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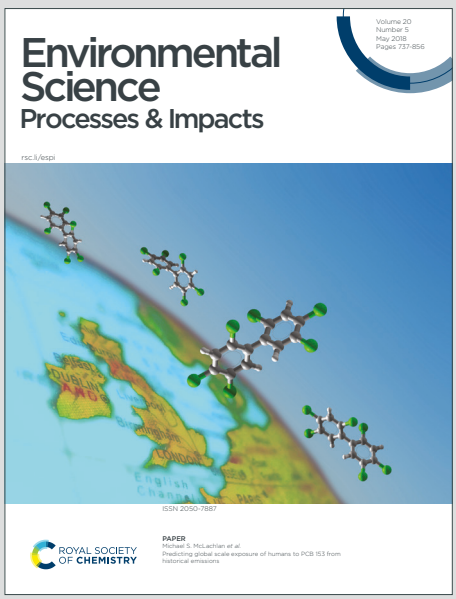

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Environmental Significance Statement

Large variabilities in biodegradation rates of organic contaminants across aquatic environments complicate persistence assessments, and limited knowledge of the factors controlling these rates restricts our ability to harness biodegradation as an ecosystem service through ecological engineering. Research on biodegradation and the environmental factors influencing its rate is often hindered by the significant time and resources required for biodegradation experiments. To address this issue, we investigated links between biodegradation rates and a rapid and cheap to measure environmental parameter, microbial respiration. Knowledge of parameters linked to biodegradation rates could aid in understanding or describing variability in observed biodegradation rates.

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ARTICLE

Linking water–sediment respiration to micropollutant biodegradation across aquatic environments

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Biodegradation makes major contributions to the removal of micropollutants from contaminated environments, but the factors that influence the removal rate in specific environments remain unclear. This study examined environmental biochemical oxygen demand (BOD) as a potential indicator of site-specific attenuation rate constants for 47 micropollutants. A modified BOD protocol for water-sediment systems was developed and applied in parallel with a modified OECD 309 biodegradation test on samples collected from 10 sites along an urban wastewater-impacted river. The biodegradation test provided attenuation rate constants for 34 compounds that met quality criteria for at least three sites. After normalizing by sediment dry weight, 23 compounds showed a significant positive correlation ($p < 0.05$) between BOD and attenuation rate constant, with an average linear regression R^2 of 0.71. Normalization by BOD significantly ($p < 0.05$) decreased the observed variability in attenuation rates. If biodegradation mainly occurs through co-metabolism, BOD may act as an integrative indicator of microbial metabolic activity relevant to micropollutant transformation. Our results suggest that a water–sediment BOD test may provide a practical indicator of relative biodegradation rate across sites for a subset of compounds.

Introduction

Micropollutants are anthropogenic chemicals occurring in aquatic environments at low concentrations (µg–ng L^{-1}), and are widely recognized to be removed primarily through biodegradation[1–3]. The significant variability in biodegradation rates across different environmental sites creates considerable uncertainty in estimating micropollutant exposure[3,4]. Improving the understanding of the variation in biodegradation rates could aid not only in assessing chemical exposure but also in mitigating micropollutant contamination, particularly in relation to wastewater treatment plants (WWTPs). Most WWTPs were not designed to remove low-concentration anthropogenic chemicals, and as such, a significant portion of chemicals entering the plant are emitted with the effluent[5]. In such cases, the responsibility for pollutant removal shifts to environmental processes, such as biodegradation. Understanding which environments have high potential for biodegradation could aid in maximizing this removal, either by directly selecting environments where many compounds exhibit fast removal as recipients or by using this information to develop ecological engineering strategies that improve recipient biodegradation. Understanding of the observed variability in biodegradation rates between sites is limited by the lack of practical testing methods[3]. Standard methods for biodegradation testing, such as the OECD 309 and OECD 308 guidelines, have been used to measure site-specific biodegradation rates in several studies[6–10]. Modified versions of standard tests have also been developed with the aim of improving environmental relevance. For example, Tian et al.[11] developed a modified OECD 309 test featuring, e.g., a shorter incubation, increased sediment concentration, and the spiking of mixtures. However, both standard and modified biodegradation tests are held back by their long duration (up to 100 days[12,13]) and reliance on advanced analytical equipment or 14C-labeled test chemicals, which limits the feasibility of large-scale testing. Developing a faster and more accessible indicator associated with variability in biodegradation rates could support site selection and hypothesis generation for subsequent biodegradation studies.

BOD is a parameter that describes the amount of organic matter (OM) available for oxidation in water by microbes[14]. It is a common water quality parameter for wastewater used to assess potential decreases in dissolved oxygen (DO) due to microbial respiration of effluent organic matter. BOD can be concisely described as the decrease in DO in a water sample over a specific time span due to microbial respiration of organic matter[14]. Although BOD and DO depletion have been used to quantify biodegradation in studies where the test chemical was the primary substrate[15–18], no previous studies have explored the relationship between the BOD of an environmental sample and the degradation of micropollutants (i.e., relating biodegradation rates to O_2 consumption from respiration of natural organic matter). Due to their low environmental concentrations, micropollutants are often assumed to be metabolized as a secondary substrate via co-metabolism[19,20].

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For co-metabolic degradation, it is reasonable to hypothesize that compounds may degrade faster in environments with higher BOD, as higher BOD reflects elevated overall microbial metabolic activity and, consequently, greater potential for co-metabolic transformation[21].

Although BOD is typically measured for water samples, oxygen demand is also measured for sediment samples, in which case it is referred to as Sediment Oxygen Demand (SOD) or Sediment Biochemical Oxygen Demand (SBOD). Given that sediments generally have higher biodegradation activity[22] and microbial biomass[23–25], sediment BOD could be expected to better correspond to micropollutant biodegradation than surface water BOD. SOD/SBOD measurements are less common and less standardized than BOD measurements, with diverse methodological approaches being employed[26]. The U.S. Environmental Protection Agency's SOD-protocol utilizes an open-bottom chamber placed on the sediment bed[27]. The chamber encloses a certain volume of water, and the decrease in DO is monitored for 2 h, from which the SOD is calculated[27]. Other approaches are more akin to BOD protocols, incubating sediment samples and dilution water in BOD bottles, and calculating SBOD from the DO-decrease after the incubation[28].

In this study, we develop and evaluate a BOD-based respiration test for water–sediment systems and assess whether it can indicate relative differences in micropollutant biodegradation rates among sampling sites for selected compounds.

MATERIALS AND METHODS

Study site and sample collection

Water and sediment samples were collected at 10 sites from Fyrisån (the Fyris river) and its tributaries in Uppsala, Sweden (Figure 1a). Fyrisån flows through the urban area of Uppsala (175,000 inhabitants) and its surrounding agricultural areas. It also receives effluent from a WWTP located just south of the city centre. Sites were selected to cover a range of micro-habitats, with the aim of collecting samples with variable microbial and sediment characteristics. Site locations are presented in Figure 1a, while site descriptions and visually ascertained sediment grain type are available in Table 1.

At each site, water was collected from 5 cm below the water surface in PTFE bottles rinsed with river water. Sediment was collected from the top 5 cm layer at each site using a corer, with a minimum of three cores per site collected and homogenized in PTFE bottles. When necessary, river water was added to prevent sediment samples from drying out, and a headspace was left to keep the sediment oxygenated. Prior to sampling dissolved oxygen (DO), pH, temperature, and electrical conductivity (EC) were measured in triplicate using a HQ2200 multi-meter (Hach Lange GmbH, Düsseldorf, Germany) at each site (Table 1). Samples were transported to the lab in insulated containers and stored overnight at 4°C. The BOD and OECD 309 experiments were started the following day.

Table 1. Description of sample sites, visually determined grain size, as well as dissolved oxygen (DO), pH, electrical conductivity (EC), and temperature as measured in the field.

Site	Description	DO	pH	EC	Temp.
		mg/ L		*µS/cm*	*°C*
F1	Fyrisån, close to shore. Clay/Silt sediment	11.9	6.95	406	5.1
F2	Fyrisån, close to shore, tree roots reach into sediment. Sandy sediment.	11.7	6.93	405	4.7
F3	Sävjeån tributary. Significant number of macrophytes. Clay sediment.	9.7	7.18	454	4.3
F4	Sävjeån/Fyrisån confluence. Significant number of reeds. Clay sediment.	8.4	7.09	482	4.1
F5	Fyrisån, close to shore, tree roots reach into sediment. Sandy sediment.	11.6	7.09	397	4.5
F6	Constructed channel discharging into Fyrisån. Significant number of macrophytes. Clay sediment.	0.7	6.92	598	5.8
F7	Fyrisån, by eroded section of the riverbank. Sandy sediment.	11.0	7.11	396	4.3
F8	Channel from an industrial area discharging into Fyrisån. Clay/silt sediment.	13.7	10.2	383	1.7
F9	Tributary to Fyrisån. Clay sediment.	12.7	7.31	412	2.5
F10	Fyrisån, by stormwater drainage outlet. Roots from surrounding trees reach into sediment. Clay sediment.	13.4	6.65	315	0.6

Sediment preparation

Sample sediments were prepared in the same way before the BOD and OECD 309 tests. Prior to addition, the sediment was aerated to remove reduced inorganic species, which have previously been suggested to interfere with SBOD results[28]. The sediment was also sieved using a 2 mm sieve and centrifuged at 1000 RPM for 2 min, discarding the supernatant, to remove bulk material and excess water, respectively. The sediments were then utilized to prepare the two experiments.

Biochemical Oxygen Demand (BOD) – Respiration test

The respiration test was based on the U.S. Geological Survey's protocol for five-day BOD (BOD_5)[14]. The protocol consists of a five-day incubation of DO-saturated sample water in BOD-bottles without headspace at 20 °C, where BOD_5 is calculated from the DO decrease during the incubation. Three major alterations were made to the protocol: (1) To ensure the results of the respiration and OECD 309 tests were comparable, both sediment and water were utilized for the respiration test. 15 g of wet sediment and 300 mL of water was added to each BOD bottle (DWK Life Sciences GmbH, Mainz, Germany), mirroring the sample material used in the modified OECD 309 incubation. (2) Test length was reduced from five days to three days. The introduction of sediment increases both the amount of organic matter and the number of microorganisms in the bottle, causing faster oxygen depletion[29,30]. Hence, to ensure a sufficient DO residual of > 1 mg/L at the end of the test, the incubation length was reduced to three days. (3) Bottles were incubated at 4 °C instead of 20 °C. The OECD 309 incubation was performed at river temperature (average site water temperature was 3.76 °C)

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to increase the environmental relevance of the results, and as such, the respiration test was changed to the same conditions.

Bottles were prepared in triplicate for each sample and sealed without headspace. A water seal was added around the stopper and wrapped with Parafilm to inhibit evaporation. The bottles were placed on a KS 501 digital orbital shaker (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 100 rpm to keep sediment suspended. DO was measured at the start and end of the incubation using a HQ2200 multi-meter (Hach Lange GmbH, Düsseldorf, Germany), allowing three-day BOD (BOD_3) to be determined from the decrease in DO (Equation S1). Additional information about the equipment and procedure is available in the Supporting Information S1.1.

Modified OECD 309 biodegradation test

The primary biodegradation of 47 micropollutants (Table S1) was studied using a modified version of the OECD 309 aerobic surface water biodegradation test developed by Tian et al.[11], and updated in subsequent studies[3,6]. Incubation bottles containing 15g of wet sediment and 300 mL of water were prepared for each site in triplicate. Abiotic and hydrolysis controls consisting of autoclave-sterilized sediment and water (or just water for the hydrolysis control) in the same amounts as the replicates were prepared for 5 and 4 sites respectively. Bottles were spiked to a concentration of 1 µg/L per compound with a mixture of 129 compounds prepared by Tian et al.[6] (Table S1). A subset of 47 compounds was selected based on prior experimental experience to include compounds that can be reliably quantified at low concentrations and are minimally affected by sorption and hydrolysis, thereby enabling robust assessment of biodegradation kinetics. The incubation was carried out at 4 °C and the flasks were placed on a KS 501 digital orbital shaker (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 100 rpm to keep sediment suspended. The experiment was conducted over 10 days during which DO, EC, pH, and temperature was monitored regularly (Figure S1). Subsamples were taken at 12 timepoints during the incubation and analysed using ultrahigh-performance liquid chromatography coupled with a Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer (UHPLC-Orbitrap-MS/MS, Thermo Fisher Scientific, San Jose, CA). The method utilized a reversed-phase LC column with methanol and water (each with 10 mM acetic acid) as mobile phases, and electrospray ionization in positive and negative mode (full MS settings in Table S3). Suspect screening analysis was performed with a suspect list consisting of the spiked chemicals. Additional information about the biodegradation experiments is available in the Supporting Information S1.2, while information about instrumental analysis is available in the Supporting Information S1.3.

Chemical analysis and data processing

Compound Discoverer 3.3 was utilized for compound detection and integration. A matrix-matched quality control sample (QC) was prepared using river water spiked with aqueous solutions of the micropollutants to achieve concentrations of 1 µg/L per compound. The QC was injected repeatedly during the measurement sequence after every six samples. QCs were used to correct for instrumental drift using the BatchCorr script[37]. The limit of quantification (LOQ) was determined as the concentration in the standard at the lower end of the linear range of a 13-point matrix matched calibration curve.

Attenuation rate constants (k) for the tested chemicals were measured for each sample using linear least squares regression of the natural logarithm of the tested chemical's peak area against time (Equation S2) using the script Chowclassifier[6]. Previous testing had shown that sorption and other abiotic processes had a negligible effect on the 47 test compounds, and so the attenuation was assumed to be primarily from biodegradation[6]. Still, to confirm that abiotic processes had minimal impact on attenuation, sorption, and hydrolysis controls were prepared for 5 and 4 of the sample sites, respectively. In line with the procedure of Tian et al.[3], the estimated k was only considered valid if it was based on at least five datapoints and was significantly different from 0 ($p < 0.05$). Of the 47 spiked compounds, 46 could be identified using the analytical method (Table S4) and 43 produced rate constants that passed the data quality criteria for at least one site. Although the rate constants that failed the data quality criteria were not used to derive the relationships between k and BOD, we did compare the derived relationships to the 95% confidence intervals of the rejected data to see if they were consistent. In cases where biphasic degradation kinetics were observed, the k of the initial phase was utilized for further analysis. Initial-phase kinetics have been argued by Tian et al. to be most environmentally relevant, since a change in kinetics indicates a departure of the experimental system from its original, most environmentally similar, state[11].

The determined BOD_3 and k were both normalized by dry weight to account for differences in sediment amounts between incubation flasks (Supporting information S1.4, Equation S3 & S4). To assess whether the dry weight normalized BOD_3, BOD_{3dry}, differed between sites, an analysis of variance (ANOVA) test was performed, followed by a Tukey test to identify which sites differed from each other. The BOD_{3dry} and dry weight normalized attenuation rate constants, k_{dry}, were compared using linear least-squares regression. For two compounds, flecainide and fluconazole, one and two outlier datapoints, respectively, were removed. For all three datapoints, biphasic kinetics were observed with rapid dissipation before the first 48 h, yielding a very high k. While these rate constants might represent some interesting process, their outsized influence on the regression analysis would mask any patterns in the rest of the data, and so they were removed before applying linear regression. Utilizing the abiotic controls, an abiotic process corrected version of k_{dry}, k_{dryS} (abiotic corrected, dry weight normalized attenuation rate constant), was calculated (Equation S5).

The ability of the respiration test to describe variability in attenuation rates was further assessed by testing if normalization by BOD_{3dry} decreased variance in k_{dry}. This was assessed by comparing the standard deviation of k_{dry} for each compound before and after normalization using a paired t-test.

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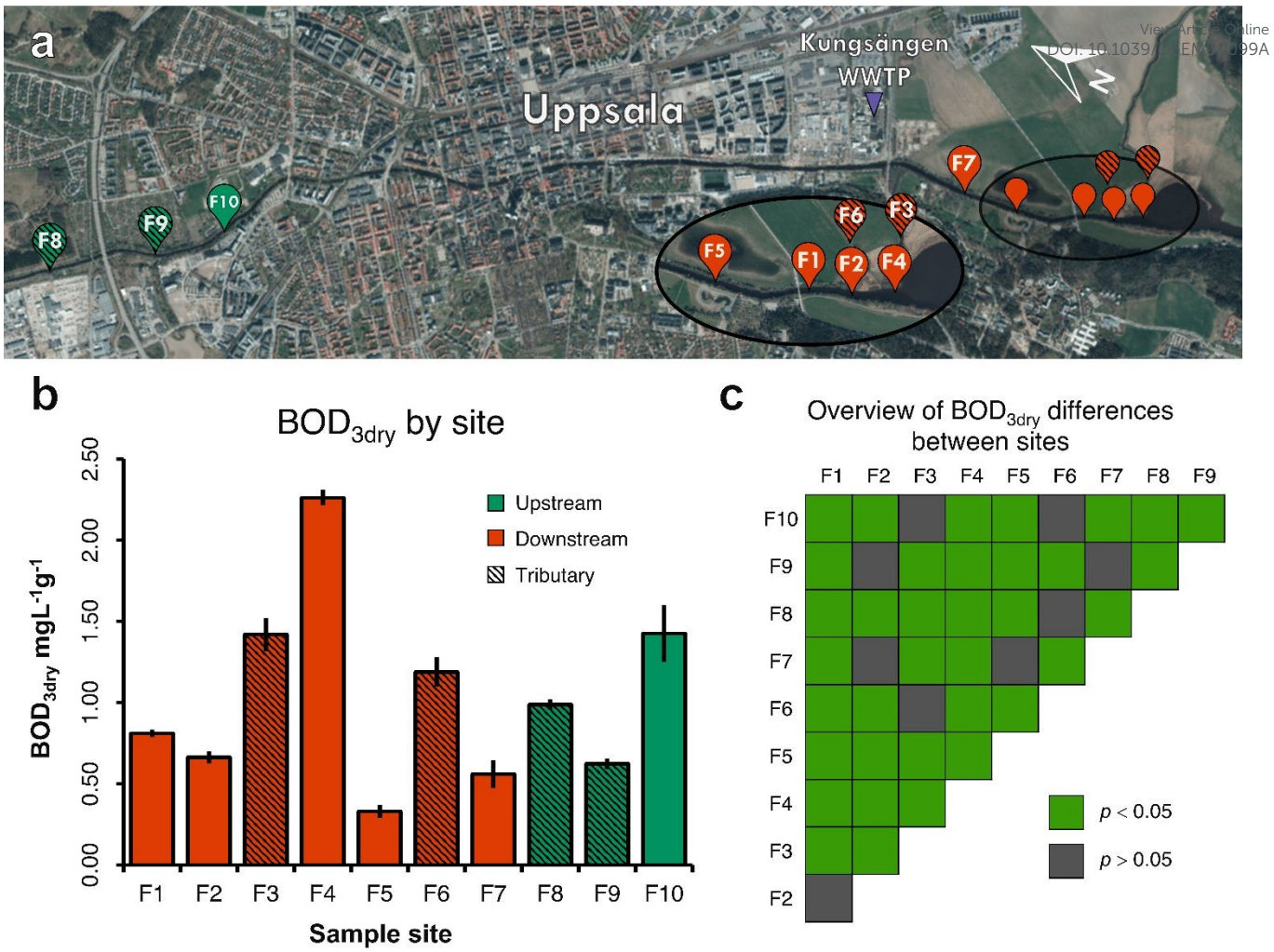

Figure 1. BOD_{3dry} variability between sites. A Map illustrating sampling sites along Fyrisån in Uppsala. The river is flowing north-to-south and the map also notes the location of the local WWTP. Basemap: 1:43000 Aerial Photography, Uppsala ©Lantmäteriet 2023. B Bar plot of the dry weight normalized three-day BOD (BOD_{3dry}) for each sample site. Error bars represent ± one standard deviation. C Results for the Tukey post-hoc test examining which sites have a significantly different BOD_{3dry}.

Further details about compound identification, data processing, and statistics are available in the Supporting information S1.4.

Structure based compound grouping

An assessment of structural similarity between test compounds was performed utilizing Molecular ACCess System (MACCS) keys as structural descriptors[32]. The MACCS key of each compound consists of 166 structural descriptors and were collected from a public repository (https://github.com/FennerLabs/pepper). Grouping was performed by complete linkage based on Euclidean distance between compound MACCS keys.

RESULTS AND DISCUSSION

Significant variability in BOD between river sites

The measured BOD_{3dry} varied between the sites, with a roughly seven-fold difference between the highest and lowest sites (Figure 1b). The ANOVA and Tukey test revealed that roughly ¾ of site pairings had significantly different BOD_{3dry} (Figure 1c). Given that microbial communities are typically more diverse in sediments as compared to water[24,25], differences between sites would likely have been smaller if water phase BOD had been measured instead. The variation in sample respiration could be caused by several factors, such as the quantity and quality of sediment organic matter, the microbial community composition, or nutrient availability[30,33]. There was no clear pattern separating sites upstream and downstream of the WWTP, or tributary sites from sites in the main river (Figure 1b). Effluent-derived organic matter, therefore, does not appear to be the main cause of variation. Sediment organic matter content was notably the most important factor controlling water-sediment respiration in a study performed by Hedin in woodland streams[33]. Sediment organic matter content can be influenced by terrestrial, aquatic, and anthropogenic OM inputs [34,35]. Hence, variability in these sources could be an important contributor to the variability in BOD_{3dry}.

Another factor that has been examined in previous research is sediment particle size, which has been found to negatively correlate with water-sediment respiration rate and microbial biomass[36]. Although particle size was only estimated visually in this study, it is noteworthy that sites assessed as having clay-

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rich sediment generally had a high BOD_{3dry}, whereas sites assessed as having sandy sediment generally had a low BOD_{3dry} (Table 1, Figure 1b). Overall, the ANOVA and Tukey tests indicate a variable distribution of the BOD_{3dry} data across the sites, even though the samples were collected in a single river system.

Attenuation was positively correlated with BOD

For 34 compounds, a k_{dry} value was determined at a minimum of three sites, enabling an assessment of the relationship between BOD_{3dry} and k_{dry} using linear regression (Table S5, Figure S3). Of these 34 compounds, 23 showed a significant positive correlation, 1 showed a significant negative correlation, and 10 showed no significant correlation at a 0.05 significance level (Figure 2). For the positively correlated compounds, R^2 ranged from 0.41-0.98, with an average value of 0.71. Still, of the 10 compounds without significant correlations, 8 had positive slopes. As for the rate constants which did not pass quality criteria, their 95% confidence intervals generally overlap with the linear regression line between k_{dry} and $BOD3_{dry}$, although for several compounds (e.g. valsartan, venlafaxine, and MCPA) there were also replicates among the rejected data that were inconsistent with the regression line (Figure S2). For ketoprofen, the rejected data contains a site for which all replicates clearly deviate from the regression line derived from the valid data (Figure S2). However, even if these datapoints were included in the linear regression, the relationship between BOD_{3dry} and k_{dry} would still be significantly positive.

As outlined in the Introduction, there is at least one possible mechanistic explanation for a positive relationship between BOD_{3dry} and k_{dry}. Micropollutants, due to their low concentrations, are generally assumed to degrade co-metabolically[19,20]. As BOD measures aerobic microbial metabolism, a high BOD should correlate to high microbial metabolic activity, potentially providing greater opportunity for micropollutant co-metabolism. An interesting comparison can be drawn to previous studies comparing biodegradation rate constants to dissolved organic carbon (DOC), as both DOC and BOD are measures related to the organic material present in a sample. Both positive and negative correlations between micropollutant biodegradation rate constants and DOC have been reported[37–41]. Several of these studies also highlighted that differences in measured biodegradation rate constants were dependent on co-substrate type and bioavailability, but with similarly contrasting results[32-36]. While Luo et al.[39] found pesticide degradation was enhanced by more assimilable carbon, Li et al.[40] found that more recalcitrant carbon pools, and associated microbial communities, promoted micropollutant degradation. In investigating variabilities in biodegradation rates across European rivers, Tian et al. found significant correlations between biodegradation rates and sediment total organic carbon (TOC), noting there was no clear mechanistic explanation[3]. As sediment organic matter is often correlated with microbial community respiration rates[33], the hypothesis we proposed here (linking respiration to co-metabolic transformation) offers one plausible interpretation of these earlier observations.

Consistent with this hypothesis, Tian et al.[3] also reported similar spatial variability in degradation rates among compounds sharing structural elements, a pattern that would be compatible with differences in co-metabolic activity driven by variation in overall community respiration. At the same time, such similarities in degradation behaviour could arise from alternative mechanisms, including co-metabolism influenced by other environmental factors or from primary metabolic processes that are not strictly compound-specific.

Thus, while these observations do not provide direct evidence for a causal link between respiration and biodegradation, they are consistent with the proposed conceptual framework. Respiration-based measurements such as BOD are potentially better predictors of biodegradation rate constants than OM concentrations because they integrate the influence of bioavailability and substrate quality by directly measuring microbial metabolism. Assessing the effects of sediment-water organic matter pools and their associated microbial communities might, however, provide interesting mechanistic insights into the factors that control environmental biodegradation rates.

Ten compounds had no significant correlation between BOD_{3dry} and k_{dry}. Since eight of these had a positive (non-significant) relationship between the parameters, it's possible that a significant relationship could be found for more compounds if more data were available. On the other hand, there might be no relationship between respiration and biodegradation for these compounds. Additionally, one compound, acesulfame, had a significant negative correlation between BOD_{3dry} and k_{dry} (Figure 2). However, the reliability of this observed relationship is questionable, as the data is heavily skewed by two sites for which there were valid k for just one of three replicates, and where said replicates had negative rate constants, indicating a concentration increase during the incubation (Figure 2). The 95% confidence intervals of the replicates that did not pass quality criteria also do not align with the pattern seen for acesulfame in Figure 2, casting further doubt on the reliability of the negative relationship (Figure S2). Given these uncertainties, we do not interpret the observed negative relationship for acesulfame as evidence of a meaningful inverse association between respiration and biodegradation.

Possible causes for differences between compounds

There are several possibilities for why 11 compounds didn't follow the observed relationships. One possibility could be that these compounds deviate because they are catabolically degraded as primary substrates[42–45] potentially causing their degradation rate to be less influenced by the presence of other carbon sources or the microbial community metabolic rate. Based on activated sludge experiments, Seller-Brison et al. suggested catabolic degradation as the dominant mechanism for acesulfame and metformin, as well as both catabolic and co-metabolic biotransformation for bezafibrate[42]. Catabolic

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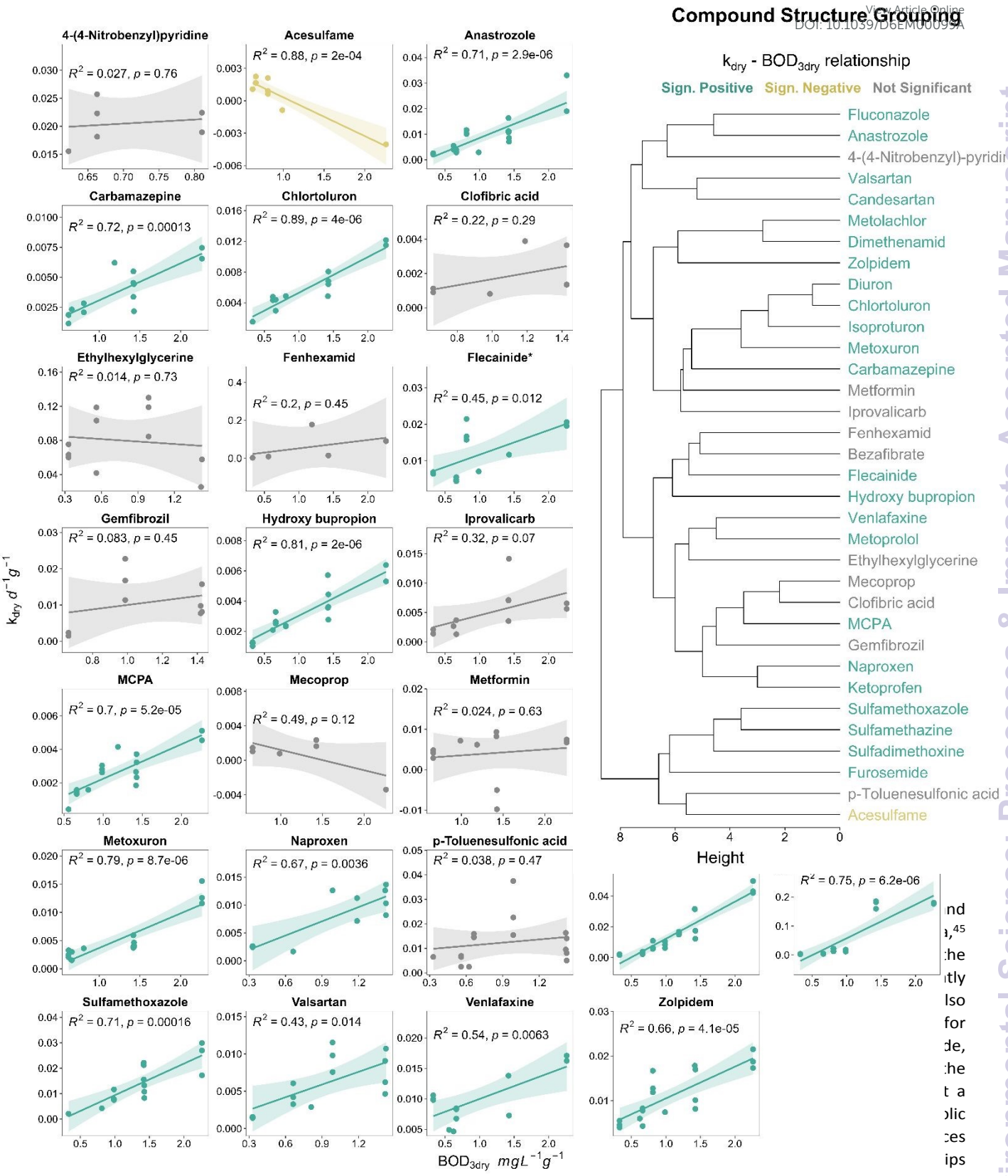

Figure 2. Relationship between BOD_{3dry} and k_{dry}. The figure displays the linear regression between BOD_{3dry} and k_{dry} for each compound for which k_{dry} could be calculated for at least three sites. Points represent the k_{dry} for the replicates of the modified OECD 309 test, BOD_{3dry} is the average for each site. Shaded areas represent the 95% confidence interval of each slope. Compounds in blue had a significant positive relationship, yellow a significant negative relationship, and grey no significant relationship at a 0.05 significance level. Compounds marked with * were subject to outlier removal (see Materials and Methods – Data processing).

and those that did not.

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carbamazepine and clofibric acid). Limited bioavailability due to sorption or charge state also does not appear to be a satisfactory explanation, as there is no clear distinction in log D_{ow} or pKa between the two groups (Figure 2, Table S1). Interestingly, despite the substantially higher pH at site F8 (BOD_{3dry} = 0.99) it doesn't appear as an outlier for the compounds with a pKa that results in greatly varying dissociation at the studied sites, e.g sulfamethazine (Figure 2, Table S1). It is possible that degradation of the 11 diverging compounds is reliant on microbes or co-substrates for which the abundance is not correlated with overall microbial community respiration. Compound-specific correlations between micropollutant degradation and specialized microbial communities[46] or specific co-substrates[47] have been observed, although the extent to which these factors influence our results is unclear.

Investigating observed relationships in relation to structural similarities between compounds could provide additional insights. Tian et al. have demonstrated correlations in degradation rates among structurally related compounds that share certain functional groups[48]. Figure 3 visualizes structural similarity between compounds via grouping based on MACCS keys[32]. As observed by Tian et al.[48], compounds with a phenylurea (chlortoluron, diuron, isoproturon and metoxuron) or sulfonamide (sulfadimethoxine, sulfamethoxazole, sulfamethazine) group cluster together and display similar behaviour, with a significant positive relationship between k_{dry} and BOD_{3dry} for all compounds in both groups (Figure 3, Figure 2). Overall, clustering does not reveal a clear structural difference between positively correlated and divergent compounds. It is, however, noteworthy that the majority of the deviating compounds cluster closest to another deviating compound, giving some credence to the idea that compound structure could explain the differing relationships between k_{dry} and BOD_{3dry} (Figure 3).

Normalizing by BOD_{3dry} decreases variability in k_{dry}

Normalization by sediment TOC has previously been assessed as a method of decreasing observed variance in degradation rates of organic contaminants in rivers, but proved unsuccessful [48]. In contrast, comparing the standard deviation in k_{dry} for each compound before and after normalization by BOD_{3dry} reveals a highly significant ($p = 1.9*10^{-5}$) decrease in mean standard deviation after normalization (Figure 4). Normalization decreased the mean relative standard deviation from 58% to 41%, and had a large Cohen's d effect size of 0.84. Normalization decreased the variance for 29 out of 34 compounds. Of the five compounds for which variance increased, the difference was minor (<10%) for bezafibrate, fenhexamid, and metformin, while ethylhexylglycerine and venlafaxine displayed a more substantial increase of 55% and 77%, respectively. The general decrease in variance after normalization indicates that variability of BOD_{3dry} can explain a substantial part of the observed variability of k_{dry} in the full dataset.

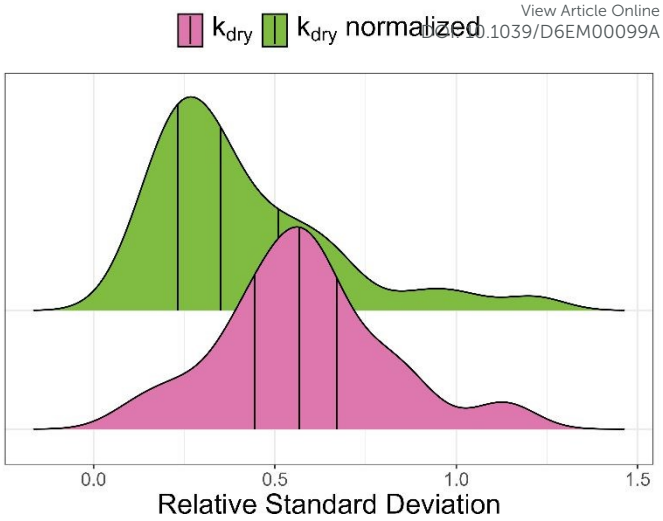

Figure 4. Density distribution of compound relative standard deviations before and after normalization of k_{dry} by $BOD3_{dry}$. Lines represent the median, first quartile and third quartile.

The influence of correcting for the abiotic control

Figure 3. Structural grouping of compounds using MACCS keys as structural descriptors. Compound label colour indicates the significance of the k_{dry} – BOD_{3dry} relationship.

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The tested compounds were selected based on the negligible influence of sorption on their dissipation in previous tests[6]. As an assurance, abiotic controls were included in the incubation for five sites, allowing the impact of abiotic processes to be examined. Peak area time series for the abiotic controls are available in the supplementary information (S2.4, Figure S4). Comparing the uncorrected and corrected rate constants for each replicate, the average ratio between k_{dry} and k_{drys} (when both passed the data quality criteria) was 0.9, with an RSD of 38%. This means that, on average, the rate constants corrected for abiotic processes were slightly larger. Rate constants increasing after correction could be the result of the dissolved fraction correction (the average dissolved fraction was 0.88), which is described in the Supporting information (S1.4.3). Moreover, for the abiotic corrected dataset 40% fewer rate constants passed the data quality criteria, which could be in part due to increased noise from incorporating two measurements into the calculations.

Due to the limited number of controls, linear regression for BOD_{3dry} and k_{drys} could only be assessed for nine compounds (Figure 5). Seven of the nine compounds had a significant positive correlation before abiotic correction, and six of these still showed a significant positive correlation after correction. The seventh, flecainide, also showed a positive correlation but it was not significant (p = 0.074), likely due in part to the smaller number of datapoints. P-toluenesulfonic acid showed no significant correlation either with or without correction. For acesulfame the relationship was negative before and after correction, whereby the reliability of the relationship is questionable, as mentioned above.

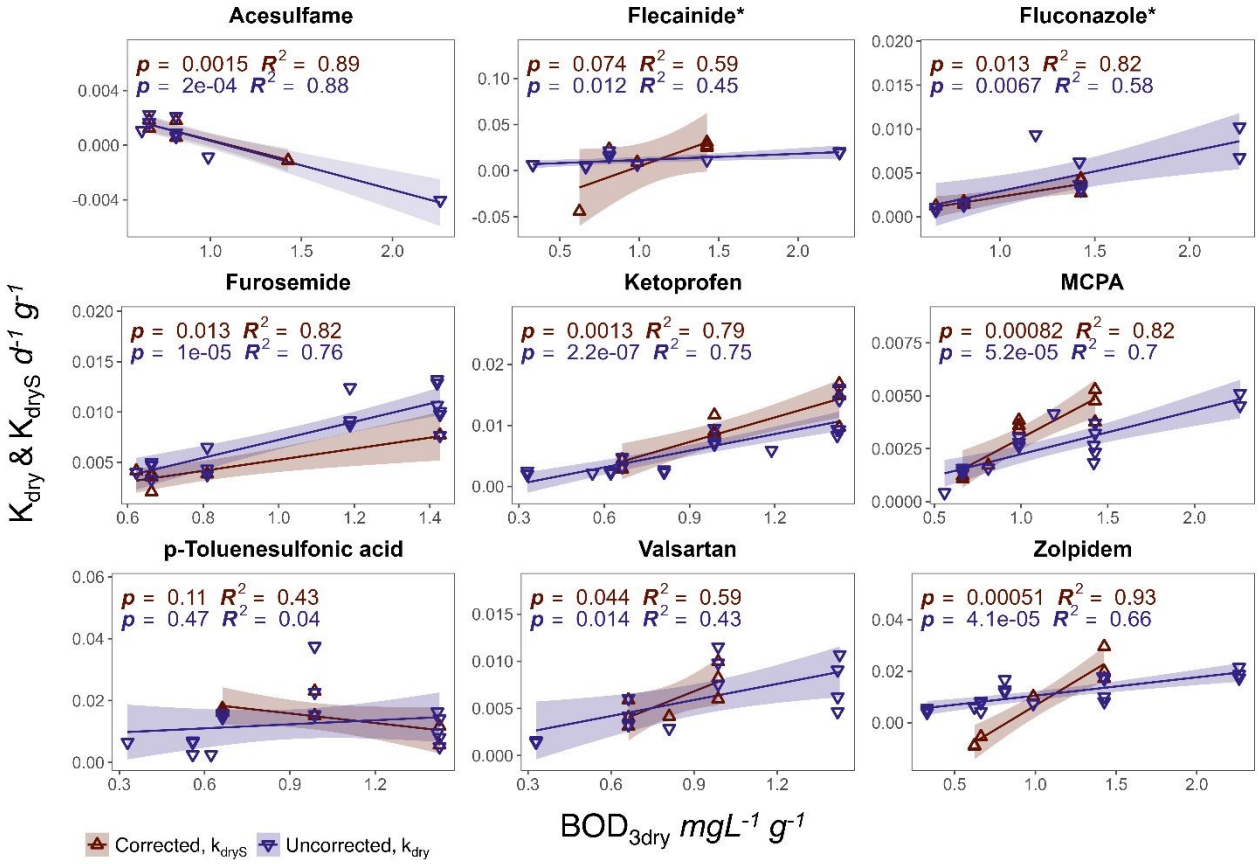

Figure 5. Relationship between BOD_{3dry}, k_{dry}, and k_{drys}. The figure displays the linear regression between BOD_{3dry}, k_{dry}, and k_{drys} for each compound for which k_{drys} could be calculated for at least three sites. Points represent the k_{dry}/k_{drys} for the replicates of the modified OECD 309 test, BOD_{3dry} is the average for each site. Shaded areas represent the 95% confidence interval of slope. Compounds marked with * were subject to outlier removal (see Materials and Methods – Data processing).

Figure 5 also reveals that while the direction of the relationship (significantly positive, negative, or not significant) between the parameters generally agrees between the corrected and uncorrected data, the slope between the parameters tends to differ to some extent between the two datasets, particularly for flecainide and zolpidem. Overall, both the uncorrected and the corrected data suggest that a positive relationship exists between attenuation and respiration for most compounds.

Implications and future prospects

The primary contribution of this work is methodological rather than predictive, demonstrating how a simple respiration-based metric can support preliminary assessments of biodegradation potential in aquatic systems. Proxies for microbial biomass have previously been suggested as a tool for faster screening of biodegradation capacity to facilitate sample site selection in biodegradation studies[3]. Here, microbial respiration of natural organic material, as measured through our modified BOD-test,

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fills a similar purpose. The observed relationships suggest that BOD-based respiration measurements may provide a useful indicator of site-specific biodegradation potential for some compounds, although their applicability is not universal. In this role, it might guide future biodegradation studies by accelerating the identification of high-priority locations for in-depth analysis. The test might also be used to aid selection of diverse inoculum for persistence assessments, potentially allowing such tests to better reflect the observed variability in degradation rates across aquatic environments[3]. Respiration-based measurements might have an advantage over TOC biomass approximations in this role as they measure microbial metabolic activity, which might be more closely correlated to biodegradation than TOC.

Still, it is worth reflecting on the assumptions underlying our results. This work assesses the capacity of the respiration test to capture the variability in a biodegradation simulation test, which itself is a simplified test attempting to describe the natural environment. Previous research has highlighted that the results of biodegradation simulation experiments don't translate directly to observations in the environment[8,49]. In particular, comparisons of rate constants modelled from field measurements and rate constants derived from laboratory experiments have revealed that simulation tests typically underestimate environmental degradation rates[8,49]. From these comparisons stream size and order has been highlighted as a key source of variability in the field not captured in the laboratory[49]. Laboratory experiments have similarly highlighted that water column depth, a parameter not included in biodegradation simulation tests, influences degradation rate constants[50]. The modified OECD 309 test is also designed to sample oxic sediment and investigate aerobic degradation, thereby purposefully omitting possible anaerobic degradation processes.

Inversely, some recent work supports the applicability of biodegradation simulation experiments to the field. A study comparing field and lab derived degradation rates utilizing a similar modified OECD 309 experiment suggested that relative differences in degradation rates between compounds are preserved between the field and lab for non- to moderately sorbing chemicals[51]. Previous work detailing the incongruence between field and laboratory derived rate constants has also noted that appropriate correction factors might be able to bridge the gap between lab and field[8]. Overall, while our test can give an indication of relative differences in degradation rates of differing environmental samples for most studied compounds, translating such predictions into the field likely necessitates hydrological data. Additionally, it is worth noting that we assess the capacity of the respiration test to indicate variabilities in degradation rates between sites, not whether or not a substance will be persistent in a given environment. A highly persistent compound is unlikely to degrade in any environment, irrespective of their BOD_{3dry}.

Additional research could build on the results of this study by incorporating more detailed characterisation of sediment and hydrological properties (e.g., water depth, sediment texture and depth of the oxic layer), which may help to better relate experimental results to in situ conditions. Since rivers are flowing and heterogenous water bodies (as can be seen in the variability observed in this experiment), assessing if the test can capture differences both between and within rivers would be important to understand its applicability. We also suggest further development of the respiration test by employing DO sensors installed internally in bottles, as has been done in previous work[52]. This would allow DO to be measured at multiple timepoints, increasing robustness and flexibility for samples with highly variable DO depletion.

Inclusion of a larger set of compounds might provide better insight into why certain compounds deviated from the relationship observed for the majority. The utilized spiking mixture was created by Tian et al.[6] for a previous study and already included additional compounds (Table S1). We selected a subset of these compounds that were reliably quantified and minimally impacted by sorption in Tian et al.'s experiments, however some of these could be included in subsequent work provided LOQs are sufficiently low and sorption can be accounted for appropriately. It's worth noting that Tian et al. found mixture effects to be negligible in previous experiments with the modified OECD 309 test, and as such we do not expect the presence of the additional compounds to affect the results of the 47 selected for analysis in this work[3,6,11].

Given that biodegradation rates are known to vary temporally[6], assessing if seasonal variations in respiration rates match variations in biodegradation would also be interesting. Finally, assessments of microbial communities and substrate (organic matter) composition could provide insights into the mechanisms underlying the observed relationships, deepening our understanding of the elusive variability in environmental biodegradation.

Conclusions

Our results indicate that respiration measurements capture aspects of site-specific variability in biodegradation for a subset of compounds, but do not provide a general predictive framework. Although this relationship is correlational, it suggests that differences in microbial metabolic activity contribute to the observed variability in biodegradation rates observed in aquatic environments. Rather than proving causation, our results show that respiration measurements may help indicate relative differences in biodegradation potential among sites for compounds showing a positive BOD–attenuation relationship. A BOD-based water–sediment respiration test could therefore be a simple and resource-efficient method to help prioritize sites and design more targeted biodegradation studies in aquatic systems.

Conflicts of interest

There are no conflicts to declare.

Data availability

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The data supporting this article have been made available in the supplementary information (SI). Supplementary information: Table S1-S5, Figure S1-S4, measured rate constants, and further experimental details. See DOI: 10.1039/x0xx00000x.

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Data Availability Statement

The data supporting this article have been made available in the supplementary information (SI). Supplementary information: Table S1-S5, figure S1 and S2, measured rate constants, and further experimental details. See DOI: 10.1039/x0xx00000x.

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