Residue and soil dissipation kinetics of chloroacetanilide herbicides on rice (*Oryzae sativa* L.) and assessing the impact on soil microbial parameters and enzyme activity

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Abstract The present investigation determines the persistence of herbicides like butachlor and pretilachlor in Indian soil, and their impact on soil biological properties including microbial biomass carbon (MBC), total microbial population numbers, and enzyme activities. Butachlor was degraded faster in autumn rice soil ($t_{1/2}$ of 10–13 days) than in winter rice soil (half-life of 16–18 days). The $t_{1/2}$ of pretilachlor in winter rice was 12–16 days. Regardless of the seasons under cultivation, no pesticide residue was detected in rice at harvest. Herbicides induced an initial decline (0–14th days after application) in MBC (averages of 332.7–478.4 g g^{-1} dry soil in autumn rice and 299.6–444.3 g g^{-1} dry soil in winter rice), microbial populations (averages of 6.4 cfu g^{-1} in autumn rice and 4.6 cfu g^{-1} in winter rice), and phosphatase (averages of 242.6–269.3 µg p-nitrophenol g^{-1} dry soil h^{-1} in autumn rice and 188.2–212.2 µg p-nitrophenol g^{-1} dry soil h^{-1} in winter rice). The

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application of herbicides favored dehydrogenase (averages of 123.1–156.7 g TPF g^{-1} dry soil in autumn and 126.7–151.1 g TPF g^{-1} dry soil in winter) and urease activities (averages of 279.0–340.4 g NH$_4$ g^{-1} soil 2 h^{-1} in autumn and 226.7–296.5 g NH$_4$ g^{-1} soil 2 h^{-1} in winter) in rice soil at 0–14th DAA. The study suggests that applications of butachlor and pretilachlor at the rates of 1000 g ha^{-1} and 750 g ha^{-1}, respectively, to control the weeds in the transplanted rice fields do not have any negative impact on the harvested rice and associated soil environment.

Introduction

Rice (*Oryza sativa* L.) is a major food crop in northeast of India (Gogoi et al., 2021). Rice is known to supply millions of farmers in India's eastern and northern regions with essential food nutrients and vitamins such as carbohydrates, calcium, iron, thiamin, pantothenic acid, folate, and energy (Baishya et al., 2016; Yadav et al., 2019). With the current increase in human populations and nutritional needs, there is an urgent requirement to boost the rice production and quality across the country. However, due to the long-term negative consequences of global warming and climate change, the frequency, intensity, and duration of abiotic stress-related factors in rice cultivation are predicted to increase in the coming years, posing a serious threat to agricultural productivity and global food security (Dar et al., 2021). Through improving our understanding of the physiology and molecular biology of rice genotypes, we may be able to cultivate and maintain higher-quality standards and stocks of rice cultivars that are more resistant to stress. Adoption of integral crop management practices using the potential of beneficial plant growth promoters (PGPs) and other plant protection formulations (PPFs) at their recommended and lawful applications to improve the rice productivity both in terms of quality and quantity and to protect the crop against devastating pests and pathogens seemed to be essential (Khan et al., 2004; Hamid & Ghazanfar, 2022). Furthermore, because transcription factors (TFs) function

as master regulators of several stress-responsive genes, they are seemed to be ideal candidates for genetic engineering to develop stress-tolerant crops including rice (Wang et al., 2016).

Weeds have remained as one of the most significant biological limiting factors to rice crops (Chauhan, 2020). Weeds have a negative impact on rice growth and development, leaf architecture, tillering capacities, crop production, and other yield-related characteristics (Haque et al., 2021). In bad scenario, weeds have been projected to lower rice yield by 15–50% or more, up to 76%, if proper weed management tactics are not to be followed. *Alternanthera sessilis*, *Ammannia baccifera*, *Cyperus iria*, *C. maritimus*, *C. rotundus*, *C. difformis*, *Cynodon dactylon*, *Echinochloa glabrescens*, *E. colona*, *E. crus-galli*, *Limnocharis flava*, *Ludwigia octovalvis*, *Marsilea minuta Monochoria vaginalis*, *Paspalum distichum*, and *Sagittaria guayanensis* are recorded as the potential weed species in the rice farming, particularly in Asia (Hossain et al., 2020). Weeds host significant pest populations, which eventually steal a substantial amount of plant nutrients from the soil system, depriving the crops from important minerals such as N, P, K, Ca, and Mg.

Herbicides in weed management in rice crop have been in practice on the regular basis at least for the last couple of decades (Singh et al., 2016). According to Hakim et al. (2021), herbicide application in rice soil can lower weed population and density and, thus, plays vital role in enhanced grain and straw yields in rice. Chemical weed control is a common and popular practice among the small-scale farmers because of its effectiveness in reducing the weed competition with minimal need of labor and cost. However, improper and illegitimate spraying with herbicides in rice fields has been documented to leave toxic agrochemicals in soil, water, and natural ecosystem (Gunstone et al., 2021). Bhattacharyya and Sarmah (2018) reported significant soil quality deterioration, air and groundwater pollution, and the formation of undesirable residues due to agrochemical application in field. Meanwhile, the resurgence of primary pests and disease development is enhanced due to inappropriate applications of herbicides and other agrochemicals (Sarmah et al., 2020). The possible explanation of all of this would be the accumulation of chemical toxicity in soil which is followed by susceptibility variation and pest resistance development (Nicolopoulou-Stamati et al., 2016). According to Alengebawy et al. (2021), soil

toxicity and decreased fertility are linked to agrochemical side effects that have a direct impact in reducing the beneficial microbial populations and functions in soil. Chemical toxicity poses serious threat to the ecological system, by altering biosynthetic pathways, protein synthesis, and cell membrane development, and influencing another essential organelle production (Hassan & Nemr, 2020). Tudi et al. (2020) advocated that chemical composition and active ingredients of a molecule, associated soil parameters, and climate plays vital roles in determining the pesticide residual activity and persistence in soil. It is therefore critical to reduce the load of chemical compounds in soil and move towards justified and authorized application of recommended doses of PPFs in order to preserve the ecosystem safe, sustainable, and environmentally pleasant.

Butachlor (*N*-(butoxy ethyl)-2-chloro-N-2, 6-dimethyl acetanilide) and pretilachlor [2-chloro-2,6-diethyl-*N*-(2-propoxyethyl)acetanilide] (Supplementary Fig. 1a and 1b) are the herbicides from the acetanilide family (Shilpakar et al., 2020; Tripthi et al., 2020) that are being used to combat annual weeds in rice fields. Since the 1960s, the chloroacetanilide class of herbicides has emerged as one of the most extensively used classes of herbicides in agriculture for the control of broad leaf weeds and annual grasses. However, large-scale and unauthorized use of these herbicides poses serious risks to the soil, air, water, and environment (Mohanty & Jena, 2019), including the non-target organisms like human beings. Chen et al. (2014) stated that high application doses of chloroacetanilide herbicides are relatively highly water-soluble and persistent in nature. According to Cheng et al. (2017), the accumulation of residues and metabolites generated by chloroacetanilide toxicity in soil and in surface and ground water potentially contribute to moderate to high levels of chronic toxicity. Ecotoxicological studies have demonstrated that these herbicides aggravate DNA damage and tumor induction in rats, fish, and human cells (Panigrahi et al., 2021). Herbicide toxicity induces disruption of several key biological processes in living organisms, including energy metabolism and Ca^{2+} homeostasis, cell signalling, and endoplasmic reticulum stress responses.

However, according to Medo et al. (2021), limited evidence on the impact of repeated applications of diverse agrochemicals including the chloroacetamide herbicides on native soil microbial populations, activity, and persistence in rice soil is yet available. Besides, herbicides at the recommended dose of application are seemed to be essential in agriculture since it aids farmers in controlling undesired pests on their field. As the chloroacetanilide herbicides dissipate rapidly from the rice fields through photodecomposition, microbial degradation, and volatilization routes at their recommended concentrations, they pose no significant difficulty or risks in terms of accumulation of chemical residue or environmental toxicity.

In the current study, the persistence of chloroacetanilide herbicides at recommended doses was evaluated to measure if they could effectively eliminate weeds in rice fields. In light of the foregoing, the present work was designed to (i) investigate the persistence of butachlor and pretilachlor in soils and plants through rapid field applications and (ii) evaluate the possible effects of target herbicides on soil health, microbial biomass carbon (MBC), native microbial communities, and important enzyme kinetics in soil.

Materials and methods

Description of the study locations and treatment design

The present field experiment is a part study of long-term trials on herbicides as a weed control measure under the All-India Co-Ordinated Research Project on Weed Management, a network project throughout India that started in the year 2000, at Assam Agricultural University's (AAU) instruction cum research farm (26°44′N, 94°12′E), Jorhat, Assam, India, at an elevation of 86.6 m above mean sea level (msl) with a mean annual rainfall of 2,124 mm. The treatments are prepared and finalized under the project where the application of 50% recommended dose of fertilizer (RDF) is considered as farmers' practice.

The experimental plot was 3400 m^2 in size (including bund area), with five blocks including all treatments and four replications per treatment. The climate at the study location was subtropical, with hot summers and cold winters. The soil in the experimental field was acid inceptisol, with 52% sand, 21% silt, and 27% clay, a cation exchange capacity (CEC) of 6.28 cmol (p+) kg^{-1}, organic carbon of 0.82%, available N of 212, P_2O_5 of 16.6, and K_2O of 136.8 kg ha^{-1}, and MBC of 365.5 µg g^{-1} soil, respectively. The experimental layout was as follows: five different treatments
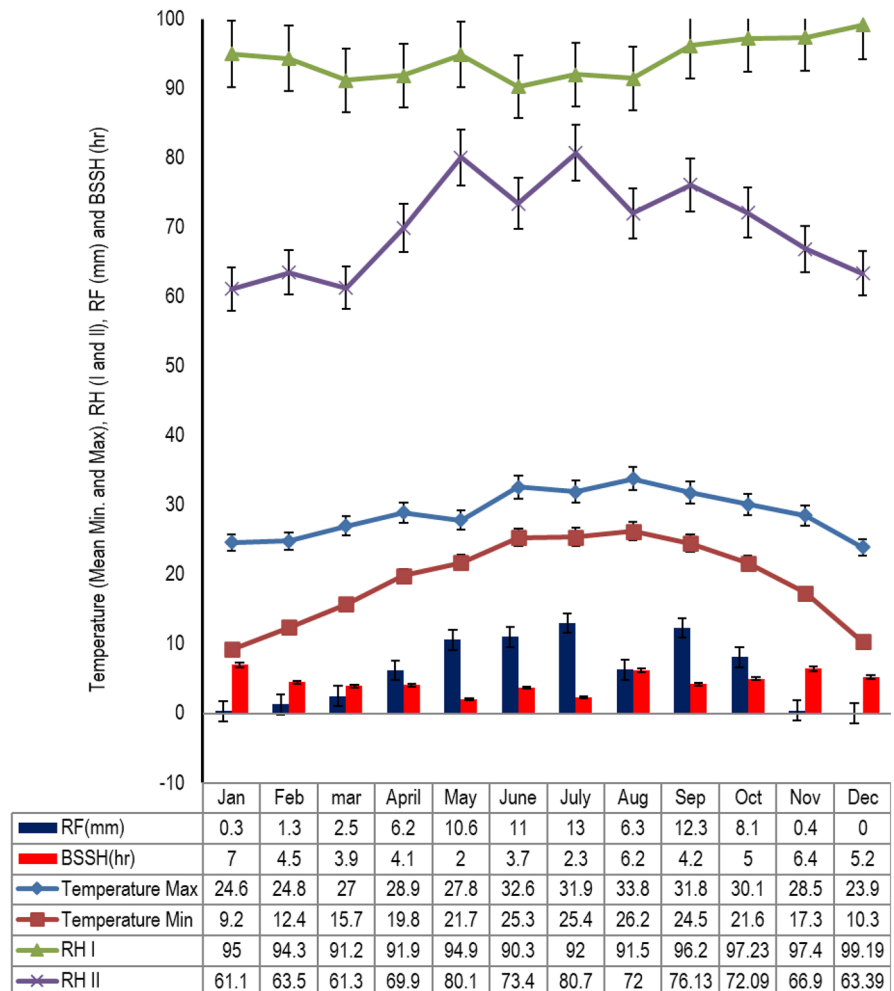
that includes T1: farmer's practice (hand weeding with 50% recommended dose of fertilizers (RDFs), T2: butachlor (at 1000 g ha^{-1}) + 2,4-D with 100% RDFs, T3: butachlor (at 1000 g ha^{-1}) + 2,4-D with 75% RDFs + 25% N through the organic source, T4: butachlor (at 1000 g ha^{-1}) + 2,4-D rotated with pretilachlor (at 750 g ha) and 100% RDFs, and T5: butachlor (at 1000 g ha^{-1}) + 2,4-D rotated with pretilachlor (at 750 g ha^{-1}) and 75% RDFs + 25% N through organic source with four replications and randomized block design (RBD). Autumn and winter rice received RDFs of 40:20:20 and 60:20:40 kg ha^{-1} NPK, respectively. To supplement 25% N, an organic source (vermicompost) comprising of total N 1.4–1.5%, P$_2$O$_5$ 1.0–1.1%, and K$_2$O 1.5–1.8% was employed. Butachlor and pretilachlor were applied as weed management practices in the rice-cultivated areas at the rates

of 1000 g ha^{-1} and 750 g ha^{-1}, respectively. Rice has been planted in the winter and autumn seasons since the year 2000, with the current data generation made during 2019–2020. The meteorological data of the study locations has been depicted in Fig. 1.

Sample collection and analysis

Soil samples were collected between 2019 and 2020 using a randomised block design (the samples were not pooled). The experiment was split into five distinct plots, one of which served as a control (no herbicide treatment) and the other four as herbicide combinations. Each plot was again divided into five blocks, with four soil samples collected at random from each block. A total of 25 composite samples of 5 plots, collected at regular intervals, including 0

Fig. 1 The meteorological and climatological data of the study locations during 2019–2020

	Jan	Feb	mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
RF(mm)	0.3	1.3	2.5	6.2	10.6	11	13	6.3	12.3	8.1	0.4	0
BSSH(hr)	7	4.5	3.9	4.1	2	3.7	2.3	6.2	4.2	5	6.4	5.2
Temperature Max	24.6	24.8	27	28.9	27.8	32.6	31.9	33.8	31.8	30.1	28.5	23.9
Temperature Min	9.2	12.4	15.7	19.8	21.7	25.3	25.4	26.2	24.5	21.6	17.3	10.3
RH I	95	94.3	91.2	91.9	94.9	90.3	92	91.5	96.2	97.23	97.4	99.19
RH II	61.1	63.5	61.3	69.9	80.1	73.4	80.7	72	76.13	72.09	66.9	63.39

(within 4 h of herbicide application), 7, 14, 30, and 60 days after application (DAA), and finally at crop harvest of each season, were homogenized and sieved (0.2 mm) to remove the stone and other plant debris. One part of the composite sample was chilled at 4 °C to analyze the MBC, soil enzyme activity, and microbial population numbers in comparison to control soil in triplicates, while the other part was dried, sieved, and placed in polypropylene bags for detection of herbicide residue. Standard methodologies for estimation of herbicide residue and soil microbiological examination were used to analyze the collected soil samples as mentioned below.

Estimation of herbicide residues in soil

Herbicide residue was detected in matrices (soil and plant) through standard protocols. For this, soil and plant samples were analyzed in triplicates at regular intervals, including 0, 7, 14, 30, and 60 DAA, and finally at crop harvest of each season. Butachlor analytical reference standard ($\geq 95.0\%$ purity, Cas No. 23184–66-9) in ampoules of 100 mg and pretilachlor reference standard ($\geq 98.7\%$ purity, Cas No. 51218–49-6) in ampoules of 250 mg (Sigma-Aldrich, Seelze, Germany) was used for calibration of gas chromatography. Florisil was heated for 12 h at 130 °C to resuscitate herbicide molecules and promote adsorption. A Thermo Scientific TG-5 MS GC column, mega pore capillary column 30 M, length × 0.25 mm, ID × 0.25-m film thickness, and a maximum temperature of 330/350 °C were used for the chromatographic separation. The oven temperature was set at 100 °C for 1 min, and then increased to 220 °C at a pace of 20 °C per minute for 10 min. The injector and detector temperatures were adjusted at 250 and 300 °C, respectively. At a flow rate of 53.6 mL min^{-1}, nitrogen was used as the carrier gas. The injection volume is set at 0.5 L, and the split is set at 100 J. The test molecules' limit of detection (LOD) was estimated using a 3:1 signal-to-noise ratio (S/N) compared to the blank matrix extract's background noise, while the limit of quantification (LQ) measured using a 5:1 S/N. Matrix-matched standards (peak area of post-extraction spike) were compared to solvent-based standards at 1 g mL^{-1}. Pesticide suppression or augmentation was used to examine the matrix effect.

The following equation was used for matrix effect (ME) evaluation:

$$ME\ (\%) = \frac{(\text{Peak area of matrix standard} - \text{Peak area of solvent standard})}{\text{Peak area of solvent standard}} \times 100$$

The collected straw and grain samples were dried in hot air oven at 65 °C–70 °C until they attained a constant weight. The chemicals and reagents used in the present experimentation were procured from Merck Pvt. Ltd., Mumbai, India.

Quantification of herbicides, extraction, and recovery study

Standard solutions of 1000 g mL^{-1} butachlor and 2500 g mL^{-1} pretilachlor were made by thoroughly dissolving 100 mg of technical grade butachlor and 250 mg of pretilachlor in 100 mL chromatographic-grade n-hexane. The standard solutions were maintained at −20 °C in a deep freezer.

The calibration curve for the herbicides was prepared by injecting known concentrations of the molecule (1.00, 0.75, and 0.25 µg mL^{-1}) into the GC. The average recoveries of butachlor in rice soil, straw, and husked rice were estimated as 88.2–94.2%, 88.6–96.4%, and 86.2–90.6%, respectively (Table 1). Similarly, the average recoveries of pretilachlor in rice soil, straw, and husked rice were 86.5–92.4%, 87.2–92.6%, and 86.4–96.2%, respectively (Table 1). For butachlor and pretilachlor, excellent linearity was obtained with all determination coefficients (R^2) more than 0.85 in the range of 0.25–1.00 µg mL^{-1}. Herbicide concentrations were linearly regressed against peak regions to create the calibration curve. The LOD of butachlor and pretilachlor in soil, straw, and husked rice was 0.016 µg g^{-1}, 0.016 µg g^{-1}, and 0.003 µg g^{-1}, respectively, in all the matrices at a signal-to-noise (S/N) ratio of 3.0. The LOQ of butachlor and pretilachlor in soil, straw, and husked rice was 0.05 µg g^{-1} and 0.01 µg g^{-1}, respectively, in all the matrices at a signal-to-noise (S/N) ratio of 10.0. Overall, the average recoveries for all of the matrixes were satisfactory, ranging from 86.2 to 98.4%.

The solvent calibration solutions were made by diluting the stock solutions with n-hexane. The matrix calibration solutions were generated by diluting the

Table 1 The average recovery, calibration curve, the limits of detection (LODs), and limits of quantification (LOQs) of the herbicides in rice soil, straw, and husked rice ($n = 5$)

Herbicides	Sample matrix	Fortified level (μg g^{-1})	Average recovery (%)	RSD (%)	Calibration curve	R^2	LOD (μg g^{-1})	LOQ (μg g^{-1})
Butachlor	Soil	0.05	92.6	0.99	$Y = -0.026x + 0.848$	0.913	0.016	0.05
		0.50	88.2	1.35				
		1.0	94.2	1.34				
	Straw	0.05	88.6	1.32	$Y = -0.022x + 0.692$	0.881	0.016	0.05
		0.50	96.4	1.70				
		1.0	92.2	1.17				
	Husked rice	0.05	86.2	1.10	$Y = -0.018x + 0.476$	0.899	0.016	0.05
		0.50	98.4	1.00				
		1.0	90.6	1.87				
Pretilachlor	Soil	0.01	86.5	1.14	$Y = -0.022x + 0.528$	0.961	0.003	0.01
		0.05	92.4	1.47				
		1.0	90.6	0.99				
	Straw	0.01	87.2	1.04	$Y = -0.018x + 0.426$	0.928	0.003	0.01
		0.05	92.6	1.01				
		0.10	90.6	0.90				
	Husked rice	0.01	94.5	1.36	$Y = -0.014x + 0.260$	0.886	0.003	0.01
		0.05	86.4	1.14				
		0.10	96.2	1.24				

stock solutions with blank extracts from all the matrices. Six concentrations from 0.001 to 1 μg mL^{-1} were made for butachlor and pretilachlor standards. The reference standards of butachlor and pretilachlor were used for quantification studies, recovery, and retention time of the herbicides.

With 150 mL of acetone/hexane ratio, 10.0 g sieved soil/5 g each of straw and husked rice were extracted. Triplicates were maintained at each case. The soil and acetone mixture was agitated on a rotary shaker for 2 h at 150 rpm at 25 °C, while the rice straw and husked rice samples in acetone were held overnight before filtration through a Buchner funnel and three washes with 20 mL acetone/hexane. Following that, all filtrates were transferred to a 250-mL separating funnel containing 30 mL of 10% NaCl solution and extracted three times with 50 mL of *n*-hexane each time. After being dried with 10.0 g anhydrous sodium sulfate and 4.0 g florisil packed in a chromatographic column, the organic phase was collected in a 250-mL flat-bottom flask (2-mm ID). The extract was then concentrated in a rotary evaporator at 45 °C to near dryness before being dissolved in 1 mL *n*-hexane for GC analysis.

Five-gram processed soil from the second part of the soil samples was put into 15-mL polytetrafluoroethylene centrifuge tubes, along with 5 g each of straw and husked rice. Seven-milliliter methanol was added to the rice and straw, and acetonitrile was added to the husked grain, and the mixture was agitated thoroughly for 10 min on a horizontal shaker. The contents were centrifuged for 10 min at 3500 rpm, with the supernatant transferred to conical flasks. The samples were re-extracted two more times, each time with fresh 5 mL of the organic solvent. The solvent extracts were combined, and PSA (25 mg) + Na$_2$SO$_4$ were used to clean them up (100 mg). The contents were combined in a vortex mixer before being centrifuged for 10 min at 3000 rpm. Filtered supernatants were dehydrated to near dryness in a rotary evaporator at 45 °C, and then reconstituted to 1 mL with hexane. Herbicide residues were determined through GC on ECD using the extract. It was injected with 1.0 L of herbicide reference standard solution. The peaks were defined based on their retention time, and the peak area was calculated.

$$\text{Residues in } \mu g \text{ g}^{-1} = \frac{(\text{Area of sample} \times \mu \text{l standard injected} \times \text{final volume} \times \text{Recovery factor})}{\text{Area of standard} \times \mu \text{l sample injected} \times \text{weight of a sample}} \times 100$$

Fortifying untreated soil and plant samples with working standard solutions (0.05, 0.10, 0.15, 0.25, and 0.5 g mL^{-1}) and processing them validated the sample preparation procedure. By injecting known herbicide standards, GC was calibrated by measuring the peak area. Recovery study with graded herbicide doses was carried out to confirm interferences and contaminants during herbicide molecule extraction and data accuracy (%). To check for contamination and interference, control and blank samples were maintained on hand using five replications.

Analysis of soil microbiological properties

Microbiological population counts (cfu g^{-1} dry soil) were performed, just before and after treatment applications. For this, 10.0 g from each of the composite soil samples was homogenized with 100 mL of sterile distilled water (SDW) and put in an orbital shaker to properly mix the soil and diluents. After that, the soil–water mixture is left to settle for 30 min. For dilution plating, a series of dilution tubes (each containing 90 mL of the SDW) representing different dilution series (i.e., 10^2 up to 10^6) were sterilized using an autoclave at 15 lb in^{-2}.

Different types of culture media like chloramphenicol rose Bengal agar (mycological peptone 5.0 g L^{-1}; dextrose 10.0 g L^{-1}; potassium dihydrogen phosphate 1.0 g L^{-1}; magnesium sulfate 0.50 g L^{-1}; rose Bengal 0.05 g L^{-1}; chloramphenicol 0.10 g L^{-1}; agar 15 g L^{-1}; pH 7.2 ± 0.2) and nutrient agar (peptone 5.0 g L^{-1}; sodium chloride 5.0 g L^{-1}; HM peptone B# 1.5 g L^{-1}; yeast extract 1.5 g L^{-1}; agar 15 g L^{-1}; pH 7.4 ± 0.2) were used for isolation of soil fungi and bacteria. Selective isolation of functional microbial categories like phosphate-solubilizing microorganisms (PSMs) and N$_2$ fixing microbial populations (NFMs) was made using specific media like Pikovskaya's agar (PKV) (yeast extract 0.5 g L^{-1}; dextrose 10.0 g L^{-1}; calcium phosphate 5.0 g L^{-1}; ammonium sulfate 0.5 g L^{-1}; potassium chloride 0.2 g L^{-1}; magnesium sulfate 0.1 g L^{-1}; manganese sulfate 0.0001 g L^{-1}; ferrous sulfate 0.0001 g L^{-1}; agar 15.0 g L^{-1}), Jensen

media (sucrose 20.0 g L^{-1}; dipotassium hydrogen phosphate 1.0 g L^{-1}; magnesium sulfate 0.50 g L^{-1}; sodium chloride 0.50 g L^{-1}; ferrous sulfate 0.10 g L^{-1}; sodium molybdate 0.005 g L^{-1}; calcium carbonate 2.0 g L^{-1}; agar 15.0 g L^{-1}), and N$_2$ free glucose mineral media (NFGMM) (KH$_2$PO$_4$ 1.0 g L^{-1}; CaCl$_2$ 1.0 g L^{-1}; MgSO$_4$·7H$_2$O 0.25 g L^{-1}; NaCl 0.5 g L^{-1}; FeSO$_4$·7H$_2$O 0.01 g L^{-1}; MnSO$_4$·H$_2$O 0.01 g L^{-1}; Na$_2$MoO$_4$ 0.01 g L^{-1}; glucose 7.0 g L^{-1}; agar 20.0 g L^{-1}), respectively. Bacteria were incubated at 28 ± 1 °C for 2 to 3 days, and fungi were grown at 25 ± 1 °C for 5 days. Phosphate-solubilizing bacteria (PSB) were incubated at 30 ± 1 °C while the plates for nitrogen-fixing bacteria (NFB) were incubated at 35 ± 1 °C for 3 days. In each of the cases, three replicates were maintained.

The microbial colonies were grown in pure culture on PDA slants and kept at 4 °C for subsequent analysis and characterization. Bacterial and fungal colonies on growth media were identified using taxonomic literature, morphology, culture, and reproductive properties (Holt, 1994; Cappuccino & Sherman, 2004; Domsch et al., 2007; Mortimer et al., 2021). HiMedia Laboratories, Pvt. Lt., Mumbai, India, provided all of the media utilized for the isolation and growth responses of soil microorganisms.

Estimation of soil MBC

The MBC of the soil was calculated using the chloroform fumigation extraction procedure (Witt et al., 2000). Field damp soil (about 25.0 g) was fumigated with ethanol-free chloroform for 24 h at 25 °C. The soil samples were extracted in a 1:4 ratio with 100 mL 0.5 M K$_2$SO$_4$ and soil. The soils were extracted without fumigation to create the control sample. Whatman No. 42 filter paper was used to filter the soil suspension.

Determination of soil enzyme activity

Casida et al. (1968) described the triphenyl tetrazolium chloride (TTC) reduction process to triphenyl formazan (TPF) for determining dehydrogenase

activity (DHA). For this, 1.0 g of field damp soil was treated with 1.0 mL of 3% TTC and incubated for 24 h at 28 °C. Sterile controls made of autoclaved soil were utilized to account for potential abiotic TTC reductions. Soil and TTC were replaced with Millipore water in spectrophotometer blanks for both autoclaved and non-autoclaved treatments. At 485 nm, the optical density was compared to triphenyl formazan standards. TPF g^{-1} dry soil 24 h^{-1} was used to calculate DHA based on dry weight. The utilization of an artificial substrate, p-nitrophenyl phosphate, was used in phosphomonoesterase (PMEase) activities (p-NPP).

The phosphatase and urease activities in the samples were determined using Tabatabai and Bremner's (1969, 1972) methodologies. Under alkaline conditions, the result of phosphomonoesterase activity, p-nitrophenol, a chromophore, was quantified calorimetrically and expressed as p-nitrophenol g^{-1} dry soil h^{-1}. Urease activity in soil was determined by incubating the soil sample with (hydroxymethyl) aminomethane (THAM) buffer, urea solution, and toluene at 37 °C for 2 h before steam distilling with MgO in 2.5 M KCl solution containing a urease inhibitor (Ag$_2$SO$_4$).

Data analysis

Using analysis of variance (ANOVA) for a RBD, the statistical significance of the treatment effects on microbial count (cfu g^{-1}) was assessed for the least significant difference (LSD) at a 5% level of significance (Panse & Sukhatme, 1967). The significant difference between the treatment means was determined using Duncan's multiple range test (DMRT) at a 5% probability level. The degradation dynamics of the herbicides in fields appeared to follow the first-order kinetic reaction and were calculated according to the following equation: $Ct = C_0 e - kt$, where Ct and C_0 are the concentrations of herbicides at time t and time 0, respectively, after spraying (µg g^{-1}), and k is the degradation rate constant (Diao et al., 2009, 2010). The half-life ($t_{1/2}$) of each herbicide was calculated using the equation: $t_{1/2} = \ln 2 / k$ (Diao et al., 2010; Liu et al., 2012). The $t_{1/2}$ values were calculated using a first-order kinetic model and regression analysis.

Results and discussion

Persistence and dissipation of butachlor and pretilachlor under field condition

Table 2 indicates the dissipation of butachlor and pretilachlor from the soil in various treatments in autumn and winter rice. The herbicides' dissipation processes followed the first-order kinetic reaction. Table 3 illustrates the dissipation half-life ($t_{1/2}$) and residue measurements of herbicides following their application in soil. Herbicide residues were not detected in field soil or control plots prior to treatment exposure. On the other hand, the residues discovered after the application of chloroacetamide herbicides varied with the treatment applications.

Initially, the butachlor residues at 0 DAA in autumn rice were in the range of 0.81–0.84 µg g^{-1}, and in winter rice, it ranged from 0.86 to 0.89 µg g^{-1}. The progressive and steady decline of herbicides in rice soil was observed on a regular basis until 60 and 75 DAA in autumn and winter rice, respectively. Butachlor decayed faster in autumn rice soil, with $t_{1/2}$ of 10–13 days, compared to 16–18 days in winter rice soil. Furthermore, butachlor breakdown in soil was affected by application rate, duration, and soil type. According to Torabi et al. (2020), this phenomenon might be due to limitation in the number of reaction sites in soil, as well as the toxic effect on native microorganisms or enzyme inhibition.

Based on the source of nitrogen supplied, pretilachlor residues in winter rice at 0 DAA were initially in the range of 0.34–0.38 µg g^{-1} with a half-life of 12–16 days and were in conformity with the findings of Murata et al. (2004). Pretilachlor at 0.75 kg a.i. ha^{-1} dissipated to below detectable level (BDL), i.e., 0.003 µg g^{-1} within 30 days after application, and it is in accordance with to the findings of Dharumarajan et al. (2011). The minimal degradation of pretilachlor was measured in the lab and found to be consistent with Adachi et al. (2007). Pretilachlor and other acetanilide herbicides break down quickly in the soil, and residues were found to be below the quantification limit of 0.01 µg g^{-1} in samples obtained from 75 DAA of pretilachlor and 0.05 g g^{-1} in samples collected from 60 to 75 DAA of butachlor in winter and autumn rice. T3 and T5 residues, which used FYM to replace 25% of nitrogen, degraded faster than T2

Table 2 Dissipation (%) of butachlor and pretilachlor from top soil (0–15 cm) in autumn and winter rice

Days after application	Dissipation (%) of butachlor and pretilachlor in soil							
	T2		T3		T4		T5	
	Autumn rice	Winter rice	Autumn rice	Winter rice	Autumn rice	Winter rice	Autumn rice	Winter rice
0	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
3	24.1 g	24 g	26.3f	25.f1	28 g	20.9 g	25.2f	23.3 g
7	62.2f	57.5f	50.8e	62.4e	61.f	34.6f	64e	38.2f
14	72.3e	72.4e	60.6d	72.5d	73.5e	42.4e	67.1d	46.3e
30	86.8c	84.1d	76e	75.4c	87.5d	56.7d	78.6c	64.2d
45	77.2d	86.1c	93.5b	88.5b	93.7c	67.6c	94.8b	76.3c
60	95.6b	90.1d	97.1a	93.9a	96.2b	82.2b	98.6a	89.3b
75	97.2a	96.3a	BDL	95.6a	98.2a	95.3a	BDL	97.5a
90	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
At harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Values within the same column followed by different letters (a, b, c) are significantly different from each another ($P < 0.05$). Four replicates are maintained at each case. *T2* butachlor + 2, 4-D with 100% NPK through chemical fertilizer, *T3* butachlor + 2, 4-D with 75% NPK through chemical fertilizer + 25% through organic source, *T4* butachlor + 2, 4-D rotated with pretilachlor with 100% NPK through chemical fertilizer, *T5* butachlor + 2, 4-D rotated with pretilachlor with 75% NPK through chemical fertilizer + 25% through organic source

and T4 residues, which used solely urea. Our results clearly showed that herbicide dissipation is strongly influenced by soil organic matter as described by Carpio et al. (2021). The study reported that 50% of butachlor residues in autumn and winter rice dissipated in soil within 7 DAA. In winter rice, 50% of the pretilachlor was dissipated in the soil within 30 DAA.

In autumn rice, the butachlor residues (about 90%) disappeared in 45 days, but in winter rice, they dissipated in 60 days. Physical characteristics such as

Table 3 Residues ($\mu g\ g^{-1}$) of butachlor and pretilachlor in autumn and winter rice-grown soil (0–15 cm)

Days after application	Residues ($\mu g\ g^{-1}$) of butachlor and pretilachlor in soil							
	T2		T3		T4		T5	
	Autumn rice	Winter rice	Autumn rice	Winter rice	Autumn rice	Winter rice	Autumn rice	Winter rice
0 DAA	0.842 ± 0.004a	0.887 ± 0.005a	0.816 ± 0.002a	0.857 ± 0.005a	0.832 ± 0.004a	0.378 ± 0.004a	0.808 ± 0.004a	0.343 ± 0.005a
3 DAA	0.634 ± 0.002b	0.679 ± 0.004b	0.612 ± 0.004b	0.645 ± 0.002b	0.622 ± 0.004b	0.306 ± 0.005b	0.608 ± 0.004b	0.266 ± 0.005b
7 DAA	0.328 ± 0.004c	0.365 ± 0.004c	0.308 ± 0.004c	0.328 ± 0.004c	0.318 ± 0.004c	0.255 ± 0.003c	0.292 ± 0.004c	0.214 ± 0.004c
14 DAA	0.228 ± 0.004d	0.264 ± 0.004d	0.215 ± 0.004d	0.238 ± 0.004d	0.228 ± 0.004d	0.228 ± 0.004d	0.204 ± 0.002d	0.187 ± 0.005d
30 DAA	0.106 ± 0.002e	0.148 ± 0.004e	0.202 ± 0.004e	0.106 ± 0.005e	0.104 ± 0.002e	0.168 ± 0.004e	0.182 ± 0.004e	0.124 ± 0.002e
45 DAA	0.065 ± 0.004f	0.108 ± 0.004f	0.058 ± 0.004f	0.095 ± 0.004f	0.062 ± 0.004f	0.124 ± 0.002f	0.045 ± 0.003f	0.087 ± 0.005f
60 DAA	0.045 ± 0.003 g	0.082 ± 0.004 g	0.024 ± 0.002 g	0.056 ± 0.003 g	0.036 ± 0.002 g	0.074 ± 0.001 g	0.016 ± 0.002 g	0.0347 ± 0.003 g
75 DAA	0.016 ± 0.002 h	0.052 ± 0.004 h	BDL	0.034 ± 0.002 h	0.014 ± 0.002 h	0.016 ± 0.002 h	BDL	0.004 ± 0.002 h
90 DAA	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
At harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
$t_{1/2}$ days	13.13	18.30	11.78	16.00	12.74	16.45	10.61	11.68

All the values are mean (±) standard deviation. Four replicates are maintained at each case. *T2* butachlor + 2, 4-D with 100% NPK through chemical fertilizer, *T3* butachlor + 2, 4-D with 75% NPK through chemical fertilizer + 25% through organic source, *T4* butachlor + 2, 4-D rotated with pretilachlor with 100% NPK through chemical fertilizer, *T5* butachlor + 2, 4-D rotated with pretilachlor with 75% NPK through chemical fertilizer + 25% through organic source

BDL below detection limit

temperature, wind velocity, and moisture level might cause faster dissipation of butachlor, with an average $t_{1/2}$ of 10–18 days (Rao et al., 2012). According to Raffa and Chiampo (2021), the rapid degradation of butachlor in soils is owing to some indigenous microorganisms adapting to butachlor after repeated applications. In treatments with or without FYM as a nitrogen supplement, more than 90% of the pretilachlor in the soil dissipated after 60 DAA. The presence of soil bacteria, in combination with aqueous photolysis, might facilitate pretilachlor dissipation (Kaur et al., 2015). Soil pH, dissolved oxygen, moisture, soil quality, and native microbial communities affect herbicide dissipation (Huang et al., 2018). Finally, regardless of the herbicides used on transplanted rice fields, no herbicide residue was identified in husked rice or rice straw.

The results revealed that herbicides at their recommended dosage on rice in open fields were safe and effective. Experiments were carried out to check the harvest time residues of butachlor and pretilachlor, as well as the persistence behavior. Herbicide residues at application rates of butachlor and pretilachlor, respectively, were found to be below the detectable limit of 0.016 μg g^{-1} and 0.003 μg g^{-1} of herbicides, according to analyses of rice field soil and rice crop samples. The residues in harvested crops including fruits and vegetables were below the maximum residue limits (MRLs) as defined in Mebdoua (2018) and may be harmless to food and the environment under existing testing conditions.

Herbicide exposure and change in microbial count (cfu g.$^{-1}$ dry soil)

The abundance and distribution of different groups of soil microorganisms were enumerated and expressed in terms of colony-forming units per gram of dry soil (cfu g^{-1}). The effect of butachlor and pretilachlor on total soil bacterial population numbers showed a decreasing trend from 0 to 7th DAA (66.5–78.6 cfu g^{-1} in autumn rice and 62.2–74.2 cfu g^{-1} in winter rice), followed by an upward trend from 14 to 60th DAA (78.2–110.5 cfu g^{-1} in autumn rice and 74.7–88.5 cfu g^{-1} in winter rice) (Fig. 2a, b). Similarly, continuous application of herbicides in soil affects the populations of total fungi. Their population showed a declining trend from 0 to 7th DAA (24.5–52.2 cfu g^{-1} in autumn rice) and

22.4–46.3 cfu g^{-1} in winter rice, followed by an increasing trend from 14 to 60th DAA (30.4–62.4 cfu g^{-1} in autumn rice) and 25.7–58.4 cfu g^{-1} in winter rice (Fig. 2c, d).

Herbicide use has also had a substantial impact on the populations of functional categories of soil microorganisms such as *Azotobacter*, *Azospirillum*, and phosphate-solubilizing bacteria (PSB). The *Azotobacter* count varied from 14.6 to 24.3 cfu g^{-1} in autumn rice and 10.5 to 22.7 cfu g^{-1} in winter rice, with an increasing tendency from the 14th to the 60th DAA, which ranged from 18.3 to 40.1 cfu g^{-1} in autumn rice and 14.4 to 32.5 cfu g^{-1} in winter rice (Fig. 3a, b). The *Azospirillum* count ranged between 12.4 and 23.4 cfu g^{-1} in autumn rice and 10.4 and 20.4 cf g^{-1} in winter rice followed by an increased trend from 14 to 60th DAA ranged between 12.3 and 36.4 cfu g^{-1} in autumn rice and 11.6 and 32.3 cfu g^{-1} in winter rice (Fig. 3c, d). Phosphate-solubilizing bacterial (PSB) populations varied from 6.4 to 13.5 cfu g^{-1} in autumn rice to 4.6–10.3 cfu g^{-1} in winter rice, with an increasing tendency from the 14th to the 60th DAA, which ranged from 7.2 to 26.5 cfu g^{-1} in autumn rice to 6.5–24.5 cfu g^{-1} in winter rice (Fig. 3e, f). Herbicide residues in soil are a major source of concern since they can persist on top soil, accumulate to critical levels, and inhibit the native microbial populations (Thiour-Mauprivez et al., 2019) while also influencing soil nutrient cycle. Depending on the dose and type of herbicide used, some microorganisms have the abilities to degrade herbicides, while others have been seriously affected (Briceno et al., 2020; Huang et al., 2018; Pileggi et al., 2020).

Furthermore, in autumn and winter rice, treatments with 25% organic nitrogen substitution had considerably larger microbial population counts than treatments with only inorganic nitrogen. This activity might be attributed to higher energy and nutrient availability released from the organic sources that eventually assists the growth of functional categories of microorganisms like *Azotobacter* spp., *Azospirillum* spp., and phosphate solubilizers. However, nutrients tend to decrease with increased intensity of rainfall, vaporization, and leaching activities.

Non-target organisms, such as native microbial populations in soil, might be affected by herbicide applications (Thiour-Mauprivez et al., 2019). When
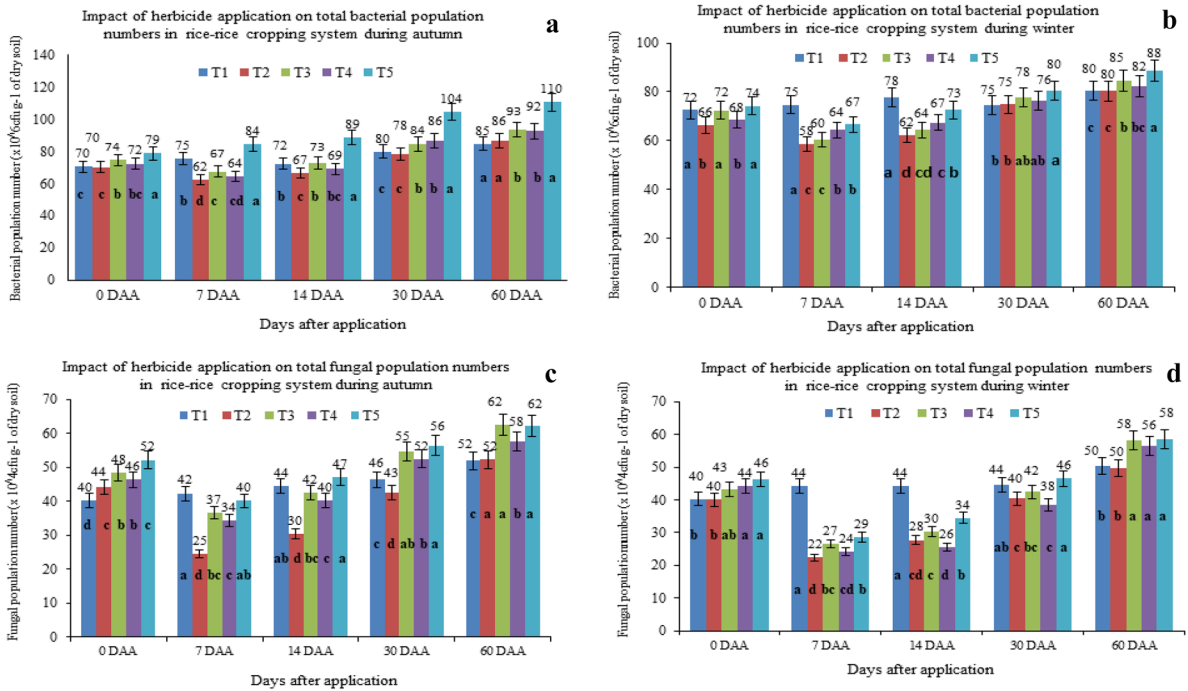
Fig. 2 a–d Impact of herbicide applications on total bacterial population numbers (cfu mL^{-1} dry soil) and total fungal population numbers (cfu mL^{-1} dry soil) in rice-rice cropping system during autumn and winter. The values in the same column that are preceded by distinct letters (a, b, c, and d) differ considerably ($P < 0.05$) as per data analysis and DMRT. Three replicates are maintained at each case. T1: control with one-hand weeding; T2: butachlor + 2,4-D with 100% NPK through chemical fertilizer; T3: butachlor + 2,4-D with 75% NPK through chemical fertilizer + 25% through organic source; T4: butachlor + 2,4-D rotated with pretilachlor with 100% NPK through chemical fertilizer; T5: butachlor + 2,4-D rotated with pretilachlor with 75% NPK through chemical fertilizer + 25% through organic source

herbicides are sprayed, a large percentage of the herbicide reaches out the soil and accumulates in microbiologically active zones such as the top soil. Chemical contamination is indicated by a change in microbial activity and relative abundance of soil microorganisms in response to herbicide inputs in soil (Streletskii et al., 2022). The microbial population grew over time, which might be attributable to herbicide adaptation or a phenomenon associated to herbicide degradation (James et al., 2021).

Furthermore, pesticide degradation is usually faster in soils with high organic matter, owing to increased microbial activity (Kaur et al., 2021). Herbicides can also produce qualitative and quantitative changes in soils and thereby influencing the populations of native microbial communities (Pose-Juan et al., 2017). Moreover, the decline in microbial population count at initial stage could be attributed to the disturbances in the biological equilibrium in soil following the application of herbicides as described by Streletskii et al. (2022) and Carpio et al. (2021). However, microbial population increased later, which might be due to microbial multiplication on increased supply of nutrients available from herbicide breakdown. Microbial catabolism and detoxifying metabolisms are the other modes of action exhibited by soil microorganisms that depends on pesticides as a major source of carbon and energy (Arora & Sahni, 2016). Furthermore, as indicated by Van Bruggen et al. (2018) and Thiour-Mauprivez et al. (2019), the initial decline in microbial count might be related to disruptions in the biological balance in soil as a result of herbicide exposure. However, microbial population increased subsequently due to microbial multiplication on enhanced supply of nutrients obtained from herbicide degradation, either chemical or microbial breakdown (Gupta et al., 2017).
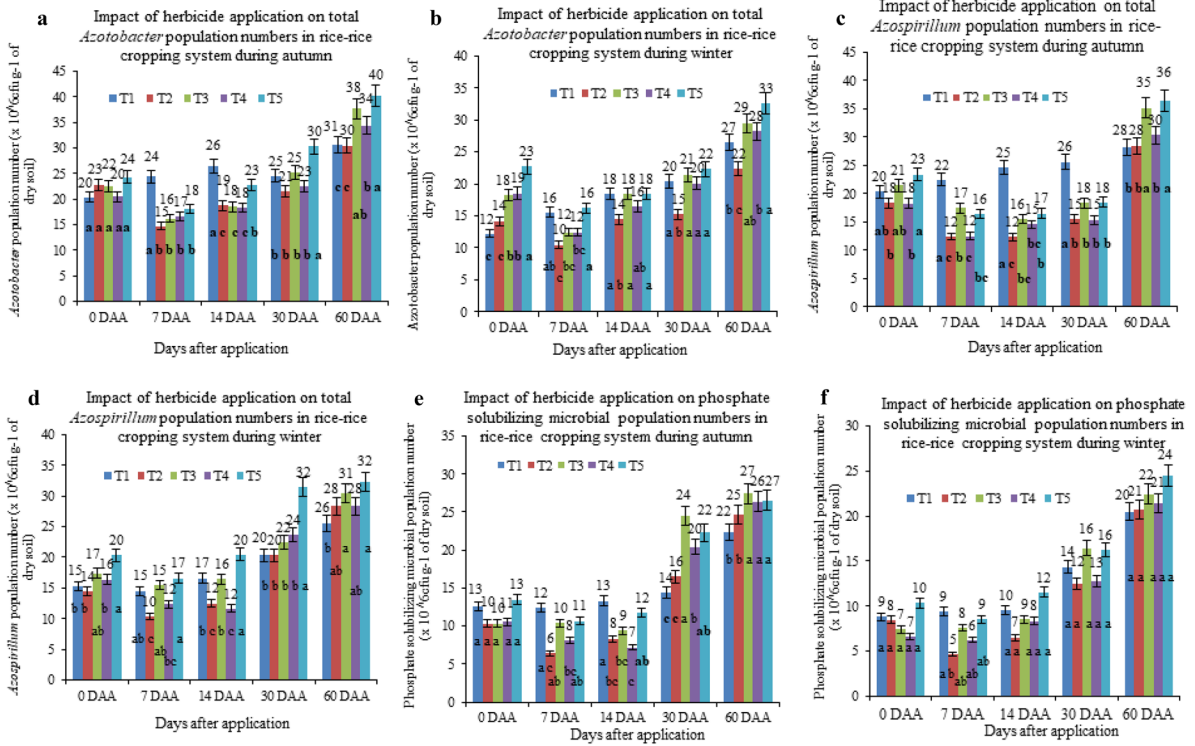
Fig. 3 **a–f** Impact of herbicide applications on functional categories of microbial population numbers (cfu mL^{-1} dry soil) in rice-rice cropping system during autumn and winter. **a, b** Total *Azotobacter* population numbers, **c, d** total *Azospirillum* population numbers, and **e, f** phosphate-solubilizing microbial population numbers. The values in the same column that are preceded by distinct letters (a, b, c, and d) differ considerably ($P < 0.05$) as per data analysis and DMRT. Three replicates are maintained at each case. T1: control with one hand weeding; T2: butachlor + 2,4-D with 100% NPK through chemical fertilizer; T3: butachlor + 2,4-D with 75% NPK through chemical fertilizer + 25% through organic source; T4: butachlor + 2,4-D rotated with pretilachlor with 100% NPK through chemical fertilizer; T5: butachlor + 2,4-D rotated with pretilachlor with 75% NPK through chemical fertilizer + 25% through organic source

Soil MBC

The soil MBC in butachlor- and pretilachlor-treated soil (Table 4) showed a declining trend from 0 to 14th DAA, ranging from 332.7 to 478.4 μg g^{-1} dry soil in autumn and 299.6–444.3 μg g^{-1} dry soil in winter rice, followed by an increasing trend from 30 to 60th DAA, ranging from 330.3 to 506.8 μg g^{-1} dry soil in autumn and 289.4–458.2 μg g^{-1} dry soil in winter rice soil. The soil MBC can be considered as a key indicator for determining soil health and nutrient allocations (Naorem et al., 2021). The temporary inhibition in MBC till 14th DAA might be attributed to the adsorption of herbicides on soil organic matter that led to lyses of microbial cells (Meena et al., 2020).

Herbicides break down in soil in several ways, releasing carbon in a simpler form that could be used as a source of nutrients (Pileggi et al., 2020), leading MBC to increase. Herbicides also have a significant impact on microbial molecular factories (MMFs) by disrupting critical functions like respiration, photosynthesis, and biosynthetic reactions, as well as cell development and division and molecular composition (Daisley et al., 2022). When compared to natural, geographical, and temporal fluctuation in soil microbial biomass, herbicide effects are usually short term and minimal. Similar to Kondratowicz (2007), the treatments (T3 and T5) with 25% organic substitutions of nitrogen resulted in significantly higher soil MBC as compared to the sole application of nitrogen. The decrease in MBC could be attributed to the rice grown in a stressed setting with insufficient soil exudates (Rahman et al., 2021). Herbicides had no effect on soil MBC

Table 4 Microbial biomass carbon in soil (µg g^{-1}) during autumn and winter rice as affected by herbicide application in rice-rice sequence

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
T1	387.34c	382.39c	419.42c	399.61c	410.41c	374.51c	367.35c	358.79c	355.19c	375.15c
T2	332.66e	319.49e	313.61e	330.26e	339.76e	299.57e	280.67e	270.57e	289.44e	304.21e
T3	452.59b	442.33b	439.39b	459.41b	461.32b	430.61b	425.29b	405.34b	412.86b	429.34b
T4	365.47d	359.21d	350.47d	368.53d	379.38d	337.62d	328.63d	318.45d	340.38d	322.49d
T5	498.43a	479.58a	478.43a	486.49a	506.79a	468.48a	463.47a	444.25a	449.53a	458.19a

Values within the same column followed by different letters (a, b, c) are significantly different from each another ($P < 0.05$). Three replicates are maintained at each case. *T1* control with one-hand weeding, *T2* butachlor + 2, 4-D with 100% NPK through chemical fertilizer, *T3* butachlor + 2, 4-D with 75% NPK through chemical fertilizer + 25% through organic source, *T4* butachlor + 2, 4-D rotated with pretilachlor with 100% NPK through chemical fertilizer, *T5* butachlor + 2, 4-D rotated with pretilachlor with 75% NPK through chemical fertilizer + 25% through organic source

in the controlled treatment with one hand weeding at 40 DAA.

Enzyme activities

A thorough examination of the data revealed a substantial correlation between weed management strategies and DAA of butachlor and pretilachlor. The effect of butachlor and pretilachlor on soil dehydrogenase activity increased from 0 to 14th DAA, ranging from 123.1 to 156.7 µg TPF g^{-1} dry soil 7 days^{-1} in autumn rice and 126.7–151.1 µg TPF g^{-1} dry soil 7 days^{-1} in winter rice, followed by a declining trend from 30 to 60th DAA ranged from 127.8 to 143.7 µg TPF g^{-1} dry soil 7 days^{-1} in autumn rice and 124.4–130.2 µg TPF g^{-1} dry soil 7 days^{-1} in winter rice (Table 5). Because of the increase in microbial community composition with the potential to utilize herbicides as carbon source, soil dehydrogenase activity in herbicide-treated soil increased from 0 to 14th DAA. The phenomenon indicates the perspectives of soil microbial diversity and enzyme dynamics in relation to development of sustainable agricultural practices (Gupta et al., 2022). Microbial O$_2$ metabolism is aided by dehydrogenase, an intracellular enzyme. Aside from that, the herbicides employed at the indicated rate were not dehydrogenase inhibitors (Mahanta et al., 2017). A similar trend was also exhibited in urease activity ranging from 279.0 to 340.4 µg NH$_4$ g^{-1} soil 2 h^{-1} in autumn and 226.7–296.5 µg NH$_4$ g^{-1} soil 2 h^{-1} in winter rice from 0 to 14th DAA followed by a declining trend that ranged from 278.5 to 336.8 µg NH$_4$ g^{-1} soil 2 h^{-1}

in autumn and 229.6–287.7 µg NH$_4$ g^{-1} soil 2 h^{-1} in winter rice at 30th–60th DAA (Table 5).

Herbicides are degraded by enzymes produced by soil microorganisms (Pileggi et al., 2020), and the metabolites are then utilized as a source of biogenous elements (Singh et al., 2017). Because of its interaction with inorganic and organic soil colloids, native soil urease is predominantly extracellular and is exceptionally persistent (Mandal et al., 2020). An enzymatic fraction located within proliferating and non-proliferating cells, or linked to or stored within cell debris, might be responsible for enhanced activity of an enzyme (Meena & Rao, 2021). Furthermore, when contrasting the treatments (T3 and T5) with a 25% organic nitrogen substitution to the sole application of nitrogen through an inorganic source in autumn and winter rice, the treatments (T3 and T5) with a 25% organic nitrogen substitution resulted in significantly higher enzyme activities.

The enzyme acid phosphatase showed a reverse trend those of enzyme dehydrogenase and urease, and reduction in activity at 0–14th DAA ranged from 242.6 to 269.3 µg p-nitrophenol g^{-1} dry soil h^{-1} in autumn and 188.2–212.2 µg p-nitrophenol g^{-1} dry soil h^{-1} in winter rice at 0–14th DAA followed by an increasing trend that ranges from 259.7 to 280.6 µg p-nitrophenol g^{-1} dry soil h^{-1} in autumn and 206.0–237.6 µg p-nitrophenol g^{-1} dry soil h^{-1} in winter rice at 30th–60th DAA (Table 5). The increase in acid phosphatase activity could be attributable to an increase in phosphate-solubilizing microbial populations in soil as a result of the decomposition of herbicides that may have acted as

Table 5 Activities of dehydrogenase (μg TPF g⁻¹ dry soil⁻¹ 7 days⁻¹), urease (μg NH$_4$ g⁻¹ soil 2 h⁻¹) and acid phosphatase (μg p-nitrophenol g⁻¹ dry soil h⁻¹) in soil during autumn and winter rice as affected by herbicide application in rice-rice sequence

Treatment	Autumn															
	Dehydrogenase enzyme					Urease enzyme					Acid phosphatase enzyme				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
T1	118.3e	123.1e	128.4e	125.8d	121.8d	270.7e	276.77e	288.48d	295.76c	281.55d	288.2a	285.6a	290.4a	287.6a	294.7a
T2	133.7d	138.8d	144.5d	134.7c	127.8c	285.54c	292.62c	268.50e	278.58d	267.59e	255.7e	248.2e	242.6e	259.7e	267.0e
T3	142.7b	149.7b	152.5b	139.7b	135.8b	315.40a	329.39a	340.49a	311.43b	303.46b	265.0c	255.3c	252.3c	268.7c	275.7c
T4	137.5c	144.5c	147.4c	135.7c	128.6c	279.04d	287.64d	294.79c	265.76e	292.43c	258.5d	250.5d	245.6d	262.5d	269.4d
T5	145.7a	154.5a	156.7a	143.7a	139.7a	309.65b	301.62b	317.03b	331.65a	336.82a	269.3b	258.4b	256.7b	272.5b	280.6b

Treatment	Winter															
	Dehydrogenase enzyme					Urease enzyme					Acid phosphatase enzyme				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
T1	108.5e	115.1e	103.8e	106.7d	110.6c	234.55d	243.76d	225.39e	242.76c	251.72e	245.8a	242.3a	249.9a	250.7a	246.8a
T2	126.7d	131.0d	136.0d	124.4c	119.5b	226.70e	255.87c	267.76c	236.71d	247.80d	202.5e	190.5d	188.2d	206.0e	219.7d
T3	136.5b	143.6b	147.2b	134.8b	128.7a	290.63a	281.89b	296.75a	276.69b	287.75a	210.4c	204.5c	202.0c	222.3c	234.4b
T4	129.7c	134.1c	138.7c	125.7c	120.7b	242.79c	230.57e	254.61d	229.61e	265.64c	204.6d	187.7e	189.1d	210.7d	225.5c
T5	140.7a	147.6a	151.1a	138.5a	130.2a	280.83b	289.50a	280.62b	295.83a	281.50b	212.2b	210.4b	205.6b	225.0b	237.6b

Values within the same column followed by different letters (a, b, c) are significantly different from each another ($P < 0.05$). Three replicates are maintained at each case. *T1* control with one hand weeding, *T2* butachlor + 2, 4-D with 100% NPK through chemical fertilizer, *T3* butachlor + 2, 4-D with 75% NPK through chemical fertilizer + 25% through organic source, *T4* butachlor + 2, 4-D rotated with pretilachlor with 100% NPK through chemical fertilizer, *T5* butachlor + 2, 4-D rotated with pretilachlor with 75% NPK through chemical fertilizer + 25% through organic source

a major source of carbon for microbial population growth and vigor. Fertilization with organic manure and nitrogen boosted microbial populations and enzymatic activity in soil (Ali et al., 2021). Herbicides had no effect on enzyme activities throughout the crop growth period in the controlled condition with one hand weeding at 40 DAA.

Conclusion

The current study concludes that using the chloroacetanilide class of herbicides like butachlor and pretilachlor at recommended doses (at 1000 g ha^{-1} and at 750 g ha^{-1}) in the field for a long period of time is an effective weed management measure that is considered a relatively safe and acceptable component for rice cultivation with no negative impact on soil, water, or associated environment. Butachlor and pretilachlor at recommended doses have little negative impact on soil biological properties, causing a transient change in soil microbial dynamics during initial exposure. The deleterious impact of herbicides on soil microbial population dynamics diminishes over time, as evidenced by the microbial population dynamics' subsequent restoration. Thus, in soils, rich in organic matter, herbicides degrade more quickly, indicating higher microbial population dynamics and function.

Author contributions   The idea, formulation and design of the research, experimentation, and plan for research communications were made by Kaberi Mahanta, Jayanta Deka, Dipjyoti Rajkhowa, and Pranaba Nanda Bhattacharyya. Kaberi Mahanta, Rajat Parit, Anshuman Kohli, and Binoy Kumar Medhi devised and carried out the experimentation procedures in laboratory and field conditions. The statistical analysis for data production was carried out by Anjan Kumar Sharma, Pranaba Nanda Bhattacharyya, and Harendra Verma. Kaberi Mahanta, Pranaba Nanda Bhattacharyya, Dipjyoti Rajkhowa, and Didier Lesueur assisted in manuscript preparation, review, and editing. The manuscript was rewritten, revised, and finally compiled and modified by Pranaba Nanda Bhattacharyya, Didier Lesueur, and Kaberi Mahanta. The findings were considered by all authors, and they all contributed to the final publication. The final manuscript has been read and approved by all the authors.

Declarations

Ethics approval   Not applicable.

Consent to participate   Not applicable.

Competing interest   The authors declare no competing interests.

References

Adachi, A., Komura, T., Andon, A., & Okano, T. (2007). Effects of spherosomes on the degradation of pretilachlor and esprocarb in soil. *Journal of Health Sciences, 53*(5), 600–603. https://doi.org/10.1248/jhs.53.600

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M. Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics, 9*(3), 42. https://doi.org/10.3390/toxics9030042

Ali, S., Liu, K., Ahmed, W., Jing, H., Qaswar, M., Kofi Anthonio, C., Maitlo, A. A., Lum, Z., Liu, L., & Zhang, H. (2021). Nitrogen mineralization, soil microbial biomass and extracellular enzyme activities regulated by long-term N fertilizer inputs: A comparison study from upland and paddy soils in a red soil region of China. *Agronomy, 11*(10), 2057. https://doi.org/10.3390/agronomy11102057

Arora, S. D., & Sahni, D. (2016). Pesticides effect on soil microbial ecology and enzyme activity-An overview. *Journal of Applied and Natural Science, 8*(2), 1126–1132.

Baishya, A., Gogoi, B., Hazarika, J., Hazarika, J. P., Bora, A. S., Das, A. K., Borah, M., & Sutradhar, P. (2016). Maximizing soil productivity and profitability through crop intensification and diversification with rice (Oryza sativa)-based cropping systems in acid soils of Assam. *Indian Journal of Agronomy, 61*, 274–280.

Bhattacharyya, P.N., & Sarmah, S.R. (2018). The role of tea soil microflora in tea cultivation. In V. S. Sharma, & K. Gunasekare (Eds.), *Global tea science: Burleigh Dodds Science Publishing* (pp 135–167). Sawston, Cambridge, UK. https://doi.org/10.19103/AS.2017.0036.24

Briceno, G., Lamilla, C., Leiva, B., Levio, M., Donoso-Pinol, P., Schalchli, H., Gallardo, F., & Diez, M. C. (2020). Pesticide-tolerant bacteria isolated from a biopurification system to remove commonly used pesticides to protect water resources. *PLoS One, 15*(6), e0234865. https://doi.org/10.1371/journal.pone.0234865

Cappucino, J.G., & Sherman, N. (2004). Microbiology–A Laboratory Manual 7th edition. Pearson Education, Dorling Kindersley (India) Pvt., Ltd.

Carpio, M. J., Sanchez-Martín, M. J., Rodríguez-Cruz, M. S., & Marín-Benito, J. M. (2021). Effect of organic residues on pesticide behavior in soils: A review of laboratory research. *Environments, 8*(4), 32. https://doi.org/10.3390/environments8040032

Casida Jr, L. E. (1968). Methods for the isolation and estimation of activity of soil bacteria. In T. R. G. Grev, & D. Parkinson (Eds.), *The ecology of soil bacteria* (DD. 97–122). Liverpool University Press, UK.

Chauhan, B. S. (2020). Grand Challenges in Weed Management. *Front. Agron., 1*, 3. https://doi.org/10.3389/fagro.2019.00003

Chen, Q., Wang, C. H., Deng, S. K., Wu, Y. D., Li, Y., Yao, L., Jiang, J. D., Yan, X., He, J., & Li, S. P. (2014). Novel three-component Rieske non-heme iron oxygenase system catalyzing the N-dealkylation of chloroacetanilide herbicides in sphingomonads DC-6 and DC-2. *Applied and Environment Microbiology, 80*, 5078–5085. https://doi.org/10.1128/AEM.00659-14

Cheng, M., Meng, Q., Yang, Y., Chu, C., Chen, Q., Li, Y., Cheng, D., Hong, Q., Yan, X., & He, J. (2017). The two-component monooxygenase MeaXY initiates the downstream pathway of chloroacetanilide herbicide catabolism in sphingomonads. *Applied and Environment Microbiology, 83*(7), e03241-e3316. https://doi.org/10.1128/AEM.03241-16

Daisley, B. A., Chernyshova, A. M., Thompson, G. J., & Allen-Vercoe, E. (2022). Deteriorating microbiomes in agriculture-the unintended effects of pesticides on microbial life. *Microbiome Research Reports, 1*, 6. https://doi.org/10.20517/mrr.2021.08

Dar, M. H., Bano, D. A., Waza, S. A., Zaidi, N. W., Majid, A., Shikari, A. B., Ahangar, M. A., Hossain, M., Kumar, A., & Singh, U. S. (2021). Abiotic stress tolerance-progress and pathways of sustainable rice production. *Sustainability, 13*(4), 2078. https://doi.org/10.3390/su13042078

Dharumarajan, S., Sankar, R., & Arun, S. (2011). Persistence and dissipation of pretilachlor in soil, plant and water of coastal rice ecosystem. *Indian Journal of Weed Science, 43*(3&4), 199–202.

Diao, J., Lv, C., Wan, X., Dang, Z., Zhu, W., & Zhou, Z. (2009). Influence of soil properties on the enantioselective dissipation of the herbicide lactofen in soils. *Journal of Agricultural and Food Chemistry, 57*, 5865–5871. https://doi.org/10.1021/jf9006856

Diao, J., Xu, P., Wang, P., Lu, D., Lu, Y., & Zhou, Z. (2010). Enantioselective degradation in sediment and aquatic toxicity to Daphnia magna of the herbicide lactofen enantiomers. *Journal of Agricultural and Food Chemistry, 58*, 2439–2445.

Domsch, K. H., Gams, W., & Anderson, T. H. (2007). *Compendium of soil fungi* (2nd ed.). IHW-Verlag.

Gogoi, B., Borah, N., Baishya, A., Nath, D. J., Dutta, S., Das, R., Bhattacharyya, D., Sharma, K. K., Valente, D., & Petrosillo, I. (2021). Enhancing soil ecosystem services through sustainable integrated nutrient management in double rice-cropping system of North-East India. *Ecological Indicators, 132*, 108262. https://doi.org/10.1016/j.ecolind.2021.108262

Gunstone, T., Cornelisse, T., Klein, K., Dubey, A., & Donley, N. (2021). Pesticides and soil invertebrates: A hazard assessment. *Frontiers in Environmental Science, 9*, 643847. https://doi.org/10.3389/fenvs.2021.643847

Gupta, A., Gupta, R., & Singh, R. L. (2017). Microbes and environment. In R. Singh (Ed.), *Principles and applications of environmental biotechnology for a sustainable future*. *Applied Environmental Science and Engineering for a Sustainable Future*. Springer, Singapore. https://doi.org/10.1007/978-981-10-1866-4_3

Gupta, A., Singh, U. B., Sahu, P. K., Paul, S., Kumar, A., Malviya, D., Singh, S., Kuppusamy, P., Singh, P., Paul, D., Rai, J. P., Singh, H. V., Manna, M. C., Crusberg, T. C., Kumar, A., & Saxena, A. K. (2022). Linking soil microbial diversity to modern agriculture practices: A review. *International Journal of Environmental Research and Public Health, 19*(5), 3141. https://doi.org/10.3390/ijerph19053141

Hakim, M. A., Juraimi, A. S., Karim, S. M. R., Khan, M. S. I., Islam, M. S., Choudhury, M. K., et al. (2021). Effectiveness of herbicide to control rice weeds in diverse saline environments. *Sustainability, 13*(4), 2053. https://doi.org/10.3390/su13042053

Hamid, M. I., & Ghazanfar, M. U. (2022). Integrated management of rice diseases. In N. Sarwar, Atique-ur-Rehman, S. Ahmad, & M. Hasanuzzaman (Eds.), *Modern techniques of rice crop production*. Springer, Singapore. https://doi.org/10.1007/978-981-16-4955-4_22

Haque, M. A., Rafii, M. Y., Yusoff, M. M., Ali, N. S., Yusuff, O., Datta, D. R., Anisuzzaman, M., & Ikbal, M. F. (2021). Recent advances in rice varietal development for durable resistance to biotic and abiotic stresses through marker-assisted gene pyramiding. *Sustainability, 13*(19), 10806. https://doi.org/10.3390/su131910806

Hassaan, M. A., & Nemr, A. E. (2020). Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. *The Egyptian Journal of Aquatic Research, 46*(3), 207–220. https://doi.org/10.1016/j.ejar.2020.08.007

Holt, J. H. (1994). Bergey's manual of determinative bacteriology, 9th ed. Lippincott, Williams & Wilkins, Philadelphia, PA.

Hossain, K., Timsina, J., Johnson, D. E., Gathala, M. K., & Krupnik, T. J. (2020). Multi-year weed community dynamics and rice yields as influenced by tillage, crop establishment, and weed control: Implications for rice-maize rotations in the eastern Gangetic plains. *Crop Protection, 138*, 105334. https://doi.org/10.1016/j.cropro.2020.105334

Huang, Y., Xiao, L., Li, F., Xiao, M., Lin, D., Long, X., & Wu, Z. (2018). Microbial degradation of pesticide residues and an emphasis on the degradation of cypermethrin and 3-phenoxy benzoic acid: A review. *Molecules, 23*(9), 2313. https://doi.org/10.3390/molecules23092313

James, T. K., Ghanizadeh, H., Harrington, K. C., & Bolan, N. S. (2021). Degradation of atrazine and bromacil in two forestry waste products. *Science Report, 11*(1), 3284. https://doi.org/10.1038/s41598-021-83052-z

Kaur, P., Kaur, P., & Bhullar, M. S. (2015). Persistence behaviour of pretilachlor in puddled paddy fields under subtropical humid climate. *Environmental Monitoring and Assessment, 187*, 524.

Kaur, R., Singh, D., Kumari, A., et al. (2021). Pesticide residues degradation strategies in soil and water: A review. *International Journal of Environmental Science and Technology*. https://doi.org/10.1007/s13762-021-03696-2

Khan, A. R., Chandra, D., Nanda, P., Singh, S. S., Ghorai, A. K., & Singh, S. R. (2004). Integrated nutrient management for sustainable rice production. *Archives of Agronomy and Soil Science, 50*(2), 161–165. https://doi.org/10.1080/03650340310001612988

Kondratowicz, M. K. (2007). Susceptibility of organic matter to oxidation and soil microbiological activity under conditions of varied crop rotation systems and fertilization. *Polish Journal of Soil Science, 40*(1), 89–99.

Liu, T., Wang, P., Lu, Y., Zhou, G., Diao, J., & Zhou, Z. (2012). Enantioselective bioaccumulation of soil-associated fipronil enantiomers in Tubifex tubifex. *Journal of Hazardous Materials, 219–220*, 50–56. https://doi.org/10.1016/j.jhazmat.2012.03.042

Mahanta, K., Dutta, P., Nath, D. J., Deka, J., Barua, I. C., Deka, N. C., & Sarma, A. K. (2017). Long-term application of herbicides on soil microbial demography in rice-rice cropping sequence of North-East India. *Indian Journal of Weed Science, 49*(4), 317–323.

Mandal, A., Sarkar, B., Mandal, S., Vithanage, M., Patra, A. K., & Manna M. C. (2020). Impact of agrochemicals on soil health. In M. N. V. Prasad (Ed.), *Agrochemicals detection, treatment and remediation* (pp. 161–187). Butterworth-Heinemann. https://doi.org/10.1016/B978-0-08-103017-2.00007-6

Mebdoua, S. (2018). Pesticide residues in fruits and vegetables. In J. M. Merillon, & K. Ramawat (Eds.), *Bioactive molecules in food. Phytochemistry.* Cham. https://doi.org/10.1007/978-3-319-54528-8_76-1

Medo, J., Makova, J., Medova, J., et al. (2021). Changes in soil microbial community and activity caused by application of dimethachlor and linuron. *Science Report, 11*, 12786. https://doi.org/10.1038/s41598-021-91755-6

Meena, A., & Rao, K. S. (2021). Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region. *India. Ecological Processes, 10*, 16. https://doi.org/10.1186/s13717-021-00288-3

Meena, R. S., Kumar, S., Datta, R., Lal, R., Vijaya Kumar, V., Brtnicky, M., Sharma, M. P., Yadav, G. S., Jhariya, M. K., et al. (2020). Impact of agrochemicals on soil microbiota and management: A review. *Land, 9*(2), 34. https://doi.org/10.3390/land9020034

Mohanty, S. S., & Jena, H. M. (2019). A systemic assessment of the environmental impacts and remediation strategies for chloroacetanilide herbicides. *Journal of Water Process Engineering, 31*, 100860. https://doi.org/10.1016/j.jwpe.2019.100860

Mortimer, P. E., Jeewon, R., Xu, J. C., Lumyong, S., & Wanasinghe, D. N. (2021). Morpho-phylo taxonomy of novel dothideomycetous fungi associated with dead woody twigs in Yunnan Province. *China. Frontiers in Microbiology, 12*, 654683. https://doi.org/10.3389/fmicb.2021.654683

Murata, T., Kazuhuro, T., Masumi, I., & Kazunari, Y. (2004). Effect of mefenacet and pretilachlor applications on phospholipids fatty acid profiles on soil microbial communities in rice paddy soil. *Soil Science and Plant Nutrition, 50*, 349–356.

Naorem, A., Maverick, J., Singh, P., & Udayana S. P. (2021). Microbial community structure in organic farming and their management. In V. S. Meena, S. K. Meena, A. Rakshit, J. Stanley, & C. Srinivasarao (Eds.), *Advances in organic farming* (pp. 47–58). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-822358-1.00004-3

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Frontiers in Public Health, 4*, 148. https://doi.org/10.3389/fpubh.2016.00148

Panigrahi, K. K., Mohanty, A., Padhan, S. R., & Guru, R. K. S. (2021). Genotoxicity and DNA Damage induced by herbicides and toxins in plants. In Z. Khan, M. Y. K. Ansari, & D. Shahwar (Eds.), *Induced genotoxicity and oxidative stress in plants*. Springer, Singapore. https://doi.org/10.1007/978-981-16-2074-4_2

Panse, V. G., & Sukhatme, P. V. (1967). Statistical methods for agriculture workers. Indian council of Agriculture Research (ICAR), New Delhi, India.

Pileggi, M., Pileggi, A. V., Michael, J., & Sadowsky, M. J. (2020). Herbicide bioremediation: from strains to bacterial communities. *Heliyon, 6*(12), e05767. https://doi.org/10.1016/j.heliyon.2020.e05767

Pose-Juan, E., Igual, J. M., Sanchez-Martín, M. J., & Rodriguez-Cruz, M. S. (2017). Influence of herbicide triasulfuron on soil microbial community in an unamended soil and a soil amended with organic residues. *Frontiers in Microbiology, 8*, 378. https://doi.org/10.3389/fmicb.2017.00378

Raffa, C. M., & Chiampo, F. (2021). Bioremediation of agricultural soils polluted with pesticides: A review. *Bioengineering (basel, Switzerland), 8*(7), 92. https://doi.org/10.3390/bioengineering8070092

Rahman Abdul, N. S. N., Abdul Hamid, N. W., & Nadarajah, K. (2021). Effects of abiotic stress on soil microbiome. *International Journal of Molecular Science, 22*(16), 9036. https://doi.org/10.3390/ijms22169036

Rao, P. C., Rama Lakshmi, C. H. S., Madhavi, M., Swapna, G., & Sireesha, A. (2012). Butachlor dissipation in rice grown soil and its residues in grain. *Indian Journal of Weed Science, 44*(2), 84–87.

Sarmah, S. R., Bhattacharyya, P. N., & Barooah, A. K. (2020). Microbial biocides-prominent alternatives of chemicals in tea disease management. *Journal of Biological Control, 34*, 144–152. https://doi.org/10.18311/jbc/2020/22689

Shilpakar, O., Karki, B., & Rajbhandari, B. (2020). Pretilachlor poisoning: A rare case of a herbicide masquerading as organophosphate toxicity. *Clinical Case Reports, 8*(12), 3507–3509. https://doi.org/10.1002/ccr3.3473

Singh, R., Kumar, M., Mittal, A., & Mehta, P. K. (2017). Microbial metabolites in nutrition, healthcare and agriculture. *3 Biotech,* 7(1), 15. https://doi.org/10.1007/s13205-016-0586-4

Singh, V., & Jat., M. L., Ganie, Z. A., Chauhan, B. S., & Gupta, R. K. (2016). Herbicide options for effective weed management in dry direct-seeded rice under scented rice-wheat rotation of western Indo-Gangetic Plains. *Crop Protection, 81*, 168–176. https://doi.org/10.1016/j.cropro.2015.12.021

Streletskii, R., Astaykina, A., Krasnov, G., & Gorbatov, V. (2022). Changes in bacterial and fungal community of soil under treatment of pesticides. *Agronomy, 12*(1), 124. https://doi.org/10.3390/agronomy12010124

Tabatabai, M. A., & Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry, 1*, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Tabatabai, M. A., & Bremner, J. M. (1972). Assay of urease activity in soils. *Soil Biology and Biochemistry, 4*, 479–548. https://doi.org/10.1016/0038-0717(72)90064-8

Thiour-Mauprivez, C., Martin-Laurent, F., Calvayrac, C., & Barthelmebs, L. (2019). Effects of herbicide on non-target microorganisms: Towards a new class of biomarkers? *Science of the Total Environment, 684*, 314–325. https://doi.org/10.1016/j.scitotenv.2019.05.230

Torabi, E., Wiegert, C., Guyot, B., Vuilleumier, S., & Imfeld, G. (2020). Dissipation of S-metolachlor and butachlor in agricultural soils and responses of bacterial communities: Insights from compound-specific isotope and biomolecular analyses. *Journal of Environmental Sciences, 92*, 163–175. https://doi.org/10.1016/j.jes.2020.02.009

Tripthi, D. K., Varma, R. K., Singh, S., et al. (2020). Silicon tackles butachlor toxicity in rice seedlings by regulating anatomical characteristics, ascorbate-glutathione cycle, proline metabolism and levels of nutrients. *Science Reports, 10*, 14078. https://doi.org/10.1038/s41598-020-65124-8

Tudi, M., Daniel, R. H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2020). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health, 18*(3), 1112. https://doi.org/10.3390/ijerph18031112

Van Bruggen, A. H. C., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., & Morris, J. G., Jr. (2018). Environmental and health effects of the herbicide glyphosate. *Science of the Total Environment, 616–617*, 255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Wang, H., Wang, H., Shao, H., & Tang, X. (2016). Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. *Frontiers in Plant Sciences, 7*, 67. https://doi.org/10.3389/fpls.2016.00067

Witt, C., Gaunt, J., Galicia, C., et al. (2000). A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. *Biology and Fertility of Soils, 30*, 510–519. https://doi.org/10.1007/s003740050030

Yadav, G. S., Lal, R., Meena, R. S., Babu, S., Das, A., Bhowmik, S. N., Datta, M., Layak, J., & Saha, P. (2019). Conservation tillage and nutrient management effects on productivity and soil carbon sequestration under double cropping of rice in north eastern region of India. *Ecological Indicators, 105*, 303–315.