Received: 7 March 2024 | Revised: 12 June 2024 | Accepted: 16 June 2024

DOI: 10.1111/sum.13086

Soil Use and Management | WILEY

RESEARCH ARTICLE

A split herbicide application strategy reduces surface runoff

Shane Scannell[1,2] | **Mark G. Healy**[1,2] | **Gustavo Sambrano**[2,3] | **John McGinley**[1,2] | **Paraic C. Ryan**[4,5] | **Per-Erik Mellander**[6] | **Liam Morrison**[2,7] | **Jenny Harmon O'Driscoll**[4] | **Alma Siggins**[2,3]

[1]Civil Engineering, University of Galway, Galway, Ireland

[2]Ryan Institute, University of Galway, Galway, Ireland

[3]School of Biological and Chemical Sciences, University of Galway, Galway, Ireland

[4]Discipline of Civil, Structural and Environmental Engineering, University College Cork, Cork, Ireland

[5]Environmental Research Institute, University College Cork, Cork, Ireland

[6]Agricultural Catchments Programme, Teagasc Environmental Research Centre, Johnstown Castle, Co., Wexford, Ireland

[7]Earth and Ocean Sciences, Earth and Life Sciences, School of Natural Sciences, University of Galway, Galway, Ireland

Correspondence
Alma Siggins, Ryan Institute, University of Galway, Galway, Ireland.
Email: alma.siggins@universityofgalway.ie

Present address
Shane Scannell, Land Sciences Department, South East Technological University, Waterford, Ireland

Abstract

Herbicides, such as MCPA and clopyralid, may be transported to surface waters via runoff, which can have unintended environmental consequences. A split herbicide application strategy, wherein applications are spread across a season, may improve herbicide effectiveness, although impacts of this strategy on runoff mitigation have not been investigated. Therefore, this study aimed to (1) quantify the impact of split-dose applications of MCPA and clopyralid on herbicide losses in surface runoff and (2) assess the impact of split-dose applications of MCPA on the quantity and classification of MCPA-degrading soil bacteria. Intact grassed soil sods were placed in 1 m-long × 0.25 m-wide × 0.1 m-deep laboratory flumes, onto which either MCPA or clopyralid were applied in one full-dose (13.5 kg MCPA ha^{-1}; 2 kg clopyralid ha^{-1}) or two split-doses (each 6.75 kg MCPA ha^{-1}; 1 kg clopyralid ha^{-1}) 42 days apart. On days 2, 7 and 21 following herbicide applications, flumes were subjected to controlled rainfall simulations at an intensity of 11 mm h^{-1}, and the herbicides in the runoff were quantified. MCPA and clopyralid concentrations in the runoff were highest immediately after the initial application. Both herbicides were below the limit of detection (0.1 µg l^{-1} for MCPA and 0.45 µg l^{-1} for clopyralid) by 44 days. No herbicides were detected in the runoff following the second split-dose application. For MCPA, this was attributed to an adaptation in the microbial community with the emergence of bacteria possessing the *tfdA* class III gene in the soil. These results support split-dose herbicide application as a strategy for agricultural management.

KEYWORDS

clopyralid, herbicides, MCPA, runoff, *tfdA* gene, water quality

1 | INTRODUCTION

Herbicides are used to prevent the spread of weeds in agricultural, residential and urban environments. Herbicides are the most common category of pesticides used worldwide, protecting up to 45% of crops from failure and associated reductions in yield (Abhilash & Singh, 2009). However, their overuse, or misuse, may have negative

impacts on environmental and public health (Mdeni et al., 2022). To prevent these, the European Union (EU) has established regulations, such as the Water Framework Directive (WFD) (2000/60/EC; EC, 2000) and the Sustainable Use Directive (SUD) (2009/128/EC; EC, 2009), to limit and control their use. In addition, the EU has set a maximum allowable concentration of individual herbicides in drinking water at $0.1\,\mu g\,L^{-1}$ and total pesticide concentration at $0.5\,\mu g\,L^{-1}$ (EU, 2020), where 'pesticides' include herbicides but also other plant protection products such as fungicides and insecticides.

The annual sale of herbicides within the EU rose from 386,400 to 439,400 tonnes between 2011 and 2016 to cope with an increasing population and demand for agricultural outputs (Pena et al., 2020). The consequential increased use of these herbicides has led to the more frequent detection of their active ingredients in the surrounding environment and drinking water (McGinley et al., 2023). Conventional herbicide application methods may result in their overapplication, leading to phytotoxicity in crops and the loss of excess herbicides to the environment (Rakhimol et al., 2019; Villamizar et al., 2020). With less than 15% of herbicides meeting their intended target (Singh et al., 2023), the remaining 85% has the potential to escape agricultural and residential areas, percolating through permeable layers in the soil or flowing through preferential pathways along the surface of the soil. Phenoxy acid herbicides are the herbicide group that is most frequently detected in European water supply zones (Muszynski et al., 2020). Both 2-methyl-4-chlorophenoxyacetic acid (MCPA) and clopyralid are highly water-soluble acid herbicides and are frequently detected in drinking water supply waterways (Khan et al., 2020). Detection of both MCPA and clopyralid in agricultural surface water has been shown to be higher in summer when application rates are at their greatest (Khan et al., 2020), and their magnitude may be increased with rainfall intensity and slope (Mu et al., 2015).

Mitigation measures to prevent the transmission of herbicides to surface and groundwaters may include multiple applications of herbicides across a season that cumulatively equal the same amount applied in one single dose (hereafter referred to as 'split-dose applications'). This has been shown to improve the efficacy of the products by ensuring a more even coverage of broadleaf weeds and thistles (Kristó et al., 2022). While maximum allowable application rates of MCPA have been shown to reduce the yield of the main crop (Karimmojeni et al., 2013), split-dose application rates have been reported to result in higher crop yields (Nath et al., 2017). In addition, split-dose applications of clopyralid combined with other herbicides have been shown to better manage the growth of target weeds than a single dose of clopyralid (Najafi et al., 2013). However, no study has yet examined the impact of split-dose applications on surface runoff and potential herbicide loss to waterways, nor has any study examined how split-dose applications impact the microbial community, specifically the species that are associated with the degradation of the applied herbicides.

Biological degradation plays a key role in the dissipation of herbicides from agricultural soils, with minimal losses being attributed to abiotic degradation factors such as photolysis, hydrolysis or oxidation (Meng et al., 2022). Specific genes associated with clopyralid degradation have not been identified, and biological degradation mechanisms appear to be the result of microbial processes (Vasic et al., 2022), but this has not been explored fully. However, the *tfdA* gene has been identified as a pathway for the bacterial degradation of MCPA (Baelum et al., 2006). The *tfdA* gene is found in various soil microorganisms, many of which are not directly associated with the degradation of herbicides (Perez-Pantoja et al., 2015), but are associated with the degradation of organic compounds to carbon and energy sources.

The *tfdA* gene has three classes (I, II and III) that are distributed among different bacterial species capable of MCPA degradation. Class III *tfdA* genes are the most studied across all MCPA-degrading species (Baelum et al., 2006) and are associated with the initial biological degradation of MCPA (Paulin et al., 2011). Bacteria that possess this class of the gene have been reported to become dominant in the presence of MCPA, particularly within soil that is newly exposed to the herbicide (Baelum et al., 2006). However, class I *tfdA* has been reported to slowly contribute to the degradation of MCPA and adapt to more efficient degradation in the absence of class III *tfdA* harbouring bacteria (Baelum et al., 2006; Paulin et al., 2011).

Therefore, the aims of this study are to (1) investigate the prevalence of MCPA and clopyralid loss by surface runoff using split-dose and full-dose application strategies and (2) assess the impact of split-dose applications of MCPA on bacteria that contain the MCPA degrading *tfdA* gene.

2 | MATERIALS AND METHODS

2.1 | Soil collection and rainfall flume preparation

Intact grassed soil sods, each measuring 0.6 m-long × 0.4 m-wide × 0.2 m-deep, were collected from mixed permanent grassland on a dry stock farm located in Killorglin, Co. Kerry in the South-West of Ireland (52°07′25.6′′N 9°50′15.6′′W). No herbicide applications had taken place

at this site in at least 10 years prior to sod collection. Sods were transported to the laboratory, where they were trimmed and placed in aluminium flumes measuring 1 m in length, 0.25 m in width and 0.1 m in depth. To prevent the formation of preferential flow paths other than surface runoff, any gaps found between the sods or along the flume edges were sealed using melted paraffin wax. The flumes were instrumented with runoff collection troughs and drainage holes (located in their bases) to prevent waterlogging. For a period of 12 weeks prior to the first application of herbicides, the flumes were exposed to natural daylight and weather conditions. To replicate routine grazing of the pasture sword, the grass was trimmed to approximately 5 cm 2 weeks before herbicide application, and every 3 weeks for the remainder of the study to replicate grazing conditions.

2.2 | Soil characterization

Soil samples, taken from the same location and depth as the soil sods, were air dried, passed through a 2 mm sieve, homogenized and analysed for physio-chemical properties. Soil moisture and bulk density were determined in accordance with BS1377-2 section 3.2.4 and 3.3.6, respectively (British Standard, 1990). Organic matter was determined using a loss on ignition test at 360°C (Schulte & Hopkins, 1996), total carbon and nitrogen (N) were assessed by combustion (McGeehan and Naylor, 1988), total phosphorus (P) was determined by the Morgan's P test (Morgan, 1941). The Mehlich-3 soil test was used to determine magnesium (Mg) and potassium (K) (Mehlich, 1984). Soil pH was measured using a pH meter, with a 1:2.5 ratio of soil: deionized water. Soil texture was determined by integral suspension pressure using sieved soil (<2 mm) and the METER Pario according to manufacturer guidelines (METER Group, 2018).

2.3 | Herbicide treatments

Laboratory-grade MCPA or clopyralid (Sigma-Aldrich, Ireland) were applied to triplicate flumes using one of two dosing strategies: (1) a single full-dose application (equivalent to 13.5 kg MCPA ha^{-1}, or 2 kg clopyralid ha^{-1}), termed 'full-dose'; or (2) two split-dose applications (equivalent to 6.75 kg MCPA ha^{-1}, or 1 kg clopyralid ha^{-1} per dose, with the two doses spaced 42 days apart), termed 'split-dose'. Three control flumes, containing the same grassed soil sods were subjected to the same experimental conditions but without herbicide application. In total, 15 flumes were used in the experiment. The application rates selected were 10 times the recommended herbicide application to ensure the detection of the herbicide in the runoff and measure the impact of excessive application rates. Herbicide applications were conducted using 750 mL trigger spray bottles to ensure uniformity of dose and to replicate typical application mechanisms.

2.4 | Rainfall simulation

The rainfall simulation was carried out as described by González Jiménez et al. (2018). Briefly, drainage holes at the base of the flumes were plugged 24 hours prior to herbicide application and the soil was saturated until water accumulated at the surface. The drainage stoppers were removed, and the flumes were allowed to reach field capacity. Once fully drained, herbicides were applied to the full- and split-dose flumes (day 0). For the split-dose flumes, the second split-dose was applied following the same procedure on day 42.

The Sustainable Use of Pesticides directive 2009/128/EC (EC, 2009) stipulates that pesticides should not be applied to land when rainfall is forecast within 48 h. This directive informed the 2-day period between herbicide application and the first rainfall event in our study. Consequently, simulated rainfall events were conducted 2, 7, 21, 44, 49 and 63 days after the initial application of herbicides. Between days 0 to 7 and 42 to 49, the flumes were stored indoors and exposed to normal laboratory conditions at approximately 15°C, to ensure initial runoff was not lost to natural rainfall events. During the simulated rainfall events, the flumes were placed in a rainfall simulator at a slope of 6°, similar to the natural slope of Irish agricultural topography (González Jiménez et al., 2018). The rainfall simulator consisted of a single ¼HH-SS14SQW nozzle (Spraying Systems Co., Wheaton, IL) attached to a 4.5 m high metal bracket surrounded by thin polythene sheeting with a rotating circuit. The simulator was calibrated to achieve an intensity of $10.2 \pm 0.1\,mm\,h^{-1}$ and a droplet impact energy of $260\,kJ\,mm^{-1}\,h^{-1}$ at 90% uniformity (Brennan et al., 2011). Each rainfall event lasted 30 min, during which water samples were collected in 10-min intervals at 10, 20 and 30 min. The water volumes were recorded, and subsamples for herbicide analysis were immediately stored at −20°C for up to a maximum of 4 weeks prior to analysis.

2.5 | Herbicide runoff analysis

Herbicides were quantified by LC–MS, where chromatographic separation was conducted using a C18 LC column in a Thermo scientific Dionex UlitMate 3000 system equipped with a binary pump, a vacuum degasser and an

autosampler. The column oven was maintained at 25°C. The mass spectrometry was conducted on a Thermo Scientific Extractive Plus Orbitrap® mass spectrometer. TraceFinder 4.1 EFS LC software was utilized for data acquisition and analysis. The flow-weighted mean concentration (FWMC) was calculated by multiplying the concentration of the captured herbicide in the surface runoff in each 10-min interval of the 30-min rainfall simulation event by the corresponding volume of flow. The resulting cumulative mass of herbicide was then divided by the total volume measured during the rainfall event.

2.6 | Microbial sample collection and analysis

Triplicate soil plugs (5 g) were extracted from the control, full-dose and split-dose flumes at weekly intervals (weeks 0–6) to assess changes quantitatively and qualitatively in the *tfdA* gene, the gene associated with the biodegradation of MCPA. Once the plugs were extracted, they were frozen at −20°C and the plug holes within the flumes were filled with paraffin wax.

DNA extractions from soil samples were performed using a Qiagen Dneasy PowerSoil Pro Kit, following the manufacturer's instructions, with the exception that 0.4 g soil was used for extractions. Following DNA extraction, DNA quantification was conducted using the Qubit dsDNA High Sensitivity Assay.

Normalized soil DNA samples (25–50 $\mu g\,L^{-1}$) were used for triplicate qPCR analysis to quantify the *tfdA* gene using the forward primer 5′-GAGCACTACGCRCTGAAYTCCCG-3′ and the reverse primer 5′-GTCGCGTGCTCGAGAAG-3′ (Baelum & Jacobsen, 2009). Standard curves from 10^7–10^1 $\mu g\,L^{-1}$ were prepared in triplicate using 280 bp gBlocks Gene Fragments produced by IDT (Integrated DNA Technologies), containing *tfdA* sequences from *Alcaligenes eutrophus*. The assay limit of quantification was 10^3 copies $\mu g\,L^{-1}$.

Quantitative real-time PCR using SYBR® Green as dye was performed using LightCycler 480 SYBR Green I Master qPCR kit (Roche) Mastermix including 0.4 μM of each primer. Five microliter aliquots of DNA extracts (~10–50 ng) were added to 20 μL reactions. The reaction conditions were as follows: 10 min at 95°C for enzyme activation, 50 cycles of 60 s at 95°C, 30 s at 64°C and 60 s at 72°C in the LightCycler 480. After amplification, a melting curve was acquired by heating the product at 4.4°C s^{-1} to 95°C, holding it for 5 s, cooling it at 2.2°C s^{-1} to 64°C, holding at 64°C for 60 s and then slowly heating at 0.1°C s^{-1} to 97°C. Measurement of florescence intensity for the qPCR was performed in the end of the 60s 72°C step, while for the melting curve assay, it was measured at the end of each increment cycle. Presence/absence determinations of three potential gene classes (I, II and III) were made based on the Tm callings using the LightCycler® 480 software, with different *tfdA* classes being identified based on their melting peaks: 91°C for class I; 85°C for class II; 88°C for class III (Baelum & Jacobsen, 2009).

2.7 | Statistical analysis

All data and statistical analysis were conducted using Microsoft Excel 365 software and data visualization was performed using GraphPad Prism 10. One-way ANOVA was used to compare the variation among different application strategies, a p-value <.05 was considered significant proof of difference in the results of statistical analysis.

3 | RESULTS AND DISCUSSION

3.1 | Soil characterization

The soil was characterized as a clay loam, which has previously been reported to have a median MCPA adsorption capacity of 10.29 $mg\,g^{-1}$ from a review of studies reporting transmission risk based on factors including adsorption and soil texture (McGinley et al., 2022). Considering MCPA's high solubility, low adsorption capacity and soil half-life, MPCA is a moderate risk for transmission to waterways via runoff (McGinley et al., 2022). Peña et al. (2015) reported that in a study of a clay loam soil, 56% of applied MCPA leached through a column, while only 7% was extracted from the soil matrix, with the rest potentially lost through degradation.

In our study, the observed soil pH was 4.7, which indicates an acidic soil (Table 1). Acidic soils have previously been negatively correlated with MCPA adsorption (Hiller et al., 2012). The acidic soils in our study may have increased the runoff of MCPA by decreasing adsorption to the soil particles. Conversely, this soil had an organic matter content (7.3%) and C:N ratio (10.2) (Table 1), which would indicate an increased sorption potential for acid herbicides (Jacobsen et al., 2008). These characteristics increase the capacity of negatively charged sites available for herbicides within the soil. K^+, Mg^+, Fe^+ and Ca^+ cations are important in regulating cation exchange processes within soil (Raman & Sathiyanarayanan, 2009), so the presence of these cations may increase the cation exchange capacity of the soil (Table 1). Finally, a high initial presence of total phosphorus (929.0 $mg\,kg^{-1}$) (Table 1) may have negatively impacted the rate of MCPA sorption within the soil, as previously reported by Hiller et al. (2012). This may be because of potential binding sites within clay soil and

TABLE 1 Soil chemical characterization prior to herbicide application. Results are averages (mean) and standard deviation with triplicate sampling.

Determinand (unit)	Average	Std dev
pH Water [1:2.5]	4.7	0
Organic Matter LOI (% w/w)	7.3	0
Total Magnesium (Mg) ($mg\,kg^{-1}$)	4057	81
Total Phosphorus (K) ($mg\,kg^{-1}$)	929	41
Total Potassium (P) ($mg\,kg^{-1}$)	1833	175
Total Carbon (C) (% w/w)	3.35	0.05
Total Nitrogen (N) (% w/w)	0.328	0.01
Total Calcium (Ca) ($mg\,kg^{-1}$)	620	176
Total Iron (Fe) ($mg\,kg^{-1}$)	40,459	1298
Carbon: Nitrogen (C: N) Ratio (:1)	10.2	0.35
Soil Texture	Clay loam	–
Soil Bulk Density[a]	$1.6\,g/cm^3$	–
Soil Moisture[a]	26%	–

[a]These measurements were taken using intact field samples.

organic matter becoming occupied by phosphate, hence reducing MCPA absorbance.

Clopyralid has been reported to have a half-life of 42 days in clay loam soil at 10°C (Smith & Aubin, 1989), indicating that it has the potential to persist in the soil over the duration of the study.

3.2 | Pesticide detection in surface runoff

Split MCPA and clopyralid applications reduced the FWMC of each herbicide lost to surface runoff, potentially by allowing time for the adsorption or the development of degradation mechanisms within the soil after herbicide application. This adaptation allowed for subsequent herbicide applications to be degraded rapidly compared with the initial application. The results are divided into three phases: phase I (day 2), phase II (days 7 and 11) and phase III (days 44, 49 and 63).

This split-dose application strategy led to, on average, a 43% decrease in the mass of MCPA contained in the surface runoff over the study period. However, this reduction was not statistically significant ($p > .05$), which may have been caused by the presence of heterogeneity and variability observed within intact soil samples (Rosemary et al., 2017). In contrast, an 82% decrease observed in the surface runoff of clopyralid between the full-dose and split-dose strategy showed a significant statistical difference ($p < .05$), indicating its effectiveness in mitigating the transport of clopyralid into nearby water bodies.

3.2.1 | Phase I: Day 2 rainfall event

Two days after the initial herbicide application, herbicide concentrations in the runoff from flumes subjected to a split-dose application were lower than the flumes subjected to a full-dose application: $10 \pm 4\,\mu g\,L^{-1}$ compared with $42 \pm 11\,\mu g\,L^{-1}$ for clopyralid, and $77 \pm 3\,\mu g\,L^{-1}$ compared with $170 \pm 99\,\mu g\,L^{-1}$ for the MCPA (Figure 1).

For both MCPA and clopyralid and for both full- and split-dose applications, a greater concentration was detected in the runoff on day 2 than the combination of all five subsequent controlled rainfall events (Figure 1). This initial high concentration may have been exacerbated by applying the herbicides at 10 times the recommended application rate. Overapplication of pesticides is typically utilized to represent a 'worst-case scenario' in agricultural management (Álvarez-Martín et al., 2017; Khorram et al., 2015). Nonetheless, the results indicate that regardless of utilizing a full- or split-dose application approach, significant herbicide contamination was present in the runoff 48 h after the initial application (Roache, 2020). This provides context to how 'persistent' and 'very persistent' herbicides, as described in a review by McGinley et al. (2023), may enter the environment and impact non-target species.

3.2.2 | Phase II: Days 7 and 21 rainfall events

The decline in MCPA and clopyralid concentrations from days 2 to 7, and again from days 7 to 21, suggests that the application strategy influenced the detection of herbicides in surface runoff over time (Figure 1). The FWMC for the full-dose MCPA was $170\,\mu g\,L^{-1}$ on day 2 and reduced to $2\,\mu g\,L^{-1}$ by day 21. A decrease was also observed for the first split-dose MCPA treatment, from $77\,\mu g\,L^{-1}$ on day 2 to $1\,\mu g\,L^{-1}$ on day 21. This decrease in the FWMC is likely the result of biological degradation within the soil combined with the loss of MCPA through runoff. The MCPA within these samples dissipated faster than the 7-day half-life reported by Saleh et al. (2016), using sterilized soil samples, suggesting that a biological pathway increased the rate of MCPA degradation. Similarly, the full-dose FWMC of clopyralid reduced from $42\,\mu g\,L^{-1}$ on day 2 to $1\,\mu g\,L^{-1}$ on day 21. A decrease was also observed for the split-dose clopyralid application, from $10\,\mu g\,L^{-1}$ on day 2 to below the limit of quantification of $0.45\,\mu g\,L^{-1}$ from day 7 onwards. Similar to the MCPA treatments a decrease in

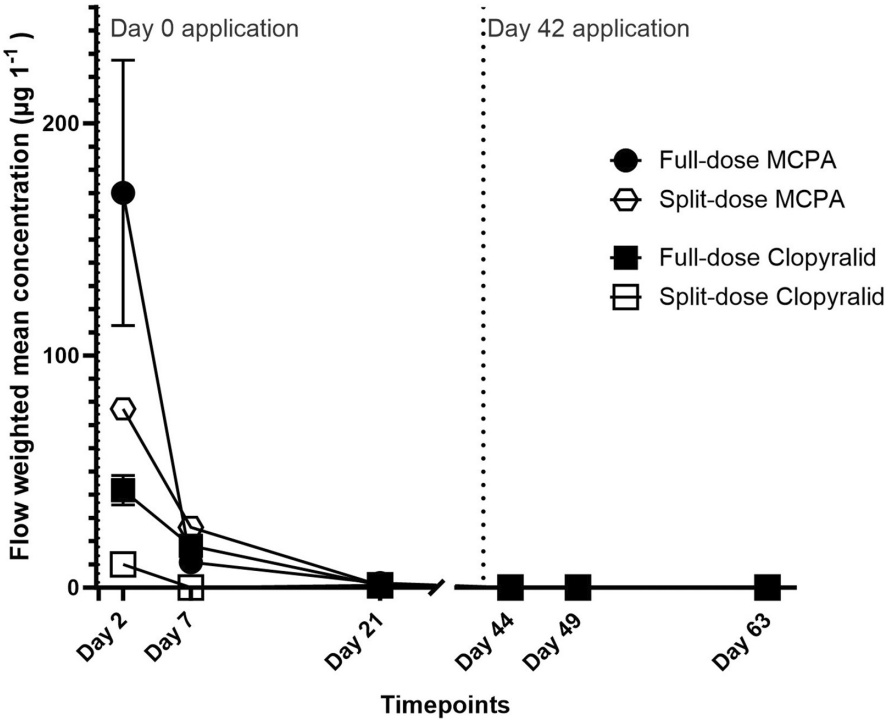

FIGURE 1 Flow weighted mean concentrations of surface runoff events for MCPA and Clopyralid ($\mu g\,L^{-1}$) over the 63-day study duration. Error bars are standard error of the mean, where $n = 3$. Herbicide applications occurred on days 0 and 42.

the FWMC of the runoff suggests the development of a biological degradation pathway for clopyralid, as the rate of degradation occurs quicker than the reported half-life of 13.4 days observed by Tandon and Singh (2022), using sterilized soil.

MCPA and clopyralid have a high affinity to adsorb to organic matter within the soil, but are also highly water soluble, making them mobile and likely to be lost to surface runoff during prolonged rainfall events (Morton et al., 2019; Tandon & Singh, 2022). This was observed in the first 21 days of the study, where the FWMC of both MCPA and clopyralid increased during each 30-min rainfall event. MCPA has been reported to have an affinity to run off the surface of soils during diffuse rainfall events, as documented by Morton et al. (2019), enhancing the potential for these herbicides to reach waterways. Morton et al. (2019) also highlighted that during these rainfall events, high water solubility of MCPA increased detection in surface runoff. This was observed to a lesser extent under the split-dose application strategy in the current study, showing the inherent ability of soil to mitigate against the herbicide losses in surface water runoff while utilizing a split-dose application strategy.

3.2.3 | Phase III: Days 44, 49 and 63 rainfall events

The lack of detection of both herbicides, particularly after the second split-dose application of MCPA and clopyralid, is unlikely to be the result of abiotic degradation mechanisms because of the controlled conditions of this study. This adaptive response to the presence of MCPA and clopyralid within the soil suggests that a biotic degradation pathway changed within the soil. No quantifiable MCPA or clopyralid were detected from any flumes from day 44 onwards (Figure 1). The MCPA detection limit of $0.1\,\mu g\,L^{-1}$ corresponds to the drinking water limit for individual pesticides, including herbicides (EU, 2020). The clopyralid detection limit of $0.45\,\mu g\,L^{-1}$ of the instrumentation used in this study is higher than the drinking water limit, so more sensitive analysis would be required to confirm that it meets these standards. While surface runoff is not directly used as drinking water, in the absence of an environmental water quality standard for herbicides, achieving a drinking water quality ensures a low environmental risk to the receiving water body. While this study has shown that regardless of a full- or split-dose application method, herbicide detection within watercourses will be observed, the amount of herbicide captured in surface runoff under a split-dose application regime is the lesser of the two.

3.3 | Biological degradation of MCPA

To assess any potential changes in the MCPA-degrading microbial community, the quantity and classification of *tfdA* genes were assessed. While amplification of the *tfdA* gene was observed, it was consistently below the limit of quantification of the assay (10^3 copies μL^{-1}). Previous studies reported *tfdA* levels greater than 10^6 copies μL^{-1}

(Baelum & Jacobsen, 2009) in MCPA-degrading soil communities. This lack of quantification suggests a relatively low abundance of native *tfdA* communities within the soil rather than contamination in the extraction inhibiting the qPCR assay and has been previously observed by White et al. (2022).

Despite the low abundance of the *tfdA* gene, the melting peaks obtained could be used to determine the presence or absence of the three different *tfdA* classes in the soil samples, which are associated with different bacterial groups (Table 2). *tfdA*-bearing bacteria within soil perform a number of key processes, such as nutrient cycling (Zhang et al., 2022) and plant pathogen suppression (Tao et al., 2020), and while their primary functions are not directly associated with herbicide degradation, they possess genes capable of MCPA degradation exist within these species. Soil communities which have not previously been exposed to MCPA, hold the innate ability to degrade acid herbicides, which has previously been attributed to tfdA class III Bradyrhizobium-Agromyces-Nitrobacter-Afipia cluster (Kitagawa et al., 2002; Table 3).

The results indicate that class I *tfdA* genes were present in all control flumes throughout the study (Table 3). The soil in these flumes did not have herbicides applied during the study, or for at least 10 years prior to our sampling, indicating that the soil had the capacity to degrade MCPA even without the selective pressure of the herbicide being present. Generally, class I *tfdA* genes are consistently detected in both split- and full-dose flumes, with the exception of one replicate split-dose flume on day 14 (Table 3).

Class II is less widely distributed, being found only within *Burkholderia* spp. (Kitagawa et al., 2002). It serves in plant growth and soil bioremediation (Elshafie & Camele, 2021), but has been shown to have a poor ability to degrade acid herbicides (Samuelsen et al., 2017). Higher doses of MCPA did not appear to act as an inhibitor to the detection of *tfdA* class III gene, as has been observed with higher doses of similar phenoxy acid herbicides, suggesting a lower cytotoxic action of MCPA towards these bacteria (Mierzejewska et al., 2019). The detection of class III *tfdA* gene harbouring bacteria emerged within 2 weeks of MCPA application, corresponding with previous work by Baelum et al. (2008), where only bacteria possessing class III *tfdA* genes were able to proliferate during the degradation of MCPA. In this study, class III *tfdA* was only observed in soil samples where MCPA had been applied, with a stronger observed presence when single full doses of MCPA were applied to the soil (Table 2).

The presence of class III did not have an impact on the detection of classes I and II *tfdA* gene bacteria, indicating that the co-existence of these different gene classes is not mutually exclusive and that they may serve distinct functions in the microbial community's response to

TABLE 2 tfdA bearing bacterial groups capable of degrading MCPA within soils.

tfdA class	MCPA degrading bacteria	Reference
I	*Achromobacter* spp.	Baelum et al. (2010)
	Burkholderia spp.	Tonso et al. (1995)
	Cupriavidus spp.	Baelum et al. (2010)
	Delftia acidovorans	Hoffmann et al. (2003)
	Halomonas spp.	Maltseva et al. (1996)
	Pseudomonas spp.	Baelum et al. (2010)
	Rhodoferax fermentans	Fulthorpe et al. (1995)
	Variovorax koreensis	Tonso et al. (1995)
	Alcaligenes xylosoxidans	Tonso et al. (1995)
II	*Burkholderia* spp.	Baelum et al., 2010
III	*Bradyrhizobium* spp.	Kamagata et al. (1997)
	Pseudomonas spp.	Baelum et al. (2010)
	Variovorax koreensis	Baelum et al. (2010)
	Cupriavidus spp.	Baelum et al. (2010)

TABLE 3 tfdA class gene detection within soil across three application rates over a period of 42 days with '1, 2 and 3' representing the detection of the tfdA gene in replicate samples.

	Day	Class I	Class II	Class III
MCPA 0 (control)	7	1,2,3	1,2	
	14	1,2,3	1,2,3	
	21	1,2,3	1,2,3	
	42	1,2,3	2	
MCPA split dose	7	1,2,3	1,2,3	
	14	2,3		1
	21	1,2,3	1,2,3	
	42	1,2,3	1,2,3	1
MCPA full dose	7	1,2,3	1,2,3	
	14	1,2,3	1,2	1,2,3
	21	1,2,3	2	1,2
	42	1,2,3	1,2,3	1,2,3

herbicide application although these functions have not been specifically identified to date (Baelum et al., 2008). This demonstrates that class III *tfdA* harbouring bacteria, while already present within the community, were able to adapt to the presence of high amounts of MCPA, which may explain the rapid dissipation of the second MCPA split-dose to the soil. While no gene capable of degrading clopyralid has been identified to date, a biological degradation pathway is likely present within the soil. As a result of our novel split application approach to MCPA and clopyralid application, a practical method to reducing the risk of herbicides being lost to surface runoff has been identified.

4 | CONCLUSION

This study confirmed that a split application strategy could mitigate losses of MCPA and clopyralid from surface runoff over a 63-day study period. Furthermore, this investigation highlighted a biological response to the presence of MCPA, with adaptation of the *tfdA* gene microbial community. Class III *tfdA* genes, known for their involvement in the initial biological degradation of MCPA, were particularly responsive to herbicide exposure. This adaptation suggests the microbial community's ability to rapidly adapt to the presence of herbicides, especially under split-dose applications.

This research contributes valuable insights into sustainable herbicide management practices. The adoption of split-dose applications emerges as a practical strategy to minimize herbicide loss to surface runoff, demonstrating its potential environmental benefits. Further exploration of microbial communities and their response to herbicide exposure will undoubtedly enhance our understanding of herbicide fate in the environment, guiding the development of more sustainable agricultural practices.

ACKNOWLEDGEMENTS

This project is funded under the Irish EPA Research Programme 2014–2020 (Project reference number 2019-HW-LS-3). The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action and Environment. It is administered by the Environmental Protection Agency, which has the statutory function of co-ordinating and promoting environmental research.

CONFLICT OF INTEREST

Although every effort has been made to ensure the accuracy of the material contained in this journal paper, complete accuracy cannot be guaranteed. Neither the Environmental Protection Agency nor the authors accept any responsibility whatsoever for loss or damage occasioned or claimed to have been occasioned, in part or in full, as a consequence of any person acting or refraining from acting, as a result of a matter contained in this journal paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are included in the tables/figures and text of the manuscript. Additional information is available from the corresponding author upon reasonable request.

REFERENCES

Abhilash, P. C., & Singh, N. (2009). Pesticide use and application: An Indian scenario. *Journal of Hazardous Materials*, *165*, 1–12.

Álvarez-Martín, A., Sánchez-Martín, M. J., Ordax, J. M., Marín-Benito, J. M., & Rodríguez-Cruz, M. S. (2017). Leaching of two fungicides in spent mushroom substrate amended soil: Influence of amendment rate, fungicide ageing and flow condition. *Science of the Total Environment*, *584*, 828–837.

Baelum, J., Henriksen, T., Hansen, H. C., & Jacobsen, C. S. (2006). Degradation of 4-chloro-2-methylphenoxyacetic acid in top- and subsoil is quantitatively linked to the class III tfdA gene. *Applied and Environmental Microbiology*, *72*, 1476–1486.

Baelum, J., & Jacobsen, C. S. (2009). TaqMan probe-based real-time PCR assay for detection and discrimination of class I, II, and III tfdA genes in soils treated with phenoxy acid herbicides. *Applied and Environmental Microbiology*, *75*, 2969–2972.

Baelum, J., Jacobsen, C. S., & Holben, W. E. (2010). Comparison of 16S rRNA gene phylogeny and functional tfdA gene distribution in thirty-one different 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid degraders. *Systematic and Applied Microbiology*, *33*, 67–70.

Baelum, J., Nicolaisen, M. H., Holben, W. E., Strobel, B. W., Sorensen, J., & Jacobsen, C. S. (2008). Direct analysis of tfdA gene expression by indigenous bacteria in phenoxy acid amended agricultural soil. *The ISME Journal*, *2*, 677–687.

Brennan, R. B., Fenton, O., Grant, J., & Healy, M. G. (2011). Impact of chemical amendment of dairy cattle slurry on phosphorus, suspended sediment and metal loss to runoff from a grassland soil. *Science of theTotal Environment*, *409*, 5111–5118.

Bristish Standard. (1990). *BS 1377–2: 1990, methods of test for soils for civil engineering purposes-part 2: Classification tests*. British Standard Institution.

Elshafie, H. S., & Camele, I. (2021). An overview of metabolic activity, beneficial and pathogenic aspects of *Burkholderia* Spp. *Metabolites*, *321*, 11.

Eu, E. U. (2020). *Directive (EU) 2020/2184 of thE European Parliament and of the Council of 16 December 2020 on THE quality of water intended for human consumption (recast)*. Official Journal of the European Union.

EUROPEAN COMMISSION, EC. (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities*, *327*, 1–72.

EUROPEAN COMMISSION, EC. (2009). Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for community action to achieve the sustainable use of pesticides. *Official Journal of the European Communities*, *309*, 71–86.

Fulthorpe, R., Mcgowan, C., Maltseva, O., Holben, W., & Tiedje, J. M. (1995). 2,4-Dichlorophenoxyacetic acid-degrading bacteria contain mosaics of catabolic genes. *Applied and Environmental Microbiology*, *61*, 3274–3281.

González Jiménez, J. L., Healy, M. G., Roberts, W. M., & Daly, K. (2018). Contrasting yield responses to phosphorus applications on mineral and organic soils from extensively managed grasslands: Implications for P management in high ecological status catchments. *Journal of Plant Nutrition and Soil Science*, *181*, 861–869.

Hiller, E., Tatarkova, V., Simonovicova, A., & Bartal, M. (2012). Sorption, desorption, and degradation of (4-chloro-2-methylphenoxy) acetic acid in representative soils of the Danubian lowland, Slovakia. *Chemosphere*, *87*, 437–444.

Hoffmann, D., Kleinsteuber, S., Müller, R. H., & Babel, W. (2003). A transposon encoding the complete 2,4-dichlorophenoxyacetic acid degradation pathway in the alkalitolerant strain Delftia acidovorans P4a. *Microbiology (Reading, England)*, *149*(Pt 9), 2545–2556. https://doi.org/10.1099/mic.0.26260-0

Jacobsen, C. S., Van Der Keur, P., Iversen, B. V., Rosenberg, P., Barlebo, H. C., Torp, S., Vosgerau, H., Juhler, R. K., Ernstsen, V., Rasmussen, J., Brinch, U. C., & Jacobsen, O. H. (2008). Variation of Mcpa, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons. *Environmental Pollution*, *156*, 794–802.

Kamagata, Y., Fulthorpe, R. R., Tamura, K., Takami, H., Forney, L. J., & Tiedje, J. M. (1997). Pristine environments harbor a new group of oligotrophic 2,4-dichlorophenox yacetic acid-degrading bacteria. *Applied and Environmental Microbiology*, *63*, 2266–2272.

Karimmojeni, H., Pirbaloti, A. G., Kudsk, P., Kanani, V., & Ghafori, A. (2013). Influence of Postemergence herbicides on Weed Management in spring-sown linseed. *Agronomy Journal*, *105*, 821–826.

Khan, M. A., Costa, F. B., Fenton, O., Jordan, P., Fennell, C., & Mellander, P. E. (2020). Using a multi-dimensional approach for catchment scale herbicide pollution assessments. *Science of the Total Environment*, *747*, 141232.

Khorram, M. S., Wang, Y., Jin, X., Fang, H., & Yu, Y. (2015). Reduced mobility of fomesafen through enhanced adsorption in biochar-amended soil. *Environmental Toxicology and Chemistry*, *34*(6), 1258–1266.

Kitagawa, W., Takami, S., Miyauchi, K., Masai, E., Kamagata, Y., Tiedje, J. M., & Fukuda, M. (2002). Novel 2,4-dichlorophenoxyacetic acid degradation genes from oligotrophic *Bradyrhizobium* sp. strain Hw13 isolated from a pristine environment. *Journal of Bacteriology*, *184*, 509–518.

Kristó, I., Vályi-Nagy, M., Rácz, A., Tar, M., Irmes, K., Szentpéteri, L., & Ujj, A. (2022). Effects of weed control treatments on weed composition and yield components of winter wheat (*Triticum aestivum* L.) and winter pea (*Pisum sativum* L.) intercrops. *Agronomy*, *12*, 2590.

Maltseva, O., Mcgowan, C., Fulthorpe, R., & Oriel, P. (1996). Degradation of 2,4-dichlorophenoxyacetic acid by haloalkaliphilic bacteria. *Microbiology*, *1996*(142), 115–1122.

Mcgeehan, S. L., & Naylor, D. V. (1988). Automated instrumental analysis of carbon and nitrogen in plant and soil samples. *Communications in Soil Science and Plant Analysis*, *19*, 493–505.

Mcginley, J., Harmon O'driscoll, J., Healy, M. G., Ryan, P. C., Mellander, P. E., Morrison, L., Callery, O., & Siggins, A. (2022). An assessment of potential pesticide transmission, considering the combined impact of soil texture and pesticide properties: A meta-analysis. *Soil Use and Management*, *38*(20), 1162–1171.

Mcginley, J., Healy, M. G., Ryan, P. C., O'driscoll, H., Mellander, P. E., Morrison, L., & Siggins, A. (2023). Impact of historical legacy pesticides on achieving legislative goals in Europe. *Science of the Total Environment*, *873*, 162312.

Mdeni, N. L., Adeniji, A. O., Okoh, A. I., & Okoh, O. O. (2022). Analytical evaluation of carbamate and organophosphate pesticides in human and environmental matrices: A review. *Molecules*, *27*, 618.

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, *15*, 1409–1416.

Meng, X., Guo, Y., Wang, Y., Fan, S., Wang, K., Han, W., & Wu, W. (2022). A systematic review of photolysis and hydrolysis degradation modes, degradation mechanisms, and identification methods of pesticides. *Journal of Chemistry*, *2022*, 1–13.

Meter Group. (2018). *PARIO soil particle analyzer—Manual*. METER Group.

Mierzejewska, E., Baran, A., Tankiewicz, M., & Urbaniak, M. (2019). Removal and Ecotoxicity of 2,4-D and MCPA in microbial cultures enriched with structurally-similar plant secondary metabolites. *Water*, *11*, 1451.

Morgan, M. F. (1941). *Chemical Soil Diagnosis by the Universal Soil Testing System*. Connecticut Agricultural Experiment Station.

Morton, P. A., Fennell, C., Cassidy, R., Doody, D., Fenton, O., Mellander, P. E., & Jordan, P. (2019). A review of the pesticide MCPA in the land-water environment and emerging research needs. *WIREs Water*, *7*, 1–16.

Mu, W., Yu, F., Li, C., Xie, Y., Tian, J., Liu, J., & Zhao, N. (2015). Effects of rainfall intensity and slope gradient on runoff and soil moisture content on different growing stages of spring maize. *Water*, *7*, 2990–3008.

Muszynski, P., Brodowska, M. S., & Paszko, T. (2020). Occurrence and transformation of phenoxy acids in aquatic environment and photochemical methods of their removal: A review. *Environmental Science and Pollution Research International*, *27*, 1276–1293.

Najafi, H., Bazoubandi, M., & Jafarzadeh, N. (2013). Effectiveness of repeated reduced rates of selective broadleaf herbicides for Postemergence weed control in sugar beet (*Beta vulgaris*) world. *Journal of Agricultural Research*, *1*, 25–29.

Nath, C. P., Das, T. K., Rana, K. S., Bhattacharyya, R., Pathak, H., Paul, S., Meena, M. C., & Singh, S. B. (2017). Weed and nitrogen management effects on weed infestation and crop productivity of wheat–Mungbean sequence in conventional and conservation tillage practices. *Agricultural Research*, *6*, 33–46.

Paulin, M. M., Nicolaisen, M. H., & Sorensen, J. (2011). (R, S)-dichlorprop herbicide in agricultural soil induces proliferation and expression of multiple dioxygenase-encoding genes in the indigenous microbial community. *Environmental Microbiology*, *13*, 1513–1523.

Pena, A., Delgado-Moreno, L., & Rodriguez-Liebana, J. A. (2020). A review of the impact of wastewater on the fate of pesticides in

soils: Effect of some soil and solution properties. *Science of the Total Environment*, *718*, 134468.

Peña, D., López-Piñeiro, A., Albarrán, Á., Becerra, D., & Sánchez-Llerena, J. (2015). Environmental fate of the herbicide MCPA in agricultural soils amended with fresh and aged de-oiled two-phase olive mill waste. *Environmental Science and Pollution Research*, *22*, 13915–13925.

Perez-Pantoja, D., Leiva-Novoa, P., Donoso, R. A., Little, C., Godoy, M., Pieper, D. H., & Gonzalez, B. (2015). Hierarchy of carbon source utilization in soil bacteria: Hegemonic preference for benzoate in complex aromatic compound mixtures degraded by *Cupriavidus pinatubonensis* strain Jmp134. *Applied and Environmental Microbiology*, *81*, 3914–3924.

Rakhimol, J. R., Thomas, S., Volova, T., & Springerlink, K. J. (2019). *Controlled release of pesticides for sustainable agriculture*. Springer.

Raman, P., & Sathiyanarayanan, D. (2009). Physico–chemical characteristics of soil and influence of cation exchange capacity of soil in and around Chennai. *Rasayanjournal*, *2*, 875–885.

Roache, M. (2020). *Sustainable Use of Pesticides Directive* [Online]. Accessed November 15, 2023 https://www.teagasc.ie/publications/2020/sustainable-use-of-pesticides-directive.php

Rosemary, F., Vitharana, U. W. A., Indraratne, S. P., Weerasooriya, R., & Mishra, U. (2017). Exploring the spatial variability of soil properties in an Alfisol soil catena. *Catena*, *150*, 53–61.

Saleh, O., Pagel, H., Enowashu, E., Devers, M., Martin-Laurent, F., Streck, T., Kandeler, E., & Poll, C. (2016). Evidence for the importance of litter as a co-substrate for MCPA dissipation in agricultural soil. *Environmental Science and Pollution Research International*, *23*, 4164–4175.

Samuelsen, E. D., Badawi, N., Nybroe, O., Sorensen, S. R., & Aamand, J. (2017). Adhesion to sand and ability to mineralise low pesticide concentrations are required for efficient bioaugmentation of flow-through sand filters. *Applied Microbiology and Biotechnology*, *101*, 411–421.

Schulte, E. E., & Hopkins, B. G. (1996). Estimation of soil organic matter by weight loss-on-ignition. *SSSA Special Publication*, *46*, 21–31.

Singh, N. K., Sanghvi, G., Yadav, M., Padhiyar, H., Christian, J., & Singh, V. (2023). Fate of pesticides in agricultural runoff treatment systems: Occurrence, impacts and technological progress. *Environmental Research*, *237*, 117100.

Smith, A. E., & Aubin, A. J. (1989). Persistence studies with the herbicide clopyralid in prairie soils at different temperatures.

Tandon, S., & Singh, A. (2022). Residue behavior of Clopyralid herbicide in soil and sugar beet crop under subtropical field conditions. *Journal of Food Protection*, *85*, 735–739.

Tao, C., Li, R., Xiong, W., Shen, Z., Liu, S., Wang, B., Ruan, Y., Geisen, S., Shen, Q., & Kowalchuk, G. A. (2020). Bio-organic fertilizers stimulate indigenous soil Pseudomonas populations to enhance plant disease suppression. *Microbiome*, *8*, 137.

Tonso, N., Matheson, V., & Holben, W. (1995). Polyphasic characterization of a suite of bacterial isolates capable of degrading 2,4-D. *Microbial Ecology*, *30*, 3–24.

Vasic, V., Hajnal-Jafari, T., Djuric, S., Kovacevic, B., Stojnic, S., Vasic, S., Galovic, V., & Orlovic, S. (2022). Effect of herbicide Clopyralid and Imazamox on dehydrogenase enzyme in soil of regenerated Pedunculate oak forests. *Forests*, *2022*(13), 926.

Villamizar, M. L., Stoate, C., Biggs, J., Morris, C., Szczur, J., & Brown, C. D. (2020). Comparison of technical and systems-based approaches to managing pesticide contamination in surface water catchments. *Journal of Environmental Management*, *260*, 110027.

White, A. M., Gonzalez Vazquez, A., Mcdaniel, E. A., Peterson, B. D., Koch, P., Remucal, C. K., & Mcmahon, K. D. (2022). Expanded diversity of *tfdA* harboring bacteria across the natural and built environment. *bioRxiv*, 2022–09.

Zhang, X., Wang, N., Hou, M., Wu, H., Jiang, H., Zhou, Z., Chang, N., Wang, Q., Wan, X., Jiang, J., Shen, Z., & Li, Y. (2022). Contribution of K solubilising bacteria (*Burkholderia* sp.) promotes tea plant growth (*Camellia sinesis*) and leaf polyphenols content by improving soil available K level. *Functional Plant Biology*, *49*, 283–294.

How to cite this article: Scannell, S., Healy, M. G., Sambrano, G., McGinley, J., Ryan, P. C., Mellander, P.-E., Morrison, L., Harmon O'Driscoll, J., & Siggins, A. (2024). A split herbicide application strategy reduces surface runoff. *Soil Use and Management*, *40*, e13086. https://doi.org/10.1111/sum.13086