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Differential herbicide persistence and shifts in soil bacterial communities in Alfisol and Inceptisol

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ABSTRACT

Weeds significantly reduce crop yields and promote herbicide use, accounting for 80% of agricultural pesticides. However, herbicide persistence and toxicity adversely affect soil microbial communities, impacting soil health and productivity. This study compared the effects of organic (Vinegar-weed-care: acetic acid) and chemical herbicides (PrimextraGold: Atrazine + S-metolachlor; Imazapyr: Isopropyl amine) on soil bacteria in Alfisol and Inceptisol from Ibadan, Nigeria. Soils were analysed for physical properties and microbial DNA, and herbicide degradation was tracked using GC-MS at 0, 4, 8, and 12 weeks. Alfisol exhibited higher fertility with pH 6.2, organic carbon 3.9 g kg^{-1}, nitrogen 0.7 g kg^{-1}, and phosphorus 25.9 mg kg^{-1}, compared to Inceptisol (pH 5.5, organic carbon 1.9 g kg^{-1}, nitrogen 0.6 g kg^{-1}, phosphorus 20.8 mg kg^{-1}). Herbicide persistence was greater in Alfisol: metolachlor (84.94%) and Imazapyr (61.00%) vs. Inceptisol (52.55% and 50.15%, respectively). Organic herbicide metabolites also persisted more in Alfisol (35.13%) than in Inceptisol (28.00%). In non-sterile Alfisol, biodegradation of PrimextraGold and Imazapyr was lower (43.46% and 10.30%) than in sterile soils (53.97% and 16.17%), while the organic herbicide biodegraded more in non-sterile (23.49%) than in sterile (17.08%). In non-sterile Inceptisol, Imazapyr degraded less (8.94%–31.72%) than in sterile (29.49%–34.75%), but atrazine degraded more in non-sterile (61.96%–68.17%). Organic acetamide degraded better in non-sterile Inceptisol (23.42%–90.5%) than in sterile (12.47%–30.7%). Chemical herbicides reduced *Candidatus Udaeobacter*, *Pedosphaera*, and *Chthoniobacter* in Alfisol, while organic herbicides enhanced them in both soils. These findings highlight the ecological benefits of soil-friendly organic herbicides.

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1. INTRODUCTION

Agricultural production is known globally to be adversely affected by the presence of weeds (Kubiak et al., 2022; MacLaren et al., 2020). Weeds are widely distributed on agricultural lands, where they compete with crops for nutrients, water, and light, leading to substantial crop losses each year (Scavo & Mauromicale, 2020). The competition between weeds and crops within the soil ecosystem reduces the overall nutrient availability of the crop, thus limiting its growth and productivity. Scarrow (2021) highlighted that the aggressive nature of weeds allows them to dominate crops, resulting in lower crop yields and threatening food security in heavily infested agricultural zones. Furthermore, many farmers struggle to control weeds early in the growing season. This leads to significant reductions in crop yield at harvest, with global economic losses due to uncontrolled weed infestations in major crops estimated at over $33 billion annually (Abouziena & Haggag, 2016).

The complexity of weed seed banks, defined as the accumulation of viable weed seeds in the soil, presents a significant challenge for weed management. These seeds persist annually and can germinate even without the addition of new seeds, making control efforts more difficult (Gioria et

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al., 2021). This resilience, coupled with their tolerance to pests and diseases, gives weeds a strong competitive advantage over crops, ultimately leading to reduced yields and significant economic losses for farmers.

To combat weeds, farmers primarily use herbicides, accounting for approximately 80% of global agricultural pesticide use (FAO, 2022). However, herbicides largely remain in the soil, affecting microbial communities vital for soil health (Schreiber et al., 2018). Accumulated herbicide residues can negatively impact soil structure and microbial diversity. Many studies have assessed the impact of pesticides on soil microbial communities, suggesting that this impact may depend on various factors such as toxicity, persistence, and fate in the soil (Wang et al., 2024). These factors determine how herbicides persist in the soil, their biodegradation rates, and their ability to disrupt microbial functions, including nutrient cycling and organic matter decomposition. The fate of herbicides in the soil is influenced by their chemical structure, adsorption-desorption processes, and interactions with microbial communities (Michael et al., 2024; Singh & Singh, 2016). Microbes are vital for herbicide degradation, primarily through conjugative reactions that convert herbicides into less harmful byproducts (Kaur et al., 2023). Additionally, abiotic degradation, such as photodegradation and chemical hydrolysis, also contributes, depending on factors like sunlight, temperature, and soil moisture (Meng et al., 2022). The rate of herbicide degradation is affected by environmental conditions, molecular structure, and soil characteristics, including pH and organic matter content (Babal et al., 2022).

Soil organic matter (SOM) and clay fraction are crucial in retaining herbicides by adsorbing them to their surface, which reduces mobility and leaching into groundwater (Bonfleur et al., 2016). This retention can lead to the formation of persistent metabolites that linger in the soil (Takeshita et al., 2019). Organic amendments, like compost or manure, enhance herbicide persistence, which is the ability of the soil to hold herbicide molecules on its particles, which slows down herbicide movement and breakdown (Glaspie et al., 2021; Tejada & Benítez, 2017).

The interaction between SOM and herbicides is complex, as the effectiveness of herbicide adsorption depends on the origin of the organic matter, soil pH, climatic conditions, and the composition of soil microbial communities (Sebastian et al., 2016). Furthermore, soil clay particles also play a role in herbicide sorption due to their negative charge, which attracts and binds positively charged herbicide molecules. The combined effect of SOM and clay particles helps immobilize herbicides in the soil, making them less available for plant uptake but also slowing their degradation (Glaspie et al., 2021).

The persistent use of chemical herbicides in agriculture has been well-documented for its detrimental effects on soil microbial communities, including significant reductions in microbial diversity and functional alterations that can lead to long-term soil degradation (Michael et al., 2024; Sim et al., 2022). While organic herbicides are increasingly considered as alternatives due to their environmental safety and shorter half-life in soils (Saini & Singh, 2019), limited studies have investigated their persistence and biodegradation rates in tropical soils, such as Alfisol and Inceptisol. This study addresses the limited knowledge of how organic herbicides affect soil bacterial communities, particularly in Alfisol and Inceptisol, which differ in their physicochemical properties. While chemical herbicides are known for their persistence and harmful effects on soil microbes, the behaviour and biodegradation of organic herbicides remain poorly understood. By comparing the persistence and microbial impact of organic versus chemical herbicides in these two soil types, the research offers new insight into the environmental suitability of organic herbicides for sustainable weed management.

2. MATERIALS AND METHODS

2.1. Materials

Three types of herbicides were used in this study, consisting of two commercial chemical herbicides, namely, PrimextraGold (with active ingredients Atrazine + S-metolachlor), produced by Syngenta, and Imazapyr (with active ingredient Isopropyl amine), produced by Badische Anilin- und Soda-Fabrik (BASF), Rainbow Chemical, CYNDA, Dow AgroSciences LLC (a subsidiary of DowDuPont), and Syngenta AG, and one organic herbicide (Vinegar (with Acetic acid) produced by EcoClean Solutions. Greater emphasis on the environmental impact of chemical herbicides has driven global interest in less hazardous, biodegradable alternatives like horticultural vinegar. Approved by the Organic Materials Review Institute, herbicides based on vinegar are considered suitable for organic farming and hence merit application in this study.

2.2. Soil Sample Collection and Experimental Design

Soil samples were randomly collected from Alfisol and Inceptisol at a depth of 0–15 cm from 10 points across six locations using a random composite sampling approach, where sampling points were evenly scattered across each field without a fixed pattern, to capture spatial variability within each site. These included the University of Ibadan (Latitude 7.4448° N, Longitude 3.8994° E), Institute of Agricultural Research and Training, Obafemi Awolowo University at Moore Plantation (Latitude 7.3778° N, Longitude 3.8731° E), National Horticultural Research Institute (Latitude 7.3764° N, Longitude 3.9116° E), and the International Institute of Tropical Agriculture (IITA) (Latitude 7.4975° N, Longitude 3.8966°E). These were previously classified as Alfisol and Inceptisol soil locations. The samples were bulked to create composite samples used for the screenhouse study. Portions of each sample were sterilized according to the method described by Pose-Juan et al. (2017) to serve as control treatments in a microcosm experiment assessing herbicide degradation. Ten kilogrammes of sterile and non-sterile soils were placed into perforated 10-litre pots in a 2×2×3 factorial arrangement, set in a completely randomized design (CRD) with three replications. The herbicides were applied based on their recommended time of application, thus, post-emergence for Imazapyr and Vinegar, and pre-emergence for PrimextraGold on non-sterilized soils, while all treatments were applied to sterilized soils, excluding the controls.

Following herbicide application, bacterial DNA was extracted using the E.Z.N.A.® Soil DNA Kit Omega Bio-tek, Inc.; Norcross, Georgia; USA following the manufacturer's protocol, excluding bead beating, with a 70 µL elution buffer. Extracted DNA was quantified using a Nanodrop spectrophotometer and Qubit™ dsDNA BR Assay Kit Thermo Fisher Scientific, Inc.; Waltham, MA; United States, then stored at – 20 °C. Libraries prepared from amplified DNA samples were subsequently sequenced at the Integrated Genomics Facility of Kansas State University. Sequence data were analysed using QIIME 2 (v.2019.7). A one-way Analysis of Variance (ANOVA) was conducted to evaluate the effect of herbicide treatments on the relative abundance of each genus within the selected bacterial phyla, Verrucomicrobium and Proteobacteria. To further explore pairwise differences among treatments, a Tukey's Honest Significant Difference (HSD) post hoc test was performed. Each genus was analysed independently using the following structure: Dependent Variable: Relative abundance (%) and Independent Variable: Treatment (categorical with 6–8 levels).

2.3. Field Layout

The field study was conducted from September to November 2019 across four agricultural research farms in Ibadan, southwestern Nigeria: the University of Ibadan, IAR&T (Moore Plantation), NIHORT, and IITA. A randomized complete block design (RCBD) with factorial arrangements was used, with three replications across 144 plots. Factors included soil types and herbicide treatments. PrimextraGold was applied pre-emergence at 2.64 kg ai Atrazine + S-metolachlor ha^{-1} (0.0380 kg/144 m²), Imazapyr was applied post-emergence at 0.3 kg ai Isopropyl amine ha^{-1} (0.0043 kg/144 m²), according to the manufacturer's instructions, and vinegar herbicide was also applied post-emergence at 1.26 kg ai acetic acid per ha^{-1} (0.0181 kg/144 m²) according to the modified version of Webber III et al. (2018). Herbicides were applied uniformly in the morning.

2.4. Physical and Chemical Soil Analyses

Soil samples were collected at a depth of 15 cm at 0, 4, and 8 weeks (and 12 weeks in biodegradation assessment) and analysed for physical and chemical properties. Soil particle size distribution was determined using the hydrometer method (Bouyoucos, 1962). Organic carbon was assessed via the potassium dichromate method (Walkley & Black, 1934), while pH was measured using a pH meter (Thomas, 1996). Available phosphorus was determined using the Bray 1 method (Murphy & Riley, 1962). Exchangeable bases (K^+, Na^+, Ca^{2+}, Mg^{2+}) were measured following extraction with 1N ammonium acetate (Okalebo et al., 2002), using a flame photometer for K^+ and Na^+, and atomic absorption spectrophotometry for Ca^{2+} and Mg^{2+}. Exchangeable acidity was determined via 1N KCl extraction (McLean, 1965). Heavy metals (Zn^{2+}, Cu^{2+}, Mn^{2+}, Fe^{3+}) were measured using ICP-OES. Total nitrogen was assessed using the Kjeldahl method (Bremner & Mulvaney, 1982).

2.5. Soil Extraction and GC-MS Analysis

Soil samples were extracted using the modified methanol-chloroform-water method (1:1:0.9) (Axelsson & Gentili, 2014) and analysed via GC-MS (Shimadzu GCMS-QP 2010SE, Shimadzu Corporation, Kyoto, Japan). The GC column used was a SH-Rxi-5MS (30 m × 0.25 mm × 0.25 µm), a non-polar column, which is ideal for separating volatile and semi-volatile organic compounds. The carrier gas used in the GC-MS analysis was helium at a flow rate of 1.2 mL min^{-1}, ensuring optimal separation of compounds. Samples were collected at 0, 4, 8, and 12 weeks. GC conditions included an oven temperature of 140°C (held for 1 min), then increased to 280°C at 4°C increments every 5 minutes.

Compound identification was performed based on ≥90% similarity with reference spectra from the NIST 14 Mass Spectral Library (National Institute of Standards and Technology, USA) for known components of Primextra Gold, Imazapyr, and vinegar-based herbicides. For validation, the identities of selected compounds were confirmed using pure analytical standards purchased from Sigma-Aldrich (USA), ensuring accurate identification and quantification.

2.6. Persistence and Biodegradation Rate of Herbicide Metabolites

The persistence of herbicide metabolites in Alfisol and Inceptisol was determined by the percentage peak area of metabolites. The biodegradation rate was evaluated using semiquantitative kinetics analysis (Co-Ct/Co × 100) following the modified version of Momoh et al. (2021).

3. RESULTS

3.1. Physical and Chemical Characteristics of Herbicide-treated and Non-treated Soil Samples from Alfisol and Inceptisol

Table 1 and 2 summarize the physical and chemical properties of herbicide-treated and non-treated soil samples from Alfisol and Inceptisol. Non-treated Alfisol had a slightly acidic pH of 6.2, while Inceptisol had a moderately acidic pH of 5.5. Organic carbon content was higher in Alfisol (3.9 g kg^{-1}) than in Inceptisol (1.9 g kg^{-1}). Total nitrogen and available phosphorus were significantly greater in Alfisol (0.7 g kg^{-1} and 25.9 mg kg^{-1}) compared to Inceptisol (0.6 g kg^{-1} and 20.8 mg kg^{-1}, respectively). Potassium was also higher in Alfisol (0.5 Cmol kg^{-1}) than in Inceptisol (0.3 Cmol kg^{-1}, $p < 0.05$). Concerning clay particles in Alfisol, 112.2 g kg^{-1} was observed, which was significantly higher than 103.9 g kg^{-1} found in Inceptisol. Calcium levels were lower (1.7 Cmol kg^{-1} in Alfisol vs. 4.8 Cmol kg^{-1} in Inceptisol, $p > 0.05$). Herbicide treatments did not significantly influence any variables. Soil pH and total nitrogen levels were significantly higher at week 8 compared to weeks 0 and 4, while available phosphorus, calcium, and potassium levels were significantly higher at week 0.

Table 1. Chemical properties of Alfisols and Inceptisols before and after herbicide application

Treatments	pH (H_2O) 1:1	OC	N	Bray P	Ca	Mg	K	Na	Zn	Cu	Mn	Fe
		g kg^{-1}	mg kg^{-1}	mg kg^{-1}	Cmol kg^{-1}	Cmol kg^{-1}	Cmol kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Soil type												
Alfisol	6.2a	3.9a	0.7a	25.9	17b	0.8	0.5a	0.1	150.4a	100.2	0.7b	3.6b
Inceptisol	5.5b	1.9b	0.6b	20.8	48a	0.6	0.3b	0.1	2.6b	98.5	175.2a	5.3a
				ns		Ns		ns		ns		
Week												
0	5.7b	2.7	0.6b	40.4a	42a	0.9	0.6a	0.1	76.6	103.2	70.1	4.5
4	5.8b	2.9	0.6b	17.2b	25b	0.6	0.4b	0.1	77.9	99.1	128.1	4.9
8	6.1a	3.1	0.8a	12.3b	29b	0.6	0.3b	0.1	74.9	95.8	65.5	3.9
		ns				ns		ns	ns	ns	ns	ns
Herbicides												
Control	6.1	2.8	0.6	19.7	32	0.6	0.4	0.1	74.6	97.7	68.3	4.7
IM	5.8	3.2	0.7	24.2	31	0.7	0.5	0.1	79.7	100.8	71.7	4.7
ORGH	5.8	2.8	0.6	16.6	30	0.6	0.4	0.1	76.5	98.0	142.7	4.2
PMG	5.8	2.8	0.7	32.8	36	0.9	0.5	0.1	75.1	101.0	69.0	4.1
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*W	*	*	*	ns	*	ns	ns	ns	ns	ns	ns	ns
S*H	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
W*H	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*W*H	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Notes: Means with the same letter (s) in a column are not significantly different at a 5 % level of probability by Duncan Multiple Range Test (DMRT),: significant at P=0.05. ns= not significant IM = Imazapyr, ORG.H = Organic (Vinegar) herbicide, PMG= PrimextraGold; S * W = interaction between soil and weeks, S * H = interaction between Soil and Herbicides, W * H = interaction between Weeks and Herbicides, S * W * H = interaction between Soil, Weeks and Herbicides.

Table 2. Physical properties of Alfisol and Inceptisol before and after herbicide application

Treatments	Sand (g kg^{-1})	Clay (g kg^{-1})	Silt (g kg^{-1})
Soil type			
Alfisol	822.2	112.2a	65.6
Inceptisol	828.9	103.9b	67.5
	ns		ns
Weeks			
0	845.0a	94.2b	60.8b
4	839.2a	102.5b	58.8b
8	792.5b	127.5a	80.0a
Herbicides			
Control	823.3	104.4	72.2
IM	825.6	107.8	66.7
ORGHs	828.9	108.9	62.2
PMG	82.44	111.1	65.0
	ns	ns	ns
S*W	*	*	*
S*H	ns	ns	ns
W*H	ns	ns	ns
S*W*H	ns	ns	ns

Notes: Means with the same letter (s) in a column are not significantly different at a 5 % level of probability by the Duncan Multiple Range Test (DMRT), ns: not significant*: significant at P=0.05. IM = Imazapyr, ORG.H = Organic (Vinegar) herbicide, PMG= PrimextraGold; S * W = interaction between soil and weeks, S * H = interaction between Soil and Herbicides, W * H = interaction between Weeks and Herbicides, S * W * H = interaction between Soil, Weeks and Herbicides *Note: Data represent mean values obtained from a factorial experiment involving soil types, herbicide treatments, and observation periods. Means are presented as combined effects unless otherwise specified. Each value reflects the interaction of soil type with other factors as analysed in the factorial ANOVA. For details on specific soil type responses, refer to the interaction effects in the results text.*

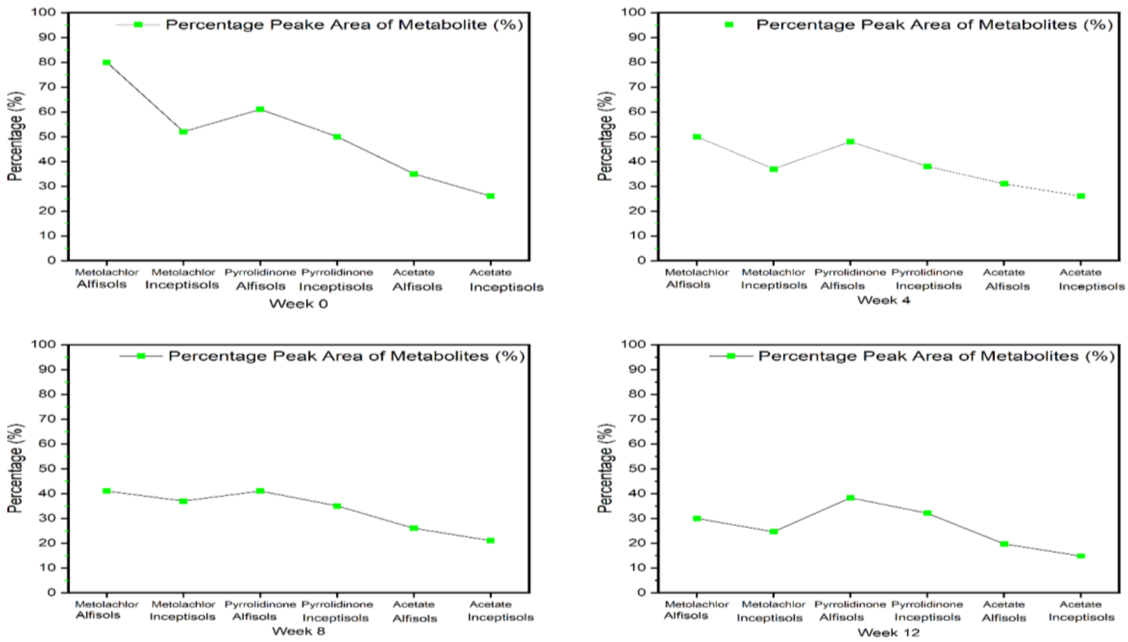

Figure 1. Effect of non-sterile Alfisols and Inceptisols on herbicide metabolites persistence at 0-12 weeks after herbicide application

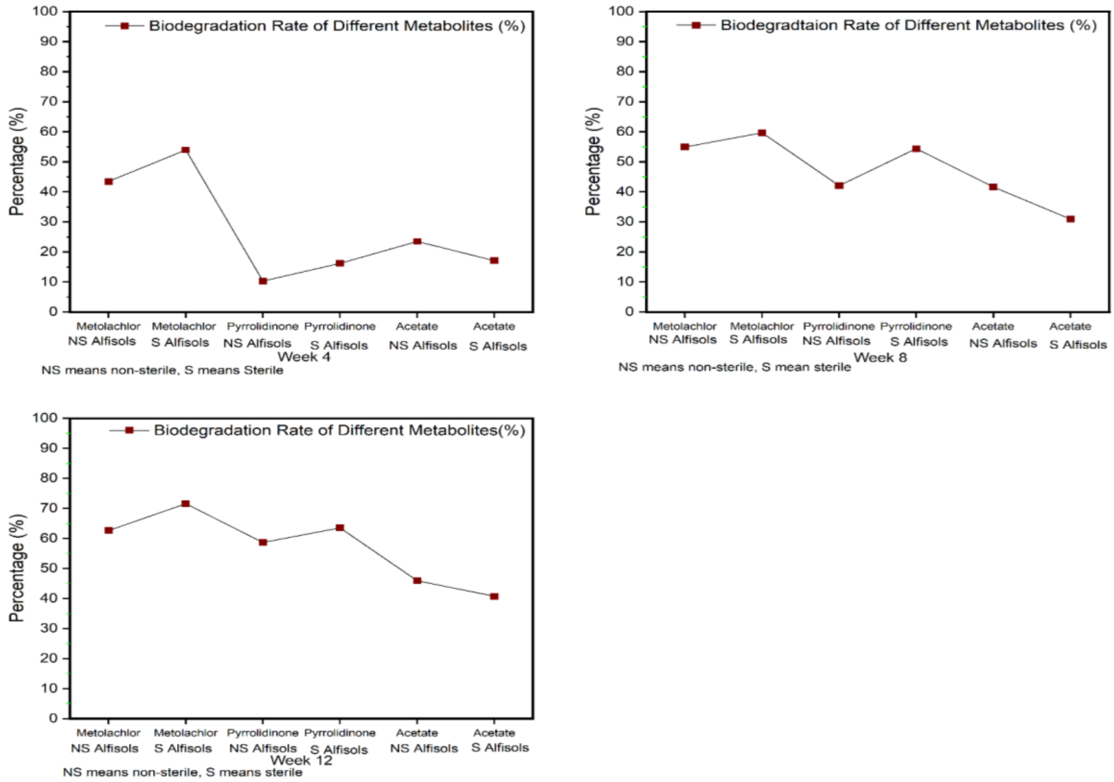

Figure 2. Biodegradation level of herbicide metabolites in Alfisol at 4-12 weeks after herbicide application

3.2 Persistence of Herbicide Metabolites in Alfisol and Inceptisol

At the onset of the study, herbicide application showed notable differences in metabolite persistence between soil types (Fig. 1). The active ingredients Atrazine and S-metolachlor (AS-M) from Primextragold demonstrated higher persistence of metolachlor in Alfisol (84.94% peak area) compared to Inceptisol (52.55%). Similarly, the metabolite 2-Amino-4,5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one from Imazapyr (IA) had greater persistence in Alfisol (61.00% peak area) compared to Inceptisol (50.15%). Acetic acid (AA) also showed higher persistence for Acetamide in Alfisol (35.13%) than in Inceptisol (28.00%). At 4 weeks, metabolites like 2-Amino-3-carboxymethyl-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one from IA and metolachlor from AS-M were more persistent in Alfisol (48.71% and 50.97%, respectively) than in Inceptisol (35.31% and 37.05%). Similarly, AA metabolites persisted more in Alfisol (30.18%) than in Inceptisol (25.00%).

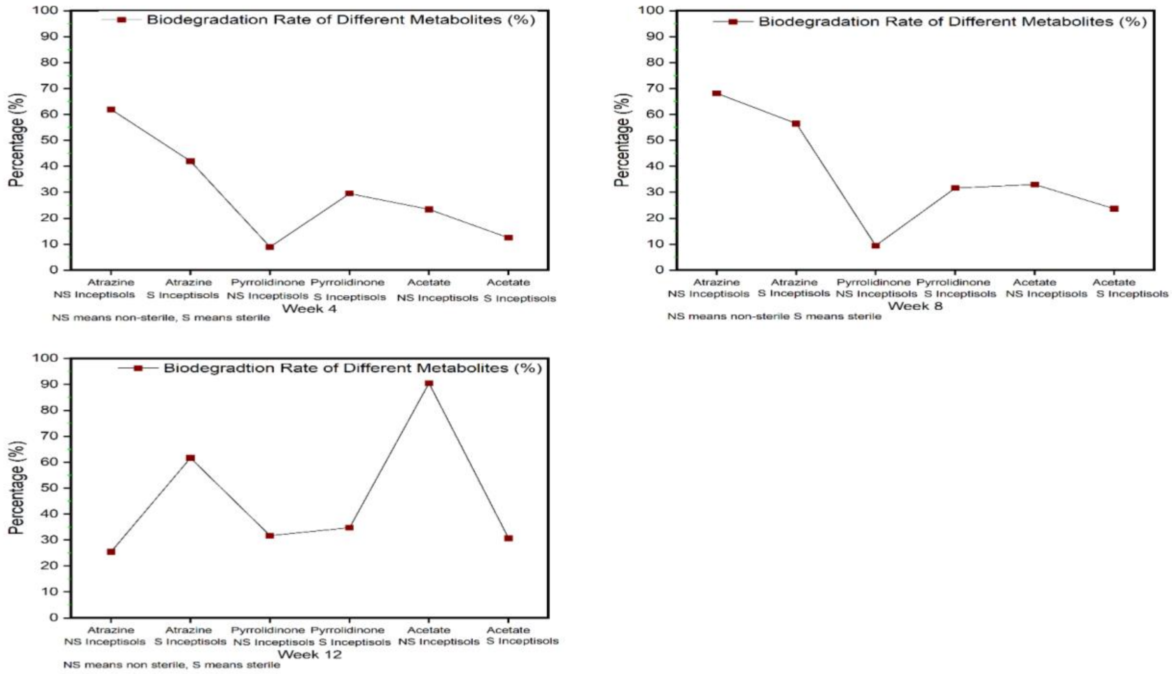

Figure 3. Biodegradation level of herbicide metabolites in Inceptisol at 4-12 weeks after herbicide application

At 8 weeks, IA and AS-M metabolites continued to show higher persistence in Alfisol (42.55% and 43.85%, respectively) compared to Inceptisol (33.20% and 35.09%), while AA metabolites remained lower in both soils (22.08% in Alfisol and 19.20% in Inceptisol). By week 12, persistence patterns were consistent, with IA, AS-M, and AA metabolites showing higher peak areas in Alfisol (38.35%, 31.75%, and 18.11%, respectively) compared to Inceptisol (30.75%, 24.29%, and 15.20%). These findings suggest that Alfisol is more prone to metabolite accumulation and toxicity than Inceptisol across different herbicide treatments.

3.3. Biodegradation level of metabolites in Alfisol at 4, 8, and 12 weeks after herbicide application

Figure 2 shows that metabolite biodegradation levels from herbicides Imazapyr (IA) and Primextragold (AS-M) applications were lower in non-sterile Alfisol than in sterile. The IA-derived metabolite, 2-Amino-3-carboxymethyl-4,5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one, had a biodegradation level of 10.30% in non-sterile compared to 16.17% in sterile Alfisol. Similarly, metolachlor from AS-M showed 43.46% in non-sterile compared to 53.97% in sterile. Conversely, acetamide from the organic herbicide (AA) had higher biodegradation in non-sterile Alfisol (23.49%) than in sterile soil (17.08%). These patterns persisted at weeks 4, 8, and 12, with organic herbicide metabolites consistently showing higher biodegradation.

3.4. Biodegradation level of metabolites in Inceptisol at 4, 8, and 12 weeks after herbicide application

Figure 3 highlights variations in metabolite biodegradation from IA, AS-M, and AA applications in both sterile and non-sterile Inceptisol. The metabolite 2-Amino-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-1 formed after IA application at Week 4 showed low biodegradation in non-sterile (8.94%) than in sterile Inceptisol (29.49%). This trend persisted at Week 8 (9.46% vs. 31.62%) and Week 12 (31.72% vs. 34.75%). Atrazine from AS-M showed higher biodegradation in non-sterile (61.96%) than in sterile Inceptisol (41.98%) at Week 4, continuing at Week 8 (68.17% vs. 56.49%). However, at Week 12, biodegradation of Atrazine in non-sterile Inceptisol decreased to 25.46%, lower than the earlier weeks, whereas sterile Inceptisol showed a similar trend with 27.14% biodegradation.

At Week 4, the metabolite Acetamide, resulting from the application of the AA, demonstrated a higher biodegradation level in non-sterile Inceptisol (23.42%) compared to sterile Inceptisol (12.47%). Non-sterile Inceptisol continued to show greater biodegradation of metabolite Acetamide at 32.98% versus 23.63% in sterile Inceptisol at Week 8. For Week 12, at this stage, biodegradation of Acetamide in non-sterile Inceptisol rose substantially to 90.5%, while in sterile Inceptisol, it was lower at 30.7%.

3.5. Relative Abundance of Different Genera in the Soil Types as Influenced by the Herbicides over Time

As indicated in the visualization labels (Fig. 4, 6, and 7), the stacked bar charts represent only the top dominant bacterial genera, which constitute approximately 8-15% of the total bacterial community across all treatments. The bacterial community composition visualization (Fig. 5A) explicitly addresses this issue by showing what percentage of the total community is represented in the analysis (green bars) versus what remains unanalyzed (orange bars). This visualization confirms that bacterial genera within the phylum Verrucomicrobiota represent approximately 9.4% of the total bacterial community, while those within Proteobacteria represent about 11.7% of the total community.

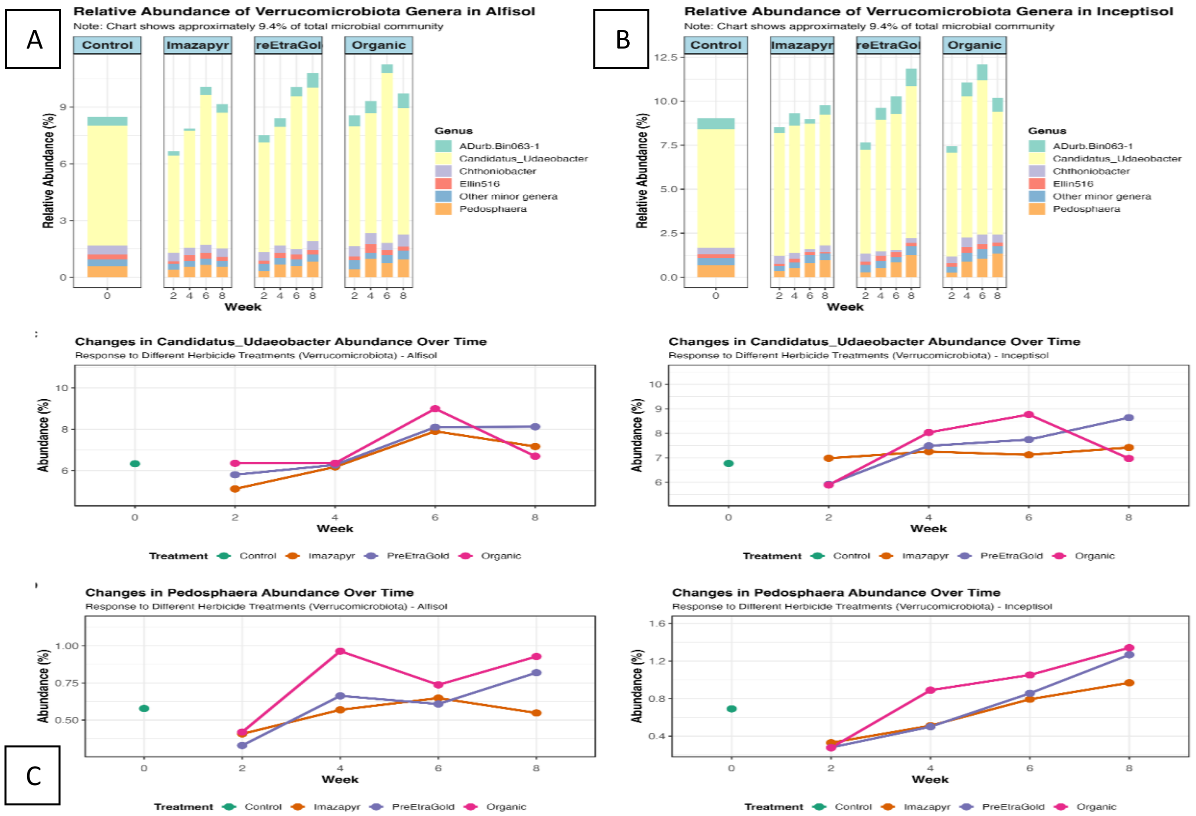

Figure 4. Effects of herbicide treatments on microbial community composition
ALF = Alfisol, INC = Inceptisol, IA = Isopropyl amine or Imazapyr, AS-M=Atrazine + S metolachlor or PrimextraGold, AA=Acetic acid or organic herbicide and Control (CNT)

The remaining ~80-85% comprises hundreds of low-abundance genera, each representing less than 0.1% of the total community, which are not individually displayed for visual clarity.

3.6. Effects of Different Herbicides on Specific Microbial Genera

The ANOVA results revealed significant effects of herbicide treatments on microbial community composition, particularly for the bacterial genus *Candidatus Udaeobacter*, the most dominant genus (Fig. 4). The Tukey's posthoc tests indicate that both PrimextraGold and Organic herbicide treatments resulted in similar increases in *Candidatus Udaeobacter* abundance compared to the control (differences of 0.71 and 0.71 percentage points, respectively, while Imazapyr had a less pronounced effect (0.34 percentage points)). This pattern is visually evident in the genus-specific response plots (Fig. 5B), where it was observed that bacterial genus *Candidatus Udaeobacter* abundance increased progressively across weeks under all treatments, but most prominently under the organic treatment in both Alfisol and Inceptisol soils. By week 6, organic treatment resulted in the highest abundance of this genus in both soil types.

3.7. Sensitivity of Microbial Genera to Chemical Herbicides

The sensitivity analysis identified several genera that were particularly vulnerable to chemical herbicides. The most sensitive taxa included members of the *Verrucomicrobiota* phylum such as the bacterial genus *Pedosphaera,* which showed reductions of up to 59.9% under certain treatments (Fig. 4C). Other highly sensitive bacterial genera included *LD29* and *Opitutus,* which declined by more than 55% under Imazapyr treatment. Importantly, the line graphs tracking bacterial genus *Pedosphaera* abundance over time (Fig. 4C) demonstrate that while chemical herbicides (Imazapyr and PrimextraGold) caused substantial initial declines in abundance, the Organic herbicide maintained or even enhanced abundance, particularly in Inceptisol soil. This pattern suggests that Organic herbicide has a less detrimental impact on sensitive microbial genera.

3.8. Soil Type-Specific Differences in Microbial Response

Significant soil type-specific differences were observed in microbial responses to herbicide treatments. In Figure 6A and 6B, which are the comparisons between Alfisol and Inceptisol, it was observed that the bacterial genus *Candidatus Udaeobacter* generally maintained higher abundances in Inceptisol (Fig. 6B) than in Alfisol (Fig. 6A) across all treatments and time points. For instance, under Organic herbicide at week 6, abundance reached approximately 9% in Inceptisol compared to about 8% in Alfisol. Similarly, the bacterial genus *Pedosphaera* showed greater sensitivity to chemical herbicides in Inceptisol than in Alfisol, with sharper initial declines followed by stronger recovery under organic herbicide in Inceptisol (Fig. 4C). This suggests that soil properties influence how microbial communities respond to herbicide applications.

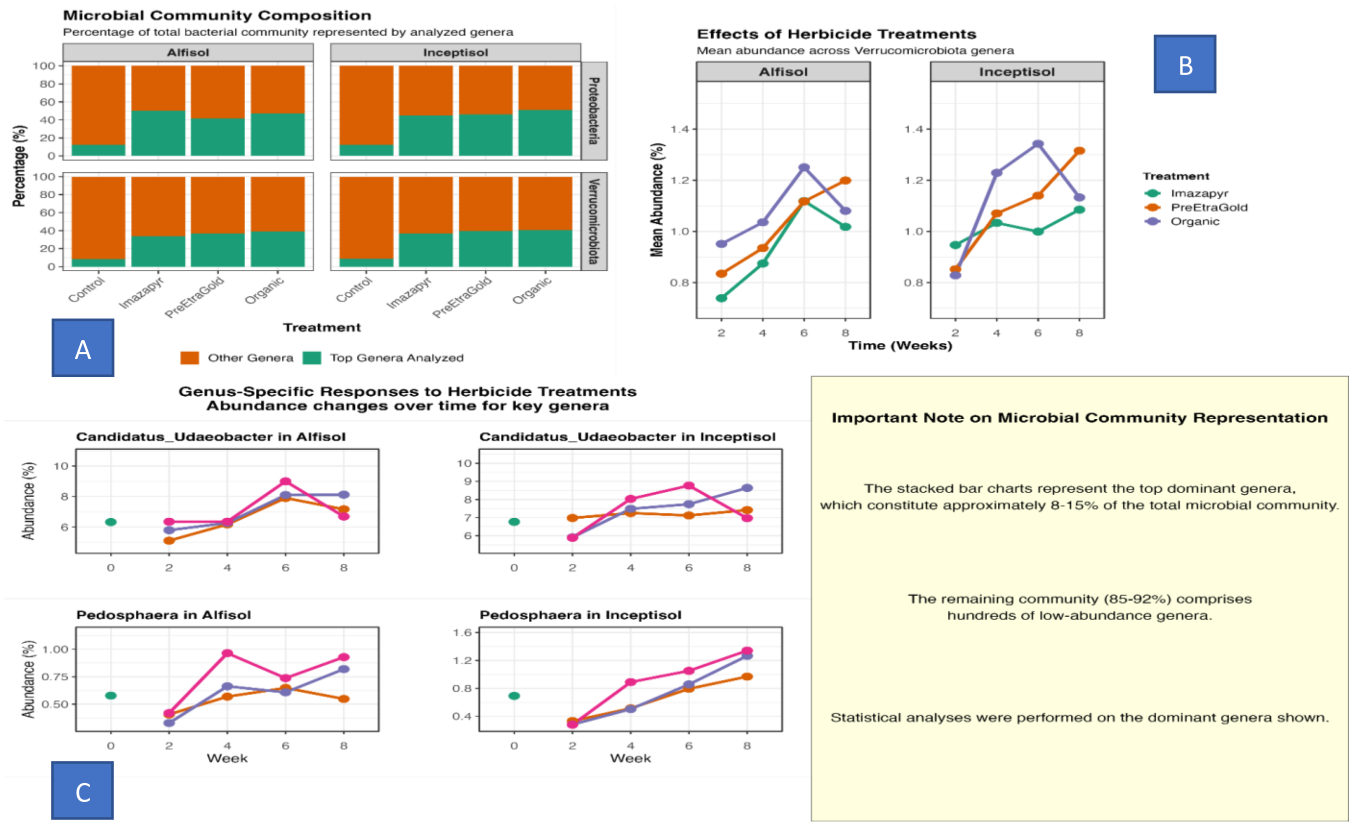

Figure 5. The bacterial community composition visualization and herbicide treatment effect

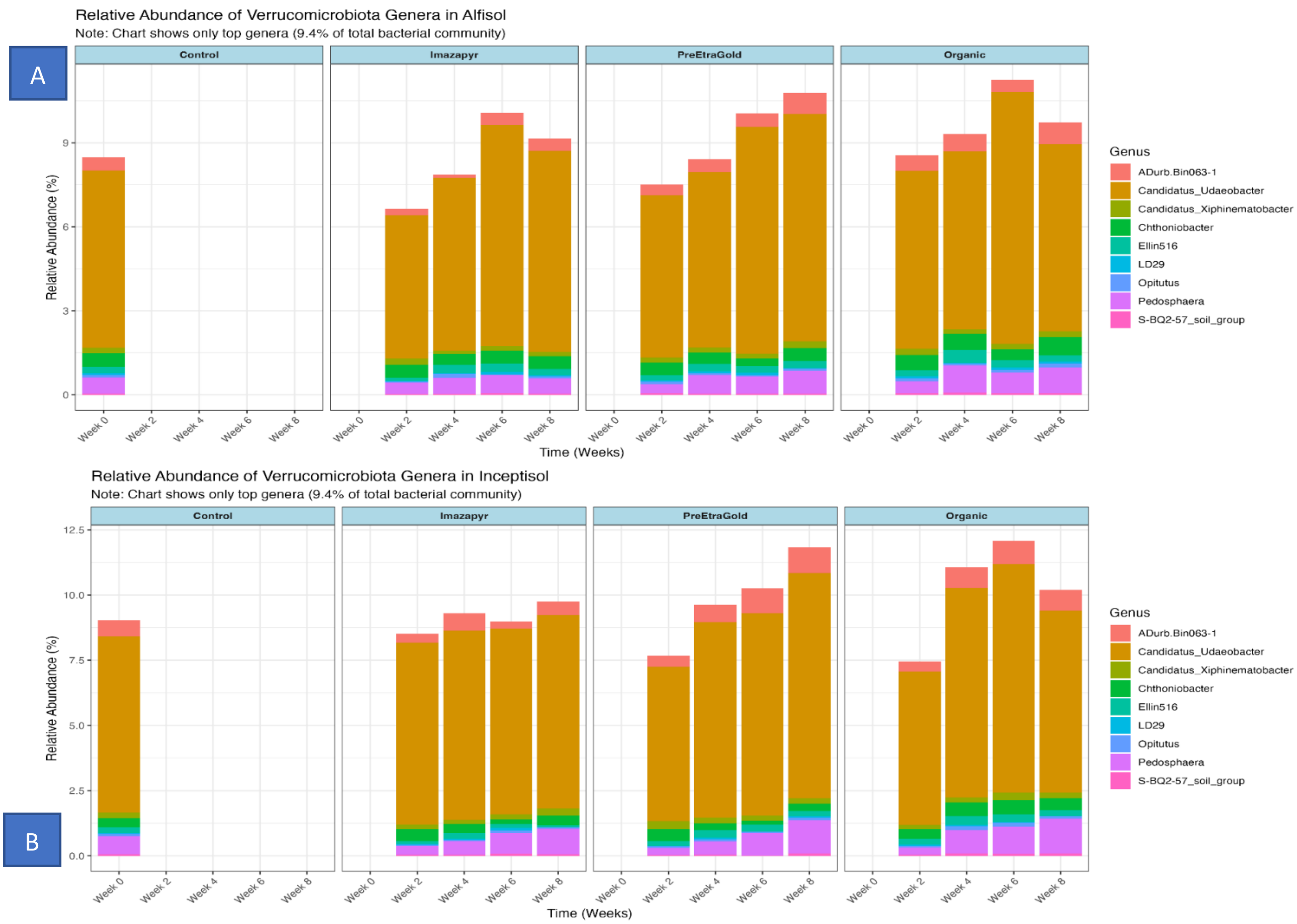

Figure 6. Soil Type-Specific Differences in Verrucomicrobiota Response

Figure 7. Soil type-specific differences in proteobacteria response

3.9. Temporal Variations in Herbicide Impacts

The time series data presented in Figures 4, 6, and 7 demonstrate that herbicide impacts vary significantly across the 8-week experimental period. For the bacterial genus *Candidatus Udaeobacter*, an initial decrease was observed at week 2, followed by recovery and increases through weeks 4-6, particularly under organic herbicides. In contrast, genera like *Pedosphaera* showed more complex temporal patterns with initial declines under chemical herbicides followed by partial recovery, while under Organic treatment, there was a consistent increase over time. (Fig. 5C). Figure 6 shows that by week 8, bacterial genus *Pedosphaera* abundance under Organic treatment was substantially higher than the initial values, indicating long-term positive effects.

3.10. Organic vs. Chemical Herbicide Effects on Microbial Diversity

The overall treatment effects plot (Fig. 5C) demonstrates that the Organic herbicide maintained a higher mean abundance of *Verrucomicrobiot*a genera compared to chemical herbicides across most time points in both soil types. This trend is particularly evident in sensitive bacterial genera like *Pedosphaera* and *Candidatus udaobacter* (Fig. 4C), where organic herbicides consistently resulted in higher abundances than either Imazapyr or PrimextraGold. The stacked bar charts (Fig. 6 and 7) further support this conclusion, showing that while the total abundance of analysed genera was similar across treatments, the community composition under organic treatment maintained greater similarity to the control, particularly for sensitive taxa. This suggests that Organic herbicide has a less disruptive effect on soil microbial ecology.

4. DISCUSSION

This study revealed that soil type significantly influenced the persistence of herbicide metabolites and the structure of bacterial communities, with implications for sustainable herbicide management. Alfisol retained more herbicide residues over time, while Inceptisol facilitated greater microbial degradation, especially under organic herbicide treatments. The distinct physicochemical properties of these soils shaped the observed microbial responses and herbicide fates, indicating the interdependence of soil characteristics, chemical behaviour, and microbial ecology.

Alfisol, compared to Inceptisol, exhibited about 7.4% higher clay content and moderate pH, contributing to its enhanced capacity to retain herbicide metabolites such as isopropylamine (IA), Atrazine + S-Metolachlor (AS-M), and acetic acid (AA) over the 12 weeks. This trend aligns with previous studies indicating that clayey soils with moderate pH tend to exhibit stronger sorption of polar herbicides, reducing their bioavailability and leaching potential (Bonfleur et al., 2016; Glaspie et al., 2021; Hussain et al., 2015). Alfisol also had about 20% more available phosphorus than Inceptisol, which could enhance microbial growth and nutrient cycling capacity. However, despite these advantages, the same properties that promote nutrient retention may also restrict microbial access to adsorbed herbicides, slowing biodegradation.

In contrast, Inceptisol, with its lower clay content and slightly more acidic pH, supported faster degradation of herbicide residues, particularly for acetic acid metabolites in non-sterile treatments (23.49% degradation), suggesting more active microbial processes. This is consistent with findings by Smith et al. (2020), who reported that reduced sorption and higher microbial mobility in less structured soils promote degradation. Interestingly, isopropylamine showed higher persistence in Inceptisol, a pattern possibly explained by the inhibitory effect of lower pH on microbial enzymatic access to the compound (Rasool et al., 2022). Although AS-M was initially degraded effectively in Inceptisol (weeks 4 and 8), the degradation slowed down by week 12, which may indicate the formation of toxic or recalcitrant intermediates that suppressed further microbial activity (Chen et al., 2021; Kaiser et al., 2016). Sterile soils showed minimal degradation, confirming the central role of microbial action, even though some abiotic processes, such as hydrolysis may contribute (Chowdhury et al., 2021).

Microbial community profiles corroborated these degradation patterns. The most abundant bacterial phyla were *Verrucomicrobiota* (9.4%) and *Proteobacteria* (11.7%), with Figure 5A showing shifts in broader community structures, while Figures 4, 6, and 7 highlighted dominant genera in the 8–15% range. *Candidatus Udaeobacter*, a well-known oligotrophic bacterial genus, was the most abundant across treatments and increased slightly under both PrimextraGold and Organic herbicide (0.71 percentage points), but showed weaker responses under Imazapyr (0.34). Its ability to thrive under moderate chemical stress reflects metabolic flexibility (Breidenbach et al., 2016; Willms et al., 2020);. In contrast, bacterial genera such as *Pedosphaera*, *LD29*, and *Opitutus* declined significantly by over 55% under chemical herbicides, particularly Imazapyr and PrimextraGold (Pertile et al., 2021). Organic herbicides helped preserve or even enhance the abundance of these sensitive genera, suggesting ecological compatibility and a lower disruption threshold (Smith et al., 2020).

Soil type further modulated these microbial shifts. Inceptisol allowed better preservation and recovery of genera like *Candidatus Udaeobacter* and *Pedosphaera*, likely due to its less compacted texture, slightly acidic pH, and higher microbial mobility. These factors collectively improve microbial resilience and nutrient cycling potential, as supported by Tripathi et al. (2018). Temporal analysis showed that microbial recovery under Organic herbicides was most pronounced between weeks 4 and 6, while chemical herbicides caused sharper initial declines and slower rebounds, particularly in nutrient-sensitive taxa (Wang et al., 2024).

Organic herbicides consistently preserved microbial community structure and diversity better than their chemical counterparts. *Verrucomicrobiota* bacterial genera such as *Chthoniobacter* and *Pedosphaera* thrived under Organic herbicides, as seen in Figures 5B, 5C, 6, and 7, while chemical herbicides caused pronounced shifts in microbial composition and abundance. Organic herbicides not only maintained microbial populations closer to control levels but also preserved essential ecosystem functions tied to decomposition and nutrient cycling (Smith et al., 2020; Zhang et al., 2015). These findings emphasize the role of organic herbicide alternatives in promoting soil health and long-term agricultural sustainability.

Despite these insights, some limitations should be acknowledged. The duration of the study, limited to 12 weeks, may not fully capture the long-term ecological impacts of herbicide use, especially under variable environmental conditions. The study also focused exclusively on bacterial communities, without examining fungi or archaeal populations that may also play key roles in soil health and herbicide degradation. Furthermore, functional implications were predicted from taxonomic data, not confirmed through metatranscriptomics or enzyme assays. Future research incorporating broader microbial profiling and functional assessments would deepen understanding of soil-herbicide-microbe interactions.

5. CONCLUSION

This study aimed to determine how soil type (Alfisol compared to Inceptisol) influences herbicide metabolite persistence and microbial community responses under organic and chemical herbicide treatments. Alfisols retained more herbicide metabolites, whereas Inceptisols promoted stronger microbial degradation, especially of organic herbicides. Organic herbicides maintained microbial diversity and function, preserving key taxa (e.g., *Candidatus Udaeobacter, Pedosphaera*). In contrast, chemical herbicides (Imazapyr, Primextra Gold) suppressed sensitive genera and shifted community composition. These results confirm that soil physicochemical properties strongly influence herbicide fate and microbial responses. The findings highlight soil type as a key factor in herbicide-microbe interactions and suggest ecological advantages of organic herbicides for sustaining microbial resilience. Herbicide management tailored to soil type could improve agroecosystem sustainability. Future research should examine long-term effects and metabolite-specific pathways using multi-omics.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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