# Supplementary material to:

**Macroinvertebrate communities respond strongly but non-specifically to a toxicity gradient derived by effect-based methods**

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**Table S1.** Physicochemical parameters.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Minimum | Mean | Maximum | Unit |
| Electric conductivity (EC) | 108 | 686 | 1,365 | µS/cm |
| Dissolved oxygen (DO) | 15.1 | 70.7 | 103 | % |
| Total organic carbon (TOC) | 2.50 | 15.2 | 35.0 | mg/l |
| Nitrite-N (NO2-N) | 5.02 | 60.1 | 165 | µg/l |
| Ammonium-N (NH4-N) | 16.5 | 253 | 3,425 | µg/l |
| Orthophosphate (PO4-P) | 0.025 | 0.15 | 1.12 | mg/l |

**Table S2.** Taxonomic diversity, functional diversity, sensitivity metrics and group abundances with mean, minimum (Min) and maximum (Max) values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Metric | Description | Min | Mean | Max |
| taxonomic diversity | Abundance | Number of individuals per m² | 518 | 3,951 | 12,265 |
| #Taxa | Number of Taxa (taxonomic richness) | 13 | 34.5 | 72 |
| Shannon | Shannon diversity index | 0.91 | 2.12 | 3.47 |
| Evenness | Evenness of Shannon-Wiener-diversity | 0.27 | 0.61 | 0.81 |
| functional diversity | FRic | Functional richness | 1.03x10-6 | 0.01 | 0.09 |
| FEve | Functional Evenness | 0.36 | 0.50 | 0.58 |
| FDis | Functional dispersion | 0.06 | 0.12 | 0.15 |
| FRed | Functional redundancy | 0.06 | 0.07 | 0.08 |
| sensitivity metrics | %EPT | % Ephemeropterans, Plecopterans, Trichopterans of total abundance | 0.00 | 8.90 | 61.1 |
| #EPTCBO | Number of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata taxa | 0 | 16 | 50 |
| ASPT | Average score per taxon | 3 | 5.09 | 6.85 |
| BMWP | Biological Monitoring Working Party | 18 | 85.5 | 185 |
| GFI | German fauna index  | -1.18 | 0.25 | 1.39 |
| GSI | German saprobic index | 1.46 | 2.04 | 2.62 |
| MMI | Multimetric index  | 0 | 0.31 | 0.79 |
| SPEARpest. | SPEcies At Risk of pesticide pollution | 0 | 0.44 | 1.15 |
| taxonomic composition (grouped) | Amphipoda | Abundance of amphipods (Ind./m²) | 4.8 | 605 | 5,726 |
| Bivalvia | Abundance of bivalves (Ind./m²) | 0 | 126 | 1,648 |
| Chironomids | Abundance of chironomids (Ind./m²) | 86.4 | 1,399 | 5,889 |
| Coleoptera | Abundance of coleopterans (Ind./m²) | 0 | 78.7 | 638 |
| Decapoda | Abundance of decapods (Ind./m²) | 0 | 0.32 | 9.60 |
| Diptera (w/o Chiro.) | Abundance of dipterans without chironomids (Ind./m²) | 0 | 111 | 697 |
| Ephemeroptera | Abundance of ephemeropterans (Ind./m²) | 0 | 122 | 650 |
| Gastropoda | Abundance of gastropods (Ind./m²) | 0 | 16.2 | 178 |
| Heteroptera | Abundance of heteropterans (Ind./m²) | 0 | 2.94 | 46.4 |
| Hirudinea | Abundance of hirudineans (Ind./m²) | 0 | 20.9 | 403 |
| Hydrazoa | Abundance of Hydrazoa (Ind./m²) | 0 | 0.05 | 1.6 |
| Isopoda | Abundance of isopods (Ind./m²) | 0 | 106 | 2,214 |
| Megaloptera | Abundance of megalopterans (Ind./m²) | 0 | 0.27 | 10.4 |
| Odonata | Abundance of Odonata (Ind./m²) | 0 | 8.34 | 63.2 |
| Oligochaeta | Abundance of oligochaetes (Ind./m²) | 10.4 | 1,224 | 10,406 |
| Plecoptera | Abundance of plecopterans (Ind./m²) | 0 | 39.0 | 689 |
| Trichoptera | Abundance of trichopterans (Ind./m²) | 0 | 82.9 | 410 |
| Turbellaria | Abundance of turbellarians (Ind./m²) | 0 | 6.33 | 45.6 |

**Table S3.** Taxa list.

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Taxon | Number ofoccurrences | Abundance (Ind./ m²) |
| Mean | Maximum |
| Amphipoda | *Crangonyx pseudogracilis* | 1 | 0.16 | 4.80 |
|  | *Crangonyx/Niphargus* sp. | 3 | 1.89 | 48.0 |
|  | Gammaroidea Gen. sp. | 3 | 1.31 | 24.0 |
|  | *Gammarus fossarum* | 10 | 210 | 5,269 |
|  | *Gammarus pulex* | 23 | 137 | 1,712 |
|  | *Gammarus roeselii* | 12 | 88.1 | 759 |
|  | *Gammarus* sp. | 25 | 153 | 773 |
|  | *Gammarus tigrinus* | 3 | 13.8 | 326 |
| Bivalvia | *Corbicula fluminea* | 5 | 5.80 | 101 |
|  | *Musculium lacustre* | 1 | 0.32 | 9.60 |
|  | *Pisidium* sp. | 16 | 108 | 1,618 |
|  | *Sphaerium corneum* | 2 | 1.79 | 39.2 |
|   | *Sphaerium* sp. | 6 | 10.6 | 250 |
| Chironomids | Chironomidae Gen. sp. | 23 | 405 | 5,674 |
|  | Chironominae Gen. sp. | 2 | 0.35 | 8.00 |
|  | Chironomini Gen. sp. | 25 | 269 | 1,493 |
|  | *Prodiamesa olivacea* | 24 | 145 | 941 |
|  | Tanypodinae Gen. sp. | 24 | 180 | 2,670 |
|   | Tanytarsini Gen. sp. | 29 | 399 | 1,819 |
| Coleoptera | *Anacaena bipustulata* Ad. | 1 | 0.16 | 4.80 |
|  | *Dryops* sp. Lv. | 3 | 1.71 | 32.0 |
|  | Dytiscidae Gen. sp. Lv. | 4 | 2.61 | 56.0 |
|  | *Elmis aenea/maugetii/rietscheli/rioloides* Ad. | 7 | 11.5 | 117 |
|  | *Elmis* sp. Ad. | 2 | 0.05 | 0.80 |
|  | *Elmis* sp. Lv. | 8 | 10.6 | 96.0 |
|  | *Elodes marginata* Lv. | 2 | 0.90 | 19.2 |
|  | *Elodes* sp. Lv. | 6 | 0.79 | 11.2 |
|  | *Esolus angustatus* Ad. | 1 | 0.96 | 28.8 |
|  | *Esolus parallelepipedus* Ad. | 1 | 0.08 | 2.40 |
|  | *Esolus* sp. Ad. | 3 | 0.40 | 4.80 |
|  | *Esolus* sp. Lv. | 2 | 2.64 | 76.8 |
|  | *Haliplus* sp. Lv. | 1 | 0.32 | 9.60 |
|  | *Helophorus brevipalpis* Ad. | 1 | 0.03 | 0.80 |
|  | *Hydraena gracilis* Ad. | 5 | 3.49 | 73.6 |
|  | *Hydraena minutissima* Ad. | 1 | 0.13 | 4.00 |
|  | *Hydraena* sp. Ad. | 6 | 4.65 | 106 |
|  | *Hydrocyphon deflexicollis* Lv. | 2 | 1.45 | 38.4 |
|  | Hydroporinae Gen. sp. Lv. | 1 | 0.38 | 11.5 |
|  | *Limnius perrisi* Ad. | 3 | 0.35 | 4.80 |
|  | *Limnius* sp. Ad. | 6 | 2.65 | 54.4 |
|  | *Limnius* sp. Lv. | 10 | 25.1 | 307 |
|  | *Limnius volckmari* Ad. | 7 | 3.47 | 38.4 |
|  | *Orectochilus villosus* Lv. | 6 | 1.65 | 15.2 |
|  | *Oreodytes sanmarkii* Ad. | 1 | 0.03 | 0.80 |
|  | *Oulimnius* sp. Ad. | 1 | 0.16 | 4.80 |
|  | *Oulimnius* sp. Lv. | 5 | 1.03 | 9.60 |
|  | *Platambus maculatus* Lv. | 2 | 1.07 | 31.2 |
|   | *Riolus* sp. Lv. | 1 | 0.32 | 9.60 |
| Decapoda | Decapoda Gen. sp. (*Orconectes immunis*) | 1 | 0.32 | 9.60 |
| Diptera (w/o Chiro.) | *Atrichops crassipes* | 1 | 0.03 | 0.80 |
|  | Ceratopogoninae/Palpomyiinae Gen. sp. | 15 | 21.4 | 163 |
|  | *Chelifera* sp. | 1 | 0.09 | 2.60 |
|  | *Chrysopilus* sp. | 1 | 0.16 | 4.80 |
|  | *Chrysops* sp. | 3 | 0.83 | 14.4 |
|  | Clinocerinae Gen. sp. | 4 | 0.93 | 9.60 |
|  | Culicidae Gen. sp. | 2 | 0.34 | 5.30 |
|  | *Dasyhelea* sp. | 2 | 2.72 | 72.0 |
|  | *Dicranota* sp. | 9 | 5.11 | 72.0 |
|  | *Dixa* sp. | 2 | 0.49 | 9.60 |
|  | Dolichopodidae Gen. sp. | 3 | 1.92 | 28.8 |
|  | *Eloeophila* sp. | 5 | 2.80 | 38.4 |

**Table S3.** Taxa list. (continued)

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Taxon | Number ofoccurrences | Abundance (Ind./ m²) |
| Mean | Maximum |
| Diptera (w/o Chiro.) | *Hemerodromia* sp. | 4 | 0.88 | 9.60 |
|  | *Ibisia marginata* | 2 | 0.57 | 14.4 |
|  | Limoniidae Gen. sp. | 4 | 0.57 | 4.80 |
|  | *Lispe* sp. | 1 | 0.16 | 4.80 |
|  | *Pedicia* sp. | 1 | 1.44 | 43.2 |
|  | *Pilaria* sp. | 3 | 0.57 | 9.60 |
|  | *Prosimulium* sp. | 9 | 26.0 | 461 |
|  | *Prosimulium tomosvaryi* | 2 | 0.25 | 4.80 |
|  | Psychodidae Gen. sp. | 6 | 1.20 | 14.4 |
|  | *Ptychoptera* sp. | 3 | 1.36 | 19.2 |
|  | *Simulium (Wilhelmia)* sp. | 1 | 0.09 | 2.60 |
|  | *Simulium latipes* | 2 | 0.48 | 9.60 |
|  | *Simulium monticola* | 1 | 0.09 | 2.60 |
|  | *Simulium ornatum*-Gr. | 4 | 0.93 | 20.0 |
|  | *Simulium* sp. | 18 | 34.7 | 259 |
|  | *Simulium vernum*-Gr. | 1 | 0.16 | 4.80 |
|  | *Stratiomyidae* Gen. sp. | 7 | 1.17 | 9.60 |
|  | *Tabanidae* Gen. sp. | 3 | 0.55 | 9.60 |
|   | *Tipula* sp. | 9 | 3.39 | 43.2 |
| Ephemeroptera | Baetidae Gen. sp. | 10 | 1.75 | 12.0 |
|  | *Baetis alpinus/lutheri/melanonyx/vardarensis* | 1 | 0.09 | 2.60 |
|  | *Baetis buceratus* | 2 | 0.10 | 2.20 |
|  | *Baetis lutheri* | 1 | 0.16 | 4.80 |
|  | *Baetis muticus* | 2 | 2.81 | 81.6 |
|  | *Baetis nexus* | 6 | 0.96 | 17.6 |
|  | *Baetis niger* | 1 | 0.32 | 9.60 |
|  | *Baetis rhodani* | 12 | 38.5 | 485 |
|  | *Baetis* sp. | 13 | 12.8 | 120 |
|  | *Baetis vernus* | 4 | 0.45 | 4.80 |
|  | *Caenis luctuosa* | 1 | 0.16 | 4.80 |
|  | *Caenis luctuosa/macrura* | 1 | 0.80 | 24.0 |
|  | *Caenis* sp. | 3 | 0.64 | 9.60 |
|  | *Centroptilum luteolum* | 3 | 0.53 | 14.4 |
|  | *Cloeon dipterum* | 2 | 7.04 | 190 |
|  | *Cloeon* sp. | 1 | 0.32 | 9.60 |
|  | *Ecdyonurus* sp. | 1 | 0.08 | 2.40 |
|  | *Ecdyonurus venosus*-Gr. | 1 | 0.21 | 6.40 |
|  | *Epeorus assimilis* | 3 | 6.36 | 187 |
|  | *Ephemera danica* | 11 | 5.81 | 72.0 |
|  | *Ephemera* sp. | 2 | 1.44 | 38.4 |
|  | *Ephemerella mucronata* | 1 | 1.71 | 51.2 |
|  | *Ephemerella* sp. | 1 | 0.48 | 14.4 |
|  | *Habroleptoides confusa* | 4 | 3.02 | 43.5 |
|  | *Habrophlebia lauta* | 1 | 1.60 | 48.0 |
|  | *Habrophlebia* sp. | 1 | 0.09 | 2.60 |
|  | *Heptagenia flava* | 1 | 0.05 | 1.60 |
|  | Heptageniidae Gen. sp. | 2 | 4.16 | 115 |
|  | Leptophlebiidae Gen. sp. | 2 | 3.07 | 81.6 |
|  | *Metreletus balcanicus* | 2 | 1.66 | 42.7 |
|  | *Paraleptophlebia* sp. | 1 | 0.16 | 4.80 |
|  | *Rhithrogena semicolorata*-Gr. | 8 | 21.1 | 178 |
|   | *Rhithrogena* sp. | 6 | 3.76 | 48.0 |
| Gastropoda | *Acroloxus lacustris* | 1 | 0.64 | 19.2 |
|  | *Ancylus fluviatilis* | 4 | 1.09 | 19.2 |
|  | *Bithynia tentaculata* | 3 | 1.27 | 28.8 |
|  | *Galba truncatula* | 2 | 0.25 | 4.80 |
|  | *Gyraulus* sp. | 1 | 0.32 | 9.60 |
|  | *Physa fontinalis* | 1 | 0.32 | 9.60 |
|  | *Physella* sp. | 4 | 2.14 | 43.2 |
|  | *Potamopyrgus antipodarum* | 5 | 8.68 | 178 |

**Table S3.** Taxa list. (continued)

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Taxon | Number ofoccurrences | Abundance (Ind./ m²) |
| Mean | Maximum |
| Gastropoda | *Radix balthica/labiata* | 5 | 1.31 | 14.4 |
|   | *Radix* sp. | 2 | 0.19 | 4.80 |
| Heteroptera | *Aphelocheirus aestivalis* | 4 | 0.59 | 15.2 |
|  | Corixidae Gen. sp. | 4 | 2.21 | 46.4 |
|  | Heteroptera Gen. sp. | 1 | 0.09 | 2.60 |
|   | *Nepa cinerea* | 2 | 0.05 | 0.80 |
| Hirudinea | *Alboglossiphonia heteroclita* | 3 | 1.12 | 14.4 |
|  | *Dina lineata* | 1 | 0.16 | 4.80 |
|  | *Erpobdella octoculata* | 7 | 12.6 | 283 |
|  | *Erpobdella testacea* | 1 | 0.16 | 4.80 |
|  | *Glossiphonia complanata* | 4 | 0.64 | 4.80 |
|  | *Glossiphonia concolor* | 1 | 0.16 | 4.80 |
|  | *Glossiphonia nebulosa/verrucata* | 1 | 0.16 | 4.80 |
|  | *Glossiphonia* sp. | 1 | 0.16 | 4.80 |
|   | *Helobdella stagnalis* | 6 | 5.79 | 120 |
| Hydrozoa | Hydrozoa Gen. sp. | 1 | 0.05 | 1.60 |
| Isopoda | Asellidae Gen. sp. | 3 | 3.36 | 57.6 |
|  | *Asellus aquaticus* | 17 | 94.0 | 2,214 |
|  | *Proasellus coxalis* | 5 | 8.91 | 149 |
| Megaloptera | Sialis lutaria | 2 | 0.37 | 10.40 |
| Odonata | Anisoptera Gen. sp. | 1 | 0.03 | 0.80 |
|  | *Calopteryx* sp. | 4 | 0.93 | 12.0 |
|  | *Calopteryx splendens* | 5 | 1.48 | 16.8 |
|  | *Calopteryx virgo* | 4 | 0.27 | 5.60 |
|  | Coenagrionidae Gen. sp. | 4 | 2.21 | 53.6 |
|  | *Cordulegaster boltonii* | 1 | 0.11 | 3.20 |
|  | *Cordulegaster* sp. | 1 | 0.08 | 2.40 |
|  | *Gomphus vulgatissimus* | 1 | 0.03 | 0.80 |
|  | *Libellula fulva* | 1 | 0.03 | 0.80 |
|  | Libellulidae Gen. sp. | 1 | 0.16 | 4.80 |
|  | *Onychogomphus forcipatus forcipatus* | 2 | 0.05 | 0.80 |
|  | *Onychogomphus/Ophiogomphus* sp. | 1 | 0.03 | 0.80 |
|  | *Ophiogomphus cecilia* | 2 | 0.05 | 0.80 |
|  | *Orthetrum* sp. | 1 | 0.03 | 0.80 |
|  | *Platycnemis pennipes* | 7 | 1.91 | 14.4 |
|   | Zygoptera Gen. sp. | 3 | 0.95 | 14.4 |
| Oligochaeta | *Branchiura sowerbyi* | 1 | 1.12 | 33.6 |
|  | *Eiseniella tetraedra* | 21 | 17.9 | 139 |
|  | Enchytraeidae Gen. sp. | 2 | 62.9 | 1,882 |
|  | *Haplotaxis gordioides* | 10 | 23.8 | 614 |
|  | Lumbriculidae Gen. sp. | 12 | 110 | 2,496 |
|  | *Lumbriculus variegatus* | 3 | 2.08 | 33.6 |
|  | Naididae/Tubificidae Gen. sp. | 28 | 415 | 2,765 |
|  | Oligochaeta Gen. sp. | 26 | 582 | 5,376 |
|   | *Stylodrilus heringianus* | 6 | 9.67 | 160 |
| Plecoptera | *Amphinemura* sp. | 2 | 7.59 | 202 |
|  | *Brachyptera risi* | 4 | 6.09 | 92.8 |
|  | *Brachyptera seticornis* | 2 | 0.40 | 11.3 |
|  | *Brachyptera* sp. | 2 | 0.82 | 19.9 |
|  | *Isoperla* sp. | 2 | 2.24 | 44.0 |
|  | *Leuctra nigra* | 2 | 1.21 | 33.6 |
|  | *Leuctra* sp. | 2 | 6.14 | 163 |
|  | *Nemoura* sp. | 2 | 2.50 | 38.4 |
|  | *Perlodes* sp. | 1 | 0.03 | 0.80 |
|  | Perlodidae Gen. sp. | 1 | 0.09 | 2.60 |
|  | *Protonemura* sp. | 6 | 6.37 | 96.0 |
|   | *Siphonoperla* sp. | 2 | 5.48 | 149 |

**Table S3.** Taxa list. (continued)

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Taxon | Number of occurrences | Abundance (Ind./ m²) |
| Mean | Maximum |
| Trichoptera | *Anabolia brevipennis* | 1 | 0.16 | 4.80 |
|  | *Anabolia nervosa* | 3 | 0.45 | 5.60 |
|  | *Athripsodes bilineatus bilineatus* | 2 | 0.40 | 7.20 |
|  | *Athripsodes* sp. | 3 | 0.83 | 15.2 |
|  | Chaetopterygini/Stenophylacini Gen. sp. | 12 | 12.3 | 126 |
|  | *Chaetopteryx villosa villosa* | 1 | 0.03 | 0.80 |
|  | *Crunoecia irrorata irrorata* | 1 | 0.09 | 2.60 |
|  | *Cyrnus trimaculatus* | 1 | 0.13 | 4.00 |
|  | *Drusus annulatus/biguttatus* | 1 | 0.03 | 0.80 |
|  | *Glossosoma conformis* | 1 | 0.19 | 5.60 |
|  | *Glyphotaelius pellucidus* | 3 | 0.35 | 4.80 |
|  | *Goera pilosa* | 1 | 0.16 | 4.80 |
|  | *Halesus* sp. | 6 | 2.30 | 24.0 |
|  | *Hydropsyche angustipennis angustipennis* | 5 | 1.08 | 12.8 |
|  | *Hydropsyche fulvipes* | 1 | 0.16 | 4.80 |
|  | *Hydropsyche instabilis* | 3 | 0.92 | 13.1 |
|  | *Hydropsyche pellucidula*-Gr. | 2 | 0.16 | 4.00 |
|  | *Hydropsyche saxonica* | 2 | 1.20 | 35.2 |
|  | *Hydropsyche siltalai* | 6 | 5.68 | 70.4 |
|  | *Hydropsyche* sp. | 10 | 4.92 | 56.0 |
|  | *Hydroptila* sp. | 7 | 8.23 | 186 |
|  | Hydroptilidae Gen. sp. | 1 | 0.16 | 4.80 |
|  | *Ironoquia dubia* | 1 | 0.03 | 0.80 |
|  | *Lepidostoma basale* | 6 | 2.61 | 32.0 |
|  | Limnephilidae Gen. sp. | 12 | 9.90 | 206 |
|  | Limnephilini Gen. sp. | 18 | 16.3 | 162 |
|  | *Lype* sp. | 7 | 1.15 | 14.4 |
|  | *Micrasema longulum* | 1 | 0.08 | 2.40 |
|  | *Mystacides azurea* | 3 | 0.31 | 4.40 |
|  | *Mystacides longicornis/nigra* | 2 | 0.41 | 9.60 |
|  | *Mystacides* sp. | 1 | 0.05 | 1.60 |
|  | *Odontocerum albicorne* | 1 | 0.21 | 6.40 |
|  | *Philopotamus montanus montanus* | 2 | 0.53 | 9.50 |
|  | *Philopotamus* sp. | 1 | 0.08 | 2.40 |
|  | *Plectrocnemia* sp. | 3 | 0.48 | 7.90 |
|  | Polycentropodidae Gen. sp. | 4 | 0.80 | 10.5 |
|  | *Polycentropus flavomaculatus flavomaculatus* | 1 | 0.03 | 0.80 |
|  | *Polycentropus* sp. | 2 | 0.19 | 4.80 |
|  | *Potamophylax cingulatus/latipennis/luctuosus* | 4 | 1.87 | 34.4 |
|  | *Potamophylax rotundipennis* | 2 | 0.21 | 5.60 |
|  | *Potamophylax* sp. | 2 | 0.19 | 4.80 |
|  | *Psychomyia pusilla* | 4 | 1.81 | 28.8 |
|  | *Rhyacophila (Rhyacophila)* sp. | 8 | 2.09 | 28.8 |
|  | *Rhyacophila* sp. | 2 | 0.38 | 10.5 |
|  | *Sericostoma* sp. | 6 | 1.55 | 14.4 |
|  | *Silo pallipes* | 5 | 1.57 | 20.0 |
|   | *Tinodes* sp. | 2 | 0.25 | 4.80 |
| Turbellaria | *Dugesia gonocephala* | 1 | 0.64 | 19.2 |
|  | *Dugesia lugubris/polychroa* | 6 | 3.18 | 38.4 |
|  | *Dugesia* sp. | 4 | 1.15 | 19.2 |
|  | *Polycelis* sp. | 1 | 0.32 | 9.60 |
|   | Turbellaria Gen. sp. | 6 | 1.04 | 7.20 |

Calculation of Aquatic lifecycle Trait:

The trait aquatic lifecycle describing the degree of which a taxon is living in water across all life stages. It was manually calculated based on the tachaqua-trait (Tachet et al. 2010). First, affinity scores across life stages were summed for each taxon depending on the life history of the taxon (**Tab. S4**). Then, sums were divided by the maximum potential affinity to calculate the affinities for a full aquatic life (*full*) cycle on a scale from 0 (no aquatic life stage) to 1 (all life stages aquatic). The affinities for a mostly non-aquatic life cycle (*part*) was calculated by subtracting 1 – *full*.

**Table S4.** Potential life stages for taxonomic groups.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Taxonomic group | Egg | Larva | Nymph/Pupa | Adult |
| Gastropoda | x |  |  | x |
| Amphipoda |  |  |  |  |
| Bivalvia | x | x |  | x |
| Decapoda | x | x |  | x |
| Ephemeroptera | x | x |  | x |
| Heteroptera | x | x |  | x |
| Hirudinea | x | x |  | x |
| Isopoda | x | x |  | x |
| Odonata | x | x |  | x |
| Oligochaeta | x | x |  | x |
| Plecoptera | x | x |  | x |
| Turbellaria | x | x |  | x |
| Coleoptera | x | x | x | x |
| Diptera | x | x | x | x |
| Megaloptera | x | x | x | x |
| Trichoptera | x | x | x | x |

**Table S5.** Trait coverage

|  |  |
| --- | --- |
| Trait group | Trait coverage (%) |
| Taxa | Genus | Family | Total |
| Direct | Aver. | Total | Direct | Aver. | Total | Direct | Aver. | Total | Direct | Aver. | Total |
| Feeding | 0.96 | 0 | 0.96 | 0.00 | 0.00 | 0.01 | 0 | 0.03 | 0.03 | 0.97 | 0.03 | 1.00 |
| Locomotion | 0.79 | 0 | 0.79 | 0.03 | 0.06 | 0.09 | 0 | 0.10 | 0.10 | 0.82 | 0.15 | 0.97 |
| Body size | 0.42 | 0 | 0.42 | 0.31 | 0.06 | 0.37 | 0 | 0.20 | 0.20 | 0.73 | 0.25 | 0.98 |
| Voltinism | 0.41 | 0 | 0.41 | 0.31 | 0.06 | 0.37 | 0 | 0.20 | 0.20 | 0.73 | 0.25 | 0.98 |
| Dispersal | 0.41 | 0 | 0.41 | 0.31 | 0.06 | 0.37 | 0 | 0.20 | 0.20 | 0.72 | 0.25 | 0.97 |
| Life duration | 0.40 | 0 | 0.40 | 0.31 | 0.06 | 0.37 | 0 | 0.20 | 0.20 | 0.72 | 0.25 | 0.97 |
| Aquatic lifecycle | 0.41 | 0 | 0.41 | 0.29 | 0.06 | 0.35 | 0 | 0.22 | 0.22 | 0.70 | 0.28 | 0.98 |
| Respiration | 0.41 | 0 | 0.41 | 0.29 | 0.06 | 0.35 | 0 | 0.22 | 0.22 | 0.70 | 0.28 | 0.98 |
| Oviposition | 0.40 | 0 | 0.40 | 0.29 | 0.06 | 0.35 | 0 | 0.22 | 0.22 | 0.70 | 0.28 | 0.97 |
| Resistance forms | 0.39 | 0 | 0.39 | 0.28 | 0.06 | 0.34 | 0 | 0.22 | 0.22 | 0.67 | 0.28 | 0.96 |

Tachet, H., Bournaud, M., Richoux, P., Usseglio‐Polatera, P., 2010. Invertébrés d’eau douce - systématique, biologie, écologie. CNRS Editions, Paris.

**Table S6.** Spearman rank correlation of PC1 and PC2 with additional environmental variables. Spearman rhos are listed in the upper right part, p values in the lower left part.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   | pH | DO | EC | TOC | NO2-N | NH4-N | PO4-P | Morph. degrad. | Baseline tox. (s) | Mutagenicity (s) | Baseline tox. (w) | Mutagenicity (s) | Dioxin. act (w) | Estrogen. act. (w) | PC1 | PC2 |
| pH | - | *0.36* | *0.20* | *0.15* | *0.09* | *-0.14* | *0.18* | **0.42** | *0.13* | *-0.34* | *-0.03* | *0.34* | *-0.05* | *0.04* | *0.10* | *0.30* |
| DO | *ns* | - | *-0.36* | **-0.47** | *-0.32* | **-0.60** | *-0.34* | *0.05* | **-0.58** | *0.05* | *-0.34* | *-0.36* | **-0.62** | **-0.62** | **-0.57** | *0.25* |
| EC | *ns* | *ns* | - | **0.75** | **0.69** | **0.56** | *0.15* | *0.31* | **0.50** | *0.09* | **0.37** | **0.58** | **0.64** | **0.49** | **0.58** | *-0.32* |
| TOC | *ns* | **\*\*** | **\*\*\*** | - | **0.43** | **0.50** | *0.21* | *0.28* | **0.46** | *-0.18* | **0.41** | **0.39** | **0.62** | **0.44** | **0.54** | *-0.12* |
| NO2-N | *ns* | *ns* | **\*\*\*** | **\*** | - | **0.74** | **0.36** | **0.44** | *0.27* | *0.13* | *0.15* | **0.37** | *0.35* | *0.31* | *0.35* | *-0.24* |
| NH4-N | *ns* | **\*\*\*** | **\*\*** | **\*\*** | **\*\*\*** | - | **0.59** | *0.16* | **0.48** | *-0.05* | **0.51** | **0.39** | **0.59** | **0.58** | **0.60** | *-0.33* |
| PO4-P | *ns* | *ns* | *ns* | *ns* | **\*** | **\*\*** | - | *0.33* | **0.44** | *-0.29* | **0.41** | *0.32* | **0.44** | **0.69** | **0.53** | *-0.16* |
| Morph. degrad. | **\*** | *ns* | *ns* | *ns* | **\*** | *ns* | *ns* | - | *0.05* | *-0.09* | *0.03* | *0.22* | *0.13* | *0.21* | *0.16* | *0.05* |
| Baseline tox. (s) | *ns* | **\*\*\*** | **\*\*** | **\*** | *ns* | **\*\*** | **\*** | *ns* | - | *-0.23* | **0.50** | **0.63** | **0.67** | **0.66** | **0.76** | *-0.24* |
| Mutagenicity (s) | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | - | *-0.16* | *-0.20* | *-0.17* | *-0.24* | *-0.25* | **-0.73** |
| Baseline tox. (w) | *ns* | *ns* | **\*** | **\*** | *ns* | **\*\*** | **\*** | *ns* | **\*\*** | *ns* | - | **0.54** | **0.83** | **0.74** | **0.85** | **-0.49** |
| Mutagenicity (s) | *ns* | **\*** | **\*\*\*** | **\*** | **\*** | **\*** | *ns* | *ns* | **\*\*\*** | *ns* | **\*\*** | - | **0.67** | **0.68** | **0.80** | *-0.12* |
| Dioxin. act (w) | *ns* | **\*\*\*** | **\*\*\*** | **\*\*\*** | *ns* | **\*\*\*** | **\*** | *ns* | **\*\*\*** | *ns* | **\*\*\*** | **\*\*\*** | - | **0.85** | **0.92** | **-0.43** |
| Estrogen. act. (w) | *ns* | **\*\*\*** | **\*\*** | **\*** | *ns* | **\*\*\*** | **\*\*\*** | *ns* | **\*\*\*** | *ns* | **\*\*\*** | **\*\*\*** | **\*\*\*** | - | **0.90** | *-0.33* |
| PC1 | *ns* | **\*\*** | **\*\*\*** | **\*\*** | *ns* | **\*\*\*** | **\*\*** | *ns* | **\*\*\*** | *ns* | **\*\*\*** | **\*\*\*** | **\*\*\*** | **\*\*\*** | - | **-0.36** |
| PC2 | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | **\*\*\*** | **\*\*** | *ns* | **\*** | *ns* | **\*** | - |

ns – non significant (p > 0.05), \* - p< 0.05, \*\* - p < 0.01, \*\*\* - p< 0.001

**Table S7.** Mantel test with Holm correction for spatial autocorrelation with 999 runs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Matrix | Distance (km) | # Distances | Pearson's *r* | *p*-value |
| Metrics | 3.65 | 100 | 0.10 | 0.024 |
| 10.0 | 122 | 0.10 | 0.048 |
| 16.4 | 160 | 0.07 | 0.119 |
| 22.8 | 100 | -0.16 | 0.056 |
| 29.2 | 154 | 0.03 | 0.288 |
| Taxonomic composition (grouped) | 3.65 | 100 | 0.05 | 0.150 |
| 10.0 | 122 | 0.00 | 0.508 |
| 16.4 | 160 | -0.02 | 0.772 |
| 22.8 | 100 | -0.12 | 0.124 |
| 29.2 | 154 | 0.02 | 1.000 |
| Taxonomic composition (original) | 3.65 | 100 | 0.04 | 0.181 |
| 10.0 | 122 | 0.03 | 0.362 |
| 16.4 | 160 | -0.11 | 0.093 |
| 22.8 | 100 | -0.15 | 0.044 |
| 29.2 | 154 | 0.01 | 0.548 |
| Trait composition | 3.65 | 100 | 0.01 | 0.357 |
| 10.0 | 122 | 0.00 | 0.714 |
| 16.4 | 160 | -0.04 | 0.816 |
| 22.8 | 100 | -0.04 | 0.916 |
| 29.2 | 154 | -0.02 | 1.000 |



**Figure S1.** Explained variance of metrics (A), grouped abundances (B), abundances at the original identification level (C) and traits by toxicity (PC1) and natural factors (distance to source and region). N=30.

**Table S8.** Scores of all taxa at first RDA axis.

|  |  |  |  |
| --- | --- | --- | --- |
| Taxon | Score | Taxon | Score |
| *Gammarus* sp. | -0.354 | *Centroptilum luteolum* | -0.021 |
| *Gammarus fossarum* | -0.313 | *Corbicula fluminea* | -0.021 |
| *Gammarus pulex* | -0.263 | *Athripsodes bilineatus bilineatus* | -0.020 |
| *Gammarus roeselii* | -0.238 | *Chrysops* sp. | -0.019 |
| *Baetis rhodani* | -0.191 | *Aphelocheirus aestivalis* | -0.019 |
| *Prodiamesa olivacea* | -0.186 | Turbellaria Gen. sp. | -0.018 |
| *Rhithrogena semicolorata*-Gr. | -0.158 | *Esolus* sp. Ad. | -0.018 |
| Chironomidae Gen. sp. | -0.147 | *Baetis vernus* | -0.017 |
| Tanytarsini Gen. sp. | -0.147 | *Calopteryx splendens* | -0.017 |
| *Pisidium* sp. | -0.141 | Tabanidae Gen. sp. | -0.017 |
| *Limnius* sp. Lv. | -0.134 | *Haplotaxis gordioides* | -0.016 |
| *Ephemera danica* | -0.125 | *Dugesia gonocephala* | -0.016 |
| *Baetis* sp. | -0.106 | *Potamophylax* sp. | -0.015 |
| *Stylodrilus heringianus* | -0.105 | *Hydropsyche angustipennis angustipennis* | -0.015 |
| *Elmis aenea/maugetii/rietscheli/rioloides* Ad. | -0.095 | *Philopotamus montanus montanus* | -0.015 |
| *Brachyptera risi* | -0.087 | *Gammarus tigrinus* | -0.014 |
| *Elmis* sp. Lv. | -0.085 | *Ecdyonurus venosus*-Gr. | -0.014 |
| *Hydropsyche siltalai* | -0.078 | *Ephemerella* sp. | -0.014 |
| *Hydraena* sp. Ad. | -0.069 | *Epeorus assimilis* | -0.013 |
| *Hydraena gracilis* Ad. | -0.069 | *Hemerodromia* sp. | -0.013 |
| *Limnius volckmari* Ad. | -0.062 | *Plectrocnemia* sp. | -0.013 |
| *Lepidostoma basale* | -0.057 | *Polycentropus* sp. | -0.013 |
| *Halesus* sp. | -0.051 | *Mystacides azurea* | -0.013 |
| *Potamopyrgus antipodarum* | -0.049 | *Goera pilosa* | -0.012 |
| *Habroleptoides confusa* | -0.047 | *Hydroptila* sp. | -0.012 |
| *Hydropsyche* sp. | -0.046 | *Brachyptera* sp. | -0.011 |
| *Potamophylax cingulatus/latipennis/luctuosus* | -0.045 | *Baetis lutheri* | -0.010 |
| *Dicranota* sp. | -0.045 | *Cordulegaster boltonii* | -0.010 |
| *Protonemura* sp. | -0.045 | *Radix balthica/labiata* | -0.009 |
| Chaetopterygini/Stenophylacini Gen. sp. | -0.045 | *Dugesia* sp. | -0.009 |
| *Ephemera* sp. | -0.044 | *Sphaerium corneum* | -0.009 |
| *Rhithrogena* sp. | -0.042 | *Cordulegaster* sp. | -0.008 |
| *Eloeophila* sp. | -0.041 | *Ecdyonurus* sp. | -0.008 |
| *Crangonyx/Niphargus* sp. | -0.038 | *Esolus parallelepipedus* Ad. | -0.008 |
| Heptageniidae Gen. sp. | -0.038 | *Micrasema longulum* | -0.008 |
| *Lype* sp. | -0.038 | *Philopotamus* sp. | -0.008 |
| *Rhyacophila (Rhyacophila)*.sp. | -0.038 | *Prosimulium tomosvaryi* | -0.008 |
| *Silo pallipes* | -0.036 | *Hydropsyche fulvipes* | -0.008 |
| Limnephilidae Gen. sp. | -0.036 | *Anabolia nervosa* | -0.008 |
| *Sericostoma* sp. | -0.035 | *Riolus* sp. Lv. | -0.007 |
| Corixidae Gen. sp. | -0.035 | *Esolus* sp. Lv. | -0.007 |
| *Hydrocyphon deflexicollis* Lv. | -0.035 | *Baetis buceratus* | -0.007 |
| *Orectochilus villosus* Lv. | -0.032 | Ceratopogoninae/Palpomyiinae Gen. sp. | -0.007 |
| *Athripsodes* sp. | -0.031 | *Cyrnus* *trimaculatus* | -0.007 |
| Baetidae Gen. sp. | -0.031 | Stratiomyidae Gen. sp. | -0.006 |
| *Dugesia lugubris/polychroa* | -0.030 | *Ophiogomphus cecilia* | -0.006 |
| *Oulimnius* sp. Lv. | -0.028 | *Tinodes* sp. | -0.006 |
| *Hydropsyche instabilis* | -0.028 | *Anacaena bipustulata* Ad. | -0.005 |
| *Limnius* sp. Ad. | -0.026 | Hydroptilidae Gen. sp. | -0.005 |
| *Ephemerella mucronata* | -0.026 | *Limnius perrisi* Ad. | -0.005 |
| Polycentropodidae Gen. sp. | -0.025 | *Elmis* sp. Ad. | -0.005 |
| *Ptychoptera* sp. | -0.025 | Anisoptera Gen. sp. | -0.005 |
| *Ancylus fluviatilis* | -0.024 | *Hydraena minutissima* Ad. | -0.004 |
| *Nemoura* sp. | -0.024 | *Heptagenia flava* | -0.004 |
| *Tipula* sp. | -0.024 | *Mystacides* sp. | -0.004 |
| *Pedicia* sp. | -0.024 | Gammaroidea Gen. sp. | -0.003 |
| *Lumbriculus variegatus* | -0.023 | *Drusus annulatus/biguttatus* | -0.003 |
| Psychodidae Gen. sp. | -0.021 | *Dina lineata* | -0.003 |

**Table S8.** Scores of all taxa at first RDA axis. (continued)

|  |  |  |  |
| --- | --- | --- | --- |
| Taxon | RDA1 | Taxon | RDA1 |
| *Glossiphonia concolor* | -0.003 | *Amphinemura* sp. | 0.001 |
| *Atrichops crassipes* | -0.003 | *Siphonoperla* sp. | 0.001 |
| *Gomphus vulgatissimus* | -0.003 | *Esolus angustatus* Ad. | 0.001 |
| *Onychogomphus/Ophiogomphus* sp. | -0.003 | *Baetis muticus* | 0.001 |
| *Polycentropus flavomaculatus flavomaculatus* | -0.003 | *Habrophlebia lauta* | 0.001 |
| *Onychogomphus forcipatus forcipatus* | -0.003 | *Chrysopilus* sp. | 0.001 |
| *Elodes* sp. Lv. | -0.003 | *Lispe* sp. | 0.001 |
| *Psychomyia pusilla* | -0.003 | *Oulimnius* sp. Ad. | 0.001 |
| *Potamophylax rotundipennis* | -0.003 | *Sialis lutaria* | 0.001 |
| *Calopteryx* sp. | -0.002 | *Pilaria* sp. | 0.002 |
| *Platycnemis pennipes* | -0.002 | Hydrozoa Gen. sp. | 0.002 |
| *Ironoquia dubia* | -0.002 | *Crangonyx pseudogracilis* | 0.002 |
| Zygoptera Gen. sp. | -0.002 | *Glossiphonia* sp. | 0.002 |
| *Nepa cinerea* | -0.002 | *Caenis luctuosa* | 0.002 |
| *Calopteryx virgo* | -0.002 | Asellidae Gen. sp. | 0.002 |
| *Anabolia brevipennis* | -0.002 | *Bithynia tentaculata* | 0.002 |
| Libellulidae Gen. sp. | -0.002 | *Glossiphonia complanata* | 0.002 |
| *Simulium ornatum*-Gr. | -0.002 | *Glossiphonia nebulosa/verrucata* | 0.005 |
| *Baetis nexus* | -0.002 | *Caenis* sp. | 0.005 |
| *Rhyacophila* sp. | -0.001 | *Radix* sp. | 0.005 |
| Limoniidae Gen. sp. | -0.001 | *Platambus maculatus* Lv. | 0.005 |
| *Brachyptera seticornis* | -0.001 | Decapoda Gen. sp. | 0.006 |
| *Libellula fulva* | -0.001 | *Cloeon* sp. | 0.006 |
| *Orthetrum* sp. | -0.001 | *Gyraulus* sp. | 0.006 |
| Clinocerinae Gen. sp. | -0.001 | *Haliplus* sp. Lv. | 0.006 |
| *Baetis alpinus/lutheri/melanonyx/vardarensis* | 0.000 | *Musculium lacustre* | 0.006 |
| *Chelifera* sp. | 0.000 | Chironominae Gen. sp. | 0.009 |
| *Crunoecia irrorata irrorata* | 0.000 | *Acroloxus lacustris* | 0.009 |
| *Habrophlebia* sp. | 0.000 | *Physella* sp. | 0.010 |
| Heteroptera Gen. sp. | 0.000 | *Dasyhelea* sp. | 0.010 |
| Perlodidae Gen. sp. | 0.000 | *Galba truncatula* | 0.011 |
| *Simulium (Wilhelmia)* sp. | 0.000 | *Branchiura sowerbyi* | 0.012 |
| *Simulium monticola* | 0.000 | Culicidae Gen. sp. | 0.015 |
| *Isoperla* sp. | 0.000 | Coenagrionidae Gen. sp. | 0.019 |
| *Mystacides longicornis/nigra* | 0.000 | *Physa fontinalis* | 0.020 |
| *Dixa* sp. | 0.000 | Sphaerium sp. | 0.021 |
| *Caenis luctuosa/macrura* | 0.000 | *Eiseniella tetraedra* | 0.021 |
| *Elodes marginata* Lv. | 0.000 | Hydroporinae Gen. sp. Lv. | 0.021 |
| Dolichopodidae Gen. sp. | 0.000 | *Simulium latipes* | 0.021 |
| *Alboglossiphonia heteroclita* | 0.000 | *Dryops* sp. Lv. | 0.031 |
| *Chaetopteryx villosa villosa* | 0.000 | *Cloeon dipterum* | 0.038 |
| *Erpobdella testacea* | 0.000 | *Metreletus balcanicus* | 0.041 |
| *Oreodytes sanmarkii* Ad. | 0.000 | Dytiscidae Gen. sp. Lv. | 0.063 |
| *Perlodes* sp. | 0.000 | Limnephilini Gen. sp. | 0.066 |
| *Ibisia marginata* | 0.000 | *Prosimulium* sp. | 0.070 |
| *Hydropsyche pellucidula*-Gr. | 0.000 | *Erpobdella octoculata* | 0.073 |
| *Paraleptophlebia* sp. | 0.000 | *Helobdella* *stagnalis* | 0.078 |
| *Simulium vernum*-Gr. | 0.000 | Enchytraeidae Gen. sp. | 0.091 |
| *Glyphotaelius pellucidus* | 0.000 | Tanypodinae Gen. sp. | 0.094 |
| *Glossosoma conformis* | 0.000 | Lumbriculidae Gen. sp. | 0.118 |
| *Odontocerum albicorne* | 0.000 | *Proasellus coxalis* | 0.126 |
| *Leuctra nigra* | 0.000 | *Simulium* sp. | 0.131 |
| *Baetis niger* | 0.000 | Chironomini Gen. sp. | 0.188 |
| *Polycelis* sp. | 0.000 | *Asellus aquaticus* | 0.266 |
| *Helophorus brevipalpis* Ad. | 0.000 | Naididae/Tubificidae Gen. sp. | 0.446 |
| Leptophlebiidae Gen. sp. | 0.001 | Oligochaeta Gen. sp. | 0.568 |
| *Hydropsyche saxonica* | 0.001 |  |  |
| *Leuctra* sp. | 0.001 |  |  |