

Testing the performance of macroinvertebrate-based indices of intermittence in rivers beyond their region of development

Running title: Testing flow intermittence indices

Authors

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Abstract

Intermittent rivers represent more than half of the length of the global river network, and their extent is predicted to increase. Although flow intermittence is a natural phenomenon, it is exacerbated by the combined and interacting effects of climate change and anthropogenic pressures. This increasing intermittence represents a major threat to freshwater biodiversity and highlights the pressing need for indices that can assess the responses of freshwater biota to drying events. However, most such indices remain untested beyond their region of origin, limiting their widespread use. We addressed this gap by testing the capacity of two macroinvertebrate-based indices that respond to flow intermittence (the Drought Effect of Habitat Loss on Invertebrates [DEHLI] index and the Monitoring Intermittent Streams index [MIS-index]), developed and evaluated in the UK, to distinguish between perennial and intermittent river sites in Italy. Scores for both indices and index-derived richness metrics (i.e. the taxa richness of groups of environmental association classified by the indices) were higher at perennial than intermittent sites. Among index-derived richness metrics, only those including rheophilic taxa were lower at intermittent sites, whereas others were comparable at perennial and intermittent sites. Our results suggest that both indices have the potential for use beyond their original region of development, with a better performance of DEHLI index due to its family-level taxonomic resolution. However, their responsiveness could be enhanced by regional adaptation, to increase the pool of scoring taxa. In addition, the development of conceptual frameworks predicting assemblage composition in intermittent rivers across different biogeographical regions could guide the future development of new indices for flow intermittence.

Keywords

IRES, biomonitoring, biotic indices, invertebrates, ecological indicators, bioassessment.

1 | Introduction

More than 50% of the global river network experiences flow intermittence, with dry periods spanning days to years (Messenger et al., 2021). While river drying is a natural phenomenon, the extent of intermittent rivers is increasing due to climate change and growing human pressures (Döll & Schmied, 2012; Mimeau, Künne, Branger, et al., 2024; Tramblay et al., 2021), with previously perennial rivers becoming intermittent (Datry et al., 2023). This increasing river drying represents a major threat to freshwater biodiversity, reducing aquatic diversity primarily through the loss of sensitive taxa (Leigh & Datry, 2017). In this context, tools are needed to monitor how aquatic biota respond to intermittence, during seasonal dry phases as well as unpredictable drought events (Skoulikidis et al., 2017; Stubbington et al., 2017). As such, new indices have been developed to assess the responses of river biota, in particular aquatic macroinvertebrate communities, to changing habitat conditions (England et al., 2019; Straka et al., 2019). However, these indices have been developed and tested in specific environmental contexts (i.e., within individual countries or ecoregions), and their performance beyond their regions of origin remains largely untested (but see Theodoropoulos et al., 2021).

Two indices developed in England, UK, characterize macroinvertebrate community responses to hydrological variability, including stream drying: the Drought Effect of Habitat Loss on Invertebrates index (DEHLI; Chadd et al., 2017) and the Monitoring Intermittent Streams index (MIS-index; England et al., 2019). DEHLI quantifies community responses to changing in-channel habitat conditions during droughts by assigning Drought Intolerance Scores (DIS) to macroinvertebrate families (or genera) within ecological groups associated with habitats lost at key stages of channel drying (i.e. flow-decrease, loss-lateral, loss-longitudinal, pool-shrinking, pool-drying, total-dry; Table S1). MIS-index characterizes ecological responses to habitat changes associated with flow intermittence, by averaging weighted taxa richness (largely at genus or species level) in six habitat-association groups (i.e. MIS-groups: lotic-fast, lotic, generalist, lentic, semi-aquatic, terrestrial; Table S1). By characterizing community responses to changing hydrological conditions, these indices could also support the interpretation of biomonitoring data in river health assessments. However, the capacity of DEHLI and MIS-index to characterize community responses to drying has only been tested within UK lowland, calcareous systems (i.e., the chalk streams; Bunting et al., 2020; England et al., 2019; White et al., 2019), whereas index performance in other contexts remains unexplored.

80 We addressed this gap by evaluating the ability of DEHLI and MIS-index to distinguish between
81 macroinvertebrate communities collected in perennial and intermittent river sites in Italy, thus testing the
82 transferability of indices across environmental contexts. Our central hypothesis was that indices developed in
83 one region can be effectively applied in other environmental contexts if there is sufficient overlap in taxa pools.
84 As such, we predicted that both DEHLI and MIS-index would distinguish between perennial and intermittent
85 sites due to the overlapping regional taxa pools (i.e. 77%, 54% and 26% at the family, genus and species levels,
86 respectively; see Figure S1). We also predicted that DEHLI would outperform the MIS-index, because it is
87 based mainly on family-level data, rather than a mixed taxonomic level dominated by genera and species, and
88 is thus more likely to share taxa with those in other ecoregions (e.g. Schmidt-Kloiber & Hering, 2015).

89

90 **2 | Methods**

91 *2.1 Test dataset: study area and design*

92 The performance of DEHLI and MIS-index was tested using a dataset collected from 22 perennial (P) and 18
93 intermittent (I) sites across five streams in the Po River basin (Emilia-Romagna region, northern Italy) in May–
94 June 2017 (Figure 1). The climate in the study area is temperate/cold (Köppen–Geiger classification Cfa, Cfb,
95 and Dfb; Beck et al., 2018), with mean annual temperature ranging from 5 to 14°C and annual precipitation
96 from 600 to 2300 mm (both calculated for the period 1961–2010 in Emilia-Romagna region (Antolini et
97 al., 2016). Site elevation varied between 174 and 820 m a.s.l. (mean $\pm$ SD, 469 $\pm$ 162 m). Land use (calculated
98 for each sub-catchment from CORINE land cover data 2018) is mainly forests or semi-natural (mean $\pm$ SD,
99 73 $\pm$ 21%, according to CORINE land cover nomenclature).

100 Rivers and streams in the study area are both surface water and groundwater-fed, with high discharge in early
101 spring and autumn and low discharge in summer. Each site's flow permanence regime was classified as P or I
102 based on regulatory authority discharge data (when available, www.arpae.it), aerial photos from Google Earth
103 and information provided by local residents. Classifications were then validated using field observations and
104 hourly water temperature data logged from late July to mid-November (Onset HOBO). We classified I-sites
105 as those that dried for ≥ 2 consecutive days in this period (Blasch et al., 2004). The number of dry days for the

106 18 I-sites varied between three and 70 days (mean $\pm$ SD, 53 ± 21 days), with flow resuming at I-sites in early
107 November. P-sites were classified as those with continuous flow.

108 Macroinvertebrates were collected from mid-May to mid-June 2017. A Surber net (0.05 m² frame area, 500-
109 μ m mesh) was used to collect six macroinvertebrate samples per site, encompassing all available habitats to
110 maximize the number of taxa collected. One site had only isolated ponds at the time of sampling; consequently,
111 only two samples were collected. Each macroinvertebrate Surber sample (236 in total) was preserved
112 with 90% ethanol and stored until processing in the laboratory. All material was processed and specimens
113 identified to family or genus level (Campaoli et al., 1994; Tachet et al., 2010), their life stage (i.e. juvenile,
114 pupa or adult) noted, and each counted.

115 2.2 Statistical analysis

116 We assigned each taxon a DIS following Chadd et al. (2017) and to a MIS-group following England et al.
117 (2019) (Table S1). Taxa without a DIS and/or a MIS-group were excluded from the dataset (see Results and
118 Table S2). The DEHLI index was then calculated as the sum of the DIS values divided by the number of
119 scoring taxa (Chadd et al., 2017). The MIS-index was calculated as the weighted mean (i.e. the sum divided
120 by the total number of scoring taxa in a sample) of the taxa in each MIS-group multiplied by its weighting
121 factor (England et al., 2019). For calculation of both indices, we aggregated taxa at the taxonomic level
122 required by each index (i.e. mainly family level for DEHLI and a mixed level for MIS-index; Table S2). We
123 also calculated the aggregated taxa richness in each DEHLI group (six metrics) and each MIS-group (six
124 metrics). Metrics were not calculated for the *total-dry* DEHLI group or the *terrestrial* MIS-group because 0–
125 1 taxa were recorded for these groups.

126 We compared index scores and richness metric values at P and I sites using linear mixed-effect models (LMM,
127 for indices) and generalized LMM (GLMM, for richness metrics), the latter fitted with a Poisson distribution
128 to account for zero-inflated count data (Zuur et al., 2009). *Flow regime* (categorical: P, I) was included as a
129 fixed term, and *site* (categorical: 40 levels) as random term. Model results were summarised with Type II
130 ANOVA (to account for the unbalanced design), and *p*-values adjusted with Bonferroni correction for multiple
131 comparisons.

132

133 3 | Results

134 We recorded 73,047 individuals in 133 taxa (87 families). Taxa with no DIS (30 taxa from 24 families) or
135 MIS-group (52 taxa from 36 families) were excluded (Figure 2, Table S2), leaving 77% and 61% of taxa
136 assigned to DIS and MIS-groups, respectively. Some excluded taxa occurred frequently, including the mayfly
137 family Ephemerellidae (excluded from DEHLI calculations), present in 67% of the samples, and the mayfly
138 genera *Ecdyonurus*, *Rhithrogena* and *Habroleptoides* (excluded from MIS-index calculations), found in 64,
139 39 and 33% of the samples, respectively. Several excluded taxa had different occurrences in P and I-sites
140 (Figure S1).

141 DEHLI index scores were significantly higher at P-sites (5.7 ± 0.7) than at I-sites (5.2 ± 0.8 , Table 1), whereas
142 MIS-index values were comparable at both site types (Figure 3, Table 1). Differences in richness metrics
143 between P and I-sites varied among DEHLI groups and MIS-groups (Figure 3, Table 1). Richness in the *flow-*
144 *decrease* and *loss-longitudinal* DEHLI groups were significantly higher at P-sites (2 ± 1 and 5 ± 2 taxa per
145 sample, respectively) than at I-sites (1 ± 1 and 3 ± 2 taxa per sample, respectively), whereas other DEHLI
146 richness metrics were comparable across sites. The *lotic-fast* MIS-group was significantly higher at P-sites (2
147 ± 1 taxa per sample) than at I-sites (1 ± 1 taxa per sample), whereas for all other MIS-groups, richness was
148 comparable at P and I-sites.

149

150 4 | Discussion and Conclusions

151 To date, the few indices developed to characterize how ecological communities respond to changing habitat
152 availability during river drying remain largely untested beyond their region of origin. Testing is required to
153 inform the adaptation and use of such indices, both to document responses to changing in-channel conditions
154 and to inform the interpretation of biomonitoring data (Stubbington et al., 2018). We thus evaluated the
155 performance of two UK indices of intermittence (DEHLI, MIS-index) in Italy, by comparing index scores and
156 richness metrics representing macroinvertebrate communities from perennial (P) and intermittent (I) river sites.
157 Differences in DEHLI scores and DEHLI and MIS-index richness metrics between P and I-sites in Italian
158 rivers suggest that these indices have the potential to describe ecological responses to intermittence beyond
159 their region of development. Metrics representing the richness of taxa associated with fast-flowing conditions

160 were particularly responsive to intermittence, suggesting their potential use for assessing responses to drying
161 events.

162 4.1 Performance of intermittence indices

163 DEHLI index scores were significantly higher at P than I-sites, suggesting the potential of this index for
164 application in regions beyond the UK. These differences between sites were detected based on
165 macroinvertebrate communities sampled in late spring and early summer, approximately seven months after
166 the preceding dry phase and rewetting of the I-sites, similar to observations in UK calcareous rivers
167 (Sarremejane et al., 2019; White et al., 2019). This suggests a ‘drying memory’ of I-sites, with persistent effects
168 on community composition, consistent with previous findings in temperate UK (Bunting et al., 2020),
169 Mediterranean (Belmar et al., 2019), and Alpine (Piano et al., 2019) streams. Furthermore, we detected
170 differences between P and I-sites for richness metrics representing groups associated with faster flow
171 conditions, i.e. the *flow-decrease* and *loss-longitudinal* DEHLI groups and the *lotic-fast* MIS-group. Taxa in
172 these groups prefer fast-flowing and well-oxygenated waters (Chadd et al., 2017; England et al., 2019) and
173 thus may be eliminated during ponded and dry phases, with their recolonization after flow resumption
174 reflecting taxon-specific dispersal capacities (Sarremejane et al., 2020). For example, many taxa belonging to
175 the families Heptageniidae (Ephemeroptera), Leuctridae (Plecoptera), and Rhyacophilidae (Trichoptera),
176 commonly found in both UK chalk streams and Italian rivers, prefer fast-flowing waters (e.g., Schmidt-Kloiber
177 & Hering, 2015) and were more abundant in P-sites, enhancing their potential as indicator taxa that represent
178 the effects of stream drying. In contrast, the richness of groups associated with later stages of drying, including
179 lentic and semi-aquatic taxa (such as the Diptera family Tabanidae and the Heteroptera genus *Micronecta*),
180 was comparable at P and I-sites. Differences in macroinvertebrate communities between P and I-sites thus
181 mostly reflect the absence of rheophilic taxa from I-sites rather than the occurrence of lentic and semi-aquatic
182 taxa in ponded and dry phases (Kelso & Entekin, 2018).

183 4.2 Factors affecting index performance

184 The MIS-index and seven DEHLI and MIS-group richness metrics were comparable at P and I-sites. This
185 difference in performance likely reflects their different taxonomic resolution, with DEHLI’s higher, family
186 level enhancing its transferability across different ecoregions (Heino & Soininen, 2007). Similarly,

187 performance of the Lotic-invertebrate Index for Flow Evaluation (LIFE; Extence et al., 1999), developed for
188 UK, performs well in its family but not species-level form in other regions, including Mediterranean Greece
189 (Theodoropoulos et al., 2021). Largely genus- and species-based indices such as the MIS-index have less
190 taxonomic overlap among regions and are affected by local endemisms and biogeographic history. The
191 resultant exclusion of 52 taxa from MIS-index calculations (39% of the dataset, compared to 18% for DEHLI)
192 likely reduced its detection of differences between P and I-sites, because several of the excluded taxa had
193 different distributions between P and I-sites (Figure S2). In addition, several rheophiles including the
194 Ephemeroptera genera *Rhithrogena*, *Oligoneuriella*, and *Ecdyonurus*, the Plecoptera genera *Perla*, *Dinocras*,
195 and *Protonemura*, and the Diptera family Blephariceridae, as well as primarily lentic taxa including
196 *Siphonurus* (Ephemeroptera), *Anax* and *Chalcolestes* (Odonata), and *Pomatinus* (Coleoptera), were present in
197 our dataset but are not MIS-index scoring taxa, potentially reducing the responsiveness of index scores and
198 richness metrics to environmental differences between P and I-sites.

199 In addition, taxa in the *total-dry* DEHLI group and in the *semi-aquatic* and *terrestrial* MIS-groups were poorly
200 represented in our Italian dataset, which was collected during flowing phases and exclusively within the wetted
201 area (i.e. not also from marginal sediments, as recommended by England et al. 2019). Including semi-aquatic
202 and terrestrial taxa may enhance characterization of ecological responses to flow intermittence (Bunting et al.,
203 2020; Sarremejane et al., 2019) but can also pose challenges, requiring specialized field and taxonomic
204 identification skills (England et al., 2019).

205 The performance of the DEHLI and MIS indices (and derived richness metrics) may also reflect contrasting
206 characteristics of the drying process in different river morphologies. Both indices were developed and tested
207 using data from chalk streams in south and east England (Chadd et al., 2017; England et al., 2019), which are
208 groundwater-dominated, narrow, single-channel rivers (Berrie, 1992). In contrast, we sampled Italian rivers
209 fed both by surface runoff and groundwater, and typified by wider, multi-channel. The wetted part of the
210 riverbed is highly mobile and lacks vegetated margins in these braided rivers (Vander Vorste et al., 2016). As
211 a result, the DEHLI stage *loss-lateral* – in which lateral connectivity is lost in chalk streams, eliminating an
212 associated group of taxa that use marginal habitats, including their vegetation, – does not occur consistently in
213 braided intermittent rivers. As such, taxa included in this DEHLI group were scarce in our dataset, likely
214 preventing its discrimination of P and I-sites.

215 4.3 Future perspectives

216 Our study highlights opportunities to adapt the tested indices to improve their performance and thus support
217 their future application in different ecoregions. For instance, index performance could be enhanced i) by
218 regional adaptation of the taxa list to better represent taxa pools, including scoring and classification of new
219 taxa; and ii) by considering differences in river morphology and drying patterns. Moreover, the dynamic
220 mosaic of aquatic and terrestrial habitats that characterizes intermittent rivers represents an opportunity to
221 include more semi-aquatic and river-associated terrestrial taxa in intermittence indices.

222 Considering the current and predicted increase in river drying (Mimeau, Künne, Devers, et al., 2024; Zipper
223 et al., 2021), indices are needed to document the ecological effects of drying on stream biota, ideally, indices
224 that remain effective across regions and river types. To overcome regional differences in taxa pool and thus
225 enhance transferability, indices calculated based on selected functional traits represent a promising solution,
226 as demonstrated by Laini et al. (2022) using their Flow-T index. Like taxonomic indices, such trait-based
227 metrics require testing beyond their region of origin, to determine their wider applicability, limits and potential
228 for improvement or regional adaptation. In addition, such indices could inform the interpretation of
229 biomonitoring indices that document biotic responses to human pressures, thus supporting ecosystem health
230 assessment in intermittent rivers.

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233 Data availability statement

234 The data that support the findings of this study are available from the corresponding author upon request.

235

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352 Tables

353

354 **Table 1.** Results of mixed models summarized with type II ANOVA comparing index scores and richness
 355 metrics (which represent macroinvertebrate taxa richness in groups associated with different habitats) at
 356 perennial and intermittent sites. Significant differences are marked in bold.

	Chi-square	Df	<i>p</i> -values	Bonferroni adjusted <i>p</i> -values
DEHLI index and metrics				
<i>DEHLI index</i>	8.68	1	0.003	0.039
<i>Flow-decrease</i>	20.58	1	<0.001	<0.001
<i>Loss-lateral</i>	<0.01	1	0.997	1.000
<i>Loss-longitudinal</i>	15.33	1	<0.001	<0.001
<i>Pool-shrinking</i>	1.74	1	0.279	1.000
<i>Pool-drying</i>	0.23	1	0.635	1.000
MIS-index and metrics				
<i>MIS-index</i>	3.54	1	0.059	0.708
<i>Lotic-fast</i>	20.33	1	<0.001	<0.001
<i>Lotic</i>	3.63	1	0.057	0.681
<i>Generalist</i>	0.34	1	0.559	1.000
<i>Lentic</i>	2.55	1	0.110	1.000
<i>Semi-aquatic</i>	0.08	1	0.768	1.000

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358 **Figure Legends**

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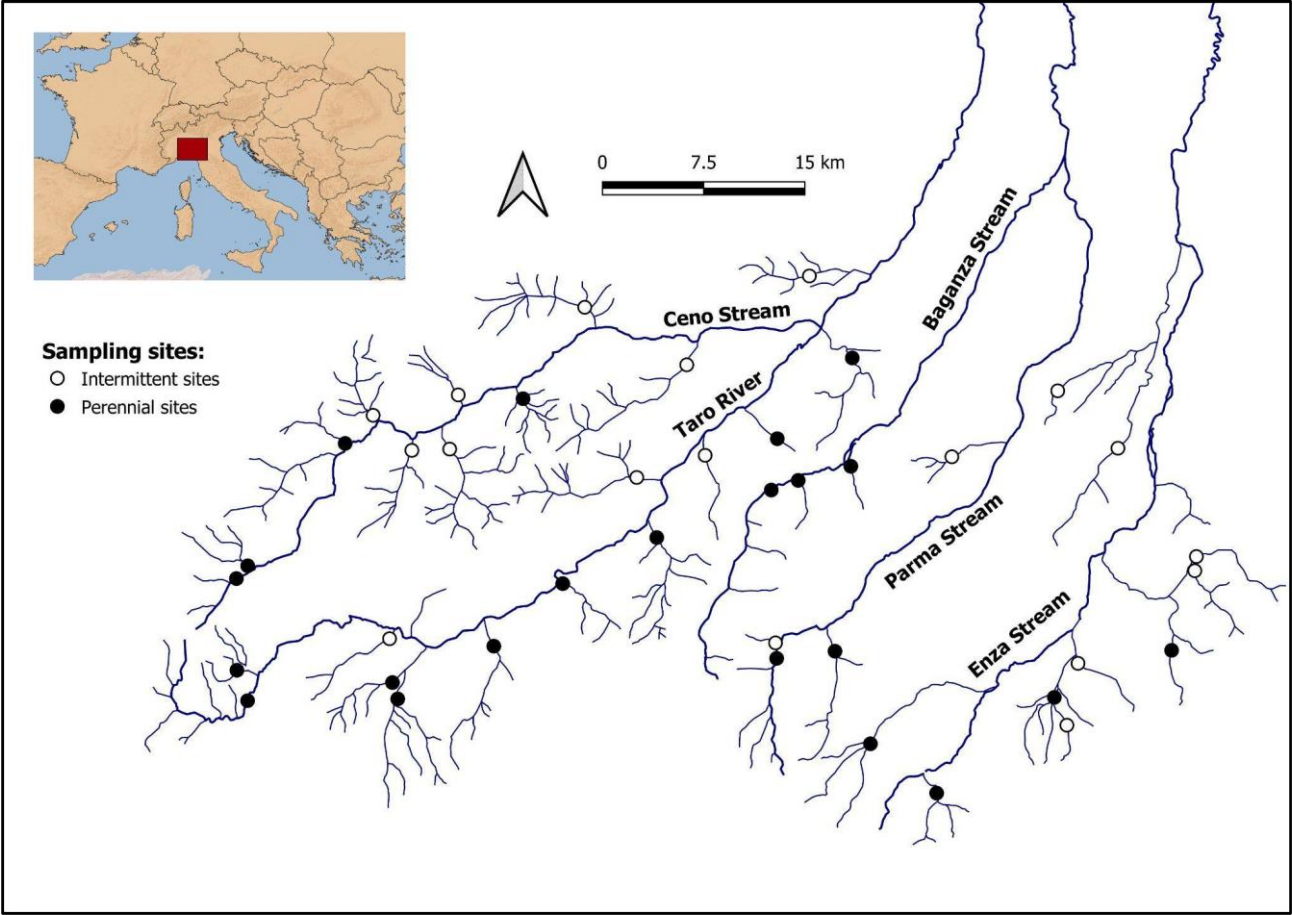
360 **Figure 1.** Location of the 22 perennial and 18 intermittent sampling sites in the Po River basin, northern Italy.

361 **Figure 2.** Taxa distribution in the original test dataset (a), taxa without a DEHLI drought-intolerance score
362 (b), and MIS-group (c), and therefore excluded from analyses.

363 **Figure 3.** DEHLI and MIS-index scores and richness metrics for habitat-association groups at perennial (P)
364 and intermittent (I) sites. *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; NS = non significant i.e. $p > 0.05$.

365 **Figures**

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368 **Figure 1.**

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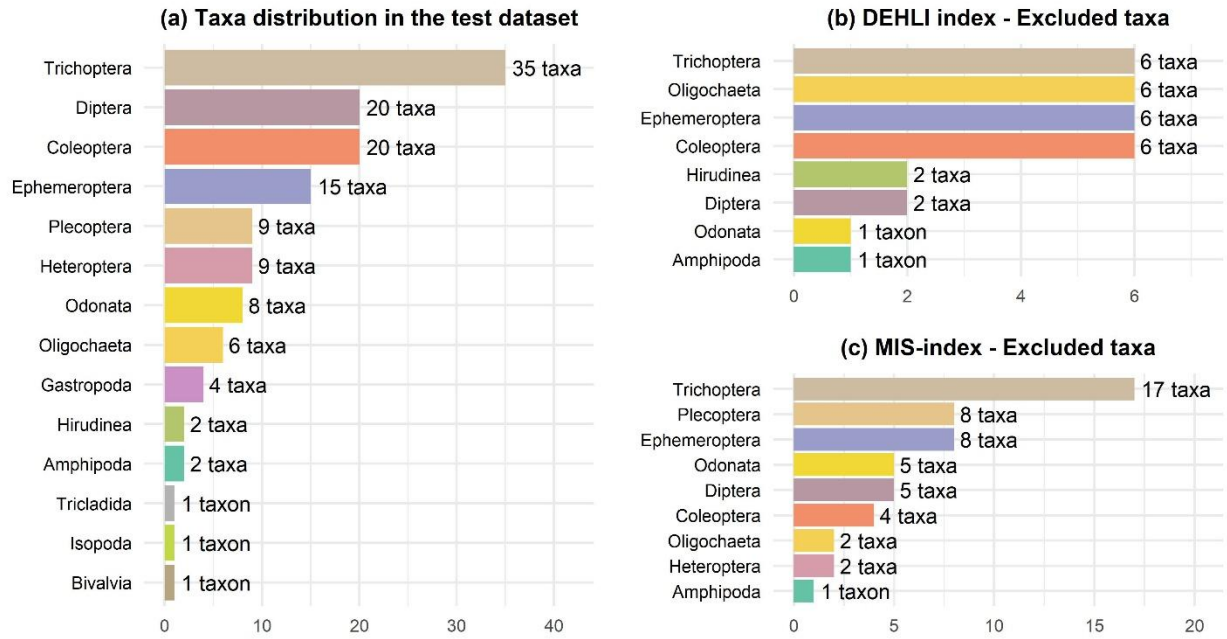


Figure 2.

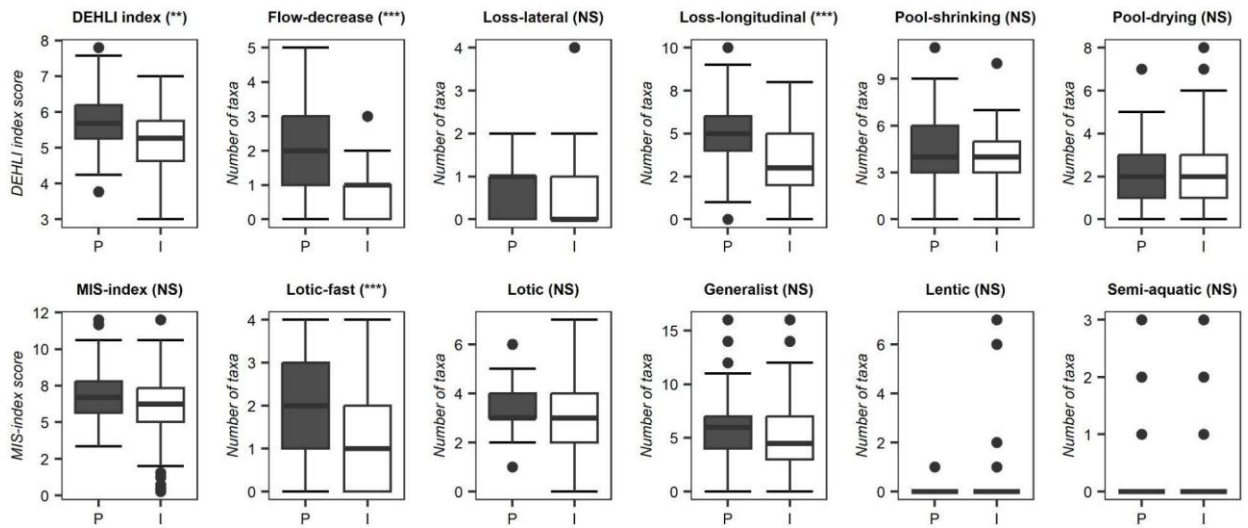


Figure 3.