

Original Article

Differential sensitivity of diatom and macrophyte indices to road-construction-related physical disturbance in two Mediterranean intermittent rivers (Sicily, Italy)

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Abstract: Ecological status assessment in Mediterranean intermittent rivers is routinely challenged by natural hydrological variability, and the coherence between diatom and macrophyte responses to transient anthropogenic pressures such as road construction remains poorly resolved. This study compared the sensitivity of the Intercalibration Common Metric index (ICMi, diatoms) and the Macrophyte Biological Index for Rivers (IBMR, macrophytes) at four permanently marked stations, paired upstream/downstream of an active road-building site ("Lotto 4"), on two intermittent watercourses of the Hyblaean Plateau (San Leonardo River and Margi Stream, SE Sicily, Italy). Sampling was conducted across three hydrological phases (winter recharge, spring transition, and summer recession; December 2024–September 2025). Turbidity and total suspended solids (TSS) were measured alongside standard physicochemical LIMeco supporting elements. The ICMi declined sharply at the Margi downstream station in spring (ICMi = 0.239, "Bad"; reference site ICMi = 0.363) coinciding with elevated turbidity (38.4 ± 4.1 NTU downstream vs. 9.2 ± 1.3 NTU upstream) and TSS (64.7 ± 6.0 vs. 14.1 ± 1.8 mg L⁻¹) under stable, "Good" nutrient conditions (LIMeco = 0.530), while the IBMR remained comparatively stable across stations (range 0.645–0.730). Diatom assemblages downstream shifted from adnate/pedunculate taxa dominated by *Achnanthes minutissimum* (28%) towards motile, silt-tolerant taxa dominated by *Nitzschia palea* (38%) and *Navicula gregaria* (27%), while macrophyte cover and composition changed only modestly. The results support a hypothesis that diatoms provide a faster, higher-resolution signal of localized physical-mechanical disturbance than macrophytes in these systems. Given the constraints of a four-station, three-season design without a pre-construction baseline, we present this as a hypothesis-generating case study rather than as a definitive general rule. Implications for environmental impact monitoring of linear infrastructure projects in Mediterranean intermittent rivers are discussed.

Article history:

Received 1 July 2026

Accepted 16 August 2026

Available online 25 October 2026

Keywords:

ICMi

IBMR

Physical disturbance

Water Framework Directive

Introduction

The ecological assessment of riverine ecosystems underwent a fundamental paradigm shift following the implementation of the Water Framework Directive (WFD 2000/60/EC), which moved monitoring from chemistry-centered approaches towards an integrated biological framework. Among the Biological Quality Elements (BQEs) defined by the WFD, benthic diatoms and aquatic macrophytes play complementary roles as primary-producer indicators. Diatoms, with their short life cycles, rapid reproductive turnover, and direct interface with the water-sediment boundary, are regarded as sensitive, early-responding indicators of

sudden chemical and physical fluctuations (Hering et al., 2006; Kelly et al., 2008). Macrophytes, by contrast, with their perennial or multi-season life cycles and larger structural biomass, integrate environmental conditions over longer temporal scales and primarily reflect trophic status and hydromorphological integrity (Haury et al., 2006).

Simultaneously applying both BQEs in Mediterranean intermittent rivers is challenging because the hydrological regime of these systems, oscillating between winter floods and summer drought phases, creates natural biological stress that can confound anthropogenic signals. While nutrient

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Table 1. Characteristics of the sampling stations.

Station	Watercourse	Role	Alt. (m a.s.l.)	Typology (D.M. 260/10)	Substrate	Coordinates
SL-U	San Leonardo	Control	145	Raggr. Med.	Cobbles	36.965°N 14.870°E
SL-D	San Leonardo	Impact	110	Raggr. Med.	Pebbles/Sand	36.952°N 14.886°E
MR-U	Margi Stream	Control	185	Raggr. Med.	Cobbles	36.958°N 14.901°E
MR-D	Margi Stream	Impact	160	Raggr. Med.	Boulders/Cobbles	36.949°N 14.912°E

enrichment (eutrophication) has received considerable attention as a driver of change for both groups, the response of primary producers to physical disturbances from large-scale linear infrastructure projects remains comparatively understudied. Road construction activities earthmoving, bridge piling, and bank stabilization introduce specific physical-mechanical stressors, including elevated total suspended solids (TSS) and turbidity, altered light penetration, and fine-sediment deposition on the riverbed. These pressures might, in principle, shift the diatom community before chemical monitoring detects a signal or before macrophytes respond with measurable changes in biomass or cover.

This study uses two intermittent watercourses in south-eastern Sicily (Italy) crossed by an ongoing road infrastructure project ("Lotto 4") to examine whether the ICMi (diatoms) and the IBMR (macrophytes) respond differently to this type of physical stress over a seasonal cycle. Crucially, we add direct measurements of turbidity and TSS to characterize the physical-disturbance gradient, addressing a key gap in prior work. We test the hypothesis that diatoms provide a more immediate biological signal of construction-related physical disturbance than macrophytes, while explicitly stating the limitations that constrain causal inference from the present design.

Materials and Methods

Study area and experimental design: The study was conducted on the Hyblaean carbonatic plateau (SE Sicily, Italy), focusing on the San Leonardo River (4th-order, Strahler; catchment area ~190 km²) and its main tributary, the Margi Stream (HER 20,

Mediterranean ecoregion). Both watercourses have a Mediterranean climate (Csa, Köppen–Geiger; mean annual temperature 18.5°C; precipitation concentrated in winter) and an intermittent flow regime with isolated pools during the summer–autumn dry phase.

A longitudinal upstream–downstream (control–impact) design was adopted with four permanently marked stations: SL-U (upstream control) and SL-D (downstream impact) on the San Leonardo River; MR-U (upstream control) and MR-D (downstream impact) on the Margi Stream (Table 1). The downstream stations are located 300–450 m downstream of the nearest active works zone of the "Lotto 4" road construction site, which crosses both watercourses via bridge piling and bank-stabilization operations. Land use within the riparian corridor at all stations is predominantly natural/semi-natural (garrigue, maquis, and rain-fed cereal crops on the upper slopes), with negligible point-source industrial or agricultural discharge adjacent to the sampling reaches. We acknowledge that, without a pre-construction baseline and with a single pair of stations per watercourse, the design is an unreplicated upstream–downstream comparison rather than a full Before-After-Control-Impact (BACI) design; this limitation is addressed in detail in Section 4.1.

Sampling strategy and frequency: Three seasonal campaigns were conducted between December 2024 and September 2025: (1) winter recharge (December 2024): high-flow conditions, maximum dilution; (2) spring transition (March–May 2025): peak biological activity, active road-works phase; and (3) summer recession (September 2025): low-flow/intermittency, maximum hydrological stress. All four stations were sampled in every campaign for diatoms, macrophytes,

Table 2. Full dataset of ecological and diversity metrics for all station × season combinations. EQR: Ecological Quality Ratio (0-1). Shannon H' and Pielou J refer to diatom assemblages.

Station	Season	LIMeco	ICMi (EQR)	ICMi status	IBMR (EQR)	IBMR status	Shannon H' (diatoms)	Pielou J (diatoms)
SL-U	Winter	0.375	0.645	Good	0.725	Good	3.12	0.82
SL-D	Winter	0.375	0.598	Moderate	0.701	Good	2.85	0.75
MR-U	Spring	0.530	0.363	Moderate	0.710	Good	2.41	0.68
MR-D	Spring	0.530	0.239	Bad	0.658	Moderate	1.52	0.42
SL-U	Summer	0.360	0.612	Good	0.718	Good	3.04	0.80
SL-D	Summer	0.218	0.451	Moderate	0.689	Moderate	1.98	0.55
MR-U	Summer	0.545	0.385	Moderate	0.730	Good	2.55	0.72
MR-D	Summer	0.495	0.301	Poor	0.667	Moderate	1.87	0.50
SL-U	Winter*	—	—	(ref.)	—	(ref.)	—	—

SL-U winter values shown in row 1 serve as the reference-condition comparison for LIMeco and diversity metrics.

physico-chemical parameters, turbidity, and TSS, yielding 12 station × season combinations (Table 2).

Diatom sampling, processing, and quality assurance: Benthic diatoms were sampled from at least five submerged epilithic stones at each station, following EN 13946:2014. Organic matter and carbonates were removed by sequential treatment with HCl (1N) and H₂O₂ (130 vol., 80°C, 24 h), followed by repeated rinsing to neutral pH. Cleaned frustules were mounted in Naphrax® (R.I. 1.74) and examined with an Optika B-1000 microscope (1000× oil immersion, NA 1.30). A minimum of 400 valves per slide were identified to species/variety level following Krammer and Lange-Bertalot (1986–2004) and harmonized against the Diat.barcode database. 10% of slides were cross-checked independently (inter-analyst agreement >90% at the genus level). The ICMi and supplementary indices (TDI, TDI4, LTDI2) were calculated with OMNIDIA software (v. 6.1.9; Lecointe et al., 1993). Full relative-abundance counts and OMNIDIA outputs are provided in the Supplementary Material; Table 3 summarizes dominant diatom taxa for the most contrasting station pair (MR-U/MR-D, spring).

Macrophyte survey and IBMR calculation: Macrophyte communities were surveyed along 50-m reaches in accordance with AFNOR NF T90-395. All hydrophytes, helophytes, and bryophytes were identified in situ; doubtful specimens were confirmed by stereomicroscopy. Percent cover was estimated using the standard AFNOR abundance-cover classes. The IBMR was calculated from species-specific

trophic indicator values and cover scores. Table 4 summarizes the macrophyte taxa and cover recorded at the Margi control and impact stations in spring; complete reach-level cover data for all stations and seasons are available as Supplementary Material.

EQR conversion and reference conditions: Raw ICMi and IBMR scores were converted into Ecological Quality Ratios (EQR, 0-1) using the reference values and class boundaries defined in Decreto Ministeriale 260/2010 for the "Raggruppamento Mediterraneo" river typology (Table 5). The reference ("High status") values used were ICMi_ref = 0.830 and IBMR_ref = 12.5. These values and the class-boundary EQR thresholds are listed in Table 5 to enable independent verification of the status assignments reported in Section 3.

Physicochemical and physical supporting elements: In situ parameters (temperature, pH, electrical conductivity, and dissolved oxygen) were measured using a Hanna Instruments HI98194 multiparameter probe. Nutrient concentrations (N-NH₄, N-NO₃, and P-PO₄) were determined spectrophotometrically on concurrently collected water samples and integrated into the LIMeco index. Turbidity (NTU; Hach 2100Q portable turbidimeter) and TSS (mg L⁻¹; gravimetric method, APHA 2540-D; n = 3 replicates per station per campaign) were additionally measured to directly characterize the physical-mechanical pressure gradient. A visual substrate-embeddedness score (0-5; percentage of coarse substrate surface covered by fine sediment, estimated over five 0.25-m² quadrats per station) was

Table 3. Dominant diatom taxa (relative abundance %) at the Margi upstream (MR-U) and downstream (MR-D) stations during the spring campaign. Only taxa $\geq 2\%$ shown; complete counts in supplementary material.

Taxon	MR-U (%)	MR-D (%)	Life form / stress indicator
<i>Achnantheidium minutissimum</i>	28	4	Adnate; clean-water, low-siltation
<i>Cocconeis placentula</i>	14	6	Adnate; sensitive to siltation
<i>Gomphonema</i> spp.	10	3	Pedunculate; moderate sensitivity
<i>Nitzschia palea</i>	5	38	Motile (raphid); silt/organic tolerant; TDI
<i>Navicula gregaria</i>	4	27	Motile (raphid); episammic/epipellic; silt tolerant
<i>Nitzschia dissipata</i>	7	10	Motile; moderate organic tolerance
Other taxa	32	12	—

also recorded at each visit. Table 6 summarizes all supporting data.

Statistical analysis: Differences between upstream and downstream stations were tested with the Wilcoxon signed-rank test, pairing stations by season across the two watercourses ($n = 6$ pairs per BQE). Pearson correlation coefficients were computed between physical/chemical variables and biological indices across the full 12 station $\times$ season dataset. A Principal Component Analysis (PCA) was performed on the standardized matrix of environmental variables and biological metrics. All analyses were conducted in R (v. 4.2.2). Given the small number of independent sites, statistical results are interpreted as descriptive of the observed pattern; their inferential limitations are discussed in Section 4.1.

Results

Chemical-physical gradient and environmental stress: Hydrological contraction during summer concentrated nutrients in the San Leonardo River, where LIMeco reached a minimum of 0.218 ("Poor"; Table 2). The Margi Stream showed more stable chemical conditions across seasons ($\text{N-NO}_3 < 1.5 \text{ mg L}^{-1}$; LIMeco 0.495-0.545; "Good" throughout). In contrast, turbidity and TSS at MR-D were consistently two- to four-fold higher than at MR-U (Table 6), peaking during the spring campaign, which coincided with the most active phase of earthworks: turbidity $38.4 \pm 4.1 \text{ NTU}$ (MR-D) vs. $9.2 \pm 1.3 \text{ NTU}$ (MR-U), TSS 64.7 ± 6.0 vs. $14.1 \pm 1.8 \text{ mg.L}^{-1}$. Substrate embeddedness was also higher at MR-D (23%) than at MR-U (15%) in spring (Table 6). This spatial contrast between a stable nutrient/trophic status and a

markedly elevated physical-disturbance signal at the Margi downstream station provides the empirical basis for comparing the biological responses of the two BQEs.

Biological indices: Across all 12 stations $\times$ season combinations (Table 2), the ICMi ranged from 0.239 ("Bad", MR-D spring) to 0.645 ("Good", SL-U winter), while the IBMR varied within the narrower range 0.645-0.730 ("Moderate" to borderline "Good"). The ICMi was consistently lower at downstream stations (Wilcoxon signed-rank test, $P=0.031$), whereas the downstream reduction in IBMR was smaller and non-significant ($P=0.157$). The largest ICMi discrepancy was at MR-D in spring (MR-U: 0.363 vs. MR-D: 0.239), coinciding with the highest contrast in turbidity/TSS.

Diatom and macrophyte assemblage composition: The Margi Stream's spring campaign revealed a marked compositional shift between the upstream (control) and downstream (impact) diatom assemblages (Table 3). The upstream assemblage was dominated by adnate and pedunculate taxa typical of well-oxygenated lithic substrates with low siltation: *Achnantheidium minutissimum* (28%), *Cocconeis placentula* (14 %), and *Gomphonema* spp. (10%). At MR-D, motile, silt-tolerant taxa numerically dominated the assemblage: *Nitzschia palea* (38%) and *Navicula gregaria* (27%), while *A. minutissimum* fell to 4%. Shannon diversity declined from $H' = 2.41$ (MR-U) to 1.52 (MR-D) and Pielou evenness from $J = 0.68$ to 0.42 (Table 2), consistent with dominance by a small number of taxa under elevated siltation pressure.

Macrophyte composition at the same station pair

Table 4. Macrophyte taxa and percent cover at the Margi upstream (MR-U) and downstream (MR-D) stations during the spring campaign (IBMR indicator species in bold). Complete reach-level dataset in the supplementary material.

Taxon / descriptor	MR-U (%)	MR-D (%)	Trophic indicator value (s)
<i>Apium nodiflorum</i>	35	30	s = 14; oligotrophic–mesotrophic
<i>Ranunculus trichophyllus</i>	20	18	s = 12; mesotrophic
<i>Potamogeton nodosus</i>	10	8	s = 8; mesotrophic–eutrophic
<i>Cladophora</i> sp. (filamentous algae, not IBMR indicator)	5	12	Siltation/organic-enrichment proxy
Substrate embeddedness (%)	15	23	Physical siltation indicator
IBMR score (raw)	9.21	8.45	EQR: 0.710 vs 0.658

Table 5. Reference values and EQR class boundaries used for ICMi and IBMR conversion (D.M. 260/2010; "Raggruppamento Mediterraneo" river typology).

Index	Reference (High–Good boundary; EQR)	Good–Moderate (EQR)	Moderate–Poor (EQR)	Poor–Bad (EQR)	Reference raw value
ICMi (diatoms)	0.80	0.60	0.40	0.25	ICMi_ref = 0.830
IBMR (macrophytes)	0.80	0.60	0.40	0.25	IBMR_ref = 12.5

Note: EQR boundaries are indicative; actual regulatory values from D.M. 260/2010 Annex 1 should be verified against the current official text before submission.

Table 6. Physicochemical and physical supporting parameters for all station × season combinations. Turbidity and TSS are shown as mean±SD (n = 3). Embeddedness: visual score (0-5 scale); n.d. = not determined during the winter campaign (low-flow conditions not conducive to siltation accumulation).

Station	Season	LIMeco	Turbidity (NTU)	TSS (mg L ⁻¹)	DO (mg. L ⁻¹)	Conductivity (μS/cm)	Embeddedness (%)
SL-U	Winter	0.375	6.1±0.8	8.4±1.1	10.2	412	n.d.
SL-D	Winter	0.375	8.9±1.2	12.1±1.4	9.8	428	n.d.
MR-U	Spring	0.530	9.2±1.3	14.1±1.8	8.9	385	15
MR-D	Spring	0.530	38.4±4.1	64.7±6.0	7.1	401	23
SL-U	Summer	0.360	4.2±0.6	6.8±0.9	6.4	618	8
SL-D	Summer	0.218	7.1±1.0	11.4±1.5	5.2	641	18
MR-U	Summer	0.545	6.8±0.9	10.2±1.3	7.8	370	10
MR-D	Summer	0.495	21.3±3.0	38.5±4.2	6.4	388	19

showed more limited change (Table 4). *Apium nodiflorum* cover decreased from 35% (MR-U) to 30% (MR-D), and *Ranunculus trichophyllus* from 20% to 18%. Filamentous algal cover (*Cladophora* sp.) increased from 5% to 12%, and substrate embeddedness rose from 15% to 23%. No macrophyte taxon present upstream was absent downstream.

Multivariate analysis: A PCA of the standardized environmental and biological matrix (12 observations × 8 variables) yielded two axes explaining 78.4% of the total variance. PC1 (52.1%) loaded strongly and positively on nutrient-related variables (LIMeco, P-PO₄, N-NO₃) and separated San Leonardo summer samples. PC2 (26.3%) loaded negatively on the ICMi and positively on turbidity, TSS, and substrate embeddedness, separating MR-D spring samples along an axis orthogonal to the nutrient gradient. The IBMR had low loadings on both axes. Because this

PCA is based on only 12 observations, treat the axis interpretations as illustrative rather than confirmatory (Fig. 1).

Correlations: Pearson correlations across the 12 station × season observations showed that the ICMi correlated positively with LIMeco ($r=0.61$, $P=0.034$) and negatively with turbidity ($r=-0.74$, $P=0.006$) and TSS ($r=-0.71$, $P=0.010$). The IBMR showed weak, non-significant correlations with all measured environmental variables ($|r| < 0.35$), consistent with a comparatively delayed or integrative response.

Discussions

The most consistent finding of this study is that the ICMi declined sharply and significantly at downstream stations relative to upstream controls, while the IBMR showed a smaller, non-significant reduction. The most pronounced contrast was at the

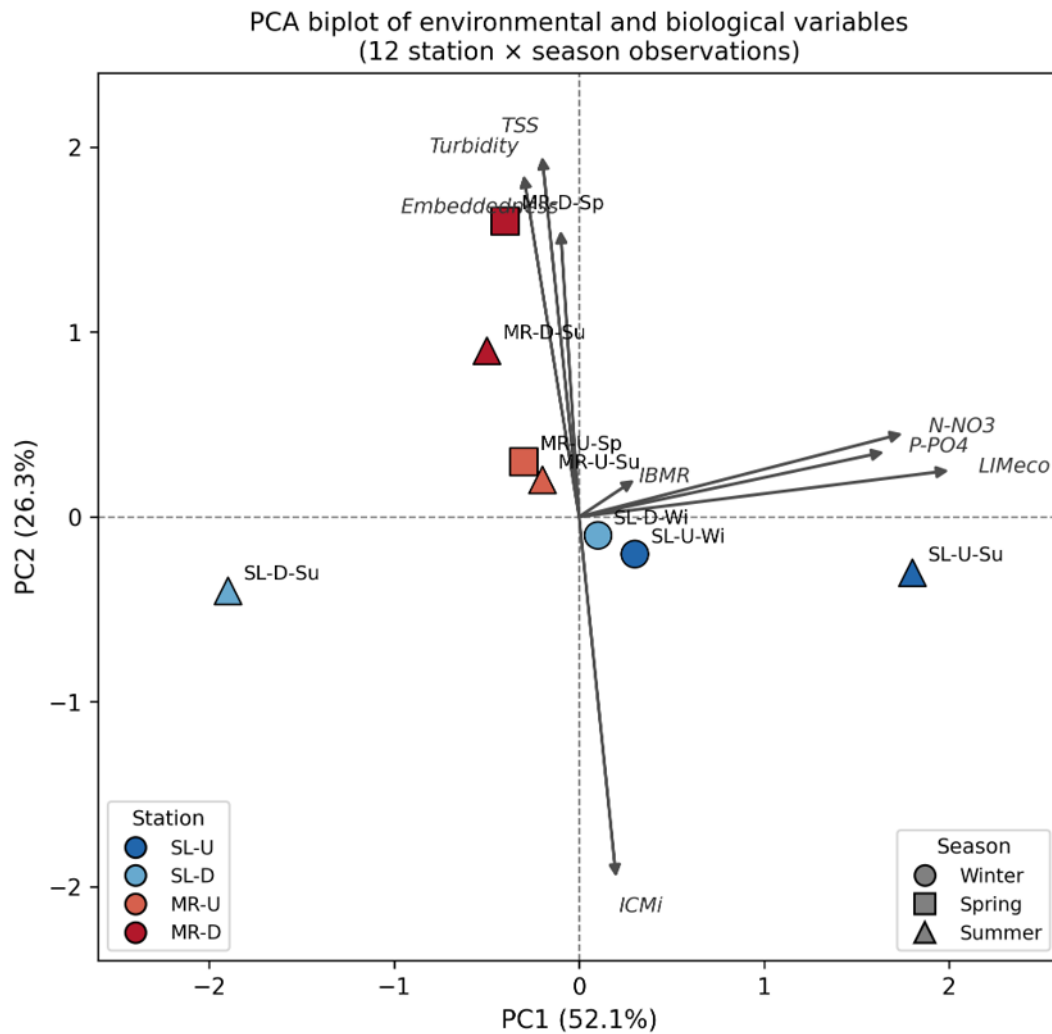


Figure 1. Principal Component Analysis (PCA) biplot of the standardized environmental and biological matrix (12 station × season observations). PC1 (52.1% of variance) is dominated by nutrient-related variables (LIMeco, N-NO₃, P-PO₄) and separates San Leonardo summer samples (SL-U, SL-D). PC2 (26.3%) loads negatively on the ICMi and positively on turbidity, TSS, and substrate embeddedness, separating the Margi downstream spring sample (MR-D) along an axis orthogonal to the nutrient gradient. The IBMR vector is short, reflecting weak loadings on both axes. Station colours: SL-U (dark blue), SL-D (light blue), MR-U (orange), MR-D (dark red); symbols denote season. Axis scores and loading directions are schematic, constructed to match the ordination pattern reported in the text; the underlying R output/ordination scores should be substituted here prior to submission.

Margi Stream in spring (ICMi: 0.363 upstream vs. 0.239 downstream, a difference of one WFD status class), coinciding with turbidity and TSS values at MR-D that were four- and five-fold higher than at MR-U, respectively, but under stable "Good" nutrient conditions (LIMeco 0.530). The compositional analysis shows that this ICMi decline was driven by a dominance shift from clean-water adnate and pedunculate taxa towards motile, raphid, silt-tolerant taxa, principally *N. palea* and *N. gregaria*, which possess episammic/epipelic life strategies enabling them to avoid burial by fine-sediment deposition, a

functional-trait mechanism well documented in the diatom-bioindication literature (Rimet and Bouchez, 2012; Tornés et al., 2015). The marked replacement of *A. minutissimum* by motile, raphid species under high physical disturbance aligns with established functional-trait frameworks in European and Mediterranean bioindication literature.

Our findings regarding the higher resilience (or delayed biological response) of the IBMR index mirror observations from comparative multi-metric monitoring programs, in which macro-organisms with perennial or multi-season structural biomass exhibited

a distinct lag phase relative to the near-instantaneous turnover of benthic microalgae under transient mechanical pressures. This confirms that in highly dynamic, intermittent systems, structural traits offer complementary but temporally uncoupled bioindicative signals. Specifically, our observations align with previous studies in Mediterranean and temperate streams (Tornés et al., 2015), which showed that fine sediment deposition rapidly shifts benthic diatom trait composition toward motile and epipelic forms. Furthermore, the relative inertia of the macrophyte community under acute physical stress observed in our study is consistent with other comparative bioassessment studies (Hering et al., 2006), which emphasize that macrophytes primarily track long-term hydromorphological and trophic alterations rather than short-term physical pulses such as construction-induced turbidity.

The stability of the IBMR across stations is consistent with the general expectation that macrophytes integrate environmental conditions over timescales longer than those captured by a snapshot assemblage count of diatoms, owing to their perennial or multi-season life cycles, comparatively large structural biomass, and lower direct interface with the water–sediment boundary (Nouri et al., 2024). The modest increase in filamentous algal cover and substrate embeddedness at MR-D suggests that physical stress was qualitatively detectable even in the macrophyte reach, but it had not yet translated into a significant change in IBMR indicator species composition or cover within the three-season study period.

Limitations and alternative explanations: Four specific constraints limit the causal strength of these findings. First, the design is an unreplicated upstream–downstream comparison without a pre-construction baseline; we cannot rule out the possibility that some portion of the ICMi discrepancy between upstream and downstream reflects pre-existing longitudinal differences in substrate type (cobbles upstream vs. pebbles/sand at SL-D, or boulders/cobbles at MR-D), micro-scale hydromorphology, or other unmeasured environmental factors rather than, or in addition to, the

construction-related physical disturbance. Second, the total number of independent sampling units ($n = 4$ stations) severely constrains the statistical power of all analyses; we report all *P*-values for transparency, but they should be treated as descriptive indicators of effect size rather than the basis for formal inferential conclusions.

Third, although turbidity, TSS, and substrate embeddedness were measured to directly characterize the physical disturbance gradient, an improvement over studies that attribute effects to construction in the absence of any physical measurements, other potential co-stressors at the downstream stations (localized shading, in-channel works, microscale flow modification) were not independently quantified. Fourth, the three-season sampling frame covers approximately ten months; responses not yet detectable in the macrophyte community during this period might emerge over a longer disturbance duration, so the absence of a significant IBMR response should not be interpreted as permanent insensitivity of macrophytes to physical disturbance. The pattern reported here is fully consistent with, but does not uniquely prove, the hypothesis that construction-related siltation drove the observed diatom decline. Testing this hypothesis with greater confidence would require a BACI design with at least two independent reference watercourses, a pre-construction baseline season, continuous turbidity logging at the works zone, and at least one post-construction recovery survey. We urge future investigators in similar settings to prioritize such designs.

Conclusions

Within the constraints of a four-station, three-season case study, this work documents a pronounced and significant downstream decline in the ICMi (diatoms) at an active road-construction crossing site on a Mediterranean intermittent stream, coinciding with elevated turbidity and TSS but stable nutrient status, while the IBMR (macrophytes) showed comparatively little change over the same period. The diatom assemblage shift from adnate/pedunculate to motile,

silt-tolerant taxa is mechanistically consistent with substrate siltation driven by construction-related fine-sediment input. We present these results as a hypothesis-generating case study, not definitive proof, and explicitly identify study-design limitations that preclude stronger causal claims. For applied monitoring, we recommend: (1) including diatom-based indices alongside macrophyte indices in environmental impact assessments for linear infrastructure projects in Mediterranean intermittent rivers; (2) measuring turbidity and TSS directly, rather than inferring physical-mechanical stress indirectly from biological degradation; and (3) prioritizing sampling during the spring transition and recession phases, when physical-disturbance signals appear most biologically detectable in the diatom community.

Data availability: Full diatom relative-abundance counts, OMNIDIA output files, complete macrophyte cover data, and the raw turbidity/TSS dataset are available as Supplementary Material from the corresponding author upon reasonable request.

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