming units per gram of dry soil (CFU g^{-1} dry soil). Microbial counts were determined at 0, 15, 30, 60, 90, 120, and 150 days, and inhibition or stimulation percentages were calculated relative to the untreated control. Because the microbial assessment was based on culture-dependent enumeration using selective media, the results represent only the culturable fraction of the soil microbiota under the experimental conditions used.

2.4 Soil physicochemical properties

The physicochemical characteristics of the studied soil are summarized in Table 1.

The soil exhibited neutral pH (7.3) and low salinity (EC = 0.58 dS m^{-1}). The low organic matter content (0.31%) indicates limited microbial biomass potential and reduced capacity for rapid biodegradation. The dominance of silt and clay fractions suggests relatively higher water retention compared to sandy soils, which may influence atrazine sorption and microbial activity.

2.5 Determination of atrazine residues

Atrazine residues in soil were determined according to the AOAC (29) method. Gas chromatography with flame ionization detection (GC-FID) was selected for atrazine residue quantification because it provides reliable separation, sensitivity, and reproducibility for pesticide residue analysis in soil extracts when supported by appropriate extraction, calibration, and recovery procedures. Although other analytical techniques, such as high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS), can also be used for atrazine determination, GC-FID remains a suitable method for routine determination of atrazine residues in extracted soil samples.

Briefly, 10 g of atrazine-treated soil was transferred into a 250 mL conical flask, followed by the addition of 10 mL distilled

water and 25 mL methanol. The contents were vigorously shaken for 3 h, filtered, and transferred into a clean beaker. Then, 10 mL dichloromethane and 10 mL saturated sodium chloride solution were added. The mixture was vigorously shaken for 5 min and allowed to stand for 3 min until phase separation occurred. The dichloromethane layer was collected, and the aqueous phase was re-extracted twice with 10 mL dichloromethane. The combined dichloromethane fractions were dried by passing them through anhydrous sodium sulfate on filter paper. The solvent was evaporated to dryness using a rotary evaporator at 70 °C, and the residue was re-dissolved in 10 mL hexane and stored at 5 °C until GC analysis.

Gas chromatographic analysis was performed using a gas chromatograph equipped with a flame ionization detector (FID) and a DB-5 fused silica capillary column of 30 m length and 0.25 mm internal diameter. The stationary phase was 5% phenyl methylpolysiloxane with a film thickness of 0.25 µm. The injector and detector temperatures were maintained at 240 °C and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 4.23 mL min^{-1}. The oven temperature program was set as follows: initial temperature of 50 °C, increased at 5 °C min^{-1} to 75 °C, then increased at 10 °C min^{-1} to 160 °C, increased at 5 °C min^{-1} to 180 °C, and finally increased at 3 °C min^{-1} to 240 °C, where it was held for 20 min. The flow rates of makeup gas helium, hydrogen, and air were 30, 40, and 400 mL min^{-1}, respectively.

Each sample extract was analyzed by duplicate injections of 2 µL. Analytical-grade atrazine standard with 99.9% purity was used for calibration. Three atrazine standard concentrations, 41, 205, and 410 ppm, were injected under the same chromatographic conditions, and the detector response was used to construct the standard curve. The available archived method record reported a limit of detection (LOD) of 2 ppm for atrazine and recovery greater than 99%, supporting the reliability of the extraction and residue determination procedure.

The reported LOD refers to atrazine concentration in the final hexane extract analyzed by GC-FID, not to the initial atrazine concentration applied to soil. Because 10 g of soil was extracted and the final residue was re-dissolved in 10 mL hexane, an LOD of 2 ppm corresponds approximately to 2 mg kg^{-1} soil, equivalent to 0.002 mg g^{-1} soil, assuming complete extraction. Therefore, the detection limit was below the atrazine concentration range used in this study (0.678–5.08 mg g^{-1} soil), supporting the suitability of the method for monitoring atrazine dissipation during incubation. Atrazine residues were determined at 0, 15, 30, 60, 90, 120, and 150 days.

2.6 Statistical analysis

Statistical analyses were performed using Statistica software (version 14.1.0, StatSoft Inc., USA). The effects of atrazine concentration and incubation time on microbial populations and atrazine degradation were assessed using two-way ANOVA, including their interaction (concentration × time). When significant differences were detected ($p < 0.05$), Tukey's HSD test was used for multiple comparisons, and differences were indicated by letter groupings. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test, with transformations applied where necessary. Results are presented as mean ± standard error (SE) for microbiological measurements and as mean ± standard deviation (SD) for atrazine degradation rate in Figure 1, based on triplicate measurements (n = 3).

Atrazine degradation kinetics were evaluated by calculating the first-order degradation rate constant (K) and half-life ($T_{1/2}$), following the method of Müller et al. (30). The degradation rate constant was determined using Equation 1:

$$K = \frac{2.303}{t} \log_{10}\left(\frac{C_0}{C_t}\right) \quad (1)$$

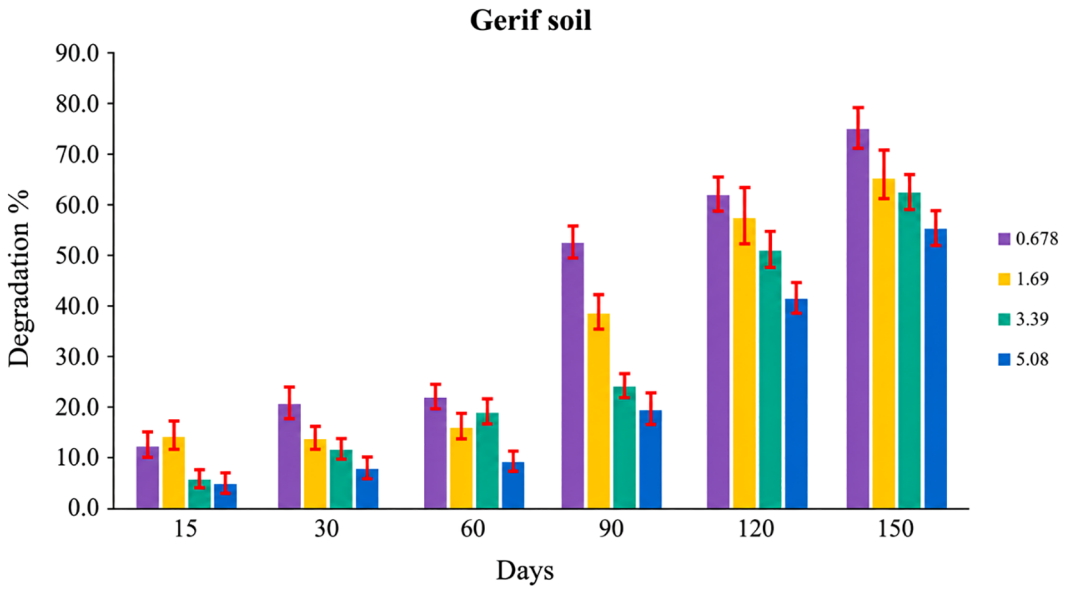

FIGURE 1
Atrazine degradation rate (%) in Gerif soil incubated at 28 °C over 150 days at different initial atrazine concentrations (0.678, 1.69, 3.39, and 5.08 mg g^{-1} soil). Error bars indicate the standard deviation of replicate measurements (n = 3).

where C_0 is the initial atrazine concentration, C_t is the atrazine concentration remaining after an incubation period t, and t is the incubation time in days. The atrazine half-life was subsequently calculated using Equation 2:

$$T_{1/2} = \frac{0.693}{K} \quad (2)$$

These parameters were calculated for each atrazine treatment to quantify the persistence and degradation behavior of atrazine in Gerif soil.

3 Results

3.1 Effects of atrazine on soil microbial populations

Atrazine application significantly affected soil microbial populations, with responses varying across microbial groups, concentrations, and incubation time.

3.1.1 Organic nitrogen-utilizing bacteria

All atrazine treatments resulted in a progressive decline in the abundance of organic nitrogen-utilizing bacteria, with inhibitory effects becoming more pronounced over time (Figure 2). The strongest inhibition was observed after 90 days of incubation, particularly at the highest concentration (5.08 mg g^{-1} soil), where microbial abundance decreased by 73.6% relative to the control. Lower concentrations exhibited similar trends but with reduced magnitude, indicating a clear concentration-dependent inhibitory effect.

3.1.2 Fungal populations

Fungal populations were consistently suppressed across all atrazine treatments throughout the incubation period (Figure 3). The most pronounced inhibition (70.0%) occurred at 3.39 mg g^{-1} soil after 15 days of incubation, suggesting that fungi were highly sensitive to atrazine exposure, particularly during the early stages. No full recovery of fungal populations was observed even after prolonged incubation (150 days), indicating persistent toxic effects.

3.1.3 Mineral nitrogen-utilizing bacteria and actinobacteria

The response of mineral nitrogen-utilizing bacteria is presented in Table 2. Atrazine caused significant reductions in bacterial abundance across all concentrations during the early and intermediate incubation stages (15–120 days), with inhibition reaching up to 80.7% at 1.69 mg g^{-1} soil after 30 days. However, a notable shift in response was observed at the later stage (150 days), where the two highest concentrations (3.39 and 5.08 mg g^{-1} soil) showed a stimulatory effect, with increases of +28.5% and +9.3%, respectively. This response may reflect delayed adaptation or selective enrichment of culturable atrazine-tolerant microbial populations rather than an overall improvement in soil microbial health. The absence of a consistent stimulatory effect at lower atrazine concentrations may be explained by the low organic matter and nutrient status of

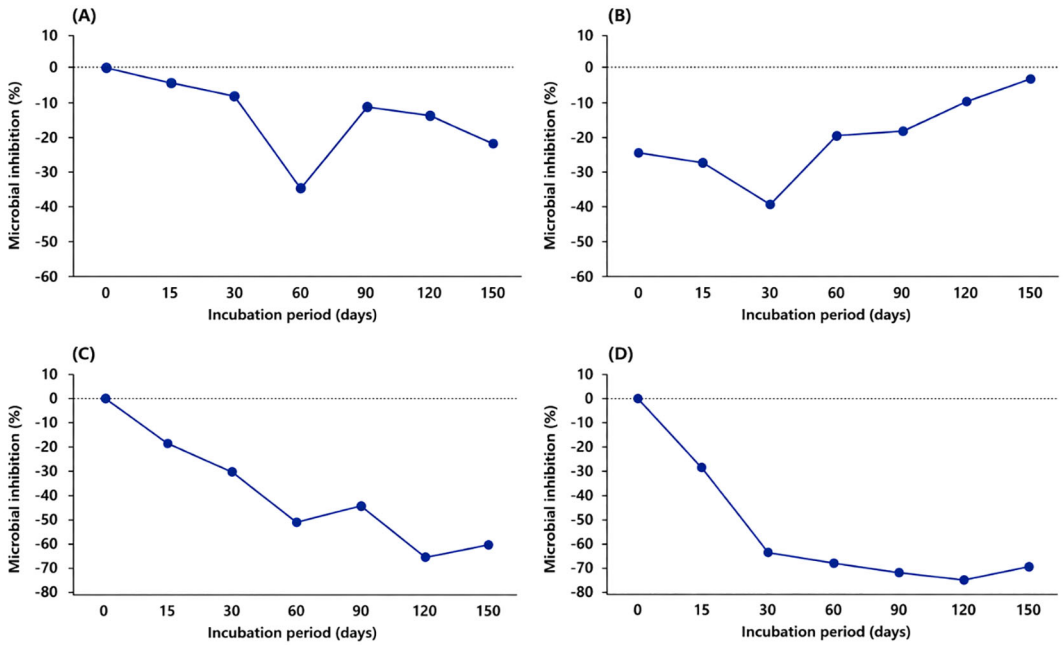

FIGURE 2
Effect of atrazine concentrations [**(A)** = 0.678, **(B)** = 1.69, **(C)** = 3.39, and **(D)** = 5.08 mg g^{-1} soil] on the inhibition (%) of organic nitrogen-utilizing bacteria in Gerif soil during incubation at 28 °C. Values represent mean inhibition/stimulation relative to the untreated control (n = 3). Negative values indicate inhibition and positive values indicate stimulation.

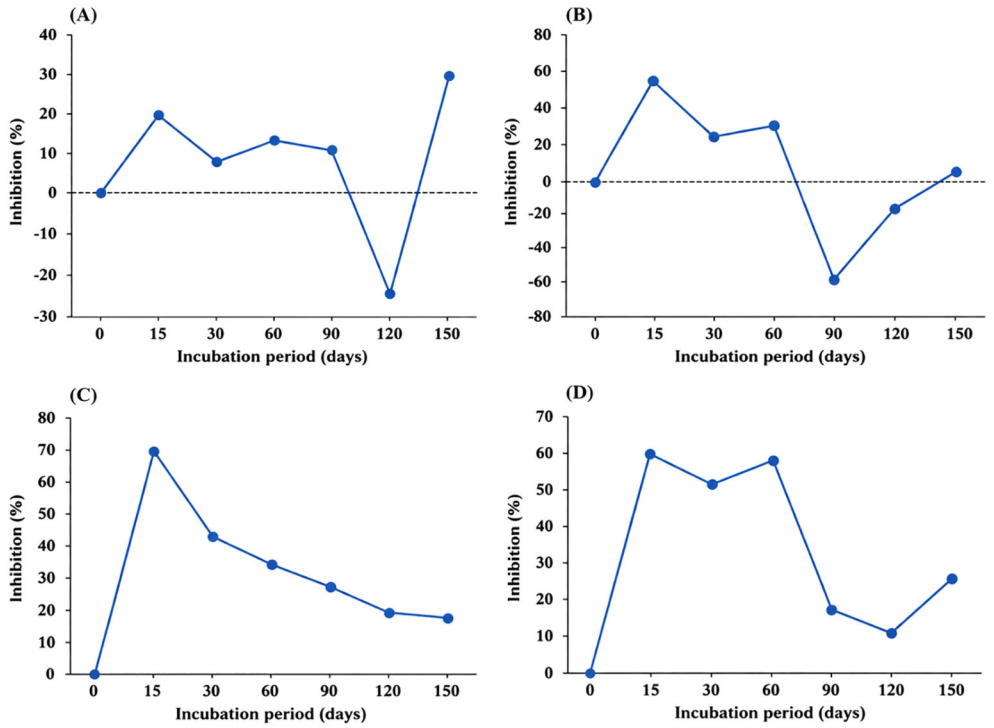

FIGURE 3
Effect of atrazine concentrations [**(A)** = 0.678, **(B)** = 1.69, **(C)** = 3.39, and **(D)** = 5.08 mg g^{-1} soil] on the inhibition (%) of fungal populations in Gerif soil during incubation at 28 °C. Values represent mean inhibition/stimulation relative to the untreated control (n = 3). Negative values indicate inhibition and positive values indicate stimulation.

Gerif soil, which likely limited microbial biomass and reduced the capacity for rapid microbial enrichment. In addition, lower atrazine concentrations may not have provided sufficient substrate availability or selective pressure to stimulate measurable growth of atrazine-tolerant microorganisms. Similar concentration-dependent microbial responses have been reported in pesticide-contaminated soils, where initial toxicity suppresses sensitive microbial groups, while prolonged exposure may enrich tolerant or pesticide-degrading microorganisms (10, 17, 23, 31, 32). In contrast, actinobacteria showed limited sensitivity to atrazine, with only minor fluctuations observed across treatments and incubation periods (Table 2).

3.2 Microbial community structure on nitrate agar

Atrazine application significantly influenced the abundance and composition of microorganisms capable of growing on nutrient-poor media (Nitrate Agar), as shown in Table 3. A general decline in total microbial counts was observed during the early incubation period (0–30 days), particularly at higher atrazine concentrations. This initial suppression phase was followed by a gradual recovery and, in some cases, a marked increase in microbial abundance at later stages (60–150 days), especially under higher atrazine concentrations.

Across all treatments and sampling intervals, Mycobacterium spp. consistently dominated the microbial community. Their relative abundance remained high even under elevated atrazine concentrations, indicating a strong tolerance to atrazine stress and a potential ecological advantage under contaminated conditions. Other genera, including *Micromonospora*, *Nocardia*, *Arthrobacter*, and *Bactoderma*, exhibited variable and generally lower abundances. Their occurrence was sporadic and often restricted to specific concentrations and time points, suggesting differential sensitivity and adaptation to atrazine exposure.

3.3 Biodegradation of atrazine in soil

Atrazine degradation occurred throughout the incubation period and was strongly influenced by initial concentration (Figure 1). Degradation was detectable as early as 15 days after application; however, the extent of degradation varied considerably among treatments. At the highest concentration (5.08 mg g^{-1} soil), only 5% degradation was observed at 15 days, increasing to 54.2% after 150 days. In contrast, the lowest concentration (0.678 mg g^{-1} soil) showed higher degradation rates, reaching 12.2% at 15 days and 75.0% after 150 days. These results demonstrate a clear inverse relationship between atrazine concentration and degradation rate, indicating that higher concentrations may inhibit microbial degradation processes or delay microbial adaptation. The progressive increase in degradation over time suggests that biodegradation is a cumulative process, likely associated with gradual microbial adaptation and enrichment of atrazine-tolerant culturable populations.

Kinetic analysis further confirmed the concentration-dependent persistence of atrazine in Gerif soil (Table 4). Based on degradation rate constant and half-life calculations, atrazine half-life ranged from 69 to 125 days under incubation at 28 °C. The highest atrazine concentration showed the longest half-life, reaching 125 days, and

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TABLE 2 Effect of atrazine concentration on mineral nitrogen-utilizing bacteria and actinobacteria in Gerif soil incubated at 28 °C.

Atrazine concentration (mg g^{-1} soil)	Total count (×10^8 CFU g^{-1} dry soil)	Inhibition/ stimulation (%)	Viable bacteria (×10^8 CFU g^{-1} dry soil)	Inhibition/ stimulation (%)	Actinobacteria (×10^8 CFU g^{-1} dry soil)	Inhibition/ stimulation (%)
At zero time						
0.0	35.0^a	0.0	35.0^a	0.0	0.0^a	0.0
0.678	36.0^a	0.0	36.0^a	0.0	0.0^a	0.0
1.69	38.0^a	0.0	36.0^a	0.0	2.0^b	0.0
3.39	34.0^a	0.0	34.0^a	0.0	0.0^a	0.0
5.08	35.0^a	0.0	35.0^a	0.0	0.0^a	0.0
P-value	***0.825***		***0.825***		***0.038***	
After 15 days						
0.0	180.0^a	0.0	180.0^a	0.0	0.0^a	0.0
0.678	54.0^b	-70.0	54.0^b	-70.0	0.0^a	0.0
1.69	36.0^c	-80.0	36.0^c	-80.0	0.0^a	0.0
3.39	84.0^d	-53.3	84.0^d	-53.3	0.0^a	0.0
5.08	60.0^e	-66.6	60.0^e	-66.6	0.0^a	0.0
P-value	***<0.001***		***<0.001***		***1.00***	
After 30 days						
0.0	186.0^a	0.0	186.0^a	0.0	0.0^a	0.0
0.678	80.0^b	-57.0	80.0^b	-57.0	0.0^a	0.0
1.69	36.0^c	-80.7	36.0^c	-80.6	0.0^a	0.0
3.39	64.0^d	-65.5	64.0^d	-65.5	0.0^a	0.0
5.08	54.0^e	-71.0	54.0^e	-71.0	0.0^a	0.0
P-value	***<0.001***		***<0.001***		***1.00***	
After 60 days						
0.0	180.0^a	0.0	180.0^a	0.0	0.0^a	0.0
0.678	76.0^b	-57.8	76.0^b	-57.8	0.0^a	0.0
1.69	42.0^c	-76.6	40.0^c	-77.7	2.0^b	0.0
3.39	79.0^b	-56.1	78.0^b	-56.6	1.0^{ab}	0.0
5.08	60.0^d	-66.6	60.0^d	-66.6	0.0^a	0.0
P-value	***<0.001***		***<0.001***		***0.045***	
After 90 days						
0.0	150.0^a	0.0	150.0^a	0.0	0.0^a	0.0
0.678	60.0^b	-60.0	60.0^b	-60.0	0.0^a	0.0
1.69	43.0^c	-71.3	42.0^c	-72.0	1.0^b	0.0
3.39	80.0^d	-46.7	80.0^d	-46.7	0.0^a	0.0
5.08	66.0^e	-56.0	66.0^e	-56.0	0.0^a	0.0
P-value	***<0.001***		***<0.001***		***0.032***	
After 120 days						
0.0	145.0^a	0.0	145.0^a	0.0	0.0^a	0.0
0.678	56.0^b	-61.3	56.0^b	-61.3	0.0^a	0.0
1.69	46.0^c	-68.2	46.0^c	-68.2	0.0^a	0.0
3.39	120.0^d	-17.2	120.0^d	-17.2	0.0^a	0.0
5.08	112.0^e	-22.7	112.0^e	-22.7	0.0^a	0.0
P-value	***<0.001***		***<0.001***		***1.00***	

(Continued)

TABLE 2 Continued

Atrazine concentration (mg g^{-1} soil)	Total count (×10^8 CFU g^{-1} dry soil)	Inhibition/ stimulation (%)	Viable bacteria (×10^8 CFU g^{-1} dry soil)	Inhibition/ stimulation (%)	Actinobacteria (×10^8 CFU g^{-1} dry soil)	Inhibition/ stimulation (%)
After 150 days						
0.0	140.0[a]	0.0	140.0[a]	0.0	0.0[a]	0.0
0.678	52.0[b]	-62.8	52.0[b]	-62.8	0.0[a]	0.0
1.69	78.0[c]	-44.2	78.0[c]	-44.2	0.0[a]	0.0
3.39	180.0[d]	+28.5	180.0[d]	+28.5	0.0[a]	0.0
5.08	153.0[e]	+9.3	153.0[e]	+9.3	0.0[a]	0.0
P-value	***<0.001***		***<0.001***		***1.00***	

Values show microbial counts and percentage inhibition (−) or stimulation (+) relative to the untreated control.
Bold values indicate the P-values for comparisons among atrazine concentrations within each incubation period. Within each column and incubation period, values followed by different superscript letters are significantly different at $p < 0.05$.

the lowest degradation rate constant, 0.00554 day^{-1}. In contrast, lower and intermediate concentrations showed shorter half-life values, indicating faster dissipation. These results confirm that atrazine degradation in Gerif soil is both time-dependent and strongly influenced by initial concentration. The kinetic pattern supports the percentage degradation results, where atrazine degradation was faster at lower concentrations and slower at the highest concentration after 150 days.

4 Discussion

4.1 Response of microbial groups to atrazine exposure

The present study demonstrates that atrazine exerts a concentration- and time-dependent influence on soil microbial communities, characterized by an initial inhibitory phase followed by partial recovery during prolonged incubation. This dynamic response reflects the resilience and adaptive capacity of soil microorganisms under chemical stress and is consistent with previous findings that pesticide effects are strongly modulated by dose, exposure duration, and environmental conditions (6, 25).

The observed concentration × time responses indicate that atrazine effects on culturable microbial groups were not constant throughout incubation but changed with exposure duration. The strong inhibition observed during the early and intermediate stages suggests acute stress on sensitive microorganisms, whereas partial recovery or stimulation at later stages may indicate reduced atrazine bioavailability, microbial adaptation, or selective enrichment of tolerant culturable populations. Therefore, the interaction between atrazine concentration and incubation time is important for understanding both short-term toxicity and longer-term microbial adjustment in Gerif soil.

At lower concentrations, atrazine stimulated the growth of both organic and mineral nitrogen-utilizing bacteria, particularly at later incubation stages. This stimulatory effect may be attributed to microbial adaptation and the potential utilization of atrazine or its metabolites as alternative nutrient sources. Similar responses have been reported for other herbicides, where low concentrations enhanced the abundance of functional microbial groups, such as phosphate-solubilizing bacteria (33, 34). In contrast, higher atrazine concentrations resulted in significant inhibition, especially during the early stages, indicating acute toxicity to sensitive microbial populations.

Fungal populations were consistently suppressed across all atrazine treatments, indicating a higher sensitivity of fungi compared to bacteria. This observation aligns with previous studies reporting prolonged inhibition of fungal growth under pesticide exposure (35–37). The greater susceptibility of fungi may be related to their slower growth rates and limited capacity for rapid physiological adaptation. Similar inhibitory effects have been observed for other agrochemicals, including thiram, which significantly reduced fungal growth and sporulation (38).

4.2 Shifts in culturable microbial genera on nitrate agar

Atrazine exposure induced notable shifts in microbial community structure, particularly among microorganisms capable of growing on nutrient-poor media. The observed increase in total microbial counts after 30 days suggests a transition from initial stress-induced suppression to the enrichment of tolerant and functionally specialized populations.

A key finding of this study is the consistent dominance of *Mycobacterium* spp. across all treatments and incubation periods. This dominance indicates a strong ecological advantage under atrazine stress conditions and suggests a high level of tolerance or adaptive capacity. Previous studies have similarly reported the predominance of *Mycobacterium* species in herbicide-contaminated soils, where they outcompete more sensitive taxa (39). This may be attributed to their metabolic versatility and potential involvement in the transformation or degradation of xenobiotic compounds.

In contrast, other genera such as *Micromonospora*, *Nocardia*, *Arthrobacter*, and *Bactoderma* showed variable and generally lower abundances, indicating differential sensitivity to atrazine exposure. These shifts in community composition reflect selective pressure imposed by the herbicide, leading to the establishment of a microbial community dominated by tolerant taxa.

TABLE 3 Effect of atrazine concentration on the abundance and relative composition of culturable microbial genera growing on nutrient-poor Nitrate Agar in Gerif soil incubated at 28 °C.

Atrazine dose (mg g^{-1} soil)	All microorganisms		Mycobacterium		Micromonospora		Nocardia		Arthrobacter		Bactoderma	
	Total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	Total count ($\times10^8$ CFU g^{-1} dry soil)	%
At zero time												
0.0	285.0^{a}	100.0	120.0^{a}	42.2	65.0^{a}	57.3	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0
0.678	280.0^{a}	98.3	280.0^{b}	100.0	0.0^{b}	0.0	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0
1.69	282.0^{a}	98.9	182.0^{c}	64.5	100.0^{c}	35.5	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0
3.39	283.0^{a}	99.3	180.0^{c}	63.6	101.0^{c}	36.1	0.0^{a}	0.0	0.0^{a}	0.0	2.0^{b}	0.3
5.08	284.0^{a}	99.7	225.0^{d}	79.2	60.0^{d}	20.8	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0
P-value	***0.712***		***<0.001***		***<0.001***		***1.00***		***1.00***		***0.032***	
After 15 days												
0.0	228.0^{a}	100.0	228.0^{a}	100.0	0.0^{a}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.678	180.0^{b}	78.9	180.0^{a}	100.0	0.0^{a}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.69	160.0^{c}	70.2	160.0^{a}	100.0	0.0^{a}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.39	138.0^{d}	60.5	120.0^{b}	87.0	18.0^{b}	13.0	0.0	0.0	0.0	0.0	0.0	0.0
5.08	96.0^{e}	30.7	80.0^{c}	83.0	16.0^{b}	17.0	0.0	0.0	0.0	0.0	0.0	0.0
P-value	***<0.001***		***<0.001***		***0.045***							
After 30 days												
0.0	224.0^{a}	100.0	220.0^{a}	98.2	0.0	0.0	0.0	0.0	4.0^{a}	1.8	0.0	0.0
0.678	160.0^{b}	71.4	140.0^{b}	87.5	0.0	0.0	0.0	0.0	20.0^{b}	12.5	0.0	0.0
1.69	155.0^{b}	69.2	145.0^{b}	93.5	0.0	0.0	0.0	0.0	10.0^{a}	6.5	0.0	0.0
3.39	136.0^{c}	60.7	116.0^{c}	85.3	0.0	0.0	0.0	0.0	20.0^{b}	12.8	0.0	0.0
5.08	93.0^{d}	37.2	80.0^{d}	86.0	0.0	0.0	0.0	0.0	13.0^{ab}	14.0	0.0	0.0
P-value	***<0.001***		***<0.001***						***0.021***			
After 60 days												
0.0	318.0^{a}	100.0	300.0^{a}	94.3	0.0^{a}	0.0	0.0	0.0	18.0	5.7	0.0	0.0
0.678	414.0^{b}	130.1	406.0^{b}	98.0	0.0^{a}	0.0	0.0	0.0	8.0	1.9	0.0	0.0
1.69	368.0^{c}	115.7	368.0^{c}	100.0	0.0^{a}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.39	568.0^{d}	178.6	560.0^{d}	92.1	80.0^{b}	14.0	0.0	0.0	0.0	0.0	0.0	0.0
5.08	608.0^{e}	191.1	602.0^{e}	99.1	60.0^{c}	9.9	0.0	0.0	0.0	0.0	0.0	0.0
P-value	***<0.001***		***<0.001***		***<0.001***							
After 90 days												
0.0	208.0^{a}	100.0	208.0^{a}	100.0	0.0^{a}	0.0	0.0	0.0	0.0^{a}	0.0	0.0	0.0
0.678	559.0^{b}	268.8	455.0^{b}	81.4	30.0^{b}	5.4	0.0	0.0	74.0^{b}	13.2	0.0	0.0
1.69	172.0^{a}	82.7	106.0^{c}	61.6	26.0^{b}	5.1	0.0	0.0	40.0^{c}	23.3	0.0	0.0
3.39	260.0^{c}	125.0	260.0^{d}	100.0	0.0^{a}	0.0	0.0	0.0	0.0^{a}	0.0	0.0	0.0
5.08	585.0^{b}	281.3	585.0^{e}	100.0	0.0^{a}	0.0	0.0	0.0	0.0^{a}	0.0	0.0	0.0
P-value	***<0.001***		***<0.001***		***<0.001***				***<0.001***			

(Continued)

TABLE 3 Continued

Atrazine dose (mg g^{-1} soil)	All microorganisms		Mycobacterium		Micromonospora		Nocardia		Arthrobacter		Bactoderma	
	Total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	total count ($\times10^8$ CFU g^{-1} dry soil)	%	Total count ($\times10^8$ CFU g^{-1} dry soil)	%
After 120 days												
0.0	370.0^{a}	100.0	100.0^{a}	27.0	130.0^{a}	35.1	14.0^{a}	37.8	0.0^{a}	0.0	1.0^{a}	0.27
0.678	540.0^{b}	145.9	182.0^{b}	33.7	400.0^{b}	74.1	140.0^{b}	25.9	16.0^{b}	3.1	2.0^{a}	200
1.69	413.0^{c}	111.6	109.0^{a}	26.4	200.0^{c}	48.4	92.0^{c}	22.8	8.0^{c}	2.0	4.0^{b}	9.7
3.39	403.0^{c}	108.9	390.0^{c}	94.6	0.0^{d}	0.0	0.0^{d}	0.0	12.0^{bc}	3.1	1.0^{a}	0.24
5.08	572.0^{b}	154.6	520.0^{d}	520.0	52.0^{e}	9.1	0.0^{d}	0.0	0.0^{a}	0.0	0.0^{a}	0.0
P-value	***<0.001***		***<0.001***		***<0.001***		***<0.001***		***<0.001***		***0.028***	
After 150 days												
0.0	311.0^{a}	100.0	150.0^{a}	48.2	494.0^{a}	158.8	140.0^{a}	45.0	71.0^{a}	22.8	0.0	0.0
0.678	282.0^{a}	90.7	109.0^{b}	38.7	160.0^{b}	56.7	68.0^{b}	24.1	62.0^{a}	21.9	0.0	0.0
1.69	480.0^{b}	154.3	373.0^{c}	77.7	0.0^{c}	0.0	27.0^{c}	5.6	80.0^{a}	16.7	0.0	0.0
3.39	442.0^{b}	142.1	161.0^{d}	36.4	182.0^{d}	41.2	60.0^{d}	13.6	23.0^{c}	5.20	0.0	0.0
5.08	911.0^{c}	292.9	341.0^{e}	34.4	533.0^{e}	58.5	26.0^{e}	2.6	14.0^{d}	1.53	0.0	0.0
P-value	***<0.001***		***<0.001***		***<0.001***		***<0.001***		***<0.001***			

Microbial counts are expressed as $\times10^8$ CFU g^{-1} dry soil. Percentages for all microorganisms indicate changes relative to the untreated control at the same incubation time, whereas percentages for individual genera indicate their relative contribution within each treatment.
Different lowercase letters within the same incubation time and microbial group indicate significant differences among treatments at $p < 0.05$.
Bold values indicate the P-values for comparisons among atrazine concentrations within each incubation period. Within each column and incubation period, values followed by different superscript letters are significantly different at $p < 0.05$.

4.3 Atrazine biodegradation dynamics

Atrazine degradation in this study exhibited a clear inverse relationship with initial concentration, with higher concentrations showing reduced degradation rates. This pattern suggests that elevated atrazine levels inhibit microbial activity or delay the development of effective degrading communities. At lower concentrations, degradation proceeded more rapidly, likely due to reduced toxicity and more favorable conditions for microbial growth and enzymatic processes.

These findings are consistent with those reported by Gan et al. (40), who observed increased persistence of atrazine at higher application rates. The progressive increase in degradation over time indicates that biodegradation is a cumulative and adaptive process, driven by the gradual enrichment of atrazine-tolerant microbial populations.

TABLE 4 Degradation kinetic parameters of atrazine in Gerif soil incubated at 28 °C for 150 days.

Atrazine concentration (mg g^{-1} soil)	Half-life, $T_1/_2$ (days)	Degradation rate constant, K (day^{-1})
0.678	70	0.0099
1.69	84	0.00825
3.39	69	0.01004
5.08	125	0.00554

Recent studies have further emphasized that pesticide degradation is often mediated by complex microbial consortia rather than individual species, with functional redundancy playing a critical role in maintaining degradation capacity under stress conditions (6, 41). The delayed yet sustained degradation observed in this study supports the concept of microbial adaptation and community restructuring as key drivers of pesticide dissipation in soil environments.

4.4 Influence of soil properties on atrazine fate

Soil physicochemical properties played a crucial role in regulating both microbial responses and atrazine degradation. The studied Gerif soil, classified as silty clay loam and characterized by low organic matter content, likely constrained microbial biomass and enzymatic activity, thereby reducing degradation efficiency and increasing atrazine persistence. Previous studies have demonstrated that pesticide degradation rates vary significantly among soil types depending on their physicochemical and biological characteristics (42). For example, Elhussein et al. (39) reported faster degradation of oxyfluorfen in more fertile soils compared with nutrient-poor soils such as Gerif, while Thapar et al. (43) attributed initial lag phases in pesticide degradation to microbial adaptation processes.

Soil composition influences pesticide fate by controlling adsorption, desorption, bioavailability, leaching, volatilization, and microbial habitat conditions. Recent studies have shown that atrazine

sorption and persistence are strongly affected by organic matter, clay minerals, temperature, and soil amendments (19–22, 44). In addition, herbicide persistence is influenced by interactions between soil texture, organic matter, and environmental conditions, which regulate adsorption, leaching, and volatilization processes (45–48). In semi-arid regions, where soils often exhibit low organic matter and are exposed to high temperature variability, these processes may enhance pesticide persistence and increase ecological risk.

Therefore, the relatively long half-life observed in Gerif soil may be attributed to its low organic matter content and limited biological activity, despite its silty clay loam texture. This interpretation is consistent with Elgaber et al. (23), who reported longer atrazine persistence in Gerif soil compared with more fertile or amended soils. Overall, the findings highlight that both herbicide properties and soil characteristics jointly control the environmental fate of atrazine, emphasizing the need for site-specific and sustainable management strategies to mitigate long-term impacts on soil health and ecosystem functioning.

4.5 Methodological scope and future research directions

This study provides important experimental evidence on the concentration-dependent effects of atrazine on culturable soil microbial groups and atrazine degradation in Gerif soil. Because microbial responses were assessed using culture-dependent enumeration, the results specifically reflect the culturable fraction of the soil microbiota under the experimental conditions used. This approach is appropriate for evaluating viable microbial groups affected by atrazine exposure; however, future studies using molecular approaches, such as 16S rRNA sequencing, metagenomics, transcriptomics, or stable isotope probing, would provide deeper insight into atrazine-degrading taxa, functional genes, and degradation pathways.

The experiment was conducted under controlled laboratory conditions using one soil type and a fixed incubation temperature of 28 °C. This design allowed clear evaluation of atrazine concentration effects while minimizing environmental variability. Future field-based studies are recommended to validate these findings under variable soil moisture, temperature, rainfall, plant–microbe interactions, repeated herbicide application, and leaching conditions. Further research should also include atrazine metabolite profiling and evaluate organic amendments, mineral fertilization, and indigenous microbial inoculants as practical strategies to enhance atrazine degradation in semi-arid Sudanese soils.

The available archived analytical method record confirms the use of GC-FID analysis, three atrazine calibration standards, duplicate injections, an LOD of 2 ppm, and recovery greater than 99%, supporting the reliability of the residue extraction and quantification procedure. The original chromatographic raw records, including the calibration equation, R^2 value, LOQ, retention-time report, and representative chromatograms, could not be retrieved because of the destruction and restricted access affecting laboratories and university facilities during the war in Sudan. Therefore, the manuscript reports only the validated analytical information available from the archived method record, while future studies should include full chromatographic validation outputs as Supplementary Material.

5 Conclusion

This study demonstrates that atrazine significantly affects culturable soil microbial groups and shows persistent behavior in Gerif silty clay loam soil from Sudan. Atrazine application produced clear concentration- and time-dependent responses, with higher concentrations causing substantial inhibition of organic nitrogen-utilizing bacteria, mineral nitrogen-utilizing bacteria, and fungi, particularly during the early and intermediate stages of incubation. In contrast, partial recovery or stimulation at later stages suggests possible microbial adaptation, reduced atrazine bioavailability, or selective enrichment of tolerant culturable microorganisms.

Microorganisms growing on nitrate agar were dominated by Mycobacterium spp., indicating a high level of tolerance under atrazine exposure and suggesting a possible role in atrazine transformation under contaminated conditions. Atrazine biodegradation followed a concentration-dependent pattern, with faster degradation at the lowest concentration, reaching 75.0% at 0.678 mg g^{-1} soil, and slower dissipation at the highest concentration, reaching 54.2% at 5.08 mg g^{-1} soil after 150 days. Kinetic analysis further confirmed this pattern, with atrazine half-life ranging from 69 to 125 days and the longest persistence observed at the highest concentration. The low organic matter content of Gerif soil likely limits microbial biomass and enzymatic activity, thereby reducing degradation efficiency and increasing atrazine persistence. These findings indicate that both herbicide concentration and soil characteristics jointly control atrazine fate in semi-arid agroecosystems.

From a management perspective, the results emphasize the need for site-specific and sustainable herbicide practices, including optimized application rates, improvement of soil organic matter, and promotion of microbial-mediated remediation strategies. Such approaches are essential to reduce long-term ecological risks, protect soil biological functioning, and support sustainable agricultural production in Sudan and similar semi-arid environments.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author contributions

AE: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing –

original draft, Writing – review & editing. EY: Formal analysis, Writing – original draft, Writing – review & editing. KC: Supervision, Writing – review & editing. AO: Supervision, Writing – review & editing. EE: Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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