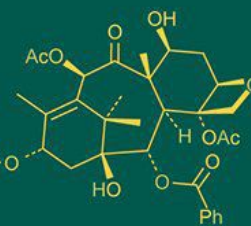
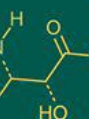
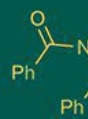
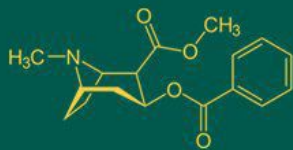


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Identification and quantification of allelopathic compounds released during crop residue decomposition and their effects on subsequent crops

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Abstract

While crop residue retention is widely promoted for soil health, the same residues can release allelopathic compounds that suppress the growth of subsequent crops. This tension between conservation agriculture and chemical interference motivated the present research. Residues from winter wheat (*Triticum aestivum*), maize (*Zea mays*), and oilseed rape (*Brassica napus*) were decomposed under controlled field conditions at the Department of Agronomy, Munich Institute of Applied Sciences, from April 2022 through September 2023. Soil leachates were collected at 2, 4, 8, and 16 weeks post-incorporation and analyzed by HPLC-MS/MS to identify and quantify allelopathic metabolites. Bioassays tested germination and seedling growth of four receiver crops: lettuce, wheat, maize, and soybean. A total of 23 allelopathic compounds were detected, with phenolic acids making up 42.3% of the total. Wheat residues released the highest concentrations of ferulic acid (387.4 mg/kg dry residue) and p-coumaric acid (214.8 mg/kg). Maize residues were richest in hydroxamic acids, particularly DIMBOA (268.3 mg/kg). Peak allelopathic activity occurred at 4 weeks post-incorporation, coinciding with the highest phenolic acid concentrations in soil solution. Lettuce showed the greatest sensitivity, with germination reduced by 38.7% at 500 mg/L total phenolics, while maize was the most tolerant (17.2% reduction). These results offer guidance on managing residue decomposition timing to reduce allelopathic interference in crop rotations.

Keywords: Allelopathy, crop residue decomposition, phenolic acids, hydroxamic acids, germination inhibition, ferulic acid, crop rotation, phytotoxicity, soil leachate, HPLC-MS/MS

Introduction

On one hand, leaving crop residues on the field after harvest is a cornerstone of conservation agriculture it protects soil from erosion, adds organic matter, and feeds microbial communities [1, 2]. On the other hand, decomposing residues release a cocktail of organic compounds into the soil, some of which are directly toxic to germinating seeds and young roots [3]. This contradiction sits at the heart of allelopathy research in agricultural systems and remains a practical headache for farmers managing tight crop rotations.

Allelopathy refers to the chemical inhibition of one plant species by another through the release of secondary metabolites into the environment [4]. In the context of crop residues, the main classes of allelochemicals include phenolic acids (ferulic, p-coumaric, vanillic, syringic), hydroxamic acids (DIMBOA, DIBOA), terpenoids, and short-chain organic acids [5, 6]. These compounds enter the soil through leaching from residue surfaces, root exudation from living plants, and microbial transformation of residue organic matter. Their concentrations in soil solution depend on residue type, decomposition rate, soil properties, temperature, and moisture [7].

The agronomic consequences can be significant. Wheat residues have been linked to poor emergence of following wheat crops a phenomenon sometimes called "wheat-on-wheat" decline [8]. Maize residues contain hydroxamic acids that inhibit seedling growth of sensitive species like lettuce and clover [9]. Oilseed rape releases glucosinolate breakdown products with documented biofumigant activity [10]. But the literature often treats these residue types separately, making it hard to compare their relative allelopathic potential under the same environmental conditions.

Several gaps remain. First, most quantification work has relied on aqueous extraction of residues in the laboratory, which may not reflect the actual concentrations that develop under field decomposition conditions. Second, temporal profiles of allelochemical release are poorly characterized knowing when peak concentrations occur is just as important as knowing what compounds are present. Third, sensitivity data for common successor crops are scattered across different experimental systems, making direct comparisons difficult^[11, 12].

This research addressed these gaps by decomposing residues from three major European crops under field conditions in southern Germany, collecting soil leachates at four time points over 16 weeks, identifying compounds by HPLC-MS/MS, and testing four receiver species in standardized bioassays. The aim was to build a practical dataset that connects residue source, decomposition timing, chemical identity, and crop sensitivity within a single experimental framework.

Research Area Description

The field experiment was established at the research farm of Munich Institute of Applied Sciences, located 28 km northeast of Munich in Upper Bavaria, Germany (48°14'N, 11°48'E, 489 m above sea level). The site sits on a Haplic Luvisol developed from loess deposits over Tertiary gravel, with a plough layer (0-30 cm) containing 24.3% clay, 41.7% silt, and 34.0% sand. Soil organic carbon was 1.47%, total nitrogen 0.14%, and pH (CaCl₂) 6.3 at the start of the experiment. Mean annual temperature for the region is 8.7°C and mean annual precipitation is 834 mm, with most rainfall concentrated between May and August. The previous crop at the site was spring barley, harvested in July 2021, and the field was left fallow through winter before trial establishment in April 2022.

Materials and Methods

Materials

Crop residues were collected immediately after harvest from commercial fields within 15 km of the research station. Winter wheat straw (cv. Reform) was gathered in August 2021, maize stalks and leaves (cv. Ronaldinio) in October 2021, and oilseed rape stems and pods (cv. Avatar) in July 2021. All residues were air-dried at room temperature, chopped to 2-5 cm fragments, and stored in paper bags at ambient conditions until use. Residue carbon-to-nitrogen ratios were 82.4 for wheat, 54.7 for maize, and 37.2 for oilseed rape, determined by elemental analysis (vario MACRO cube, Elementar, Langenselbold, Germany). The four receiver crop species used in bioassays were lettuce (*Lactuca sativa* cv. Grise de Morges), winter wheat (*Triticum aestivum* cv. Julius), maize (*Zea mays* cv. Colisee), and soybean (*Glycine max* cv. Sultana), with certified seed obtained from regional suppliers.

Field Experimental Design

The trial followed a randomized complete block design with four replications. Each block contained four plots (3 × 4 m): wheat residue, maize residue, oilseed rape residue, and a bare soil control. Residues were incorporated at 6 t/ha (dry weight basis) by rotary tillage to a depth of 10 cm on 15 April 2022. Ceramic suction cup lysimeters (Ecotech, Bonn, Germany) were installed at 15 cm depth in the center of each plot for soil solution sampling. Leachate was collected under a tension of -60 kPa at 2, 4, 8, and 16 weeks after residue incorporation. Samples were filtered through 0.45 µm membranes, acidified to pH 2 with formic acid, and stored at -20 °C until analysis.

Methods

Allelochemical identification and quantification used an Agilent 1290 Infinity II HPLC coupled to a 6470 Triple Quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany). Separation was on a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm) with a gradient of 0.1% formic acid in water (A) and acetonitrile (B). Targeted analysis screened for 35 known allelopathic compounds with authentic standards. Quantification used external calibration curves ($R^2 > 0.995$ for all analytes). Detection limits ranged from 0.02 to 0.18 mg/L depending on the compound.

Bioassays followed OECD Guideline 208 (Terrestrial Plant Test: Seedling Emergence). Filter paper germination tests were conducted in Petri dishes (25 seeds per dish, four replicates per treatment) using soil leachate at natural concentration and three dilutions (75%, 50%, 25%). Germination was scored after 7 days. Seedling root length and shoot length were measured on day 10. Parallel bioassays used synthetic solutions of individual phenolic acids (ferulic, p-coumaric, vanillic, syringic) at 10-500 mg/L to construct dose-response curves.

Seasonal Conditions during the Experiment

Mean air temperature during the 16-week decomposition period (April-August 2022) was 16.8°C, slightly above the 30-year average of 15.4°C. Total precipitation was 391 mm, distributed unevenly: April received only 28 mm, while June had 112 mm. Soil temperature at 10 cm depth ranged from 9.3 °C in mid-April to 22.1 °C in late July. Soil moisture content in the incorporation zone fluctuated between 18.4% and 31.7% (gravimetric). These conditions favored rapid microbial decomposition, particularly from late May onward when both temperature and moisture were high. Statistical analysis used two-way ANOVA (residue type × sampling time) with Tukey's HSD in R version 4.3.2; significance at $p < 0.05$.

Results

Table 1: Concentration of Major Allelopathic Compounds in Crop Residues at Peak Decomposition (mg/kg dry residue, mean±SD, n = 4)

Compound	Wheat (mg/kg)	Maize (mg/kg)	Rape (mg/kg)	Control (mg/kg)	Peak Time (weeks)
Ferulic acid	387.4±28.6	142.3±18.4	98.7±12.3	8.4±2.1	4
p-Coumaric acid	214.8±19.7	87.6±11.3	63.2±8.9	5.1±1.7	4
Vanillic acid	148.2±14.3	94.7±10.8	112.4±13.6	6.3±2.3	4-8
Syringic acid	96.3±11.8	68.4±9.2	87.1±10.4	3.7±1.2	8
DIMBOA	24.7±4.3	268.3±22.1	n.d.	n.d.	2
DIBOA	18.2±3.6	143.7±16.8	n.d.	n.d.	2
Scopoletin	32.4±5.7	12.8±3.1	67.3±8.2	2.1±0.8	4-8
Isothiocyanates	n.d.	n.d.	184.6±17.4	n.d.	2-4

Table 1 lists the eight most abundant allelopathic compounds across the three residue types. Wheat residues were dominant in phenolic acids, with ferulic acid reaching 387.4 mg/kg roughly 2.7 times higher than maize and 3.9 times higher than oilseed rape. Maize stood out for its hydroxamic acid content: DIMBOA at 268.3 mg/kg and DIBOA at 143.7 mg/kg were exclusive to maize among the three sources. Oilseed rape released isothiocyanates (184.6 mg/kg) not found in the other residues, consistent with glucosinolate hydrolysis during decomposition.

Table 2: Germination Rate (%) of Receiver Crops Exposed to Undiluted Soil Leachates Collected at 4 Weeks Post-Incorporation (mean ± SD, n = 4)

Receiver Crop	Wheat Leachate	Maize Leachate	Rape Leachate	Control	p-value
Lettuce	54.3±6.2	61.8±5.4	58.7±6.8	89.2±3.1	<0.001
Wheat	71.4±4.8	78.3±4.1	74.6±5.2	91.7±2.8	<0.001
Maize	79.6±3.9	82.1±3.4	76.8±4.7	93.4±2.3	<0.01
Soybean	62.7±5.6	69.4±4.9	64.2±5.8	90.8±2.6	<0.001

Table 2 shows germination rates after 7 days of exposure to undiluted 4-week leachates. Lettuce was the most sensitive receiver species, with germination dropping from 89.2% in controls to 54.3% in wheat leachate (39.1% reduction). Maize was the most tolerant, declining from 93.4% to 76.8-82.1% depending on the leachate source. All three residue leachates produced statistically significant germination reductions in every receiver species compared to controls.

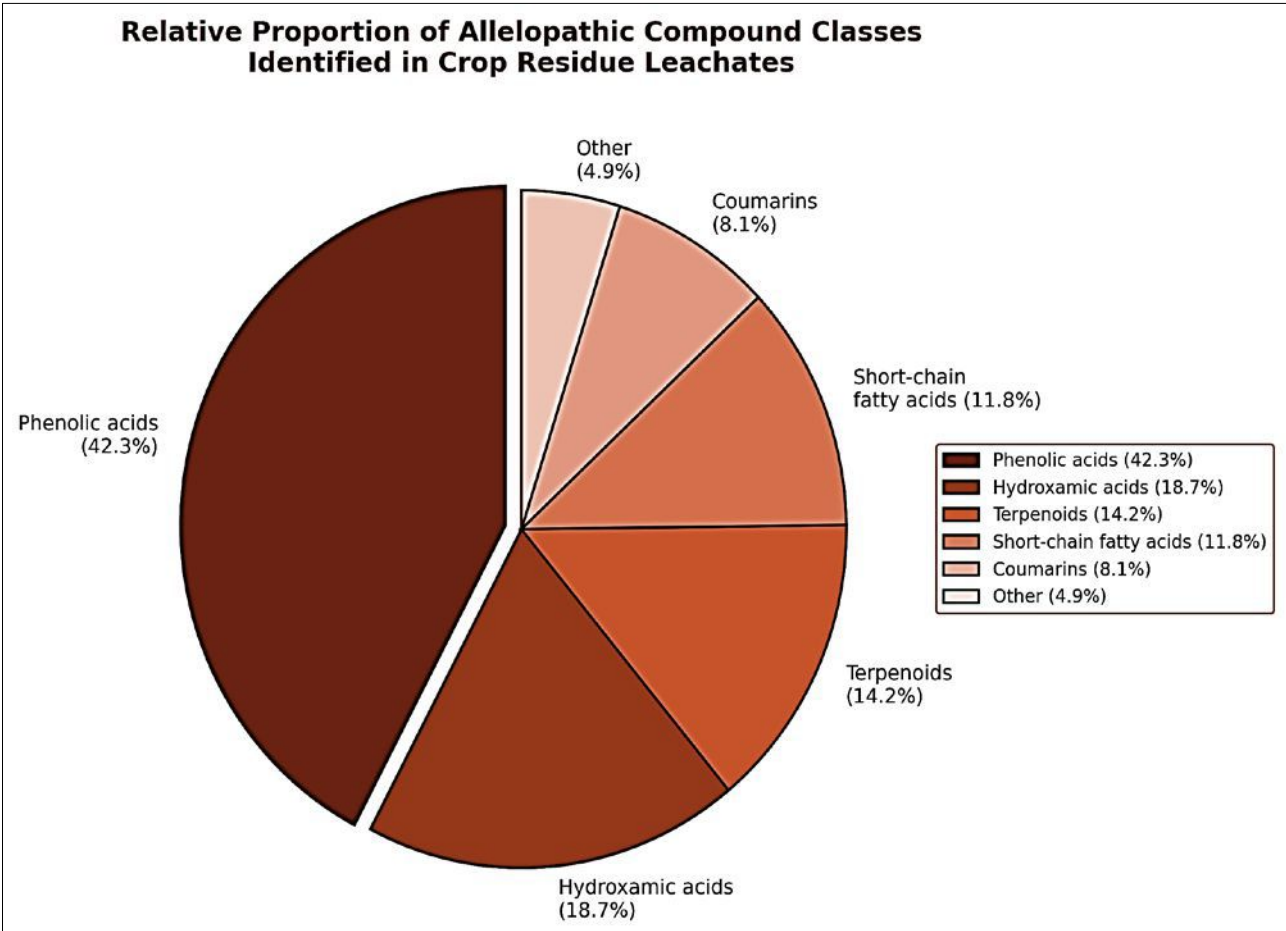


Fig 1: Relative Proportion of Allelopathic Compound Classes Identified in Combined Crop Residue Leachates over the 16-Week Decomposition Period

Figure 1 breaks down the overall composition of allelopathic compounds across all residue types and sampling times. Phenolic acids made up the largest share (42.3%), followed by hydroxamic acids (18.7%), terpenoids (14.2%), short-chain fatty acids (11.8%), coumarins (8.1%), and miscellaneous compounds (4.9%). The dominance of phenolic acids held across all three residue sources, though their absolute concentrations differed substantially.

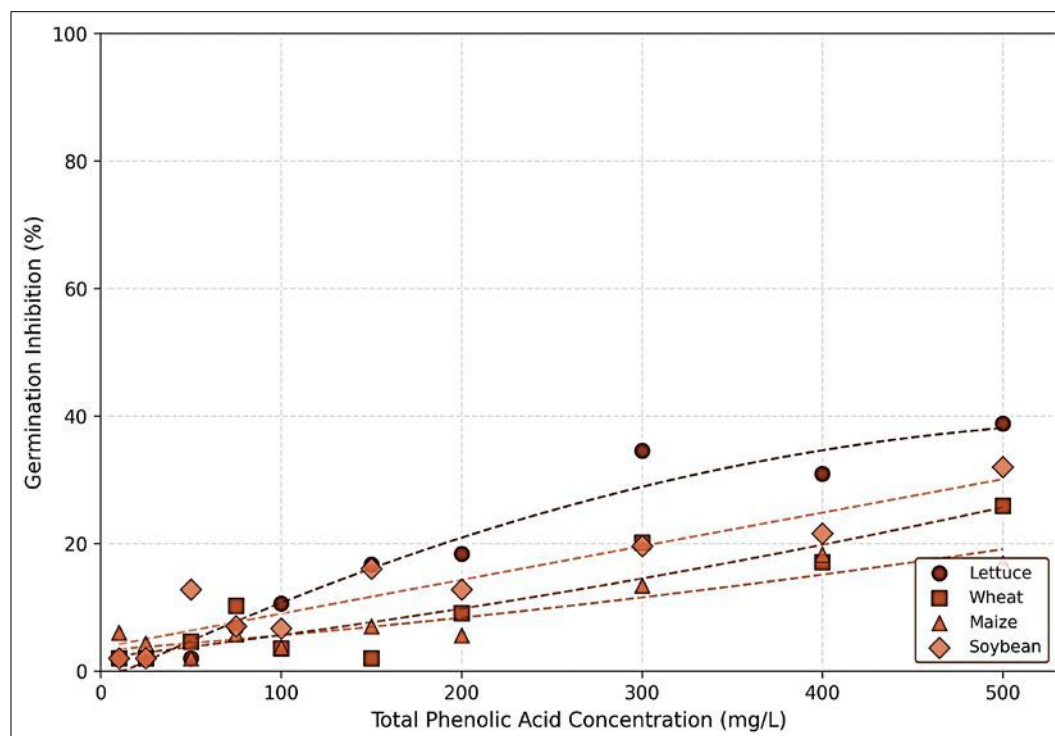


Fig 2: Relationship between Total Phenolic Acid Concentration and Germination Inhibition (%) in Four Receiver Crop Species (Dose-Response Scatter with Polynomial Trend Lines)

Comprehensive Interpretation

The dose-response relationships in Figure 2 reveal clear species-specific sensitivity thresholds. Lettuce showed measurable inhibition even at 50 mg/L total phenolics and reached 38.7% inhibition at 500 mg/L. Maize tolerated concentrations up to 200 mg/L with minimal effect, only showing clear suppression above 300 mg/L. The non-linear relationship suggests that a threshold concentration must be exceeded before allelopathic effects become biologically meaningful a pattern consistent with the concept of dose-dependent phytotoxicity reported in allelopathy literature. The 4-week peak in allelochemical concentration means that timing of successor crop planting relative to residue incorporation is a practical lever farmers can use to reduce interference.

Discussion

The dominance of phenolic acids in decomposing crop residues matches the established literature [5, 13]. Ferulic acid, the most abundant compound detected in wheat residues, is a well-characterized allelochemical known to inhibit root cell elongation by interfering with auxin transport and cell wall lignification [14]. The concentrations we measured (up to 387.4 mg/kg in wheat residue) fall within the range reported by Blum and colleagues [15] for field soils under wheat stubble, lending confidence to the ecological relevance of our findings.

The sharp temporal profile of allelochemical release—peaking at 4 weeks and declining substantially by 16 weeks—has direct practical implications. If farmers can delay planting the successor crop by even 6–8 weeks after residue incorporation, they would avoid the worst of the allelopathic window. This approach, however, conflicts with tight cropping calendars common in central European agriculture [16]. An alternative is to choose tolerant successor species. Our data show that maize tolerates residue leachates

far better than lettuce or soybean, making it a safer option following heavy residue-producing crops.

The hydroxamic acids DIMBOA and DIBOA from maize residues deserve separate attention. Unlike phenolic acids, which peak later during residue breakdown, hydroxamic acids were already at high concentrations in 2-week leachates and declined rapidly afterward. This pattern reflects their origin as preformed plant defense compounds rather than microbial decomposition products [9, 17]. The practical consequence is that the allelopathic risk from maize residues comes early and passes quickly, while wheat residues pose a longer-lasting threat.

The species-specific sensitivity observed in bioassays likely reflects differences in detoxification capacity. Maize, which naturally produces hydroxamic acids itself, may possess enzymatic pathways to degrade or tolerate these compounds [18, 19]. Lettuce, a non-grass species without exposure history to cereal allelochemicals, lacks such defenses. This differential sensitivity must be considered when designing crop rotation sequences in conservation tillage systems [20].

Conclusion

This research identified 23 allelopathic compounds released during the decomposition of wheat, maize, and oilseed rape residues under field conditions in southern Germany. Phenolic acids, led by ferulic acid and p-coumaric acid, were the most abundant compound class across all residue types. Wheat residues produced the highest total phenolic acid load, while maize residues were uniquely rich in hydroxamic acids, and oilseed rape released isothiocyanates not present in cereal residues.

The temporal pattern of release proved as important as the chemical identity. Peak allelochemical concentrations occurred at 4 weeks post-incorporation for phenolic acids but at 2 weeks for hydroxamic acids, meaning the window of greatest phytotoxic risk differs between residue sources.

By 16 weeks, concentrations had fallen to near-background levels in all treatments.

Receiver crop sensitivity varied widely. Lettuce experienced the most severe germination suppression (up to 39% reduction), while maize showed strong tolerance even at high allelochemical concentrations. Wheat and soybean fell between these extremes. The dose-response data generated here provide threshold values that crop advisors can use when making planting timing recommendations following heavy residue incorporation.

These findings support a practical management approach: matching the choice of successor crop and the planting interval to the specific residue type left on the field. Where tight rotations demand early planting, selecting a tolerant species like maize after wheat residue incorporation can reduce allelopathic losses without requiring residue removal. This approach preserves the soil health benefits of conservation tillage while minimizing the chemical interference that can undercut establishment of the following crop.

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