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Short title: Herbicide fate in cover crops

Influence Of Cover Crop Use on Soil Microbial Activity and Fate of Sulfentrazone, *S*-Metolachlor, Cloransulam-Methyl, Atrazine, and Mesotrione

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Residual herbicides are primarily degraded in the soil through microbial breakdown. Any practices that result in increased soil biological activity, such as cover cropping (between cash crop seasons), could lead to a reduced persistence of herbicides in the soil. Furthermore, cover crops can also interfere with herbicide fate by interception. Field trials were conducted between 2020 and 2023, in a corn-soybean rotation, to investigate the influence of cover crop [cereal rye (*Secale cereale* L.) and crimson clover (*Trifolium incarnatum* L.) use on soil enzyme activities [β-glucosidase (BG) and dehydrogenase (DHA)], its effect on the concentration of residual herbicides (sulfentrazone, *s*-metolachlor, cloransulam-methyl, atrazine, and mesotrione) in the soil, and the interception of herbicides by cover crop residue. The use of cover crops occasionally resulted in increased BG and DHA activities relative to the fallow treatment. However, even when there was an increase in the activity of these two enzymes, increased degradation of the residual herbicides was not observed. The initial concentrations of all residual herbicides in the soil were significantly reduced due to interception by cereal rye biomass. Nevertheless, significant reductions in early season weed biomass were observed when residual herbicides were included in the tank mixture applied at cover crop termination relative to the application of glyphosate plus glufosinate. Results from this research suggests that the use of cereal rye or crimson clover as cover crops (between cash crop seasons) do not impact the persistence of residual herbicides in the soil nor reduce their efficacy in controlling weeds early in the growing season.

Keywords: Cover crop, herbicide fate, soil microbial activity, soil residual herbicide.

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Introduction

The use of cover crops has increased significantly across the U.S. within the last decade. The state of Indiana has been a leader of cover crop adoption, with a little over 650,000 ha planted to cover crops in 2022 in comparison to the 498,000 ha in 2014 (ISDA 2023). Despite all the incentives from federal and state agencies as well as the crop production industry, this represents only 12.8% of Indiana's cropland (USDA-NASS 2023). Cover crops have been recommended as one strategy to improve the physical, chemical, and biological properties of soil (Baumhardt et al. 2015; Chen et al. 2022; Du et al. 2022; Muhammad et al. 2021), and reduce ground and surface water contamination (Lacey and Armstrong 2015; Ruffatti et al. 2019). Furthermore, some cover crop species such as cereal rye (*Secale cereale* L.) can suppress weed emergence and growth, being a valuable integrated weed management tool (Hodgskiss et al. 2020; Loux et al. 2017; Petersen et al. 2023).

One of the most frequently documented benefits of cover crop use is the increase in the soil organic matter (SOM) content (Moore et al. 2014; Poeplau and Don 2015; Villamil et al. 2006). Soil organic matter is the primary source of energy used by microorganisms to survive and multiply (Fontaine et al. 2003; Gunina and Kuzyakov 2022). Fungi and bacteria are examples of microorganisms that are constantly producing enzymes and releasing some of them into the soil solution. These enzymes are categorized into indicators of overall microbial activity (e.g. dehydrogenase – intracellular) or specific to certain nutrient cycles (e.g. hydrolases – extracellular). Dehydrogenase (DHA) is classified within the oxidoreductases, the largest enzyme group, and responsible for catalyzing redox reactions of organic compounds (e.g., pesticides) in the soil (Dixon et al. 1979). The hydrolases are responsible for catalyzing the carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycles in the soil. Within hydrolases, β-glucosidase (BG) decomposes SOM, which will ultimately result in the production of glucose, an energy source for soil microbes (Deng and Tabatabai 1994).

Among the several plant species currently used as cover crops, cereal rye is the most commonly used and is known for producing large amounts of above and belowground biomass as well as scavenging residual N left in the soil by the previous crop or applied as fertilizer in fall (Kaspar et al. 2007; Kladivko et al. 2014; Lacey and Armstrong 2015; Ruffatti et al. 2019). Once terminated, the cereal rye residue is slowly mineralized and incorporated into the SOM. Although not known for producing large amounts of biomass, leguminous cover crop species

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such as crimson clover (*Trifolium incarnatum* L.) also contribute to the SOM pool primarily by fixing N from the atmosphere (Smith et al. 1982), which is released into the soil upon degradation of the plant residue. More recently, the vast majority of the initiatives that promote the use of cover crops list soil health as one of the main benefits from this practice (Chami et al. 2023; Myers et al. 2019). Although the term "soil health" is subjective, increased microbial activity is a principal component documented in several studies (Adetunji et al. 2020; Brennan and Acosta-Martinez 2019; Finney et al. 2017; Kim et al. 2020; Nevins et al. 2018, 2020). The increased microbial activity are, for the most part, related to the increases in the SOM content as result of cover crop residue decomposition, moisture conservation, enhanced aggregate stability, and improved drainage (Dinesh et al. 2009; Hu et al. 2023; Mendes et al. 1999; dos Santos Cordeiro et al. 2021).

In addition to the mineralization of SOM, soil microorganisms can also utilize herbicides and other commonly used pesticides as an alternative source of C and N (Qiu et al. 2009). In fact, most of the herbicide degradation that occurs in soils is promoted by microbes (Van Eerd et al. 2003). Currently, there is no consensus about the effect of herbicides on soil enzymes. While some studies have reported no effect (Niemi et al. 2009; Omar and Abdel-Sater 2001), others have described either negative (Du et al. 2018; Mukherjee et al. 2016) or positive impacts (Kucharski et al. 2016; Singh and Ghoshal 2013) of herbicides on β-glucosidase activity. Unlike β-glucosidase, dehydrogenase generally shows reduced activity in the presence of herbicides (Bennicelli et al. 2009; Sebiomo et al. 2010; Tomkiel et al. 2019). However, this reduction is temporary and the activity increases as the population of microbes that are capable of degrading the herbicide increases (Cole 1976; Robertson and Alexander 1994; Sebiomo et al. 2010; Tyagi et al. 2018). Research conducted by Weaver et al. (2007) demonstrated that, even when applied at threefold label rates, glyphosate did not cause microbial community shifts in the soil. Furthermore, under laboratory settings, these authors concluded that the application of glyphosate caused only small and transient (< 7 days) effects on the soil microbial community. In general, the response of a soil enzyme to a given pesticide is practically unpredictable because different pesticides can either increase, decrease, or result in no effect to the enzyme. In addition, the response of soil enzymes to the presence of pesticides also vary by pesticide rate and soil type (Schaffer 1993).

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Establishing cover crops as the sole weed management strategy rarely results in acceptable season-long weed control (Burgos and Talbert 1996; Teasdale 1993; Teasdale et al. 2005). The competition for light, water, and nutrients, as well as the release of allelochemicals are the primary ways of weed suppression during cover crop growth. Once terminated, cover crops can suppress weeds through the physical barrier created by the residue left on the soil surface. The more biomass that is accumulated by the cover crop, the greater amount of weed suppression is achieved (MacLaren et al. 2019; Osipitan et al. 2019). However, one disadvantage of late cover crop termination (i.e., more biomass accumulation) is the interference with cash crop development. Corn, for instance, is very sensitive to late termination of cover crops, showing nutrient deficiencies and stunted growth due to nutrient (primarily N) immobilization during mineralization of the cover crop residue (Nevins et al. 2020; Reed et al. 2019; Rosa et al. 2021). Thus, in most cases, the recommendation is to terminate the cover crop at least two weeks prior to corn planting (Acharya et al. 2017). At this stage, biomass accumulation by the cover crop is usually not enough to physically suppress weed emergence throughout the entire growing season. Therefore, the inclusion of soil residual herbicides at cover crop termination is essential to extend the period of weed control (Whalen et al. 2020).

In order to provide adequate weed control, soil residual herbicides must have a proper placement, movement into the weed germination zone of the soil, and length of residual control. The length of residual control is affected by some soil properties such as SOM and clay content and environmental conditions such as temperature and rainfall volume. Furthermore, the residual activity of herbicides is also influenced by the overall microbial activity of the soil (García-Delgado et al. 2019). Therefore, the adoption of agronomic practices that have the potential to increase the activity of soil microorganisms, such as the use of cover crops, could also lead to an increased degradation of herbicides in the soil. However, to date, there is no evidence in the literature that supports this hypothesis.

Another way cover crops can influence the fate of soil residual herbicides is by interception. This interception occurs at the time of application and is directly related to the amount of cover crop biomass present, with more biomass resulting in more interception (Nunes et al. 2023). Once intercepted, residual herbicides can only move onto the soil surface with rainfall or irrigation. This movement, or leaching, of the herbicide is affected by the volume of water that falls onto the cover crop and also by the chemical properties of the herbicide. The

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more rainfall or irrigation, the greater amount of herbicide will leach onto the soil (Khalil et al. 2019). Herbicides with higher water solubility (e.g., mesotrione, cloransulam-methyl) have a tendency to be washed off of the plants more easily than those with lower solubility (e.g., *S*-metolachlor, trifluralin) (Khalil et al. 2019). In addition to the water volume and herbicide solubility, the maturity of the cover crop also affects the herbicide leaching from the plants onto the soil, with older plants having a lower ratio of cellulose:lignin than younger plants (i.e., during plant residue decomposition, enzymes such as β-glucosidase will breakdown cellulose molecules, thus exposing lignin molecules). Lignin is considered a recalcitrant cell wall component (Vanholme et al. 2010) and, in some plant parts, can account for 60 to 80% of the secondary cell wall composition (Musha and Goring 1975). Research conducted by Dao (1991) suggested that most of the herbicide binding onto the plant surface occurs at the exposed lignin sorption sites, while herbicide binding to cellulose is minimal. The lack of rainfall or irrigation after the application of a residual herbicide at cover crop termination results in reduced concentrations of these pesticides in the soil. Not only because the herbicide that was intercepted by the cover crop residue and will not leach onto the soil but also because the herbicide that reaches the soil at the time of application will not be incorporated into the top layer of soil. Ultimately, lower concentrations of residual herbicides in the soil leads to a greater reliance on postemergence herbicides to achieve acceptable weed control during the critical weed-free period (Loux et al. 2011), which goes against the principles of herbicide resistance management.

Most of the research conducted thus far has focused on the weed control efficacy of residual herbicides when applied at cover crop termination. However, there are still confounding factors such as microbial degradation and interception by cover crop residue that could be negatively impacting the efficacy of these herbicides. Knowing how these two factors affect herbicide fate in cover cropping systems is essential to further improve this practice. Therefore, the objectives of this research were (1) to evaluate the effect of cover crop use on soil microbial activity, (2) to measure the concentration of residual herbicides in the soil when applied at cover crop termination, (3) to correlate microbial activity and herbicide concentration in the soil, (4) to investigate herbicide interception by cover crops and further leaching onto the soil, and (5) to assess early- and late-season weed biomass as influenced by cover crop and herbicide treatments.

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Materials and Methods

Field trials were established at Throckmorton (40.29°N, 86.90°W) and Pinney (41.44°N, 86.92°W) Purdue Agricultural Centers (TPAC and PPAC, respectively) near Lafayette and Wanatah, IN, respectively, in September of 2019. Both trials remained in the same location until October of 2023 when final data were collected. Fields for each trial were managed as a conventional corn-soybean rotation prior to the initiation of the study, and were planted to soybeans during the 2019 growing season. The soil at TPAC consisted of a Drummer silty clay loam (22% sand, 53% silt, and 25% clay). The soil at PPAC consisted of a Tracy sandy loam (70% sand, 17% silt, and 13% clay). Soil samples were taken in March of each year and used to determine fertility parameters of the soil in each trial (Table 1). Soil from each site was tilled prior to first cover crop planting, in September of 2019, using a rotary tiller, at 10 cm deep and then managed as a transitional no-till system following a corn-soybean rotation during the subsequent years.

Treatments were arranged in a split plot design and included two cover crop species, cereal rye and crimson clover, as well as a no cover crop control as main plots. The soil residual herbicides tested were randomized in each main plot, replicated four times, and divided into no residual, medium residual and heavy residual (residual load based on the number of herbicides applied) (Table 2) for a total of 36 experimental units. Plots were 3 m by 8 m in size. Cover crops were planted in the fall of each year using a no-till drill (John Deere 1590, John Deere Co., Moline, IL) at 19 cm row spacing, at seeding rates of 112 and 22.5 kg ha^{-1} of cereal rye (Hazlet, Cisco Company, Indianapolis, IN) and crimson clover (Dixie, Cisco Company, Indianapolis, IN), respectively (Table 3). In the spring of 2020 and 2022, herbicide resistant corn (SmartStaxTM DKC 62-52RIB, Bayer Crop Science, Saint Louis, MO) was planted at 86,450 seeds ha^{-1}, in 76 cm row spacing (Table 3). In both years, starter fertilizer was applied at corn planting at 34 kg N ha^{-1} (19-17-00) and a sidedress application was made near V6 growth stage at 200 kg N ha^{-1} (UAN 28-00-00). In the spring of 2021 and 2023, herbicide resistant soybean (Enlist 3 P26T57E, Corteva, Johnston, IA) was planted at 350,000 seeds ha^{-1}, in 38 cm row spacing (Table 3).

Herbicides were applied traveling at 4.8 km h^{-1} using a CO_2-pressurized spray boom equipped with eight AIXR 110015 (TeeJet Spraying Systems Co., Wheaton, IL) nozzles spaced 38 cm apart and calibrated to deliver 140 L ha^{-1} and operating at 165 kPa. Glyphosate (Roundup PowerMax, Bayer Crop Science, Saint Louis, MO) and glufosinate (Liberty 280 SL, BASF,

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Research Triangle Park, NC) were applied in tank mix at 1,750 g ae ha^{-1} and 737 g ai ha^{-1}, respectively, at four weeks after corn or soybean planting to all plots. Non-ionic surfactant (Class Act Ridion, WinField Solutions, LLC, St. Paul, MN) and ammonium sulfate (AMSOL, WinField Solutions, LLC, St. Paul, MN) were added to all herbicide applications, at 0.25 and 5% v/v, respectively (cover crop termination and postemergence application).

Cover crop and weed biomass were determined separately one day before spring termination using a 0.25 m^2 quadrat that was randomly placed within the first 1 m (length wise) of each plot. All aboveground plant material inside the quadrat was harvested by cutting the plants at the base (1 cm above soil surface) with scissors and placed in separate bags for cover crop and weed biomass samples. Bags were placed in a forced-air oven at 80 C for 96 h. Dry weights were recorded and converted to kg ha^{-1}.

Weed biomass was also determined at 4 (from 2021 until 2023) and 18 weeks after termination (WAT) (from 2020 until 2023). Two 0.25 m^2 quadrats were randomly placed between the two center rows of the cash crop, one in the front and one in the back of each plot. All plant material within each quadrat was harvested by cutting the plants at the base (1 cm above soil surface) with scissors. Samples were placed in a forced-air oven at 80 C for 96 h. Dry weights were recorded and converted to kg ha^{-1}. Starting in 2021, weed biomass was also determined following the same method, at four weeks after cash crop planting.

In 2020, soil samples were collected five days before cover crop termination and at 21, 28, 56, 84, and 112 days after termination (DAT) to determine soil enzyme activity and the concentrations of residual herbicides. For all subsequent years, soil samples were collected five days before cover crop termination and at 0, 10, 14, 28, 56, 84, and 112 DAT. Soil samples taken before cover crop termination were used to determine the base levels of soil microbial activity. Fourteen soil cores were collected at 0 to 5 cm deep for each plot, using a 2 cm diameter probe. The cores were homogenized to form one composite sample per plot and refrigerated at 4 C until processing. The soil probe was cleaned with a 50% acetone solution between plots to avoid sample contamination. No more than 1 d after collection, soil samples were passed through a 2 mm sieve and thoroughly homogenized, and then an aliquot of approximately 60 g was placed in a 50 ml Falcon tube and stored at – 20 C prior to analysis of herbicide concentration. A 50% acetone solution was used to clean the sieve between samples. The remainder of the soil sample was kept at 4 C and then used to measure the activity of BG and DHA. Sample storage time was

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kept constant prior to each enzyme assay across sampling events, sites, and years and was never more than 72 h after sampling. Soil moisture from each sample was determined prior to the enzyme assays from a 5 g subsample that was placed in a forced-air oven at 105 C for 48 h.

β-glucosidase activity was measured according to the method described by Eivazi and Tabatabai (1988), with adaptations. The method used in this study differs from the standard bench scale method by reducing all chemicals and soil amount by 90%. All glassware was substituted by 2-ml microcentrifuge tubes and the filtration step was substituted by centrifuge. Briefly, 0.1 g (± 0.01) of soil was weighed (in triplicate plus one control sample) into 2-ml microcentrifuge tubes. Twenty µl of toluene (Fisher Scientific, Fair Lawn, NJ) were added to all samples. After 15 min, 100 µl of PNG solution [50 mM p-nitrophenyl-β-d-glucopyranoside (Acros Organics, Pittsburgh, PA)] and a modified universal buffer solution (MUB; pH 6) were added to the samples, except for the control samples that received only MUB. Samples were then thoroughly mixed and incubated at 37 C (± 1 C) for one hour. After incubation, 100 µL of calcium chloride (0.5 M; Fisher Scientific, Fair Lawn, NJ) and 400 µL of THAM [tris(hydroxymethyl)aminomethane buffer (100 mM, pH 12)] were added to all samples. PNG was added to the control samples only after the addition of THAM, which stops the enzymatic reaction. Eight blank samples, without soil, were included in the assay and treated like the experimental samples (same chemicals added and same incubation conditions). Tubes were then placed in a centrifuge and spun at 13,000 rpm for eight min. Two hundred µL of the supernatant were transferred to a 96 well microplate (costar flat-well medium binding polystyrene; cat no. 9017; Corning, Corning, NY). Absorbance of the supernatant was measured at 405 nm with a microplate reader (Multiskan Sky 96-well Microplate Reader; Thermo Scientific, Waltham, MA). Amount of *p*-nitrophenol (pNP 1 mM; Sigma Chemicals, St. Louis, MO) released from each sample was calculated from a calibration curve that was prepared using a 10 mM pNP solution diluted in water to achieve concentrations equivalent to 0, 0.01, 0.02, 0.03, 0.04, 0.05 mM of pNP. β-glucosidase activity was then expressed in µmol pNP g^{-1} dry soil hr^{-1}.

Dehydrogenase activity was measured according to the method described by Benefield et al. (1977) and adapted by von Mersi (1996), von Mersi and Schinner (1991), and Shaw and Burns (2006). Additional adaptations were made to improve efficiency of the process and reduce the use of chemicals and laboratory supplies. This assay was conducted in the dark to avoid photodegradation of INT [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride;

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Sigma Chemicals, St. Louis, MO]. Briefly, 0.1 g (± 0.01) of soil was weighed (in triplicate plus one control sample) into 2-ml microcentrifuge tubes. Control samples were autoclaved at 120 C for 20 min. Then, 200 µL of INT solution (0.5% w/v; Alfa Aesar, Ward Hill, MA) and 150 µL THAM buffer (1 M; pH 7) were added to all samples. Tubes were closed, shaken vigorously, and placed in the incubator for two h at 37 C (± 1 C). Immediately after incubation, 1 ml of an extractant solution (1:1 N,N-dimethylformamide and ethanol) was added to all tubes that were then left at room temperature for another incubation of one hour, being shaken (vortex mixer) every 20 min. Tubes were centrifuged at 13,000 rpm for eight min after the last incubation. Then, 200 µL of supernatant were transferred to a 96 well plate (polypropylene; Eppendorf, Hamburg, Germany). Absorbance of the supernatant was measured at 464 nm with a microplate reader (Multiskan Sky 96-well Microplate Reader; Thermo Scientific, Waltham, MA). Amount of INTF (iodonitrotetrazolium formazan; Sigma Chemicals, St. Louis, MO) released from each sample was calculated from a calibration curve prepared using INTF standard (100 mg INTF ml^{-1}; dissolved in extractant solution) concentrations equivalent to 0, 25, 50, 100, 200, 300, and 500 µg of INTF in 6.75 ml of solution. Dehydrogenase activity was expressed in µg INTF g^{-1} dry soil hr^{-1}.

The concentration of soil residual herbicides in the soil was determined following the QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) extraction method (Anastassiades et al. 2003), with modifications. Briefly, soil samples were thawed and a 3-g aliquot was weighed into 50-ml Falcon tubes. Fifteen ml of deionized water and acetonitrile (OptimaTM LC/MS grade with 1% formic acid; Fisher Scientific, Fair Lawn, NJ) and 10 µL of internal standards were added to the tubes that were then shaken (vortex mixer) for one minute. After shaking, 6 g of $MgSO_4$ and 1.5 g of NaOAc were added to the samples. Subsequently, tubes were placed in a Geno/Grinder 2010 (SPEX Sample Prep, Metuchen, NJ) and shaken at 1000 rpm for 3 min and then centrifuged at 2500 rpm for 10 min. Twelve ml of the supernatant were transferred into 15-ml dispersive solid phase extraction tubes (Part No. 5982-5158; Agilent Technologies, Palo Alto, CA) that were then shaken at 1100 rpm for 5 min on the Geno/Grinder. Tubes were centrifuged at 4000 rpm for 5 min and the entire supernatant was transferred into a 15-ml centrifuge tube. Samples were dried in a SpeedVac (SPD1030, Thermo Scientific, San Jose, CA) over night. Pellets were resuspended with 150 ml of 100% acetonitrile (OptimaTM LC/MS grade, Fisher Scientific, Fair Lawn, NJ) and vortexed until fully dissolved. Tubes were placed in a centrifuge

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at 4000 rpm for 5 min and 130 µL of the supernatant were transferred into a 96-well microplate (polypropylene; Eppendorf, Hamburg, Germany) prior to the analysis in the UHPLC. All samples were analyzed in an Agilent 1290 Infinity II ultra-high performance liquid chromatography (UHPLC) with a 6470 triple quadrupole mass spectrometry and a EclipsePlus C18 RRHD 1.8µm, 2.1x50mm column (Agilent technologies, Santa Clara, CA) at the Bindley Bioscience Center at Purdue University. Recoveries from fortified untreated soil samples indicated that recovery was 112, 80, 74, 113, and 70% for sulfentrazone, *s*-metolachlor, cloransulam-methyl, atrazine, and mesotrione, respectively.

All data were subjected to an Analysis of Variance (ANOVA) using the PROC GLIMMIX procedure in SAS 9.4. There was a significant treatment by year interaction for the early- and late-season weed biomass, residual herbicide concentration, and enzyme activities. Therefore, results were presented separately by year. The interaction between cover crop and herbicide treatments for early- and late-season weed biomass was non-significant, therefore, data were combined over cover crop and fallow treatments within each year. Assumptions of normality and homogeneity of variance were evaluated by visual assessment of residual plots. Data were log or square-root transformed when needed. However, original mean values are presented. Means were separated using Tukey's honest significant difference (HSD) (enzyme activity and herbicide concentration data) or Fisher's protected LSD (early- and late-season weed biomass data) ($\alpha = 0.05$). Pearson's correlation coefficients (PROC CORR procedure in SAS) were used to identify significant ($\alpha = 0.05$) relationships between soil microbial activity and concentration of residual herbicides in the soil.

Results and Discussion

Cover crop and weed biomass at termination. Cereal rye and crimson clover biomass accumulation was, on average, greater at TPAC than PPAC (Figure 1). Although the cover crop planting and termination dates are similar between sites, the overall soil fertility parameters (Table 1) from TPAC were better than those from PPAC, which resulted in greater cover crop growth. Furthermore, average monthly temperatures during the cover crop growing season were slightly higher at TPAC (data not shown). The average cereal rye biomass accumulation at TPAC and PPAC, across all years, was 4,210 and 2,623 kg ha^{-1}, respectively. The average crimson clover biomass accumulated in that same period was 1,342 and 1,099 kg ha^{-1} at TPAC

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and PPAC, respectively. In general, crimson clover biomass accumulation was fairly low in both sites. According to the midwest cover crop decision tool, the cutoff date for crimson clover planting in Indiana is September 18 (MCCC 2024). With the exception of the first cover crop planting in 2019, all other planting dates were between the first and second week of October. In the fall of 2022, the combination of a late planting date with exceptionally dry weather conditions (Figure 2) resulted in the germination of very few crimson clover plants in the fall. Hodgskiss et al. (2020) conducted field trials at TPAC in the two years prior to this research and reported up to 1,476 and 3,709 kg ha^{-1} of crimson clover and cereal rye biomass, respectively. Also, these authors did not have success in establishing crimson clover in one of the two years of their study.

Fallow plots were not kept weed-free during the study. At the time of cover crop termination, the predominant weed species present in the fallow plots and plots with low crimson clover stands were: common chickweed [*Stellaria media* (L.) Vill.], henbit (*Lamium amplexicaule* L.), purple deadnettle (*Lamium purpureum* L.), and shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.]. Cereal rye plots were, for the most part, free of winter-annual weed species at the time of spring termination.

Early-season weed control. The primary weed species present in the trial areas were: giant ragweed (*Ambrosia trifida* L.; TPAC only), waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.), horseweed [*Conyza canadensis* (L.) Cronquist; syn. *Erigeron canadensis* L.; TPAC only), giant foxtail (*Setaria faberi* Herrm.), yellow foxtail [*Setaria pumila* (Poir.) Roem. & Shult.], barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], and fall panicum (*Panicum dichotomiflorum* Michx.). The overall weed density was much higher at TPAC compared to PPAC. The experimental area at PPAC was used for commercial grain production for several years prior to the beginning of the study, while at TPAC the field was maintained as a weed science research area for at least 10 years prior to the study initiation.

No cover crop by herbicide interactions were observed for early-season weed biomass, while the main effect of herbicide was significant. Therefore, data was pooled over cover crop and fallow treatments within each year (Table 4). The use of residual herbicide at cover crop termination consistently reduced weed biomass at 4 WAP relative to the termination without residual herbicides. Between 2021 and 2023, the application of residual herbicides resulted in an

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average of 88 and 81% reduction in weed biomass at 4 WAP at PPAC and TPAC, respectively. At PPAC, the heavy residual herbicide program increased the weed control relative to the medium herbicide program only in 2023. In 2022 and 2023, at TPAC, the inclusion of a third residual herbicide in the tank mixture applied at cover crop termination improved the weed control by an average of 18% compared to the termination with two residual herbicides. This improved weed control observed at TPAC was likely the result of adding either cloransulam-methyl (2023) or mesotrione (2022) to the tank mixture, which resulted in greater control of *A. trifida* (data not shown). Investigating the control of *A. trifida* following the application of several residual herbicides in combination or not with mesotrione, Westrich et al. (2024) reported up to 84% reduction in *A. trifida* biomass as result of mesotrione application (alone or in tank mixture with the other herbicides evaluated). Similarly, other authors have observed up to 94% control of ALS-susceptible *A. trifida* four weeks after the application of cloransulam-methyl (Follings et al. 2013). In this study, the use of cereal rye and crimson clover cover crops resulted in similar weed control to the fallow treatment at 4 WAP in both locations and all years of data collection.

Late-season weed control. Late-season biomass was estimated in all four years of the study. However, in 2021 and 2023, when soybean was planted as cash crop, all plots from both locations were weed-free at 18 WAP. The primary weed species present in the trial areas in 2020 and 2022 were *S. media* (predominant species), common purslane (*Portulaca oleracea* L.), *A. tuberculatus, A. retroflexus, C. album, S. faberii, S. pumila, E. crus-galli,* and *P. dichotomilforum*.

No cover crop by herbicide interactions were observed for late-season weed biomass, while the main effect of herbicide was significant. Therefore, data was pooled over cover crop and fallow treatments within each year (Table 5). For the 18 WAP evaluation timing, the use of residual herbicides at cover crop termination did not result in greater weed control relative to the no residual program. One exception was at PPAC, in 2020, when the application of three residual herbicides improved the weed control at 18 WAP by 74% in comparison to the application of glyphosate plus glufosinate only. In the same location and year, the use of two residual herbicides at cover crop termination provided similar weed control to the no residual program. The average weed biomass for the whole trial area was 119 and 676 kg ha^{-1} in 2020 and 4 and 36 kg ha^{-1} in 2022 at PPAC and TPAC, respectively, at 18 WAP. Although crop yield was not

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determined in this study, other researchers have observed corn yield losses ranging from 11 to 74% in the presence of 2,220 to 5,900 kg ha^{-1} of *A. tuberculatus* biomass (Steckel and Sprague 2004). Fausey et al. (1997), investigated the impact of increasing ***S. faberi*** densities in corn yield and reported losses ranging from 14 to ~50% when densities were increased from 10 to 100 ***S. faberi*** plants m^{-1} of corn row. Similar to what was observed at 4 WAP, the use of cereal rye and crimson clover cover crops did not provide greater weed control than the fallow treatment later in the growing season at PPAC and TPAC, between 2020 and 2023.

Soil enzyme activities

β-glucosidase. The effect of cover crop use on BG activity is shown in Figure 3 and the detailed means separation for each site and year can be found Tables S1 and S2 (available in the supplemental material). No cover crop by herbicide interactions were observed for BG activity, while the main effect of cover crop was significant. Therefore, data was pooled over herbicide treatments within each year and sample timing (Figure 3). In general, BG activity was 38% higher at TPAC compared to PPAC. This result was expected because the average SOM content from TPAC (2.7%) was 1.7-fold greater than that from PPAC (1.6%) (Table 1). β-glucosidase has a critical role in the organic matter cycling in soils, thus being responsive to changes in SOM content (Bandick and Dick 1999; Debosz et al. 1999; Eivazi and Tabatabai 1988; Monreal and Bergstrom 2000; Sinsabaugh et al. 2008; Turner et al. 2002). β-glucosidase activities measured in our field studies are comparable to those reported in previous studies with similar SOM contents. Bandick and Dick (1999) measured BG activities ranging from 0.3 to 1.5 µmol pNP g^{-1} hr^{-1} in soils with SOM varying from 2.3 to 3.8%. Similarly, Eivazi and Tabatabai (1988), who developed the assay to measure BG activity in soils, reported activities from 0.07 to 2.12 µmol pNP g^{-1} hr^{-1} in soils with SOM ranging from 0.8 to 9.4%.

The use of crimson clover as cover crop for four growing seasons resulted in increased BG activity relative to the fallow in an average of 34 and 32% of the soil sample timings at TPAC and PPAC, respectively. However, significant amounts of crimson clover biomass were achieved only in the first year of the study with an average of 3,145 kg ha^{-1} of biomass across TPAC and PPAC. For the subsequent years, the average crimson clover biomass for both sites was 580 kg ha^{-1}. Therefore, data from this research is not enough to provide meaningful conclusions regarding the effect of crimson clover on BG activity. Nevertheless, other researchers have found increased BG activity as result of crimson clover use as cover crop when

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average biomass accumulation reached 5,972 kg ha^{-1} (Tyler 2020). On the other hand, when cereal rye was used as cover crop, we observed increased BG activity relative to the fallow in an average of 32 and 86% of the soil sample timings at TPAC and PPAC, respectively, across four years of data collection (Tables S1 and S2, available in the supplemental material). When treatment differences occurred, the use of cereal rye resulted in an average of 14 and 27% increase in BG activity relative to the fallow, at TPAC and PPAC, respectively. These results are consistent with those of previous research by Eivazi et al. (2024) that evaluated the effect of cereal rye management strategies on soil enzyme activities and reported up to 39% greater BG activity in plots with cereal rye relative to the no cover crop control. In respect to the frequency of increased BG activity with cereal rye, other researchers have found contrary results to our study. Tyler (2020) assessed the effect of cereal rye (average biomass: 3,840 kg ha^{-1}) on BG activity in soils with SOM contents above 4.9%. These authors found consistent increases in BG activity in the soil of plots with cereal rye in comparison to the no cover crop control for all samples timings during three years. However, our results showed that the consistent increase in BG activity with cereal rye was achieved only in our low SOM site (1.6%; PPAC) and after two years of cover crop adoption. In the moderate SOM site (2.7%; TPAC), cereal rye use resulted in increased BG activity relative to the fallow in no more than 5 out of 8 sample timings during four years of data collection. We suggest that the consistent BG activity increase as result of cereal rye use at PPAC is due to the low SOM background levels from that site. In other words, the C:N input (not excluding other elements present in the biomass but in lower concentrations) from cereal rye use was enough to result in significant differences in BG activity between the cereal rye and fallow treatments in a short period of time only where the starting point for the SOM content was low. Whereas at TPAC, where the SOM levels were higher (relative to PPAC) to begin with, the C:N input from cereal rye was not enough to result in significant differences between those treatments. Nevertheless, significant differences in BG activity could also happen in moderate-high SOM sites, but most likely only after several years of cereal rye use.

Dehydrogenase. Effect of cover crop use on DHA activity is shown in Figure 4 and the detailed means separation for each site and year can be found Tables S1 and S2. No cover crop by herbicide interactions were observed for DHA activity, while the main effect of cover crop was significant. Therefore, data was pooled over herbicide treatments within each year and sample timing (Figure 4). The average activity of DHA was 29% higher at TPAC (3.8 µg INTF g^{-1} hr^{-1})

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compared to PPAC (2.7 µg INTF g^{-1} hr^{-1}). We also attribute this difference in enzyme activity to the greater SOM from TPAC. More SOM means more substrate to support microbial growth and, therefore, more enzyme activity (Yuan and Yue 2012). In respect to the effect of cover crop use on enzyme activity, the pattern of DHA and BG activities were similar for each site, with cover crop use resulting in more instances of increased activity relative to the fallow at PPAC than at TPAC. For example, at TPAC, out of the 30 soil sample timings within the four years of the study, the use of cereal rye increased DHA activity relative to the fallow in only seven timings. At PPAC that number increased to 25 sample timings. After four years of cover crop use at PPAC, the average DHA activity in the soil of plots with cereal rye (3.3 µg INTF g^{-1} hr^{-1}) was 42% higher than the activity measured in the soil of fallow plots (1.9 µg INTF g^{-1} hr^{-1}), whereas at TPAC, the increase in DHA activity was of only 5%. Results from PPAC are in agreement with those observed by Eivazi et al. (2024), that reported a 47% increase in DHA activity in the presence of cereal rye in comparison to the no cover crop control.

Correlation between herbicide concentration and enzyme activities. The Pearson's correlation analysis in Table 6 shows that BG was not strongly correlated with herbicide concentration in the soil. Conversely, DHA was strongly correlated with the concentration of sulfentrazone, *S*-metolachlor, and cloransulam-methyl in 2021, and atrazine and *S*-metolachlor in 2022. In 2021, there was a clear trend of DHA activity increase from 0 to 112 DAT (Figure 4) while the concentrations of the herbicides used that year were decreasing through the growing season. Conversely, in 2022, a positive and strong correlation was observed between DHA activity and atrazine and *S*-metolachlor concentrations in the soil, when both enzyme activity and herbicide concentrations declined through most of the growing season. In that year in particular, the cover crop termination at TPAC was followed by frequent rainfall events within 14 DAT (Figures 2 and S1; month of May), which resulted in a sharp decline in the concentration of the herbicides in the soil soon after application. However, an extended period of drought between June and July (Figures 2 and S1) impacted DHA activity that only begun to recover in August of that year. Therefore, the correlations between DHA and the concentrations of atrazine and *s*-metolachlor in the soil are explained by environmental factors that affected each variable separately, rather than the direct effect of the herbicides in the enzyme activity. In agreement with some previous reports (Cole 1976; Davies and Greaves 1981; Dennis et al. 2023; Tomkiel

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et al. 2015; Tyler 2022), results from this research suggested that none of the five residual herbicides used impacted BG or DHA activity when applied at cover crop termination and within the recommended label rates.

Concentration of residual herbicides in the soil. The effect of cover crop on the concentrations of sulfentrazone, *S*-metolachlor, cloransulam-methyl, atrazine, and mesotrione is shown in Figures 5-7 and detailed means separation are shown in Tables S3-S5. The use of cereal rye as cover crop reduced the concentration of sulfentrazone in the soil relative to the fallow only at 0 DAT at PPAC in 2021 and 10 DAT at TPAC, in 2023. For all other sample timings within 2021 and 2023, the concentration of sulfentrazone was similar for all treatments. As mentioned previously, the fallow plots were no kept weed-free during our study. Thus, the presence of weeds in those plots at 0 DAT resulted in the interception of residual herbicides, similar to what happened in plots with cereal rye. The concentration of cloransulam-methyl in the soil of plots with cereal rye was lower than fallow plots only at 10 and 14 DAT at TPAC and PPAC, respectively, in 2023. No significant effect of cover crop use on cloransulam-methyl concentration was identified for all other samples timings in 2021 and 2023. Among all herbicides evaluated, *S*-metolachlor was the only one that had reduced concentrations in the soil of plots with cereal rye relative to the fallow in 20 out of the 28 sample timings between 2021 and 2023. With most of the reductions observed at PPAC (up to 5 out of 7 sample timings). When differences occurred, the concentration of *S*-metolachlor was, on average, 34% lower in plots with cereal rye in comparison to the concentration measured in fallow plots. Cereal rye biomass intercepted 39% more atrazine than the weeds present in the fallow plots at PPAC, in 2021. However, for all other sample timings, the concentration of atrazine in plots with cereal rye was either similar or higher than the concentration from fallow plots. In addition to the interception by cover crop or weed biomass, the herbicides tested in this study were also likely intercepted by the crop residue (e.g., corn and/or soybean stubble) left on the soil surface after previous year's harvest. Even though this factor was not taken in consideration in this study, we must recognize that the crop residue laying on the surface does affect residual herbicide fate by interception and retention (Banks and Robinson 1986; Bauman and Ross 1983; Dao 1991; Ghadiri et al. 1984; Reddy et al. 1995). Furthermore, aged crop residue tends to adsorb more herbicide than fresh residue, giving that there are more sorption sites (lignin) exposed upon degradation of the residue (Dao 1991).

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In general, although the use of cereal rye resulted in increased BG and DHA activities, we did not identify any specific trends that would suggest an increase in the degradation of sulfentrazone, *S*-metolachlor, cloransulam-methyl, atrazine, or mesotrione. Similarly, Reddy et al. (1995) investigated the effect of no-till and conventional till in the activity of soil enzymes and degradation of chlorimuron. No-till adoption resulted in increased enzyme activity compared to the conventional till, but only minimal changes in the pattern of chlorimuron degradation were detected (Reddy et al. 1995). On the other hand, previous studies have demonstrated that the use of ryegrass as cover crop in no-till or conventional till systems can increase the soil microbial activity and lead to enhanced degradation of fluometuron in comparison to the same systems without ryegrass (Zablotowicz et al. 2007).

Herbicide interception by cereal rye residue. Herbicide interception was calculated as the percent reduction from the expected concentration of the residual herbicide in the soil (considering complete incorporation of the herbicide applied into the 0-5 cm of soil) based on the actual concentration measured at 0 DAT (Table 7). The use of cereal rye as cover crop did not affect residual herbicide fate by increasing the activities of BG and DHA in the soil (Figures 3 and 4). However, cereal rye biomass did intercept substantial amounts of the herbicides applied at termination, leading to significant reductions in the initial concentration of all herbicides in the soil. The average amount of herbicide interception across the three years of data collection and two locations was 77%, with *S*-metolachlor being the herbicide with the least amount of interception (55%) and mesotrione the herbicide with the highest amount (91%). Although the interception of herbicides by cereal rye biomass results in lower concentrations in the soil, the early-season weed biomass data (Table 4) from this research showed that even under reduced initial concentrations in the soil, the use of residual herbicides at cover crop termination improved the weed control relative to the treatment without residual herbicides. This improved weed control was, in part, due to rainfall events that occurred on the days following the application (Figures 5-7), thus washing off the herbicide from the biomass and incorporating it into the soil. The total rainfall volume for the seven days following the herbicide application between 2021 and 2023 and across the two locations was, on average, 37 mm (Figure S1). Previous studies have demonstrated that 50 mm of rainfall were enough to move 90% of the atrazine initially intercepted by wheat straw into the soil, thus increasing the concentration of the herbicide in the soil by more than 2-fold (Ghadiri et al. 1984). In the lack of rainfall, low concentrations of residual herbicides in the soil have the potential to increase the selection pressure for herbicide resistant weed biotypes (Busi et al. 2012; Neve and Powles 2005), which has been the main issue in weed science for decades. For example, a multiple-resistant *Lolium rigidum* population

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that was subjected for three generations to recurrent low doses of pyroxasulfone had more than 30% survival rate at 240 g ai ha^{-1} (2.4-fold the label rate) (Busi et al. 2012). In that same study, Busi et al. (2012) suggested that only full label rates of pyroxasulfone would be enough to provide adequate weed control.

Our study demonstrates that cover crops occasionally increase activity of BG and DHA during the growing season, in comparison to the fallow control. Even in times when there was an increase in the activity of these two enzymes, increased degradation of any of the residual herbicides was not observed. Furthermore, results from TPAC in 2021, showed a strong correlation between increased DHA activity and decreased concentrations of sulfentrazone, *s*-metolachlor, and cloransulam-methyl, suggesting that the herbicides were being used by that enzyme as a substrate to sustain population growth. Our results also indicated that the initial concentrations all residual herbicides in the soil were significantly reduced due to interception by cereal rye biomass. However, our results showed significant reductions in early season weed biomass when residual herbicides were included in the tank mixture relative to the application of glyphosate plus glufosinate. The inclusion of two or three residual herbicides in the tank mixture applied at cover crop termination resulted in similar early season weed biomass reductions in two and one out of three years, at PPAC and TPAC, respectively. In this study, for the most part, the application of residual herbicide at cover crop termination did not improve the late season weed control relative to the no residual herbicide program. Season-long weed control was achieved only in 2021 and 2023, most likely due to the narrower row-spacings adopted for soybean planting (38 cm). The use of cereal rye or crimson clover as cover crops did not improve the control of summer annual weed species relative to the fallow treatment in any of the locations and years of the study. Overall, results from this study suggest that soil residual herbicides can and should be included in the tank mixture applied at cover crop termination without risks of increased degradation. Furthermore, even with significant interception by the cover crop biomass, soil residual herbicides still reduced weed biomass early in the season. The combination of cover crops and soil residual herbicides is, therefore, one excellent alternative to improve the weed control following an integrated management approach. Future research could investigate the adoption of more comprehensive herbicide programs that include split applications of residual herbicides along with POST applications in order to achieve season-long weed control.

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Competing interests: The author(s) declare none.

Table 1. Chemical properties and bulk density of the soil from each cover crop and fallow treatments, at PPAC and TPAC, at 0-10 cm depth, for all four years of the study[a].

Site	Year	Cover crop	pH	OM	CEC	Bulk density
				%	meq 100 g^{-1}	g cm^{-3}
PPAC[b]	2020	Cereal rye	6.7	1.5	4.1	1.47
		Crimson clover	6.5	1.5	5.5	1.47
		Fallow	6.8	1.4	4.4	1.47
	2021	Cereal rye	5.9	1.7	5.7	1.25
		Crimson clover	5.8	1.9	5.4	1.28
		Fallow	6.3	1.9	5.9	1.26
	2022	Cereal rye	6.2	1.6	5.7	1.30
		Crimson clover	6.1	1.5	5.6	1.35
		Fallow	6.8	1.4	5.8	1.35
	2023	Cereal rye	6.9	1.5	5.3	1.37
		Crimson clover	6.8	1.7	6.1	1.43
		Fallow	6.9	1.5	5.7	1.43
TPAC	2020	Cereal rye	7.0	2.8	9.1	1.32
		Crimson clover	7.1	2.7	10.1	1.32
		Fallow	6.9	2.7	10.2	1.32
	2021	Cereal rye	6.5	3.0	10.0	1.21
		Crimson clover	6.7	3.1	11.3	1.24
		Fallow	6.8	2.9	10.3	1.22
	2022	Cereal rye	7.0	2.8	10.2	1.09
		Crimson clover	7.1	2.8	10.2	1.13
		Fallow	7.2	2.7	10.3	1.16
	2023	Cereal rye	6.9	2.3	9.9	1.22
		Crimson clover	6.9	2.4	10.9	1.23
		Fallow	6.9	2.4	11.2	1.23

[a]Soil samples were taken in March of each year.

[b]Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN); OM, organic matter; CEC, cation exchange capacity.

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Table 2. Herbicide treatments and rates applied at cover crop termination[1].

Cash crop	Herbicide	Trade name	Rate	Manufacturer	
	No residual		g ai ae ha^{-1}		
	Glyphosate	Roundup PowerMax	1,750	Bayer CropScience	
	Glufosinate	Liberty 280 SL	737	BASF Corporation	
	Medium residual				
	Glyphosate	Roundup PowerMax	1,750	Bayer CropScience	
	Atrazine	AAtrex 4L	2,241 (TPAC) 1,681 (PPAC)	Syngenta Protection	Crop
Corn 2020/2022	*S*-metolachlor	Dual II Magnum	1,790 (TPAC) 1,420 (PPAC)	Syngenta Protection	Crop
	Heavy residual				
	Glyphosate	Roundup PowerMax	1,750	Bayer CropScience	
	Atrazine	AAtrex 4L	2,241 (TPAC) 1,681 (PPAC)	Syngenta Protection	Crop
	S-metolachlor	Dual II Magnum	1,790 (TPAC) 1,420 (PPAC)	Syngenta Protection	Crop
	Mesotrione	Callisto	104	Syngenta	Crop

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				Protection
	No residual			
	Glyphosate	Roundup PowerMax	1,750	Bayer CropScience
	Glufosinate	Liberty 280 SL	737	BASF Corporation
	Medium residual			
	Glyphosate	Roundup PowerMax	1,750	Bayer CropScience
	Sulfentrazone	Spartan 4F	280 (TPAC) 210 (PPAC)	FMC Corporation
Soybean 2021/2023	*S*-metolachlor	Dual II Magnum	1,790 (TPAC) 1,420 (PPAC)	Syngenta Crop Protection
	Heavy residual			
	Glyphosate	Roundup PowerMax	1,750	Bayer CropScience
	Sulfentrazone	Spartan 4F	280 (TPAC) 210 (PPAC)	FMC Corporation
	S-metolachlor	Dual II Magnum	1,790 (TPAC) 1,420 (PPAC)	Syngenta Crop Protection
	Cloransulam-methyl	FirstRate	44	Dow AgroSciences

Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

[1] Herbicides were applied in tank mixture at two weeks before cash crop planting each year.

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Table 3. Cover crop planting and termination dates and cash crop planting dates at TPAC and PPAC from 2019 until 2023

	Cover crop			Cash crop		
Year	Planting dates	Year	Termination dates	Year	Planting dates	
2019	TPAC: Sep-06th PPAC: Sep-07th	2020	TPAC: May-04th PPAC: May-01st	2020 (Corn)	TPAC: May-24th PPAC: May-22nd	
2020	TPAC: Oct-14th PPAC: Oct-15th	2021	TPAC: May-05th PPAC: May-05th	2021 (Soybean)	TPAC: May-23rd PPAC: May-21st	
2021	TPAC: Oct-03rd PPAC: Oct-04th	2022	TPAC: May-14th PPAC: May-13th	2022 (Corn)	TPAC: June-01st PPAC: May-30th	
2022	TPAC: Oct-03th PPAC: Oct-04th	2023	TPAC: May-11th PPAC: May-11th	2023 (Soybean)	TPAC: May-25th PPAC: May-25th	

Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

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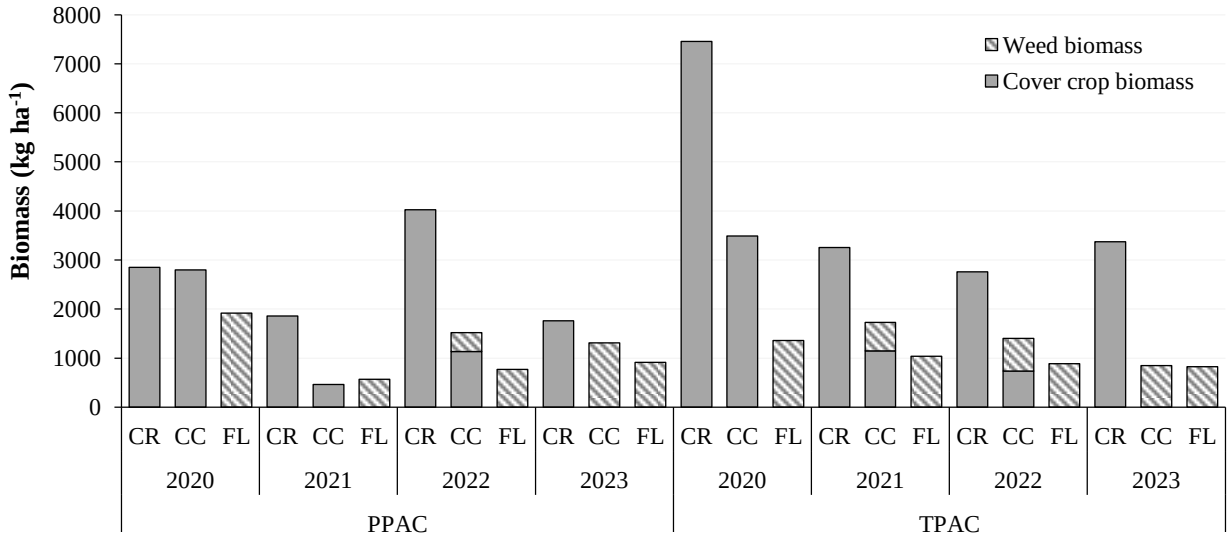

Figure 1. Cover crop and weed biomass one day before cover crop termination. Weed biomass was collected in all four years in the fallow plots and whenever there were weeds present in the cover crop plots. Abbreviations: CR, cereal rye; CC, crimson clover; FL, fallow.

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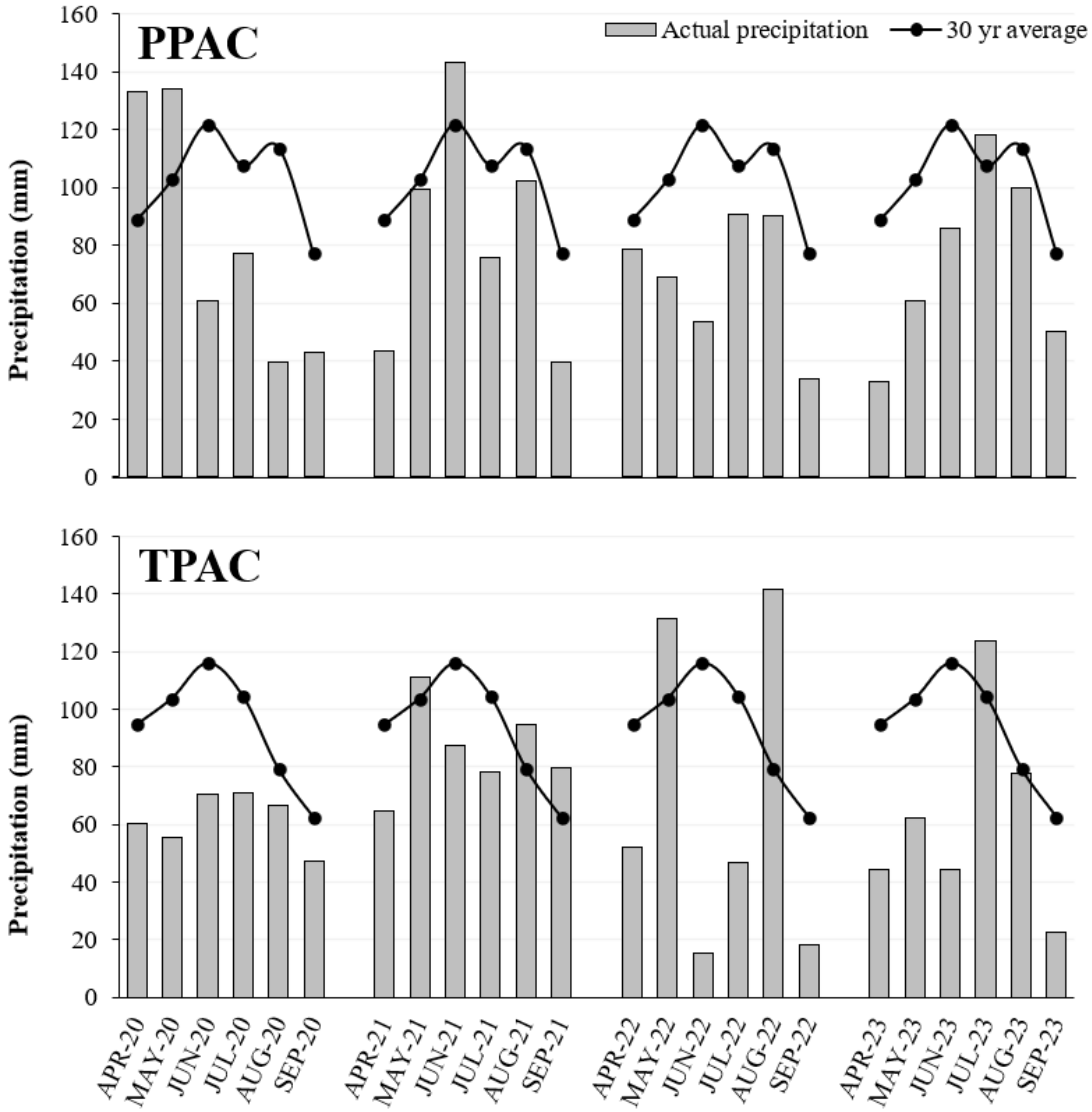

Figure 2. Thirty-year average and actual precititation for each month that data were collected over the four years of study period at PPAC and TPAC

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Table 4. Weed biomass at four weeks after cash crop planting, from 2021 until 2023.

Location	Herbicide treatment[a]	2021 Soybean		2022 Corn		2023 Soybean	
		Weed biomass		Weed biomass		Weed biomass	
		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}	
PPAC	Heavy	58	b[b]	5	b	6	c
	Medium	71	b	17	b	35	b
	No	334	a	187	a	205	a
TPAC	Heavy	124	b	117	c	1	c
	Medium	208	ab	456	b	51	b
	No	304	a	1467	a	379	a

[a] Herbicide treatments: Heavy, three residual herbicides; Medium, two residual herbicides; NO, no residual herbicides.

[b] Data were log transformed. However, original mean values are presented. Numbers followed by the same letter within year and location are not significantly different according to Fisher's protected LSD ($P < 0.05$).

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Table 5. Weed biomass at 18 weeks after cash crop planting, in 2020 and 2022[a].

Location	Herbicide treatment[b]	2020 Corn		2022 Corn	
		Weed biomass		Weed biomass	
		kg ha^{-1}		kg ha^{-1}	
PPAC	Heavy	50	b[c]	2	a
	Medium	112	ab	4	a
	No	195	a	6	a
TPAC	Heavy	466	a	33	a
	Medium	727	a	39	a
	No	835	a	37	a

[a] All plots were weed free at 18 WAP at PPAC and TPAC, in 2021 and 2023. Therefore, only data from 2020 and 2022 are being shown.

[b] Herbicide treatments: Heavy, three residual herbicides; Medium, two residual herbicides; NO, no residual herbicides.

[c] Data were log transformed. However, original mean values are presented. Numbers followed by the same letter within year and location are not significantly different according to Fisher's protected LSD ($P < 0.05$).

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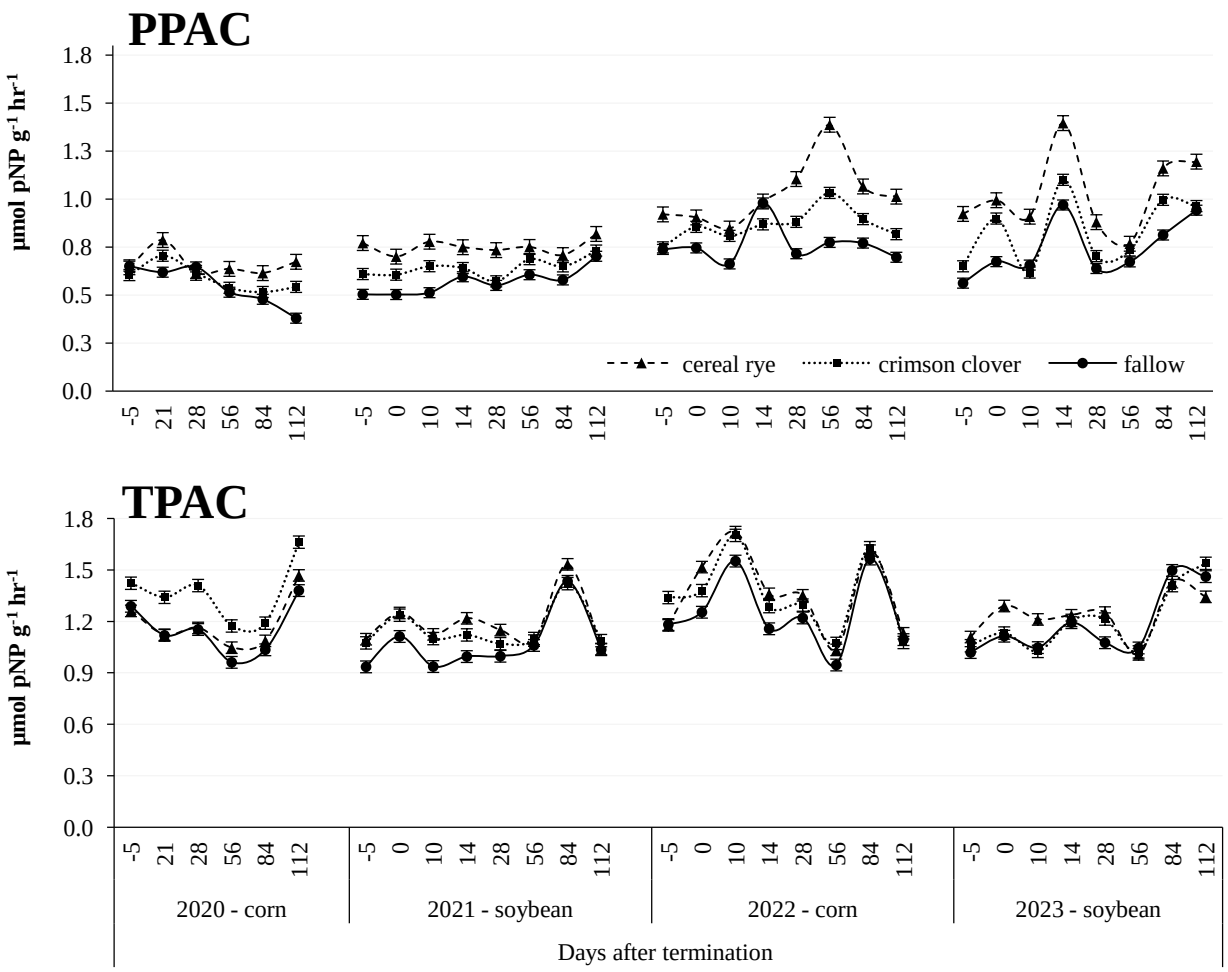

Figure 3. β-glucosidase activity in the soil (0 to 5 cm depth), from 5 days before until 112 days after cover crop termination, from 2020 until 2023. Data points represent mean ± standard error of four replications.

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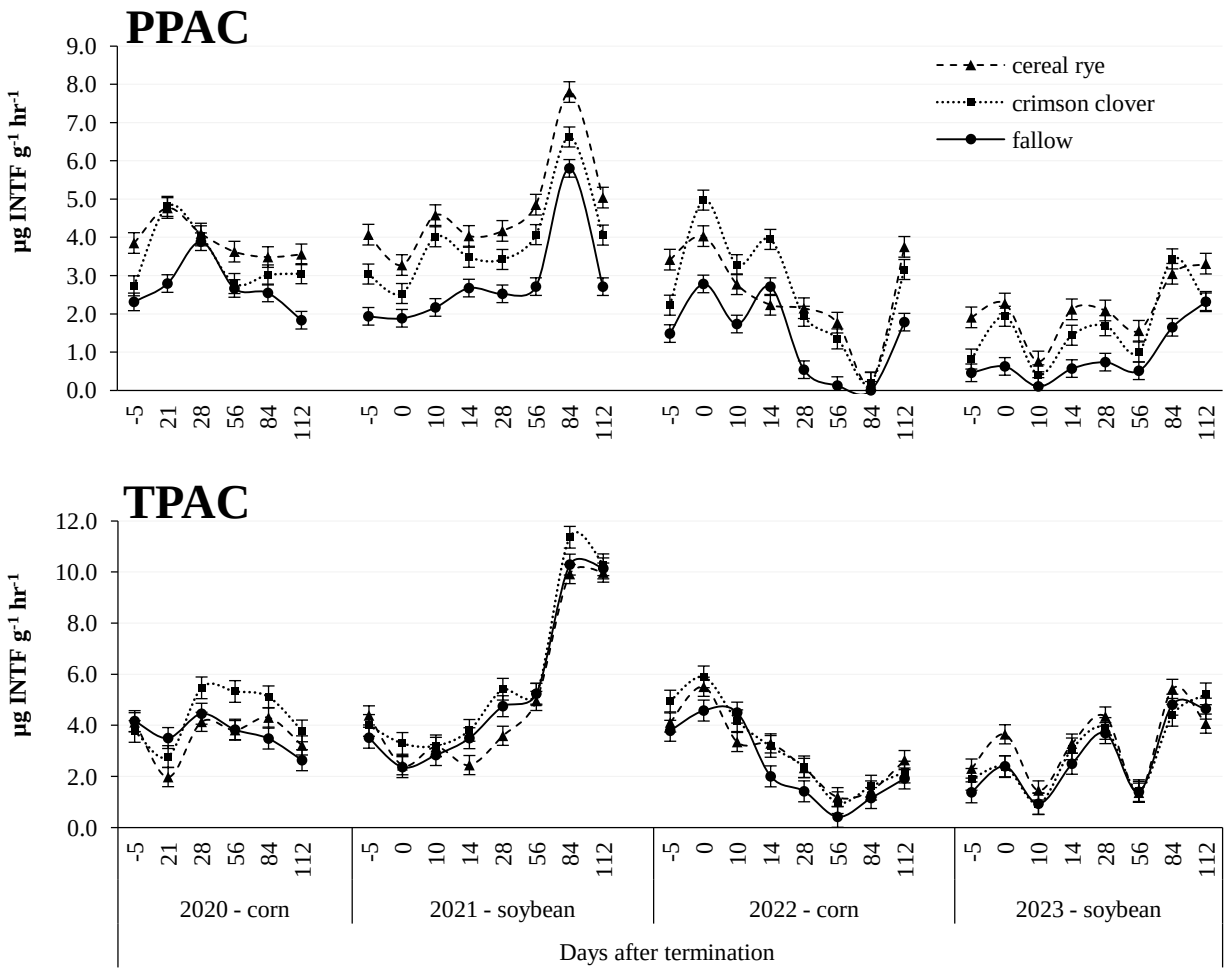

Figure 4. Dehydrogenase activity in the soil (0 to 5 cm depth), from 5 days before until 112 days after cover crop termination, from 2020 until 2023. Data points represent mean ± standard error of four replications.

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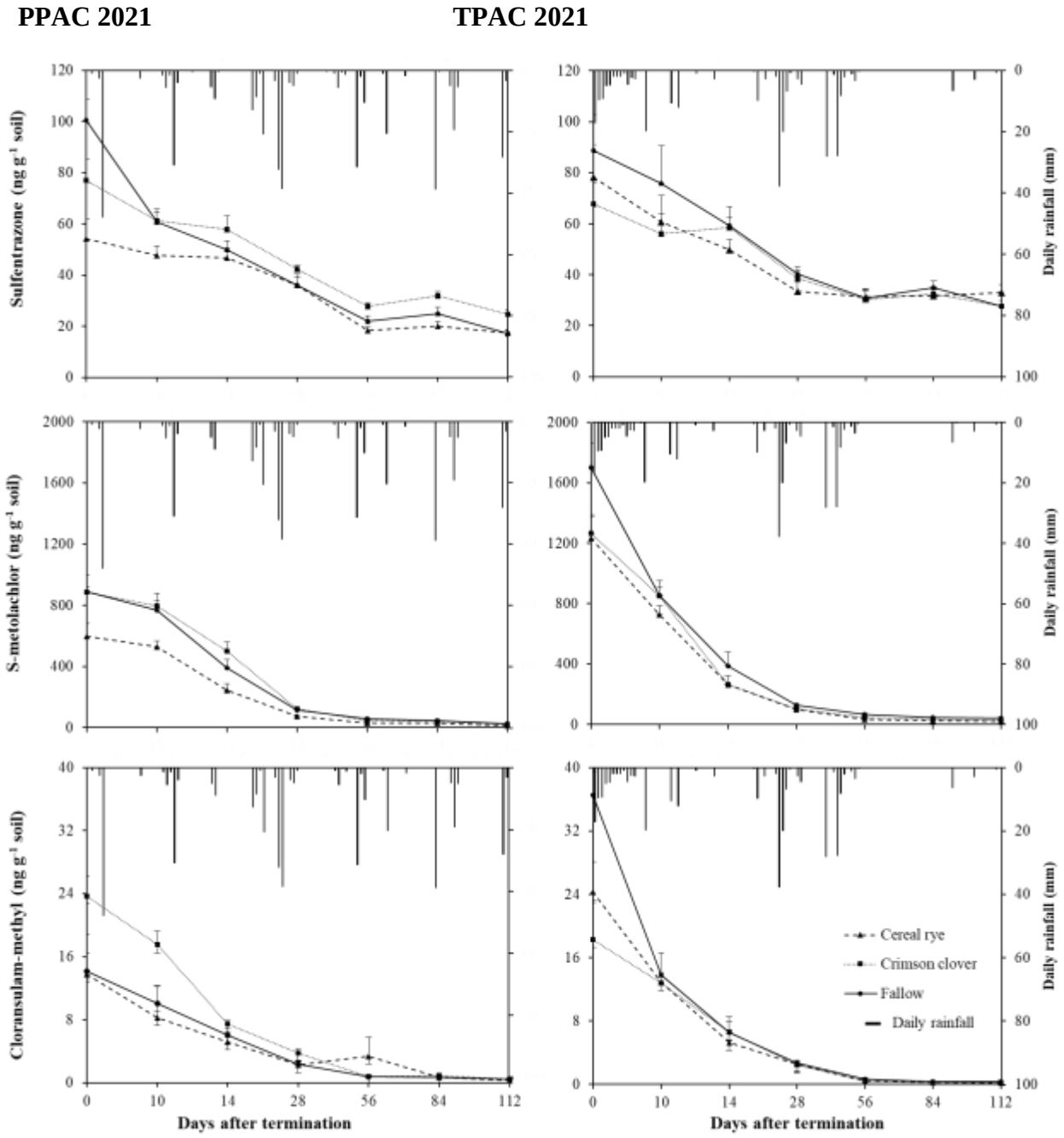

Figure 5. Sulfentrazone, *s*-metolachlor, and cloransulam-methyl concentration in the soil (0 to 5 cm depth) and daily rainfall amounts, from zero until 112 days after cover crop termination, at PPAC and TPAC, in 2021. Data points represent mean ± standard error of four replications.

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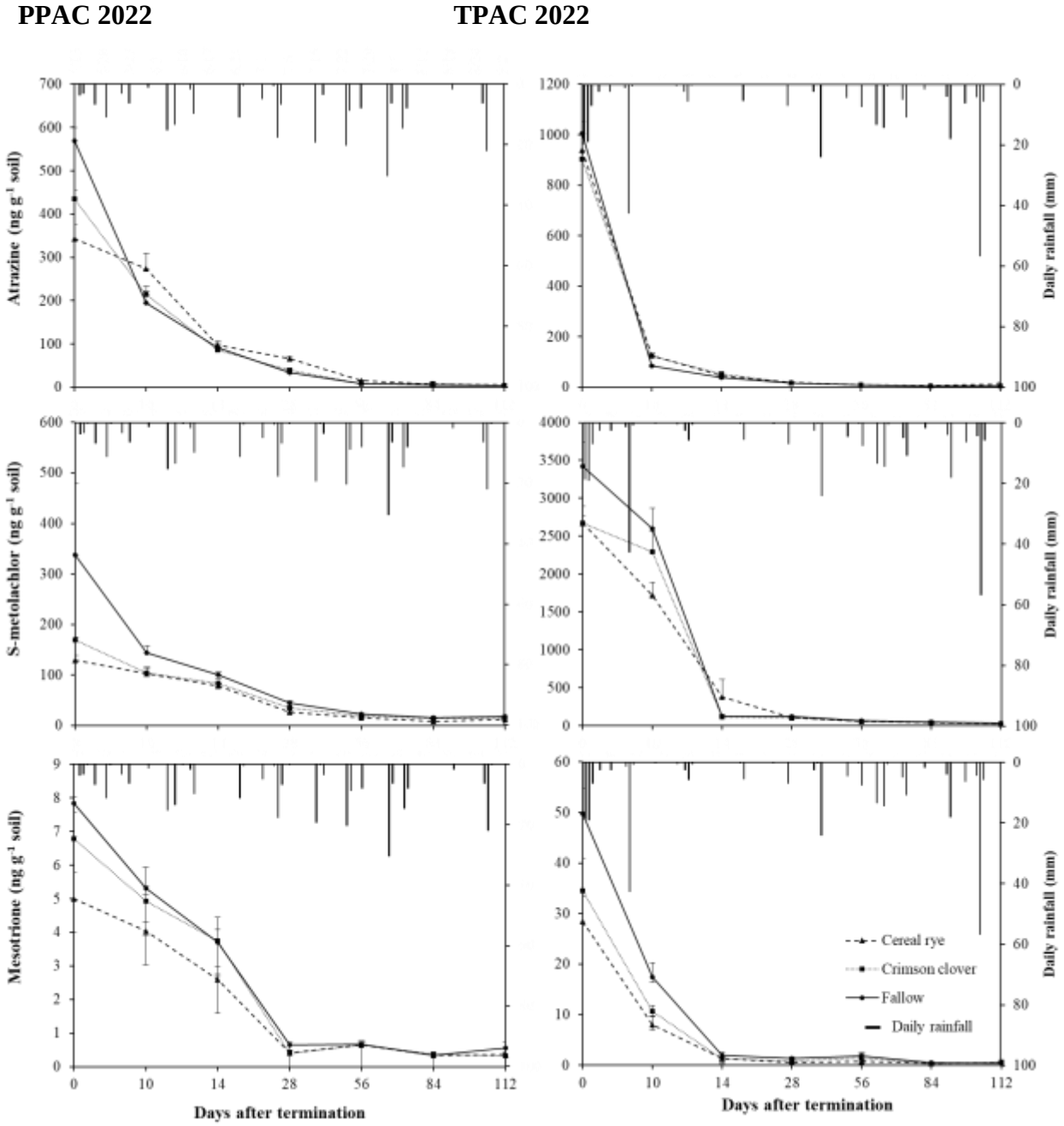

Figure 6. Atrazine, *s*-metolachlor, and mesotrione concentration in the soil (0 to 5 cm depth) and daily rainfall amounts, from zero until 112 days after cover crop termination, at PPAC and TPAC, in 2022. Data points represent mean ± standard error of four replications.

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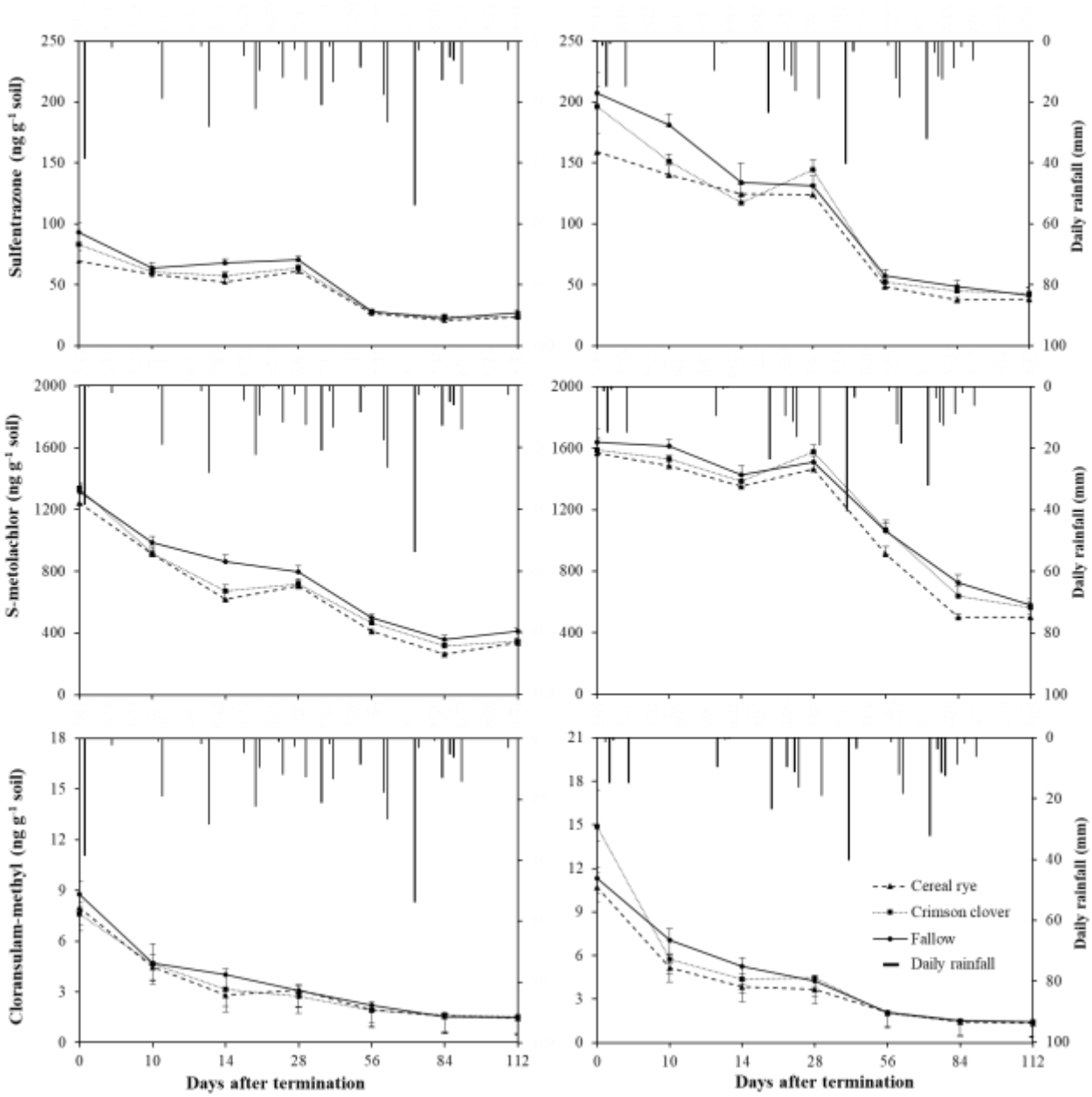

Figure 7. Sulfentrazone, *s*-metolachlor, and cloransulam-methyl concentration in the soil (0 to 5 cm depth) and daily rainfall amounts, from zero until 112 days after cover crop termination, at PPAC and TPAC, in 2023. Data points represent mean ± standard error of four replications.

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Table 6. Pearson's correlation coefficients between herbicides concentrations in the soil and enzyme activities, from 2021 until 2023[a].

		PPAC		TPAC	
Year	Herbicides[b]	β-glucosidase	Dehydrogenase	β-glucosidase	Dehydrogenase
2021	Sulfentrazone	-0.3925 *	-0.4125 **	-0.1237 ns	-0.5926 ***
	S-metolachlor	-0.2911 ns	-0.4197 **	-0.0796 ns	-0.6239 ***
	Cloransulam-methyl	0.0447 ns	-0.3893 ns	-0.0175 ns	-0.58084 **
2022	Atrazine	-0.1410 ns	0.4638 ***	0.0664 ns	0.5689 ***
	S-metolachlor	-0.0431 ns	0.2178 **	0.2600 ***	0.6156 ***
	Mesotrione	-0.2277 *	0.4383 ***	0.1265 ns	0.4846 ***
2023	Sulfentrazone	-0.1280 ns	-0.1868 *	-0.2808 ***	-0.3054 ***
	S-metolachlor	-0.3621 ***	-0.2356 ns	-0.4259 ***	0.2937 *
	Cloransulam-methyl	-0.1513 ns	-0.2860 **	-0.2032 ns	-0.2732 *

[a] Correlation coefficients were nonsignificant (ns) or significant at $*P \leq 0.05$, $**P \leq 0.01$ or $***P \leq 0.001$.

[b] n = 168 for sulfentrazone, *S*-metolachlor, and atrazine. n = 84 for cloransulam-methyl and mesotrione.

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Table 7. Expected and actual concentrations of residual herbicides in the soil (0 to 5 cm depth) and interception by cereal rye at the time of cover crop termination, from 2021 until 2023, at PPAC and TPAC.

Year	Herbicide	Site	Expected concentration[1]	Actual concentration at 0 DAT[2]	Interception[3]
			ng g^{-1}	ng g^{-1}	%
2021 Soybean	Sulfentrazone	PPAC	336.00	54.28	83.85
		TPAC	462.81	78.15	83.11
	S-metolachlor	PPAC	2272.00	596.83	73.73
		TPAC	2958.68	1232.74	58.33
	Cloransulam-methyl	PPAC	70.40	13.78	80.43
		TPAC	72.73	24.31	66.57
2022 Corn	Atrazine	PPAC	2586.15	343.69	86.71
		TPAC	4111.93	940.36	77.13
	S-metolachlor	PPAC	2184.62	129.86	94.06
		TPAC	3284.40	2670.42	18.69
	Mesotrione	PPAC	160.00	5.00	96.88
		TPAC	190.83	28.48	85.08
2023 Soybean	Sulfentrazone	PPAC	306.57	69.71	77.26
		TPAC	459.02	159.17	65.32
	S-metolachlor	PPAC	2072.99	1240.99	40.14
		TPAC	2934.43	1568.22	46.56
	Cloransulam-methyl	PPAC	64.23	7.99	87.56
		TPAC	72.13	10.71	85.15

[1] Expected concentration of the herbicide in the soil (i.e., 100% of the applied herbicide is incorporated into the top 5 cm of soil). Equivalent to the herbicide application rate in ng ha^{-1} divided by the soil weight in g ha^{-1} (0-5 cm depth).

[2] Herbicide concentration measured in the UHPLC, at 0 DAT (days after cover crop termination).

[3] Percentage reduction from the expected concentration to the actual herbicide concentration measured at 0 DAT.

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