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Water flow plays a key role in determining chemical biodegradation in water-sediment systems

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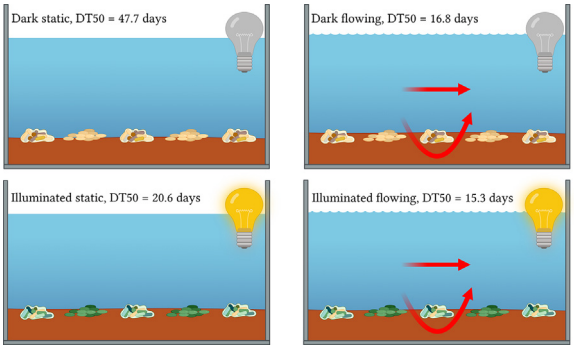
HIGHLIGHTS

- Isopyrazam dissipation was slow in dark static microflumes.
- Quicker dissipation in dark flowing and illuminated static and flowing microflumes.
- Flow may increase dissipation by increased hyporheic exchange and microbial contact.
- Non-UV light increases dissipation in static systems due to phototrophic metabolism.
- Microflumes provide greater environmental realism than OECD test systems.

GRAPHICAL ABSTRACT



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ABSTRACT

Before agrochemicals can be registered and sold, the chemical industry is required to perform regulatory tests to assess their environmental persistence, using defined guidelines. Aquatic fate tests (e.g. OECD 308) lack environmental realism as they are conducted under dark conditions and in small-scale static systems, which can affect microbial diversity and functionality. In this study, water-sediment microflumes were used to investigate the impact of these deficiencies in environmental realism on the fate of the fungicide, isopyrazam. Although on a large-scale, these systems aimed to retain the key aspects of OECD 308 tests. Tests were carried out under both a non-UV light-dark cycle and continuous darkness and under both static and flowing water conditions, to investigate how light and water flow affect isopyrazam biodegradation pathways. In static systems, light treatment played a significant role, with faster dissipation in illuminated compared to dark microflumes (DT50s = 20.6 vs. 47.7 days). In flowing systems (DT50s = 16.8 and 15.3 days), light did not play a significant role in dissipation, which was comparable between the two light treatments, and faster than in dark static microflumes. Microbial phototroph biomass was significantly reduced by water flow in the illuminated systems, thereby reducing their contribution to dissipation. Comprehensive analysis of bacterial and eukaryotic community composition identified treatment specific changes following incubation, with light promoting relative abundance of Cyanobacteria and eukaryotic algae, and flow increasing relative abundance of fungi. We conclude that both water velocity and non-UV light increased isopyrazam dissipation, but the contribution of light depended on the flow conditions. These differences may have resulted from impacts on microbial communities and via mixing processes, particularly hyporheic exchange. Inclusion of both light and flow in studies could improve the extent they mimic natural environments and predict chemical environmental persistence, thus bridging the gap between laboratory and field studies.

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1. Introduction

Agrochemicals help improve crop yield and quality (Hazell, 2002), however, they also have the potential to cause adverse effects on the environment and human health (Carter, 2000). Prior to regulatory approval, the chemical industry is responsible for carrying out regulatory tests to determine how chemicals transform in the environment and to ensure products pose as little risk as possible (Davies et al., 2013a). The frameworks for performing agrochemical regulatory tests are provided by the Organisation for Economic Co-operation and Development (OECD) and tiered tests are used to assess biodegradation processes. Simulation tests are designed to mimic defined environmental compartments, for example OECD 308 uses water and sediment inoculum to represent an aquatic environment (OECD, 2002; Kowalczyk et al., 2015). Although these tests mimic specific environments, they are conducted in simplistic laboratory scale microcosm systems (OECD, 2002; Gartiser et al., 2017; Redman et al., 2021; Davenport et al., 2022), therefore it is not possible to accurately replicate the biotic and abiotic characteristics of the real environment.

Within OECD tests, microbial communities are treated like a 'black box' and currently there is little understanding of how they change during incubation and the subsequent implications for biodegradation processes (Kowalczyk et al., 2015). Recent evidence suggests that the diversity and composition of microbial communities which develop in OECD 308 tests change markedly during incubation and they are not predictable, despite the standardised test design and incubation conditions (Southwell et al., 2020). If microbial populations incubated in microscale laboratory tests do not reflect those typical of the environment, biodegradation rates generated from regulatory tests may not represent those in the field (Sturman et al., 1995; Carpenter, 1996; Clements and Newman, 2002; Coll et al., 2020).

In nature, sunlight may be a key factor determining microbial community composition, especially in the water column and on the sediment surface. For instance, increased UV radiation exposure can reduce primary production and metabolism (Lindell et al., 1996) or, contrastingly, photolysis may increase the available biological substrate pool and therefore increase microbial productivity (Kieber, 2000). Furthermore, light stimulates growth of phototrophic microorganisms which utilise light as an energy source (Southwell et al., 2020). Phototrophic communities are metabolically capable of biodegradation of a wide range of chemicals (Stravs et al., 2017). Nevertheless, degradation is compound specific, as was shown by Davies et al. (2013a). As well as direct metabolism, phototrophs could promote heterotrophic degradation by altering environmental parameters, such as carbon availability and pH (Davies et al., 2013a). Indeed, mixed systems of both phototrophs and heterotrophs can significantly increase degradation rates relative to systems with exclusively phototrophic or heterotrophic communities (Thomas and Hand, 2012), emphasising the importance of synergistic relationships for determining biodegradation (Borde et al., 2003). Whether the role of phototrophs is direct or indirect, by excluding light in OECD 308 tests, there is no consideration of its impacts on microbial communities and their biotransformation potential. This may be detrimental to the production of environmentally relevant data.

Importantly, OECD 308 tests are typically carried out statically, meaning they are more representative of ponds and drainage ditches, where there is no or low flow, even though the data can be used for flowing water body risk assessments. Water flow and sediment dynamics are key attributes of many aquatic systems (Gartiser et al., 2017) and they have the potential to affect microbial processes both directly and indirectly. Therefore, the extent to which OECD 308 tests represent stream and river systems is uncertain (Jaeger et al., 2019). Water flow causes mixing within the water column and exchange of water across the sediment-water interface, into and out of the hyporheic zone (Bonanni et al., 1992; Rusch et al., 2001; Gualtieri, 2004). Both mixing within the water column and hyporheic exchange are determined by complex interactions between flow rate, channel morphology, and water depth (Packman and Salehin, 2003) and this can also be influenced by environmental factors, such as wind, rain, and runoff (Reynolds, 1994).

Mixing within the water column determines mass transport and stimulates microbial growth, including that of chemical degraders (Bauer et al., 2008). Spain et al. (1984) proposed that higher chemical biodegradation rates could be linked to faster mixing in large-scale systems due to increased transportation. Additionally, Oya and Valocchi (1998) concluded that chemical biodegradation only occurred in mixing zones due to promotion of microbial growth. Hyporheic exchange processes are of particular importance because they determine the extent to which chemicals carried in the water column come into contact with the sediment, governing sorption characteristics and bioavailability. They also determine contact of chemicals with biofilms and degrader communities at the sediment-water interface and within the sediment (Smith, 2005; Sánchez-Pérez et al., 2013; Posselt et al., 2020), as well as the flow of oxygen and nutrients into the sediment, controlling microbial growth and community composition (Bonanni et al., 1992; Rusch et al., 2001; Gualtieri, 2004; Jaeger et al., 2019; Cook et al., 2020).

The importance of phototrophs in chemical biodegradation has already been shown in small-scale OECD 308-type static systems (Hand and Oliver, 2010; Thomas and Hand, 2011; Southwell et al., 2020), but how flow rate influences the proliferation of these microorganisms and subsequently their biodegradation potential remains unclear. Therefore, in the current study, we used microflume water-sediment systems to investigate the extents to which water flow and the presence of light impacted microbial community dynamics and dissipation of the fungicide isopyrazam. Microflumes were incubated under either a non-UV light-dark cycle (to exclude photolysis but allow phototroph proliferation) or continuous darkness and with either static or flowing water. The dark static microflumes acted as a larger scale version of an OECD 308 test system and were used as a control against the light and flowing treatments. Changes in nutrient availability and microbial characteristics were assessed in order to understand variation in dissipation rates across treatments and also to identify regimes which retained the microbial characteristics of the initial inoculum.

2. Materials and methods

2.1. Environmental inoculum

River water and sediment were obtained from the River Dene at Wellesbourne, United Kingdom (52°12′02.5″N, 1°36′30.4″W) (Supplementary Material (SM); Fig. S1) in August 2016. Sediment was sampled within the top 5 to 10 cm of the riverbed. It was analysed in the same way as in Southwell et al. (2020) and was predominantly sandy (18.0, 9.2, and 72.8 % silt, clay, and sand, respectively). Water was collected by submerging containers under the water surface while facing upstream. Additionally, the following parameters were measured at the sample site (SM; Table S1): water temperature using a Total Immersion thermometer (Fisher Scientific, UK), light intensity using a RS-105 light meter (RS Components Ltd., UK), and water depth and velocity using an 801 EM flow meter (Valeport, UK). Water pH was measured in the laboratory using an Accumet basic AB15 pH meter (Fisher Scientific, UK). Particulates and large protozoa were filtered from the river water using a 106 μm sieve (Fischer Scientific, UK), as detailed in OECD 309 regulatory guidelines (OECD, 2004). Sediment was homogenised by wet-sieving through a 20 mm sieve (Endecotts Ltd. UK). Samples were refrigerated at 4 °C and used within 24 h.

2.2. Test chemical

Studies were performed using isopyrazam (99.4 % purity) supplied by Syngenta, Jealott's Hill International Research Centre, United Kingdom (Fig. 1 and SM; Table S2). This fungicide has a slow degradation rate in regulatory OECD 308 studies (EFSA, 2012) and would therefore exceed persistence criteria, but is susceptible to phototrophic metabolism (Hand and Oliver, 2010; Southwell et al., 2020). As isopyrazam typically degrades slowly under dark conditions, this would allow the impact of flow and the interactions between flow and light conditions to be assessed.

2.3. Experimental set up

Microflume systems (Fig. 2 and SM; Fig. S2) were made from toughened glass (Three Spires Glass Company Ltd., UK). They comprised six flowing

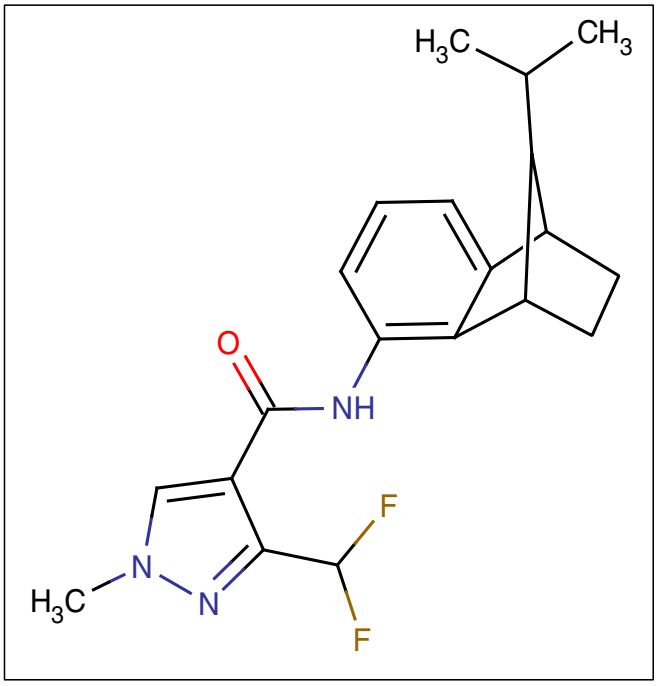

Fig. 1. Structure of isopyrazam. The compound was 99.4 % pure and the mixture was made up of 89.7 % *syn*-epimer and 9.7 % *anti*-epimer. Created using ChemDraw (PerkinElmer, US).

systems (2.36 × 0.2 × 0.2 m) with associated stainless steel plumbing (Pipestock, UK) which recirculated water, as described by Cook et al. (2020), and six static systems without the plumbing (2.0 × 0.2 × 0.2 m). Flowing microflumes were also fitted with a Clarke TAM105 pump (Clarke International, UK), a Hailea HC-300A aquarium water chiller (Hailea Group Co., China), and a GPI TM Series electronic flow meter (Great Plains Industries, Inc., US). Full details of the microflume design and preliminary tests demonstrating that sorption to the systems was minimal are shown in the SM (Method S1).

The systems were located in a controlled environment room at 20 ± 2 °C and the chillers on the flowing systems were similarly set at 20 °C to ensure that they stayed at the same temperature as the static systems. Temperature was monitored throughout the experiment using an NTC030WP00 temperature sensor (Carel, UK) in each middle microflume bank. Dark treatment microflumes were covered with DMP black damp-proof membrane 1200GA (Capital Valley Plastics, UK) so that no light could penetrate the systems. As the purpose of illumination was to investigate the contribution of phototrophic communities to metabolism under static and flowing conditions, illuminated treatment microflumes were covered with LEE226 filter (Transformation Tubes, UK) covers. These covers inhibited UV light with minimal transmission below 390 nm, preventing degradation from both indirect and direct photolysis (isopyrazam absorbs light only to 315 nm (Hand and Oliver, 2010)), but still allowing phototroph proliferation (SM; Fig. S2). Fluorescent 70 W daylight bulbs (F70W/865 T8 6ft, Fusion Lamps, UK) were used with LEE226 filters on a 16-hour light and 8-hour dark cycle. Further details of the light transmission spectra can be found in Method S2.

2.4. Environmental inoculum addition

The following treatments were set up using triplicate channels; dark static, dark flowing, illuminated static, and illuminated flowing. Prior to sediment addition, latex free stoppers from 100 mL syringes (BD Plastipak, US) were placed at allocated sediment sampling sites. Sieved sediment was then added along the length of the channel to 3 cm depth (12,000 cm^3), taking care not to disturb the stoppers, before leveling with a customised tool. River water was transferred into each microflume by the water inlet, to

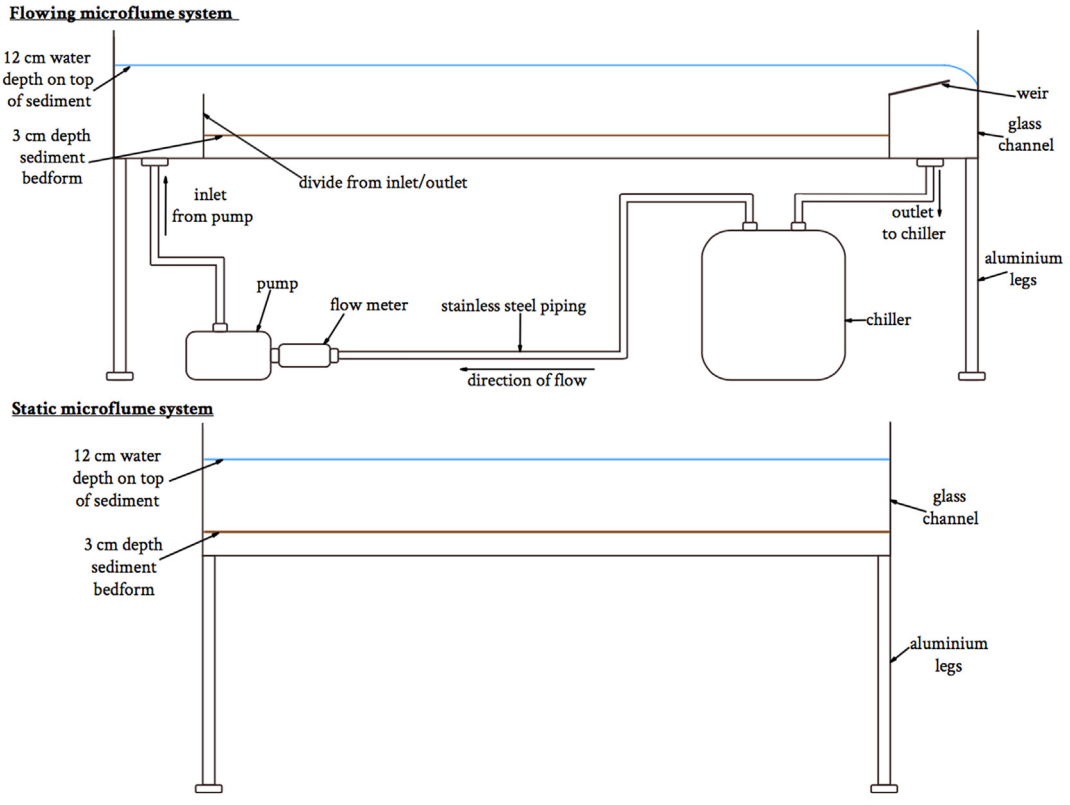

Fig. 2. The flowing (top) and static (bottom) microflume systems. Microflume systems comprised a glass channel supported by an aluminium frame with legs. The flowing microflume system had connecting stainless steel piping, along which a pump, flow meter, and chiller were attached. Diagram created using site.youidraw.com.

minimise sediment disturbance, to a 12 cm depth on top of the sediment bed (48,000 cm^3 within the main tank, excluding pipe work), which ensured a 4:1 volume ratio of water to sediment, as stated in the OECD 308 guidelines (OECD, 2002).

2.5. Establishment of water flow conditions

All microflumes were left static for two days to allow sediment particulates to settle. Uniform flow was established in each flowing system, using a Vernier depth gauge, which meant that the flow depth was constant along the channel (Chow, 1959). Although this is rarely seen in nature (Chanson, 2004) it ensured that systems could be comparable. Pumps and chillers were turned on in the flowing systems at the maximum flow rate, which equated to an average water velocity in the flowing systems of 0.03 m/s (compared with 0.08 m/s at the sampling site, standard deviation = 0.02, also see SM; Method S3). Each system was left in its respective light treatment for a further seven-days prior to chemical addition.

2.6. Isopyrazam addition

A solution of isopyrazam was prepared for each microflume in 160 mL sterile distilled water and 40 mL acetonitrile (HPLC grade, Fischer Scientific, UK) such that, when added to each respective microflume, the concentration would be 0.1 mg/L isopyrazam, with acetonitrile comprising 0.08 % of the total microflume water volume. This represents an environmentally relevant concentration (OECD, 2004). Immediately prior to application, the pumps and chillers were turned off in the flowing systems and stock solutions were applied to the top of the water column, ensuring the whole length of the microflumes was covered for a homogenous application. The pumps and chillers were left off for four days before being turned back on again, to allow initial sorption to the sediment. Systems were incubated for 52 days after chemical addition.

2.7. Sampling

Samples were removed for analysis at 10, 24, 34, 45, and 52 days after treatment (DAT). No physical samples were taken at 0 DAT, due to the potential for errors before the chemical was fully distributed throughout the water column. Instead, a nominal 0 DAT value, assuming that 100 % of the applied chemical remained in the water column, was used. The fresh samples obtained from the river were used for microbial and water chemistry analyses at 0 DAT. Microbial, water chemistry, and isopyrazam concentration analyses were carried out on all subsequent samples taken from the microflumes. DNA extraction and bacterial, phototrophic, and eukaryotic community analyses were only carried out at 0 and 52 DAT. Pumps and chillers were turned off while sampling occurred to minimise disturbance of the sediment. For each analysis, there were three replicates for each microflume treatment. For sediment sampling, a core technique (SM; Fig. S3) was used. Two samples were taken in this way from each replicate system at each time point, one each for microbial and isopyrazam analyses. From each system, 10 mL of water was sampled at each time point using a glass pipette (Type 2, Fischer Scientific, UK) submerged into the water column. Once filled, a finger was placed over the top to create a vacuum. This sample was for the isopyrazam analysis and was mixed with 2 mL acetonitrile (HPLC grade, Fischer Scientific, UK). A further 50 mL of water was collected using a falcon tube for microbial analyses.

2.8. Isopyrazam analysis

2.8.1. Water fraction

Water (plus acetonitrile) samples were sonicated to extract any chemical adsorbed to particulate matter. Centrifugation at 1000 rpm for 10 min was performed for sample clean up. Samples were analysed by LC-MS using a Poroshell 120 EC-C18 μm column (2.1 × 50 mm, Agilent Technologies, US), an Ultimate 3000 LC system (Dionex, US), and an amaZon SL ion trap (Bruker, US). DataAnalysis (version 4.2, Brucker, US) software was used to integrate isopyrazam peaks – the isomeric forms (*syn* and *anti*) of isopyrazam eluted as two separate peaks and these were analysed together to assess the total peak area. The LC-MS gradient, mass transitions, and LC-MS conditions are shown in Tables S3 to S5 of the SM. Chromatogram, standard curve, and recovery calculation examples are provided in the SM (Figs. S4 and S5 and Table S6).

2.8.2. Sediment fraction

Isopyrazam was extracted from the sediment using 30 mL 80 % acetonitrile (HPLC grade, Fischer Scientific, UK). Samples were shaken at 300 rpm for 1 h before centrifugation for 10 min at 1000 rpm. The supernatant was collected and the pellet subject to the same extraction method twice more. This method had been validated in previous studies using ^{14}C material (Southwell et al., 2020). The combined sediment extract was analysed using the LC-MS method described in Section 2.8.1.

2.9. Water chemistry analysis

System water pH was analysed using an Accumet basic AB15 pH meter (Fischer Scientific, UK). NI-14 and PO-14 test kits (Hach, UK) were used to analyse the nitrate and phosphate ion concentrations in the water.

2.10. Microbial analysis

2.10.1. Chlorophyll a analysis

Chlorophyll *a* analysis was used as an indication for phototroph proliferation, as it is the most abundant chlorophyll pigment (Morançais et al., 2018). Water samples were filtered as in Sartory (1982). A modified method from Ritchie (2006) and described in Davies et al. (2013b) was used to extract chlorophyll *a* from the water and sediment fractions using 90 % acetone (Fischer Scientific, UK) and absorbance measurements were taken before and after acidification. Absorbance was measured using an Ultrospec 1100 pro UV/Visible spectrophotometer (GE Healthcare, UK). Calculations were performed as described by the American Public Health Association (1995).

2.10.2. Viable plate counts to determine culturable bacterial communities

A serial dilution of microflume water was set up (10^0 to 10^{-5}) and 20 μL of each dilution was spread onto a quarter of a 9 cm R2A agar (Oxoid, UK) Petri dish. Plates were incubated for 2 days at 29 °C and colonies counted to determine the number of bacterial colony forming units (CFU) per μL of water.

2.10.3. Molecular microbial community analysis to evaluate populations in water and sediment

Fresh water and sediment from the sample site and water and sediment samples from the microflumes at 52 DAT were analysed. DNA isolation, quantification, PCR, purification, and normalisation methods, as well as sequence data processing, were similar to those used in Álvarez-Martín et al. (2016) and Southwell et al. (2020). Primers to amplify bacterial (Caporaso et al., 2011), phototrophic (Sherwood and Presting, 2007), and eukaryotic (Stoeck et al., 2010) communities were used. Full details are described in Method S4 in the SM. Raw sequence data and metadata are stored under the study accession number, SRP132456, in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA).

2.11. Statistical analyses

Prism (version 7, GraphPad Software, Inc., US) was used to perform statistical analyses and create figures. Significance of differences between treatments for isopyrazam concentration, water chemistry, chlorophyll *a*, and water bacteria count data were determined using a two-way ANOVA on the entire time course. Significance of differences in the relative abundance of bacterial phyla, phototrophic taxa, and eukaryotic classes between the initial inoculum and the microflume treatments were evaluated using two-way ANOVA. Multiple comparison tests were corrected using the Tukey method (Haynes, 2013). The phyloseq package in R (McMurdie and Holmes, 2013) was used to calculate the alpha (α) diversity using Fisher's method, followed by one-way ANOVA to investigate significance of differences in diversity between

the original inoculum and the microflume treatments. Beta (β) diversity was analysed using Bray Curtis similarity matrices which were visualised using non-metric multidimensional scaling (NMDS) and cluster analysis in Primer software (version 6, Primer-E Ltd., UK). PERMANOVA using the vegan package in R (Oksanen et al., 2017) was used to investigate the significance of differences in β diversity between the original inoculum and the microflume

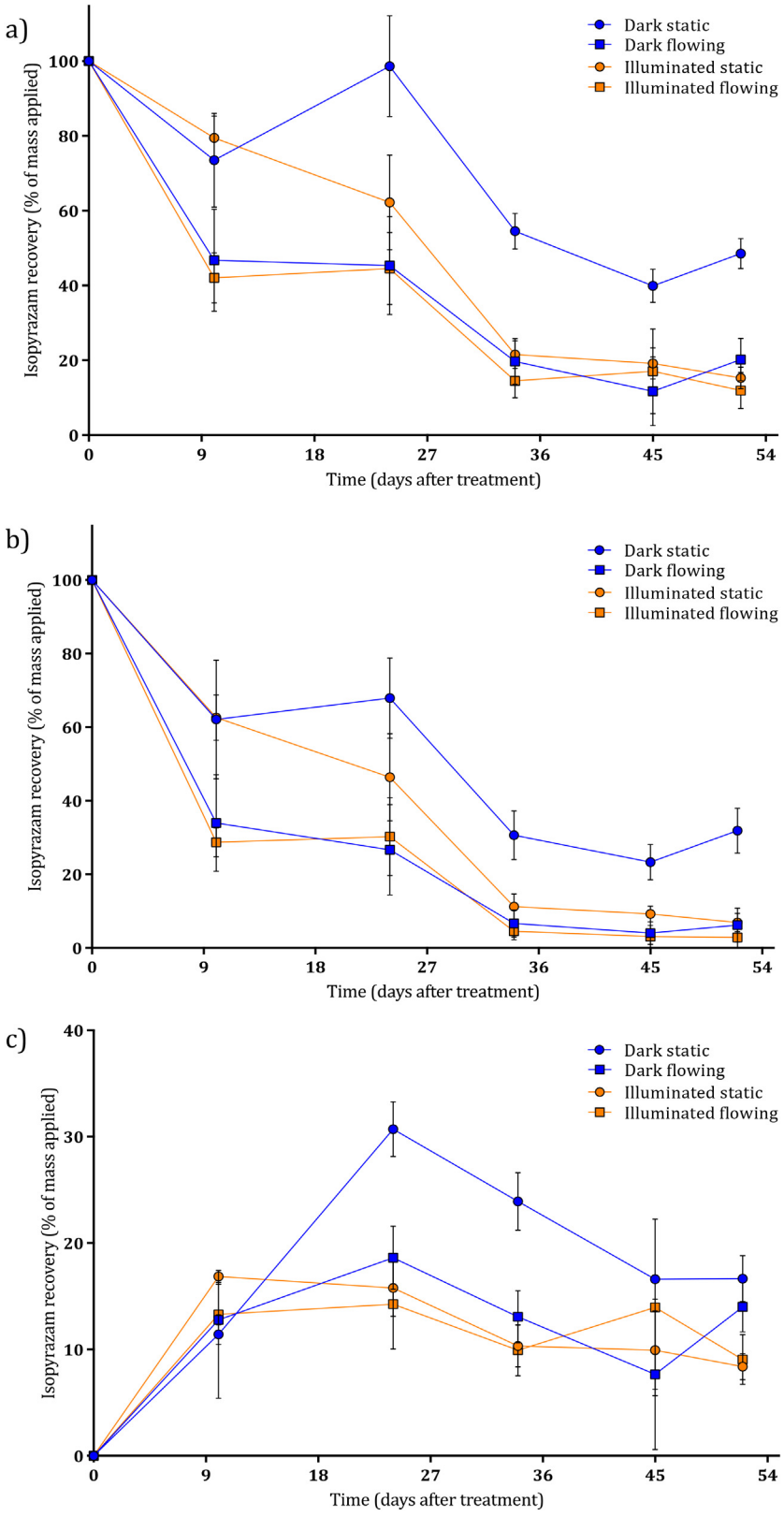

Fig. 3. Dissipation of isopyrazam in microflume systems as a percentage of the mass originally applied in the total system (a), partitioned to the water (b), and partitioned to the sediment (c). Microflumes treatments were dark static (blue circles), dark flowing (blue squares), illuminated static (orange circles), and illuminated flowing (orange squares). Error bars show ± standard deviation.

treatments, using rarefied data, with a modified script to make pairwise comparisons (Arbizu, 2015). Isopyrazam dissipation kinetics (DT50 of total system) were estimated using Single First-Order kinetics in Computer Aided Kinetic Evaluation (CAKE) (version 3.2, Tessella Ltd., UK), a modeling software which conforms to FOCUS requirements.

3. Results

3.1. Isopyrazam dissipation in microflumes

Isopyrazam dissipated in all microflumes, but with significant differences in the rate of dissipation between the treatments ($p \leq 0.0004$, Fig. 3a). DT50 estimates (SM; Table S7) decreased in the following order for the four treatments: dark static (47.7 days) > illuminated static (20.6 days) > dark flowing (16.8 days) > illuminated flowing (15.3 days). Total dissipation (i.e., water and sediment combined) in dark static systems was significantly slower ($p \leq 0.001$) compared to the other microflume treatments, with 48.5 % of the applied mass of isopyrazam remaining by 52 DAT. The total dissipation rate in illuminated static microflumes was significantly faster ($p \leq 0.01$) compared to the dark static microflumes, showing that light conditions increased dissipation rate in static water.

The addition of flow increased total isopyrazam dissipation rate in the dark microflumes ($p \leq 0.001$) relative to the dark static treatment, but there was no significant difference in dissipation rate between the dark and illuminated flowing treatments. In both flowing treatments, isopyrazam concentration was 18 % of the applied amount by 34 DAT, with no further decline by 52 DAT. The dissipation rate in illuminated static treatments was slower than the flowing treatments up to 24 days but, after

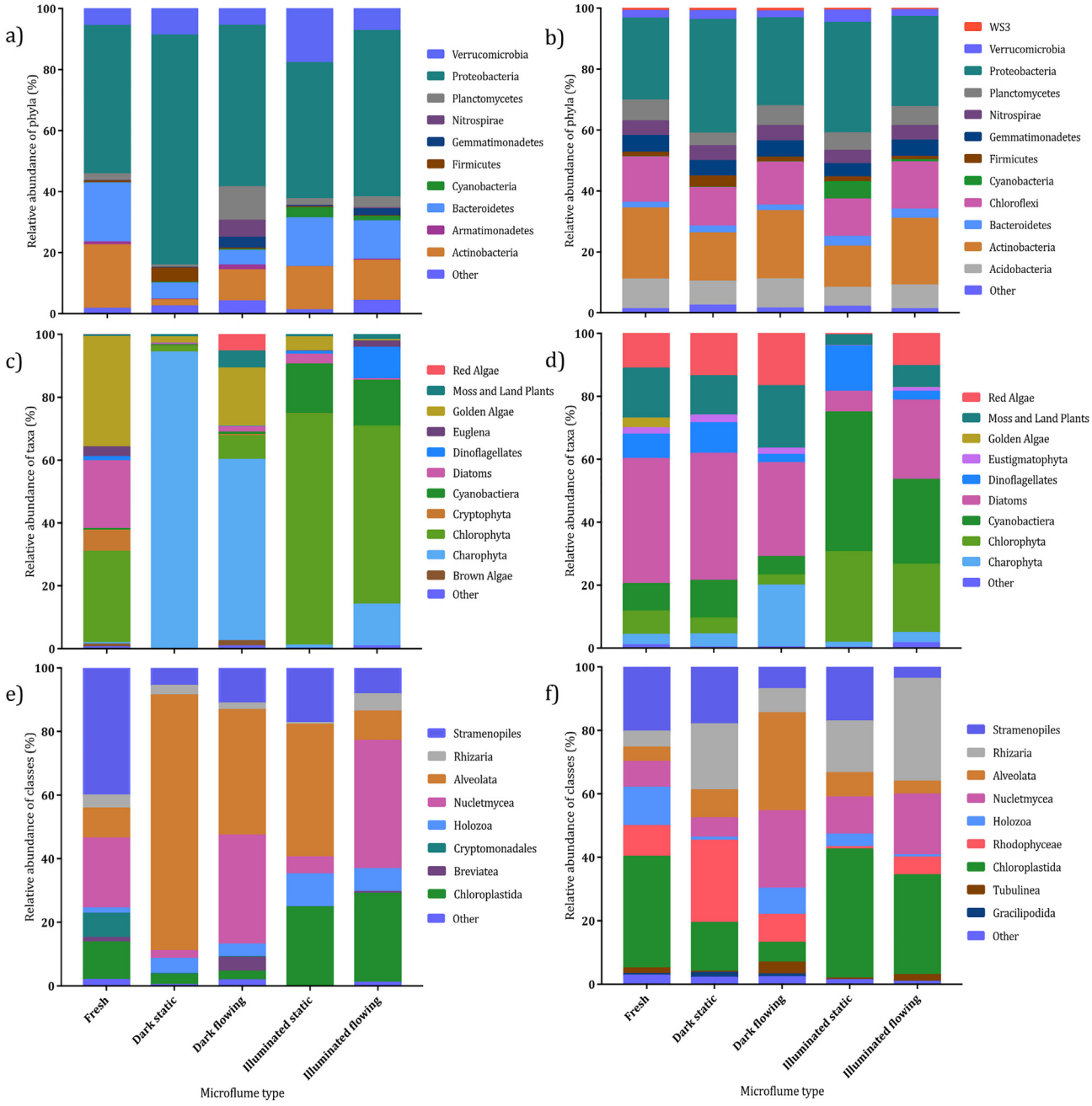

Fig. 4. Relative abundance of bacterial phyla in the water (a) and sediment (b), phototrophic taxa in the water (c) and sediment (d), and eukaryotic classes in the water (e) and sediment (f) between microflume systems. Different phyla, taxa, and classes are denoted by different colours and those making up <1 % of the relative abundance are listed under *other*. Analysis was carried out on freshly collected water and sediment and materials collected from microflumes at 52 DAT.

34 DAT, a similar percentage of isopyrazam remained in the illuminated static microflumes relative to both flowing systems; across the whole time course, there were no significant differences in dissipation between these three microflume treatments.

Dissipation dynamics between the water and sediment fractions were different between treatments (Fig. 3b and c). Dissipation from the water column was significantly faster in flowing microflumes compared to static microflumes ($p \leq 0.05$). Dissipation from the water in the illuminated static microflumes was, in turn, significantly faster than in the dark static microflumes ($p \leq 0.05$). A higher percentage of the applied isopyrazam partitioned to the sediment in the dark static microflumes (30.7 %, $p \leq 0.01$) by 24 DAT relative to the other treatments, but this gradually declined over the time course.

3.2. Microflume water chemistry

There was a significant difference in water nitrate concentrations between the microflume treatments ($p \leq 0.0001$, SM; Fig. S6a). Nitrate concentrations in the dark and illuminated flowing systems were the same; rapidly increasing to over 40 mg/L by 24 DAT and remaining at this concentration for the duration of the experiment. In contrast, nitrate concentrations in dark static microflumes ranged between 5 and 15 mg/L throughout the study duration, which was significantly lower than the other systems ($p \leq 0.001$). Nitrate concentrations in the illuminated static systems were initially similar to the dark static systems but increased to over 30 mg/L after 34 days. Phosphate concentrations initially declined in all treatments between 0 and 24 DAT (SM; Fig. S6b), but in the dark static microflumes, it significantly increased after this, especially when compared to the illuminated treatments (1.9 mg/L by 45 DAT, $p \leq 0.05$). pH (SM; Fig. S7) remained broadly constant throughout the time course for all microflume treatments (6.9 to 8.2), with only significant differences between the two dark treatments ($p \leq 0.05$).

3.3. Microflume microbial community

3.3.1. Water bacterial counts, chlorophyll a, and biofilm development

Water bacterial counts (SM; Fig. S8) increased in all treatments from 10 DAT onwards but then gradually decreased over the time course, with no significant differences between treatments, although there was a trend for higher counts in the dark static treatment. There was a significant difference in the concentration of chlorophyll *a* between the different microflume systems ($p \leq 0.0009$, SM; Fig. S9). Illuminated static microflumes had a significantly higher chlorophyll *a* concentration than all other systems (21.33 mg/m^3 by 52 DAT, $p \leq 0.01$), which were not significantly different to each other (<1 mg/m^3). Biofilm differences between treatments could also be seen by visual examination of the sediment bed (SM; Fig. S10). In both static systems, floating biofilms were observed on the water surface, whereas in flowing systems, biofilm was uniform along the sediment bed. In both illuminated systems, a build-up of green biofilm was observed on the sediment surface. In the flowing systems, this was uniform as mentioned above, whereas in the static systems it was more heterogenous; some areas had minimal growth, while others had dense patches.

3.3.2. Microbial community composition

Bacterial α diversity was not significantly different between fresh samples or microflumes at 52 DAT in the water or sediment fractions (SM; Fig. S11a and b, $p \leq 0.1307$). In contrast, phototroph (SM; Fig. S11c and d) and eukaryote (SM; Fig. S11e and f) α diversity was significantly lower ($p \leq 0.0002$ and $p \leq 0.0001$, respectively) in microflumes relative to fresh water and sediment samples, with α diversity in water samples declining by over 45 %, and in sediment samples by over 25 %. There was no significant difference in microbial α diversity between microflume treatments, with the exception of sediment eukaryote communities, which were significantly lower in the illuminated static microflumes relative to the dark flowing microflumes. PERMANOVA (SM; Tables S8 to S10) and NMDS (SM; Fig. S12) analysis showed that for each microbial group, at the OTU level, community composition in both water and sediment fractions was overall significantly different ($p \leq 0.001$) between treatments. There were no significant differences in sediment bacterial, phototroph, or eukaryote communities between the fresh material and microflume treatments, and communities in sediment from the microflume treatments were not significantly different to each other. In water, bacterial, phototroph, and eukaryote communities in fresh material was significantly different to the microflume treatments, with the exception of the bacterial community in illuminated static microflumes, and the eukaryote community in the dark static microflumes.

At the phylum level, bacterial composition was significantly different ($p \leq 0.0001$) between both water and sediment in each of the microflume treatments (Fig. 4a and b) and between the microflume treatments and fresh samples, although composition was considerably more variable in the water fraction relative to the sediment fraction. In the water fraction, the illuminated microflume samples were most similar to fresh water. Dark static microflume water diverged most from the fresh water, with a significantly increased relative abundance of Firmicutes (4.7 %, $p \leq 0.001$), and particularly of Proteobacteria (75.4 %, $p \leq 0.0001$), compared to all other treatments. Nitrospirae (5.6 %, $p \leq 0.0001$) and Planctomycetes (11.0 %, $p \leq 0.0001$) significantly increased relative abundance in dark flowing microflumes relative to the other treatments. Cyanobacteria relative abundance increased in illuminated microcosm water, with illuminated static microcosms having significantly (3.3 %, $p \leq 0.05$) more compared to fresh water and both dark microflume treatments. In the sediment, bacterial composition was less variable between the fresh samples and the microflume treatments. Generally, microflumes with the same flow treatment had similar community profiles and flowing microflume samples were more similar to fresh sediment. In line with the chlorophyll *a* analysis, relative abundance of Cyanobacteria significantly increased (5.7 %, $p \leq 0.0001$) in illuminated static microflume sediment compared to all other treatments. Overall, illuminated flowing microflumes were most similar to the fresh river samples.

For phototroph communities (Fig. 4c and d), there was considerable divergence in the water fraction between the fresh river samples and the microflumes at 52 DAT. Compared to fresh water and illuminated treatments, Charophyta increased relative abundance in dark incubated microflumes ($p \leq 0.0001$), while Chlorophyta increased relative abundance in illuminated microflumes ($p \leq 0.0001$). Dinoflagellates specifically increased relative abundance in the illuminated flowing treatment (10.0 % vs. <1.4 %) and Diatoms and Golden Algae showed reduced relative abundance in microflumes relative to fresh water ($p \leq 0.01$). Similarly in the sediment fraction, relative abundance of Cyanobacteria and Chlorophyta increased in illuminated microflumes compared to the fresh sediment and dark treatments ($p \leq 0.05$). Notably Red Algae declined markedly in the illuminated static treatment (0.7 % vs. >10.0 %). The dark static microflume retained similar community composition to the fresh sediment, but with reduced relative abundance of Golden Algae (3.1 % vs. <0.03 %).

For eukaryote communities (Fig. 4e and f), there was marked divergence in the composition of water and sediment between the fresh river samples and the microflumes at 52 DAT. In the water fraction of illuminated microflumes, Chloroplastida increased relative abundance compared to fresh water and dark treatments ($p \leq 0.001$). Stramenopiles had lower relative abundance in all microflumes than in fresh water ($p \leq 0.0001$), while for Nucletmycea, which includes fungi, this was true only in the static microflumes ($p \leq 0.0001$), with Nucletmycea also having significantly higher relative abundance in flowing microflumes compared to static microflumes ($p \leq 0.0001$). Lastly, relative abundance of Alveolata was considerably higher in dark static microflumes compared to all other samples (80.4 %, $p \leq 0.0001$). In the microflume sediment fraction, Rhizaria generally increased relative abundance compared to fresh sediment ($p \leq 0.0001$), while Chloroplastida showed lower relative abundance in the dark microflumes relative to the illuminated microflumes and fresh sediment ($p \leq 0.0001$).

4. Discussion

Isopyrazam dissipation was faster in flowing relative to static large-scale OECD 308-type microflumes, regardless of light treatment. The inclusion of

non-UV light-dark cycles only increased isopyrazam dissipation rates in static microflumes. Although illuminated static microflumes initially had a slower isopyrazam dissipation rate relative to flowing microflumes, by the end of the experiment there were no significant differences between these treatments. This data suggests that flow drives dissipation and minimises the impact of phototrophic metabolism, but that phototrophic metabolism is still dominant in static systems. Both flow and illumination impacted microbial community composition of the microflume water and sediment fractions. Light drove marked reductions of phototroph and eukaryote diversity and community composition, associated with selection of specific phototrophs, particularly Cyanobacteria and Chlorophyta. Specific evidence for the effect of flow on community composition was less clear, although flow appeared to increase relative abundance of fungi in both the water and sediment fractions. As isopyrazam dissipated in all systems, these results suggest that a range of microbial communities or physical processes are important for chemical fate and transformation in the environment.

In static microflumes, dissipation was significantly faster in the illuminated microflumes than the dark microflumes. This showed that proliferation of phototrophic communities was an important factor determining isopyrazam dissipation, i.e., phototrophic metabolism was dominant when water velocity was low. The dissipation rate was similar between the two flowing systems, regardless of light treatment, which suggested that phototroph metabolism was not a dominant process under flowing conditions. The microbial analyses also show that illuminated static systems contained significantly more chlorophyll *a* compared to all other systems and they had higher relative abundances of phototrophic bacteria and eukaryotes, namely Cyanobacteria, Chlorophyta, Chloroplastida, and Stramenopiles; this suggests that phototrophic organisms are unable to proliferate as easily in illuminated flowing conditions. Phototrophic communities have been shown to have the potential to degrade a range of chemicals (Roldán et al., 1998; Lima et al., 2003; Thomas and Hand, 2011; Davies et al., 2013a). Indeed, previous studies have shown the importance of phototrophic metabolism specifically for isopyrazam (Hand and Oliver, 2010; Hand and Moreland, 2014; Southwell et al., 2020). Phototrophs may either be able to metabolise compounds directly or have a more indirect role by stimulating activity of heterotrophic organisms (Davies et al., 2013a).

Water flow was a major contributor to isopyrazam dissipation, as loss was rapid regardless of light treatment. The faster flow rate and turbulence will increase mixing of isopyrazam, both within the water column and in the hyporheic zone within the sediment (Spain et al., 1984; Naudin et al., 2001; Packman and Salehin, 2003; Gualtieri, 2004; Higashino et al., 2004). This will enhance mass transport, increase the chance that microorganisms will come into contact with nutrients and contaminants, and increase aeration, supporting growth of heterotrophs. These processes could result in microbial community proliferation, increasing metabolism and nutrient mineralisation, as well as chemical biodegradation rates (Gantzer et al., 1988; Bonanni et al., 1992; Rusch et al., 2001; Thullner et al., 2002; Williams et al., 2003; Gualtieri, 2004; Kunkel and Radke, 2008; Sánchez-Pérez et al., 2013; Boano et al., 2014; Jaeger et al., 2019).

In flowing systems, nitrate concentration increased rapidly in the water fraction; this increase was delayed in static systems, suggesting that flow stimulated microbial mineralisation processes. Treatment differences in water fraction nitrate dynamics mirrored differences in isopyrazam dissipation, suggesting that enhanced isopyrazam dissipation in flowing systems resulted from increased microbial activity. Although nitrate is a known photosensitiser (Hand and Oliver, 2010), indirect photolysis is unlikely to have had a key role in isopyrazam dissipation in this study because of the light and filter transmission spectrum. Furthermore, if indirect photolysis had played a key role in dissipation, the dissipation in the illuminated flowing microflumes would be expected to be faster than the dark flowing microflumes, but this was not the case.

Dark static microflumes had an increase in water fraction phosphate concentration after 24 DAT, which could be due to a decrease in redox potential resulting from lower aeration due to a combination of low rates of mixing and low phototroph growth in these microflumes (Ann et al., 1999). This reduction in mixing and exchange may explain the higher mass of isopyrazam in the dark static sediment fraction, with low bioavailability of isopyrazam to microbial degraders in the water column or water-sediment interface.

Biofilms act as bioreactors with high rates of chemical and microbial turnover (Schaper et al., 2018) and the water-sediment interface acts as a platform for biofilm growth. Higher bacterial growth rates and degradation have been shown to occur at this interface (Xia and Wang, 2008) and algae, in particular, have been shown to have the potential to adsorb contaminants, including pesticides (Crum et al., 1999), heavy metals (Sandau et al., 1996), and hydrocarbons (Headley et al., 2008); therefore future work may benefit from also analysing biofilm fractions separately to water and sediment. Biofilm development is heavily impacted by water velocity, with biofilm thickness being lower when water velocity is higher (Wetzel, 1993; Battin et al., 2003). Biofilm was more uniform in illuminated flowing microflumes and those systems also had lower amounts of chlorophyll *a* compared to illuminated static systems; this is in line with previous research (Kugaorasatham et al., 1992). Due to the low water velocity in illuminated static microflumes, phototrophic biofilms were able to proliferate more efficiently, with higher amounts of chlorophyll *a* and dense heterogenous patches of phototrophic biofilm along the sediment surface.

Despite the fungicidal activity of isopyrazam, flow increased relative abundance of fungal communities in both the water and sediment fractions, suggesting differential toxicity of this compound across environmental fungal communities. Fungi are well known to be capable of chemical degradation, including pesticides, particularly through production of non-specific cytochrome P450 monooxygenases (Bending et al., 2002). Bacterial plate counts indicated that dark static microflumes supported 10 to 100 times more culturable bacteria than the other treatments, indicating that increased bacterial biomass does not necessarily promote isopyrazam dissipation. This is potentially due to the low microbial diversity in this treatment, as the water was heavily dominated by a single bacterial phyla, phototrophic taxa, or eukaryotic class in the relative abundance analyses (Proteobacteria, Charophyta, or Alveolata, respectively, 75.4 to 94.7 %); other studies also suggest that functional diversity is more important for degradation than increased biomass (Jaeger et al., 2019; Coll et al., 2020). While heterotrophic degradation of pesticides has been established (Thomas and Hand, 2012), the relative importance of bacterial and fungal degraders is unclear, and it is possible that the dissipation patterns of isopyrazam we observed reflected differences in biomass of fungi across the treatments.

As the fit to Single First-Order kinetics was not clearly demonstrated, future studies would benefit from additional earlier sampling points. Similarly, considering the study aimed to compare static and flowing conditions, a shorter period of static conditions directly after application in the flowing microflumes would have been preferable, in view of the short DT50s. Despite this, the dissipation was noticeably slower in the static systems by the first sampling point at 10 DAT, so it can be assumed that the 4 days of static conditions did not impact the comparison to a great extent.

Earlier work using inoculum collected at 8 time points over a 2-year period from the same river location as in this study, showed that isopyrazam degradation was consistently minimal under continuous darkness (DegT50s >100 days) when using microcosm systems containing 80 mL of water and 20 g of sediment (Southwell et al., 2020). In the current study, using microflumes containing a similar ratio of water to sediment but scaled up by 365-fold, dissipation under continuous darkness was much faster (DT50 <47.7 days). A direct comparison cannot be made as the two studies were carried out at different times and studies were performed in different test systems (i.e., size and geometry), both of which can greatly influence dissipation rates (Shrestha et al., 2016; Southwell et al., 2020; Seller et al., 2021). Nevertheless, these findings indicate that test system scale could affect the outcome of OECD regulatory tests. This could reflect a wider variety of microsite environmental gradients, such as pH and aeration in a larger system volume, and differences in the

biomass and diversity of microbial communities and therefore their metabolic potential (Sturman et al., 1995; Kowalczyk et al., 2015; Gartiser et al., 2017). In particular, biodegradation can reflect the activity of microbial consortia rather than one single species, with co-metabolism and genetic transfer playing a role, and these interactions may become more stochastic as sample size reduces (De Schrijver and De Mot, 1999; Sørensen et al., 2002; Lima et al., 2003; Hoskeri et al., 2014; Jaeger et al., 2019).

Non-UV light and flowing conditions are currently excluded from OECD tests, although our results indicate they may both have substantial effects on isopyrazam dissipation, although not an additive effect. This calls into question the relevance of these tests to real aquatic environments, particularly those with flowing water. This work provides support for considering the inclusion of a further range of environmental variables within higher tier laboratory testing regimes to enable better prediction of chemical behaviour in the field. Additionally, as there was little difference in dissipation between the flowing and illuminated static microflumes, it is likely that if flowing systems were used in future, then there would be no need for studies to be incubated under light conditions to provide environmentally relevant data for isopyrazam; further work on other compounds would be required to determine if this is universal.

Even with the large scale microflumes used in this study, however, accurate representation of the microbial communities active in the natural environment may prove challenging. Light had a bigger effect on microbial community composition than flow, largely through selection of phototrophic bacteria and eukaryotes, although notably flow increased relative abundance of fungi. Bacteria and eukaryote communities, including phototrophic taxa, diverged from the fresh samples and, for some groups, α diversity decreased as specific taxa were selected by the environmental conditions within the microflumes. Despite this, even if the communities present in the river cannot be completely mimicked in the microflumes, relative abundance of the bacterial communities in the fresh sediment were similar to those in the flowing microflumes. In addition, the illuminated flowing microflume water best represented the fresh water, showing that overall flowing and illuminated conditions, which are excluded from regulatory testing, can help add environmental realism to a greater extent compared to the current testing regime, which is most similar to the dark static microflumes in this study.

5. Conclusion

This study showed that in a water-sediment microflume system, water velocity had a key role in isopyrazam dissipation, especially under dark conditions. Although the effect was less pronounced in illuminated microflumes, phototrophs, which are key isopyrazam metabolisers, were not able to proliferate as rapidly as in illuminated static microflumes, indicating flow played a key role in the illuminated flowing treatments. This may have been because flow promoted mixing and increased exchange across the water-sediment interface and hyporheic zone, which became the dominant zone for microbial degradation by heterotrophs, which were able to grow unhindered due to the reduced biomass of phototrophic degraders. Nevertheless, non-UV light did play a role in isopyrazam dissipation in static microflumes, in which low turbulence allowed proliferation of phototrophic communities which could degrade isopyrazam via direct or indirect mechanisms. In flowing microflumes, however, there was a significantly higher relative abundance of fungi relative to static systems. Our findings therefore indicate that isopyrazam dissipation can result from both heterotrophic and phototrophic pathways, with the importance of each determined by flow rate. Lastly, in terms of the study design, the dark static treatment was as close to the requirements of regulatory OECD 308 studies as possible. Comprehensive analysis of bacterial, phototrophic, and eukaryote communities indicated that this treatment was no better than the others at retaining microbial community composition of the original fresh water and sediment inoculum and showed the slowest isopyrazam dissipation rate, despite promoting bacterial abundance relative to the other treatments. Indeed, the dark static microflumes were largely dominated by a single group of microorganisms in the water fraction for all analyses, suggesting that the conditions used in the OECD tests can increase divergence from the original inoculum. For the bacterial analyses, the fresh inoculum most closely resembled the illuminated flowing microflumes. Although testing on additional compounds would be beneficial, this indicates that increasing the scale and complexity of studies could improve the degree to which tests mimic natural environments, and the extent to which they are able to predict the environmental persistence of chemicals.

CRediT authorship contribution statement

GDB, LHH, and JMP conceived the study; RVS, GDB, LHH, and JMP designed the sampling and analysis programme strategy; RVS conducted the experimental work; SLH advised on molecular and bioinformatic analyses; RVS, GDB, and LHH wrote the manuscript; all authors contributed to revisions.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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