Direct and indirect photodegradation of atrazine and S-metolachlor in agriculturally impacted surface water and associated C and N isotope fractionation†

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† Electronic Supplementary Information (ESI) available: Complete list of chemicals, description of the photobleaching control and, PNA/Pyr actinometer system, characterization of the photoreactor system, details on the analytical methods for pesticide quantification, CSIA measurement and TPs elucidation, calculation of short-lived reactive intermediates, steady state concentrations, of light absorption rates and screening factors and raw results.
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Abstract

Knowledge of direct and indirect photodegradation of pesticides and associated isotope fractionation can help to assess pesticide degradation in surface waters. Here, we investigated carbon (C) and nitrogen (N) isotope fractionation during direct and indirect photodegradation of the herbicides atrazine and S-metolachlor in synthetic water, mimicking agriculturally impacted surface waters containing nitrates (20 mg L\(^{-1}\)) and dissolved organic matter (DOM, 5.4 mg C L\(^{-1}\)). Atrazine and S-metolachlor were quickly photodegraded by both direct and indirect pathways (half-lives <5 and <7 days, respectively). DOM slowed down photodegradation while nitrates increased degradation rates. The analysis of transformation products showed that oxidation mediated by hydroxyl radicals (\(\text{HO}\cdot\)) predominates during indirect photodegradation. UV light (254 nm) caused significant C and N isotope fractionation, yielding isotope enrichment factors \(\varepsilon_C = 2.7 \pm 0.3\) and \(0.8 \pm 0.1\%\), and \(\varepsilon_N = 2.4 \pm 0.3\) and \(-2.6 \pm 0.7\%\) for atrazine and S-metolachlor, respectively. In contrast, photodegradation under simulated sunlight led to negligible C and slight N isotope fractionation, indicating the influence of the radiation wavelength on the direct photodegradation-induced isotope fractionation. Altogether, this study highlights the relevance of using simulated sunlight to evaluate photodegradation pathways in the environment and the potential of CSIA to distinguish photodegradation from other dissipation pathways in surface waters.
Environmental significance

Ubiquitous pesticide contamination of surface waters is a crucial environmental issue. Little is known about pesticide photodegradation in agriculturally impacted surface waters, where nitrates and dissolved organic matter (DOM) co-occur. This study addresses this bottleneck by examining pesticide photodegradation in agriculturally impacted surface waters with nitrates and DOM. It provides reference isotope enrichment factors for direct and indirect photodegradation of atrazine and S-metolachlor in surface waters. We show that compound-specific isotope analysis (CSIA) can help to differentiate photodegradation from other dissipation pathways in surface waters. Our results also advocate for more systematic use of simulated sunlight when characterizing photodegradation mechanisms in laboratory experiments.

Introduction

The ever-increasing use of pesticides, mainly for agricultural purposes, has led to ubiquitous contamination of surface waters,\(^1\) which may affect environmental biodiversity and human health.\(^2\) Thus, understanding pesticide transformation in surface waters is crucial to achieve accurate persistence predictions, anticipate the formation of transformation products (TPs) and help to mitigate detrimental effects of further pollution. While biodegradation is a major pathway of pesticide degradation in the environment, photodegradation also plays a prominent role in surface waters.\(^3\) Pesticide photodegradation is compound- and condition-specific, which often limits the interpretation of photodegradation kinetics and pathways in various types of surface waters.\(^4\),\(^5\) In particular, the influence of the hydrochemical composition, the nature of dissolved
organic matter (DOM) as well as the light spectrum on pesticide photodegradation remains poorly understood.\(^6\),\(^7\)

Pesticides undergo photodegradation by direct and indirect pathways. During direct photodegradation, pesticide molecules absorb light, resulting in bond cleavage. Indirect photodegradation involves reactions with short-lived reactive intermediates, such as hydroxyl radical (HO\(^•\)) or DOM excited triplet states (\(^3\)DOM\(^∗\)).\(^8\) Nitrate photolysis produces HO\(^•\) that can react with pesticides in surface waters, even at nitrate concentrations as low as 0.02 mg L\(^{-1}\).\(^5\) DOM has inhibitory and/or photosensitizing effects, depending on its concentration and composition.\(^9\)

On the one hand, DOM absorbs light, reducing direct photodegradation of pesticides and HO\(^•\) generation from nitrate photolysis. On the other hand, upon light absorption, DOM generates \(^3\)DOM\(^∗\) which is the precursor of singlet molecular oxygen (\(^1\)O\(_2\)) in surface waters. Both \(^3\)DOM\(^∗\) and \(^1\)O\(_2\) can react with pesticides. DOM is also a major sink of HO\(^•\) in surface waters, reducing HO\(^•\) reactions with pesticides. DOM can also reduce pesticide oxidation intermediates back to the parent compounds, and limit pesticide photodegradation.\(^10\) The effect of DOM on pesticide photodegradation is, however, compound-specific and mostly involves unknown mechanisms.\(^11\)

While the combined effects of nitrates and DOM on pesticide photodegradation is relevant for surface waters in agricultural areas, few studies on the topic have been conducted.\(^4\),\(^12\)

Compound-specific isotope analysis (CSIA) has been used in diverse environmental compartments to investigate micropollutant degradation, including pesticides and pharmaceuticals.\(^13\) Pollutant molecules with different ratios of light over heavy stable isotopes are degraded at slightly different rates. This results in a kinetic isotope effect (KIE) quantifiable by CSIA.\(^14\) In contrast, dilution, such as transport or sorption, generally does not alter significantly stable isotope ratios (e.g., \(^2\)H/\(^1\)H, \(^13\)C/\(^12\)C, and \(^15\)N/\(^14\)N) within pollutant molecules.\(^15\) The KIE reflects the rate-limiting step of the
involved pathway. Each degradation pathway displays a specific isotope fractionation pattern. This enables to differentiate the contribution of co-occurring degradation pathways in the environment. For example, CSIA was used to distinguish direct photodegradation from other degradation pathways, including biodegradation, abiotic oxidation and dilution, affecting the dissipation of diclofenac in riverine systems. Although CSIA has been applied to characterize pesticide degradation in the environment, little is known about stable isotope fractionation of pesticides during direct and indirect photodegradation in surface waters.

To the best of our knowledge, only Hartenbach et al. have evaluated isotope fractionation for direct and indirect photodegradation of atrazine under specific conditions of irradiation and water chemistry. However, isotope fractionation may depend on the irradiation source and the DOM nature. Negligible carbon (C) isotope fractionation of the antibiotic sulfamethoxazole was observed in experiments with UVC light, while significant isotope enrichment factor ($\varepsilon_C = -4.8 \pm 0.1\%$) was observed when UVB and UVA prevailed. Slight C isotope enrichment factor ($\varepsilon_C = -0.7 \pm 0.2\%$) was also observed during direct photodegradation of diclofenac under sunlight. Differences in C and nitrogen (N) isotope fractionation patterns suggest distinct pathways associated with photodegradation of the nitrile herbicide bromoxynil when irradiated either with a UV lamp or exposed to sunlight under environmental conditions. Isotope fractionation may also depend on the nature of DOM and its propensity to favor HO• and $^3$DOM* short-lived reactive intermediates oxidation. This has been observed for both methyl and ethyl tert-butyl ether. Although these results emphasize the potential of CSIA to evaluate photodegradation in laboratories and in natural systems, reference isotope enrichment factor to characterize pesticide photodegradation in agriculturally impacted surface waters are currently missing.
In this context, the purpose of this study was to examine typical patterns of photodegradation and associated isotope fractionation for atrazine and S-metolachlor in agriculturally impacted surface waters. We irradiated atrazine and S-metolachlor with simulated sunlight (λ from 270 to 720 nm) under hydrochemical conditions representative of surface waters in agricultural settings (DOM = 5.4 mg C L⁻¹; NO₃⁻ = 20 mg L⁻¹). We hypothesized that the hydrochemical composition of surface waters differently affects direct and indirect pathways of pesticide photodegradation and associated isotope fractionation. In particular, irradiation of nitrates and DOM may lead to the formation of short-lived reactive intermediates controlling underlying photodegradation pathways. Thus, direct and indirect photodegradation of pesticides were tested separately and concomitantly, in the presence of nitrates and/or DOM. C and N isotope enrichment factors were derived for direct and indirect photodegradation of atrazine and S-metolachlor. Complementary experiments were conducted under UV light (λ = 254 nm) to evaluate the effect of the irradiation wavelengths on C and N isotope fractionation during pesticide photodegradation.

Materials and methods

Chemicals and preparation of solutions

All chemicals were at least HPLC grade (>97%) (more detail in ESI†). Atrazine and S-metolachlor (Pestanal, >99.9%) stock solutions were individually prepared at 5 g L⁻¹ in dichloromethane (DCM) and aliquots were stored at −20°C in brown glass vials. Before irradiation, pesticide stock solutions were spiked and stirred for one hour, until complete DCM volatilization. Suwannee River Fulvic Acid (SRFA - 2S101F) was obtained from the International Humic Substances Society (IHSS) and selected as a source of DOM representative of headwater rivers.²⁰ Stock solutions of SRFA were prepared at a concentration of 50 mg L⁻¹ by dissolving 10 mg of SRFA in 100 mL of ultrapure water.
(UW; Resistivity >15MΩ, dissolved organic matter (DOM; <0.2 mg C L⁻¹), followed by 15 min sonication (Branson 5510, 40 kHz). The solutions were filtered through sterile 0.22 µm pore diameter cellulose acetate membranes and stored at 4°C in brown glass vials. The synthetic surface water was prepared to target the ionic composition of typical soft surface waters.²¹

Photodegradation experiment
Atrazine and S-metolachlor were selected as representatives of widely used and ubiquitously detected triazine and chloroacetanilide pesticides,¹ and based on expected degradation kinetics. Direct photolysis experiments (DIR) were carried out at room temperature (20 ± 5 °C) independently for atrazine and S-metolachlor in UW with a 50 mM phosphate buffer (KH₂PO₄/Na₂HPO₄) at pH = 7.9 ± 0.2, as it has no significant effects on photodegradation rates and isotope fractionation.²² The effect of nitrates (NIT) on pesticide photodegradation was investigated in buffered synthetic water by adding 331 ± 2 µM of sodium nitrate salts (NO₃⁻ = 20 mg L⁻¹), representative of agriculturally impacted surface waters in Europe.²³ The effect of DOM (SRFA) was studied by adding 5.4 ± 0.2 mg C L⁻¹ of SRFA, considered as a representative concentration of rivers worldwide.²⁴ The combined effect of nitrates and DOM (TOT) was investigated in synthetic water, spiked with sodium nitrates, SRFA and atrazine (5 µM) or S-metolachlor (3 µM) (Table S1 in the ESI†).

The DIR, NIT, SRFA, and TOT experiments were conducted in a 500 mL quartz tube (⌀ = 5 cm) irradiated until the non-degraded fraction (Cₜ/C₀) was below 10% for atrazine and S-metolachlor, corresponding to an irradiation duration from 7 to 600 hours. Aliquots from 15 to 200 mL were sequentially collected during the experiments. Although the light path changed over repetitive samplings and affected the screening factor (eq. S9 in the ESI†), it did not affect kinetics rates and
the contribution of the different processes. Insignificant degradation (<5%) in sterile and dark controls for all experimental conditions indicated insignificant hydrolysis and biodegradation during the photodegradation experiments. Photo-bleaching of DOM by HO• only slightly decreased the initial DOM content (<18%) after more than 310 h of irradiation (Fig. S1 in the ESI†). Irradiation conditions in the experiments with DOM were thus assumed constant. DIR, NIT, SRFA, and TOT irradiations were carried out under simulated sunlight with a stand-alone lighting system (Lambda LS, Sutter Instrument) fitted with a 300 W xenon (Xe) arc lamp (P/N PE300BUV, Cermax). A liquid optical fiber transmitted the light to a quartz tube covered with an aluminium foil with a cut-off of UV radiations below $\lambda = 270$nm. The light spectrum (Fig. S4 in the ESI†) obtained through the quartz tube, characterized with a calibrated spectroradiometer ILT 900C (International Light), was in the range 270 to 720 nm. The mean photon fluence rate was estimated to be 7 $\mu$E m$^{-2}$ s$^{-1}$ in the 290 to 400 nm range using a p-nitroanisole (PNA; 30 $\mu$M)/pyridine (10 mM) actinometer system prepared as previously described. Up to date values of wavelength-independent quantum yields for PNA, $\phi_{PNA} = 3.19 \times 10^{-3}$ mol E$^{-1}$ were used (detailed in ESI†). Due to long irradiation times, fluctuations of light intensity were monitored for the ranges of UVA (320<$\lambda$<400 nm), UVB (280<$\lambda$<320 nm) and visible light (VIS, 360<$\lambda$<830 nm) using a calibrated SOLAR light PMA2200 radiometer. The Xe arc lamps were systematically replaced when the total light intensity dropped or whenever shift in the UVA/UVB/VIS ratios exceeded 5% of the original value (Table S2 in the ESI†).

Additional direct photolysis experiments were carried out at 254 nm (DIR254) to investigate the effect of the irradiation wavelength on direct photodegradation. The photodegradation of pesticides was examined using a light-proof box (P/N 701 435, Jeulin) with black material equipped with a low-pressure mercury lamp (LP Hg; P/N TUV G6T5, Phillips – nominal power 6W) lamp
providing a major band at 254 nm with 22% of secondary bands (light band intensities are provided in Table S3 in the ESI†). 50 mL beakers (int. \( \varnothing = 37 \) mm) in borosilicate type 3.3 were filled with 50 mL of similar buffered solution as above and spiked either with atrazine (90 \( \mu \)M) or S-metolachlor (70 \( \mu \)M). Beakers were placed into the light-proof box, irradiated on the top, and sequentially removed to determine pesticide degradation rates. Light intensity within the box was homogeneous (83<\( I_{\text{average}} <121\% \); Fig. S2 in the ESI†). Control experiments without pesticides showed no cross-contamination. In this case, the photon fluence rate was not determined because the experiments were designed to evaluate the effect of the irradiation wavelength on atrazine and S-metolachlor isotope fractionation induced by photodegradation and not to derive degradation rates.

**Analytical section**

**Pesticide extraction.** Solid phase extraction (SPE) of pesticides was carried out using SolEx C18 cartridges (1 g, Dionex, CA, USA) and an AutroTrace 280 SPE system (Dionex, CA, USA) as described elsewhere.\(^{27}\) This procedure led to quantitative extraction (\( \eta = 100\% \)) and did not result in significant C and N isotope fractionation for atrazine and S-metolachlor (\( |\Delta \delta^{13}C| = |\delta^{13}C_{EA-IRMS} - \delta^{13}C_{GC-IRMS}| = 0.6 \pm 0.2\% \) and \( |\Delta \delta^{15}N| = 0.3 \pm 0.2\% \).\(^{27}\)

**Chemical analysis and pesticide quantification.** The ionic composition of irradiated solutions was determined by ion-chromatography (ICS-51000, Dionex) for main anions and cations and by Total Organic Carbon analyzer (TOC-V CPH, Shimadzu) for the C content in DOM. \( pH \) was measured using a 350i WTW \( pH \)-meter and a SenTix electrode. Absorption spectra of pesticides, NIT and DOM solutions were measured using a UV-VIS Schimadzu UV 1700 spectrophotometer over the
range 200 to 500 nm with a 1 nm resolution or taken from the literature whenever available (Fig. S3 in the ESI†).

Atrazine and S-metolachlor were quantified in selected-ion-monitoring (SIM) mode by gas-chromatography (GC, Trace 1300, Thermo Fisher Scientific) coupled with a mass-spectrometer (MS, ISQ™, Thermo Fisher Scientific), as previously described and detailed in the ESI†. Atrazine and S-metolachlor TPs were identified by target screening using a liquid-chromatography coupled with a quadrupole time of flight high-resolution mass spectrometer (LC/Q-TOF) following the methodology described elsewhere. A suspected list of molecules used for screening of TPs was generated using pathway prediction systems (UM-PPS, Metabolite Predict 2.0, META Ultra 1.2, Meta PC 1.8.1) and by reviewing the literature. Tentative candidate molecules were assigned using the criteria of mass deviation (Δm/z) below 3 ppm and mSigma value below 30. Whenever available, suspected molecule identifications were confirmed by matching residence times (RT <0.2 min) using analytical standards.

**Analysis of C and N stable isotope composition of pesticides.** C and N stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of atrazine and S-metolachlor were measured using a GC-C-IRMS system consisting of a gas chromatograph (TRACE™ Ultra, ThermoFisher Scientific, Germany) coupled via a GC IsoLink/Conflow IV interface with an isotope ratio mass spectrometer (DeltaV Plus, ThermoFisher Scientific, Germany), and configured as described elsewhere and detailed in the ESI†.

$\delta^{13}C$ and $\delta^{15}N$ values were normalized by the Vienna Pee Dee Belemnite (VPDB) standard for C and by air for N as follows:

$$\delta^h X = 1000 \times \left( \frac{R_{sample}}{R_{standard}} - 1 \right)$$ (1)
where $\delta^h X$ is expressed in per thousand ($\%\text{o}$) and $R$ refers to the ratio of heavy ($h$) to light ($l$) isotopes of the element $X$ ($^h X / ^l X$) in the analyzed samples and the international standards. Samples were injected in triplicate and $\delta^{13} C$ and $\delta^{15} N$ values are reported as the arithmetic mean. Each measurement was within the linearity ranges for $C$ and $N$. A set of in-house benzene, toluene, ethylbenzene and xylene (BTEX) (for $C$), caffeine (IAEA 600, for $N$) and pesticide (for $C$ and $N$) standards with known isotopic composition was measured at least every ten injections to control the measurement quality. Reference $\delta^{13} C$ and $\delta^{15} N$ values of BTEX, caffeine and pesticide standards were determined using an elemental analyzer-isotope ratio mass spectrometer (Flash EA IsoLink™ CN IRMS, Thermo Fisher Scientific, Bremen, Germany). An analytical uncertainty of $1 \sigma_{\delta^{13} C} \leq 0.5\%\text{o}$ ($n = 43$) and $1 \sigma_{\delta^{15} N} < 0.6\%\text{o}$ ($n = 72$) was attributed to each measurement, corresponding to the long-term accuracy and reproducibility of pesticide standards measured across the analytical sessions.

**Data Analysis**

Pesticide degradation followed the linearized pseudo-first order equation ($R^2 > 0.82$, $p < 0.05$, $n > 5$). Degradation rates ($k_{deg}$) were normalized by the mean irradiation intensity (Table S2 in the ESI†) according to eq. 2, allowing comparison among experiments. Degradation rates presented below refer to the normalized value, $k_{eff}$:

$$k_{eff} = \frac{l_{exp}}{l_{max}} \times k_{deg} \tag{2}$$

where $l_{exp}$ and $l_{max}$ stand for the light intensities measured during each experiment and the maximal inter-experiment value used as the reference for normalization.
The Rayleigh equation (eq. 3) was used to relate pesticide degradation to changes in stable isotope ratios of the non-degraded fraction of atrazine and S-metolachlor. Bulk isotope enrichment factor ($\epsilon_{\text{bulk}}$) for C and N ($\epsilon_C$ and $\epsilon_N$) were derived from the linearized Rayleigh equation and were not forced through the origin.\(^{30}\) Isotope enrichment factors were only reported when the regression with the linearized Rayleigh equation was significant ($p<0.05$).

$$\frac{\delta^hX_t + 1000}{\delta^hX_0 + 1000} = \frac{C_t}{1000} \frac{\epsilon_{\text{bulk}}}{C_0}$$

(3)

$\delta^hX_0$ and $\delta^hX_t$ are expressed in ‰ and refer, respectively, to the initial and current isotope composition of atrazine or S-metolachlor (eq. 1). $C_t/C_0$ refers to the non-degraded fraction of atrazine or S-metolachlor. Correction of $\epsilon_{\text{bulk}}$ accounting for repetitive sampling in batch experiments were deemed irrelevant here as they systematically fell within the regression confidence interval.\(^{31}\)

In addition to $\epsilon_{\text{bulk}}$, dual-isotope plots with $\delta^{15}N$ versus $\delta^{13}C$ ($\Lambda_{N/C}$, eq. 4), reflecting changes of the isotope ratios of each element, were established to compare transformation pathways in laboratory experiments and the environment.\(^{13, 32}\) $\Lambda_{N/C}$ values were estimated using the York regression (R package geostats v1.3) accounting for uncertainty measurements of both C and N.\(^{33}\)

$$\Lambda_{N/C} = \frac{\Delta \delta^{15}N}{\Delta \delta^{13}C} \approx \frac{\epsilon_N}{\epsilon_C}$$

(4)

All statistical analysis and regressions were performed in R version 3.6.3.\(^{34}\) Data from linear regression (i.e., $k_{\text{eff}}$, $\epsilon_C$ and $\epsilon_N$) are reported with a 95% confidence interval.
Results and discussion

Effects of hydrochemistry on photodegradation rates under simulated sunlight

Nitrates and DOM under simulated sunlight affected atrazine and S-metolachlor photodegradation rates. Direct photodegradation in UW (DIR) exhibited the fastest degradation rates for both pesticides with $k_{ATZ,DIR} = (6.6 \pm 0.4) \times 10^{-6} \text{ s}^{-1}$ (half-life; $DT_{50} = 29.0 \pm 1.7$ h) and $k_{SMET,DIR} = (3.3 \pm 0.2) \times 10^{-6} \text{ s}^{-1}$ ($DT_{50} = 58.8 \pm 3.2$ h). Atrazine was slightly more sensitive to direct photodegradation than S-metolachlor. Degradation half-lives ranged between 7 to 40 h for atrazine and between 30 to 87 h for S-metolachlor, and were within the same order of magnitude as previously reported half-lives.\(^5,7,12,35\)

Differences in pesticide half-lives among the experiments may be due to different light spectra and power in the experiments where atrazine and S-metolachlor absorbed light in the near UV, overlapping with the lamp radiation spectrum (<320 nm; Fig. S3 and Fig. S4 in the ESI†).

In contrast, the photodegradation rates of atrazine and S-metolachlor were, respectively, 4.1 and 3.0 times lower in the experiments with DOM (5.4 mg C L\(^{-1}\)), with or without nitrates (20 mg L\(^{-1}\)), than in the UW experiments. This supports the notion that SRFA reduce atrazine and S-metolachlor photodegradation in surface waters at typical DOM contents.\(^9,10\) However, photodegradation rates were similar in experiments with nitrates only and with direct photodegradation ($k_{ATZ,NIT}/k_{ATZ,DIR} = 0.8$ and $k_{SMET,NIT}/k_{SMET,DIR} = 1.0$). The reaction rates increased in the experiment with both nitrates and SRFA ($k_{ATZ,TOT}/k_{ATZ,SRA} = 2.1$ and $k_{SMET,TOT}/k_{SMET,TOT} = 1.2$). This indicates an oxidation of atrazine and S-metolachlor with HO• originating from nitrate irradiation. DOM and nitrates in surface waters have a similar photosensitizing or inhibitory effect on atrazine and S-metolachlor photodegradation. Indeed, atrazine and S-metolachlor have similar absorption spectra in the near UV range and very similar
one-electron oxidation potentials ($E_{1S-metolachlor} = -2.40$ V vs NHE and $E_{1atrazine} = -2.41$ V vs NHE). It is worth noting that the one-electron oxidation potential is often used as an indicator of the reaction potential of organic contaminants with $^3$DOM$^*$ and HO•.$^9,11,36$

Photochemical predictions (eq. 5) allowed to infer the contribution of direct and indirect photodegradation. The equation 5 teases apart the contribution of direct and indirect photodegradation from the measured degradation rates (i.e., HO• and $^3$DOM$^*$ mediated).$^5,37,38$

Carbonate radicals (CO$_3$•) were not included as potentially relevant photosensitizers because oxidation of atrazine and anilines with CO$_3$• under simulated sunlight remains limited, even in carbonate-rich surface waters ([HCO$_3$•] and [CO$_3^{2-}$] $\approx$ 10 times higher than in our conditions).$^{39}$ However, carbonates were considered as potential quenchers of HO•.$^5$ The calculation procedure and parameters are summarized in the ESI† Table S4.

$$\frac{dC}{dt} = -k_{obs} \times C$$

$$= -\left(k_{dir} + k_{HO\cdot} \times [HO\cdot]_{SS} + k_{3DOM^*} \times [^3DOM^*]_{SS} + k_{1O_2} \times [^1O_2]_{SS}\right) \times C$$

(5)

$C$ stands for pesticide concentration, and $k_{obs}$ for the measured degradation rate (s$^{-1}$), which can be expressed as the sum of both direct ($k_{dir}$) and various indirect pathways ($k_{HO\cdot}$, $k_{3DOM^*}$ and $k_{1O_2}$). The latter degradation rates are second-order, and depend on the steady-state concentrations of the short-lived reactive intermediates ([HO•]$_{SS}$, [^1O_2]$_{SS}$ and [^3DOM^*]$_{SS}$).

The observed and predicted degradation rates were similar. This suggests that the dominant photodegradation pathway could be identified in all experiments (Fig. 1; Table S5 in the ESI†).

Nitrate-mediated photodegradation contributed to 60% of atrazine and 90% of S-metolachlor photodegradation in TOT conditions. Nitrate-mediated photodegradation is thus expected to
dominate in agriculturally impacted surface waters. Although competing for UV light and limiting
direct photodegradation, SRFA favored indirect photodegradation with $^3$DOM* and HO•. Accordingly, atrazine was slightly more sensitive than S-metolachlor to oxidation by $^3$DOM*. In contrast, HO• affected mostly S-metolachlor. Nitrate-mediated photodegradation was hampered in the TOT experiment in comparison with the NIT experiment. Indeed, DOM at 5.4 mg C L$^{-1}$ not only competes for light irradiance with nitrates but also quenches HO•. This is expected to reduce nitrate photosensitizing, as observed in the NIT experiment.$^{10, 40}$ In addition, direct photodegradation rates of both atrazine and S-metolachlor were 10 to 30 times slower in the SRFA than in the DIR experiments. Indeed, UV light absorption by DOM limited direct herbicide photodegradation. Finally, $^1$O$_2$ stemming from reactions between dissolved oxygen and $^3$DOM* did not significantly contribute to herbicide degradation rates (<2% for atrazine and <4% for S-metolachlor). In aqueous solutions, $^1$O$_2$ reaction with pesticides remains limited since water immediately scavenges most of the produced $^1$O$_2$.38
Fig. 1. Observed and predicted half-lives ($DT_{50}$) of A) atrazine and B) S-metolachlor under simulated sunlight. Predicted contributions of direct and indirect pathways (HO•, $^{1}$O$_2$ and $^{3}$DOM•) to the total pesticide photodegradation is displayed within the stacked bars. Photolysis condition: DIR: direct photodegradation, NIT: effect of nitrates, SRFA: effect of DOM and TOT: combined effects of nitrates and DOM. $DT_{50}$ values were calculated from degradation rates according to $DT_{50} = ln(2)/k$. Error bars correspond to the 95% confidence interval.
Altogether, both direct and nitrate-mediated photodegradation can transform atrazine and S-metolachlor in agriculturally impacted surface waters. However, DOM competes for UV light absorption with pesticides and nitrates, which greatly reduces the transformation potential of pesticides. Pesticides with a light absorption spectrum in the near UV, such as atrazine, are thus less impacted. On the other hand, nitrates (20 mg L\(^{-1}\)) promote the generation of HO•, which partly compensate for the UV light competition caused by DOM absorption.

**Formation of transformation products during photodegradation**

Transformation products (TPs) were analyzed in samples displaying a similar extent of photodegradation (≈80%). Atrazine irradiation led to desethylatrazine (DEA) and desisopropylatrazine (DIA) in all experiments. In contrast, 2-hydroxyatrazine (A-OH) was only detected in the direct photodegradation experiment (Fig. 2A).\(^4\) A-OH is also reported during biotic hydrolysis.\(^4\) Indirect photolysis may proceed through atrazine oxidation at the N-ethyl and N-isopropyl group by \(^3\)DOM* and HO•.\(^4\) Non-selective attacks of HO• on the N-ethyl and N-isopropyl groups may thermodynamically favor the formation of DEA since weaker N–C bond dissociation energy is associated with the N-ethyl group.\(^4\) A steric effect is also expectedly favoring the formation of DEA as the isopropyl group is less reactive than the ethyl group.\(^4\) On the other hand, the intermediates formed during the hydrogen abstraction by HO• can electronically favor DIA formation due to the weaker C–H bond, and weaker intermediate stabilization by the isopropyl group than by the ethyl group.\(^4\)
Four TPs of S-metolachlor were identified (Fig. 2B). Hydroxymetolachlor was observed in neither the DIR254 nor DIR experiments, although it was reported in previous studies\textsuperscript{12,47,48} as a major TP formed during direct photodegradation of metolachlor. We postulate that hydroxymetolachlor was further degraded into secondary TPs after 80% degradation of S-metolachlor. In the SRFA and DIR experiments, only metolachlor oxalinic acid (OXA) was observed, whereas in NIT and TOT experiments, no TPs could be detected. This suggests that S-metolachlor was easily oxidized by \ensuremath{^3}\text{DOM}^+ and \text{HO}\cdot into S-metolachlor OXA, its acidic form. In the presence of nitrates, the large and constant generation of nonselective \text{HO}\cdot may favor fast degradation of S-metolachlor OXA.\textsuperscript{12} This can explain the absence of TPs in the NIT and TOT experiments. The absence of TPs in the NIT and TOT experiments also supports the idea that nitrates mainly contribute to S-metolachlor photodegradation, even with 5.4 mg C L\textsuperscript{-1} of DOM. It is worth noting that the diversity of detected TPs was the highest for both atrazine and S-metolachlor during irradiations at $\lambda = 254$ nm in the DIR254 experiments (Fig. 2B). In the case of S-metolachlor, metolachlor CGA 37735, metolachlor CGA 50267 and MET-G\textsuperscript{46} were specific suspected TPs in the DIR254 experiments. However, only metolachlor CGA 37735 could be confirmed with analytical standards. This suggests that the spectrum of degradation pathways associated with monochromatic UV light is wider than that with simulated sunlight. In addition, specific TPs may be produced from the degradation of the first generation of TPs.
Fig. 2 Transformation products for A) atrazine and B) S-metolachlor in photolysis experiment. Relative intensity refers to the peak amplitude of transformation product normalized by the intensity of the dominant transformation product peak for each sample. Photolysis condition are: DIR254: direct photodegradation at 254 nm, DIR: direct photodegradation under simulated sunlight, NIT: effect of nitrates, SRFA: the effect of DOM, and TOT: concomitant effects of nitrates and DOM together. TPs are: DIA: desisopropyl atrazine, DEA: desethyl atrazine, A-OH: hydroxyl atrazine and A-DOH: desethyl 2 hydroxy atrazine, OXA: metolachlor oxalinic acid, MET-G: 1-(1-methoxypropan-2-yl)-5,9-dimethyl-1,5-dihydro-4,1-benzoazepin-2(3H)-one found by Lui et al., CGA37735: metolachlor CGA 37735 and CGA50267: metolachlor CGA 50267.
Altogether, direct and indirect photodegradation pathways produce slightly different patterns of TPs. While the dechlorinated compound A-OH features direct photodegradation for atrazine, oxidized compounds (e.g., atrazine DEA and DIA and S-metolachlor OXA) are more likely to predominate in agriculturally impacted surface waters. However, TPs are likely transient and rapidly degraded into secondary and unidentified TPs, as shown during S-metolachlor photodegradation. TP patterns were identical for atrazine in the NIT and SRFA experiments, although $^3$DOM$^+$ oxidation is expected to prevail over HO•. Similarly, indirect photodegradation of S-metolachlor did not generate specific patterns of TPs. Interestingly, S-metolachlor ESA was not detected during either direct or indirect photodegradation. Since metolachlor ESA is frequently measured in the environment and is neither a transformation product associated with hydrolysis$^{27}$ or photodegradation, its recurrent detection may indicate S-metolachlor biodegradation.

C and N isotope fractionation to trace atrazine and S-metolachlor photodegradation

*Direct photodegradation experiments.* Direct photolysis of atrazine in the DIR254 experiment with a low-pressure mercury lamp caused an inverse isotope fractionation for both C and N ($\Lambda_{N/C} = 1.17 \pm 0.11$; Table 1). This is consistent with previous observations from Hartenbach et al.$^{17}$ ($\Lambda_{N/C} = 1.05 \pm 0.14$). However, isotope enrichment factors ($\varepsilon_C = 2.7 \pm 0.3^{\circ}$ and $\varepsilon_N = 2.4 \pm 0.3^{\circ}$) were less pronounced in the present study than in Hartenbach et al.$^{17}$ The lamp emission spectra and light path lengths may affect stable isotope fractionation. Indeed, the cut-off of light emission is $>254$ nm and the light path length in Hartenbach et al.$^{17}$ was smaller (1.4 cm) than in our study
(light path length = 4.9 cm). Hence, the contribution of the secondary band wavelength (>300 nm; Table S3, ESI†) may be higher in the DIR254 experiment. Assuming that wavelengths above 254 nm do not cause significant isotope fractionation, different cut-offs and light path lengths in the two setups can lead to slightly different extents of isotope fractionation ($\Delta \varepsilon_C$ of 1.3).

The inverse C isotope fractionation of atrazine cannot be explained by the cleavage of the C–N bond at the N-ethyl or N-isopropyl group. Indeed, cleavage at the N-ethyl or N-isopropyl group leading to DEA and DIA should reflect a normal primary isotope effect. Two non-exclusive hypotheses may explain the isotope fractionation patterns in the DIR254 experiments. First, successive steps of intersystem crossing before atrazine dechlorination can lead to an inverse isotope fractionation during direct photolysis of atrazine. The inverse C isotope fractionation for atrazine in the DIR254 experiment is due to the generation of a singlet state radical stabilized by the ring delocalization. The excited singlet state then undergoes an intersystem crossing toward a triplet state and hydrolyzes to form A-OH. The likelihood of recombination back to their original state is higher for the light C isotope in the C–Cl bond, leading to an inverse C isotope fractionation. The same mechanism has been suggested to result in an inverse N isotope fractionation for atrazine. Second, a magnetic mass-independent isotope effect (MIE) involving spin carrying nuclei and unpaired electrons may also cause an inverse isotope fractionation.

In contrast, S-metolachlor featured a less pronounced and inverse C and stronger N isotope fractionation ($\Lambda_{N/C} = -4.24 \pm 0.22$) than atrazine. A similar nucleophilic substitution of the chlorine atom by a hydroxyl group at the C–Cl bond is expected for the formation of the MET-OH. However, it is a priori unlikely because the reactive site of S-metolachlor is too far from the ring.
Table 1. C and N isotope difference ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$), isotope enrichment factors ($\varepsilon$) and lambda values ($\Lambda_{N/C}$) for atrazine and S-metolachlor.

<table>
<thead>
<tr>
<th>pesticide</th>
<th>experiment ($C_{t=\text{end}}/C_0$)</th>
<th>$\Delta\delta^{13}C$ (%)</th>
<th>$\Delta\delta^{15}N$ (%)</th>
<th>$\varepsilon_C$ (%)</th>
<th>$\varepsilon_N$ (%)</th>
<th>$\Lambda_{N/C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>atrazine</td>
<td>DIR 254 (0.01)</td>
<td>-8.1</td>
<td>-10.8</td>
<td>+2.7 ± 0.3*</td>
<td>+2.4 ± 0.3*</td>
<td>+1.17 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>DIR (0.10)</td>
<td>-0.3</td>
<td>-0.5</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>NIT (0.01)</td>
<td>0.4</td>
<td>-2.0</td>
<td>n.s.</td>
<td>+0.7 ± 0.3</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>SRFA (0.03)</td>
<td>-0.7</td>
<td>-0.8</td>
<td>n.c.</td>
<td>+0.6 ± 0.2</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>TOT (0.02)</td>
<td>-0.6</td>
<td>-4.1</td>
<td>+0.1 ± 0.1</td>
<td>+0.9 ± 0.6</td>
<td>+6.0 ± 2.2</td>
</tr>
<tr>
<td>S-metolachlor</td>
<td>DIR 254 (0.01)</td>
<td>-2.3</td>
<td>4.5</td>
<td>+0.8 ± 0.1*</td>
<td>-2.6 ± 0.7*</td>
<td>-4.24 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>DIR (0.2)</td>
<td>0.2</td>
<td>1.0</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>NIT (0.02)</td>
<td>0.5</td>
<td>3.3</td>
<td>n.s.</td>
<td>-0.8 ± 0.1</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>SRFA (0.06)</td>
<td>-0.6</td>
<td>1.8</td>
<td>n.c.</td>
<td>-0.7 ± 0.4</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>TOT (0.06)</td>
<td>0.2</td>
<td>1.8</td>
<td>n.c.</td>
<td>-0.7 ± 0.1</td>
<td>n.c.</td>
</tr>
</tbody>
</table>

$\Delta \delta = \delta(t) - \delta(t=0)$, isotope enrichment factors ($\varepsilon$) are reported with their uncertainties corresponding to the 95% confidence interval from the regression analysis. n.s.: not significant ($p>0.05$). n.c.: not computed.

Rayleigh plots are presented in the ESI† Fig. S5 & S6. * $\varepsilon$ calculated for data with $C_t/C_0>0.2$ only as recommended by$^{50,51}$

Direct photodegradation under simulated sunlight (DIR) led to non-significant C and N isotope fractionation for both atrazine and S-metolachlor (Table 1). This suggests that C isotope fractionation associated with direct photodegradation under simulated sunlight of organic micropollutants remains limited. This is in line with preliminary evidence from diclofenac photolysis.$^{16}$ Most importantly, our results indicate that photo-induced C and N isotope
fractionation depends on the irradiation wavelength. Similar results have been reported by Willach et al.\textsuperscript{18} for the antibiotic sulfamethoxazole under broad and cut-off light spectrums. The Xe lamp emits light over a broad and continuous range of wavelengths and energies from the near UV ($\lambda>$270 nm) to the near-infrared ($\lambda<$600 nm), whereas the Hg lamp generates a single-band UV light ($\lambda=254$ nm). Consequently, the Xe lamp emission may generate a miscellaneous population of excited triplet states. This presumably affected the photolytic dechlorination of 4-Cl-aniline and led to varying and compensating C and N stable isotope fractionation caused by a spin selective isotope effect.\textsuperscript{50} Hence, the average isotope composition of the residual fraction of atrazine and S-metolachlor may reflect multiple and co-occurring photodegradation reactions under simulated sunlight. Interestingly, atrazine direct photolysis quantum yield also depended on the wavelength, with values ranging from 0.0158 to 0.0196 for simulated sunlight and from 0.035 to 0.060 for 254 nm irradiation.\textsuperscript{7} This emphasizes that the photodegradation pathways are wavelength-dependent, while each pathway is characterized by a specific isotope fractionation and associated quantum yield.

\textit{Indirect photodegradation experiments.} In the indirect photodegradation experiments (NIT, SRFA and TOT), only slight C and N isotope fractionation were observed, even at the latest stage of the reactions (>80% of degradation; Fig. S5 & S6 in the ESI). Thus, the uncertainties associated with the isotope enrichment factors ($\varepsilon$) were larger for the indirect than for the direct photodegradation experiments, and corresponding $\varepsilon$ should be interpreted with caution. C isotope fractionation was insignificant in both the NIT and TOT experiments under simulated sunlight, except for atrazine under in the TOT experiment. Similar N isotope fractionation patterns were observed in both the NIT and TOT experiments ($\varepsilon_N^{TOT}/\varepsilon_N^{NIT} = 1.3 \pm 0.5$ for atrazine.
and $\varepsilon_{N}^{\text{TOT}} / \varepsilon_{N}^{\text{NIT}} = 0.9 \pm 0.2$ for S-metolachlor). However, the N isotope fractionation was inverse for atrazine and normal for S-metolachlor. Similar N isotope fractionation for atrazine and S-metolachlor supports the idea that nitrate-mediated photodegradation predominates in agriculturally impacted surface waters, even in the presence of 5.4 mg C L$^{-1}$ of DOM. Accordingly, $\varepsilon_{N}$ for atrazine and S-metolachlor in the NIT experiments represent reference factors for H$\text{O}^\bullet$ oxidation in the presence of nitrates.

In the SRFA experiments, where $^3$DOM$^\ast$ oxidation and H$\text{O}^\bullet$ presumably drove the indirect photodegradation, only N isotope fractionation was observed for atrazine ($\varepsilon_{N} = 0.6 \pm 0.2\%$) and S-metolachlor ($\varepsilon_{N} = -0.7 \pm 0.4\%$). Low N isotope fractionation upon $^3$DOM$^\ast$ oxidation is consistent with the absence of C–N bond cleavage during S-metolachlor dechlorination leading to OXA. In addition, similar N fractionation in the NIT and SRFA experiments ($\varepsilon_{N}^{\text{SRFA}} / \varepsilon_{N}^{\text{NIT}} = 0.9 \pm 0.5$) for both atrazine and S-metolachlor suggest a significant contribution to the overall photodegradation of H$\text{O}^\bullet$ radicals, generated by DOM irradiation (H$\text{O}^\bullet$ = 54% and $^3$DOM$^\ast$ = 33%). The inverse N isotope fractionation for atrazine also agrees with an oxidation by $^3$DOM$^\ast$ at the N-ethyl or N-isopropyl side chain, leading to either DEA or DIA through single electron transfer.$^{20,53}$ Accordingly, an inverse C isotope fractionation would be expected, which is not the case. The large collection of chromophores in natural DOM (i.e., SRFA) results in a wide panel of excited state reduction potentials for the oxidation of molecular bonds involving irrespectively heavy or light stable isotopes.$^{10}$ This hypothesis could be confirmed experimentally using Cs$^+$ as a quencher of excited singlet states to enhance the contribution of excited triplet states. The corresponding decrease of $\varepsilon_{C}$ and $\varepsilon_{N}$ could then be followed up, as previously shown for 2-Cl-anilines.$^{50}$
Implication for tracing photodegradation using CSIA in the environment

Photodegradation contributes to pesticide degradation to a similar or greater extent than biodegradation\(^3\) in static surface waters (e.g., ponds, lakes, etc.) and rivers with long transit time.\(^{54}\) However, the contribution of photodegradation depends on the hydrochemistry and targeted pesticides. The direct photodegradation of atrazine and S-metolachlor was particularly fast, although it was slowed down by the UV light absorption caused by DOM. However, nitrate concentrations higher than 20 mg L\(^{-1}\) can significantly enhance oxidation by producing HO•, counterbalancing the photodegradation inhibition caused by DOM in surface waters. Under simulated sunlight and environmentally-relevant hydrochemistry (\(pH = 8; 20 \text{ mg L}^{-1}\) of nitrates and 5.4 mg C L\(^{-1}\) of DOM), half-lives of atrazine and S-metolachlor were as short as a few days (\(< DT_{50} < 10\) days). However, strong variations that occur naturally in solar irradiance in the UV region either due to gaseous light absorption by ozone at different altitudes or geographic locations, or to the rapid absorption of UV lights in the water, restrict our conclusions to shallow surface waters (i.e., <50 cm deep).\(^{55}\) Altogether, this advocates to take more systematically into account the local irradiation spectrum and hydrochemical conditions to estimate the kinetics and the contribution of pesticide photodegradation.\(^{56}\)

While CSIA offers a new opportunity to evaluate pesticide degradation in surface waters, identifying photodegradation pathways of micropollutants in the environment remains challenging.\(^{16,18}\) Under simulated sunlight irradiation, the C and N isotope compositions of atrazine and S-metolachlor are not significantly affected by photodegradation, although photodegradation contributes significantly to pesticide degradation. Accordingly, changes in the C stable isotope ratios may mostly reflect atrazine or S-metolachlor biodegradation. Indeed, biodegradation is typically characterized by significant isotope fractionation (Fig. 3) in surface waters with high
nitrate and DOM concentrations. This is illustrated by the dual-element plots comparing N/C isotope enrichment factors determined in this study with those from the literature (Fig. 3).

**Fig. 3** Dual C and N isotope plot for A) atrazine and B) S-metolachlor. Contrasted isotope fractionation patterns are represented under biotic oxidative dealkylation by the bacterial strain *Rhodococcus* sp. NI86/21, biotic hydrolysis with *Arthrobacter aurescens* TC1,56 abiotic acid (pH=4) and alkaline (pH=12) hydrolysis (hyd.)27 and photodegradation for DIR254 and TOT condition (this study).

However, relying solely on changes of N isotope ratios may not enable a univocal differentiation of degradation pathways. For instance, atrazine photodegradation under DIR254 condition ($\varepsilon_N = 2.4 \pm 0.3\%$) and biodegradation ($\varepsilon_N = 2.3 \pm 0.3\%$)56 have identical $\varepsilon_N$. This emphasizes the need for multi-element isotope analysis (e.g., H, C, N and Cl) to elucidate degradation pathways.

Photodegradation pathways of atrazine and S-metolachlor mostly involve C–H and C–Cl bonds.16.
In the future, multi-element isotope analysis of pesticides by CSIA from environmental samples may help to distinguish photodegradation pathways and evaluate the contribution of photodegradation to the overall dissipation.

Conclusion

This study highlights the relevance of direct and indirect photodegradation of pesticides in agriculturally impacted surface waters with nitrates and DOM. The irradiation source strongly influences the C and N isotope fractionation patterns while the hydrochemistry (i.e., nitrate, DOM) affects the degradation rates. Consequently, reference isotope enrichment factors to evaluate photodegradation in the environment with CSIA should be derived from laboratory experiments using simulated sunlight. In addition, the nature of DOM under different environmental contexts can affect the production of HO•, and thus alter stable isotope fractionation of pesticides. Although SRFA can serve as a model DOM representative of headwater rivers, the effect of DOM characteristics on stable isotope fractionation during pesticide photodegradation should be further studied. Finally, recent developments on Cl-CSIA offer promising insights to investigate pesticide degradation pathways in surface waters using multi-element CSIA.

Author Contributions


Conflicts of interest

There are no conflicts to declare.
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References


41 A. Torrents, B. G. Anderson, S. Bilboulian, W. E. Johnson and C. J. Hapeman, Atrazine photolysis: Mechanistic investigations of direct and nitrate mediated hydroxy radical processes and the


