

## Supplementary Data

**Table S1:** Time series information for the four Countries.

| Countries       | Total number of time series | Duration of time series | Number of sampling years | Number of sampled species | Number of non-native species | Sampling method                        |
|-----------------|-----------------------------|-------------------------|--------------------------|---------------------------|------------------------------|--|
| The Netherlands | 28                          | 2003–2019               | 17                       | 301                       | 17                           | Standard invertebrate net <sup>1</sup> |
| England         | 171                         | 2007–2018               | 12                       | 306                       | 10                           | UK RIVPACS protocol <sup>2</sup>       |
| Hungary         | 60                          | 2007–2017               | 11                       | 277                       | 21                           | AQEM <sup>3</sup>                      |

<sup>1</sup> Sampling method in a river stretch (approximately 50 m), 10 subsamples of 0.5 m are taken with a net (net opening width 30 cm x height 20 cm, mesh size 0.5mm) divided over the microhabitats present in the stretch (multihabitat). These samples are pooled into a 5 m sample of the site (<https://www.stowa.nl/publicaties/handboek-hydrobiologie>: book iii).

<sup>2</sup> A 3-minute kick sample (25 x 25 cm net opening with 1 mm mesh net) of the wetted channel bed with effort distributed between in-stream habitats in proportion to their areal coverage, followed by a 1-minute hand search of any other habitats not effectively sampled by the kick method (Furse et al., 1981).

<sup>3</sup> Sampling using AQEM method, in each stream 20 sampling units were collected with a pond net (25x25 cm), over a length of approximately 25 cm per sampling unit, from one or two habitats (Clarke et al., 2006).

**Table S2:** Table summarizing the non-native species found in our time series

| Species                           | Occurrences in countries          | Taxonomic group |
|-----------------------------------|-----------------------------------|-----------------|
| <i>Dikerogammarus haemobaphes</i> | England, Hungary                  | Amphipoda       |
| <i>Dikerogammarus villosus</i>    | the Netherlands, Hungary          |                 |
| <i>Orchestia cavimana</i>         | the Netherlands, Hungary          |                 |
| <i>Synurella ambulans</i>         | Hungary                           |                 |
| <i>Corophium robustum</i>         | Hungary                           |                 |
| <i>Crangonyx pseudogracilis</i>   | the Netherlands, England          |                 |
| <i>Echinogammarus ischnus</i>     | Hungary                           |                 |
| <i>Gammarus tigrinus</i>          | the Netherlands, England          |                 |
| <i>Obesogammarus obesus</i>       | Hungary                           |                 |
| <i>Branchiura sowerbyi</i>        | the Netherlands, Hungary          | Annelida        |
| <i>Hypania invalida</i>           | the Netherlands, England, Hungary |                 |
| <i>Corbicula fluminea</i>         | the Netherlands, England, Hungary | Bivalvia        |
| <i>Dreissena polymorpha</i>       | the Netherlands, England, Hungary |                 |
| <i>Sinanodonta woodiana</i>       | Hungary                           |                 |
| <i>Orconectes limosus</i>         | the Netherlands, Hungary          | Decapoda        |
| <i>Pacifastacus leniusculus</i>   | England                           |                 |
| <i>Lithoglyphus naticoides</i>    | Hungary                           | Gastropoda      |

|                                     |                                   |             |
|-------------------------------------|-----------------------------------|-------------|
| <i>Physella acuta</i>               | the Netherlands, England, Hungary |             |
| <i>Potamopyrgus antipodarum</i>     | the Netherlands, England, Hungary |             |
| <i>Piscicola haranti</i>            | Hungary                           | Hirudinea   |
| <i>Jaera istri</i>                  | Hungary                           | Isopoda     |
| <i>Proasellus coxalis</i>           | the Netherlands,                  |             |
| <i>Katamysis warpachowskyi</i>      | Hungary                           | Mysida      |
| <i>Limnomysis benedeni</i>          | the Netherlands, Hungary          |             |
| <i>Potamothenix bavaricus</i>       | the Netherlands                   | Oligochaeta |
| <i>Potamothenix heuscheri</i>       | the Netherlands                   |             |
| <i>Potamothenix moldaviensis</i>    | the Netherlands                   |             |
| <i>Dendrocoelum romanodanubiale</i> | Hungary                           | Tricladida  |
| <i>Dugesia tigrina</i>              | the Netherlands, England          |             |

**Table S3:** Table summarizing the range of all predictors for each country

| <b>Predictors</b>                               | <b>the Netherlands</b> | <b>England</b> | <b>Hungary</b>  |
|---|------------------------|----------------|-----------------|
| <b>Slope (°)</b>                                | 0–0.09                 | 0–0.37         | 0–0.04          |
| <b>Distance to outlet (m)</b>                   | 110394–257486          | 6070–310924    | 1518659–2165312 |
| <b>Distance to the next upstream barrier(m)</b> | 79.01–207.36           | 1.21–407.68    | 280.0–1781.2    |
| <b>%-Urban areas</b>                            | 0–0.44                 | 0–0.50         | 0–0.21          |
| <b>%-Water bodies</b>                           | 0–0.005                | 0–0.80         | 0–0.02          |
| <b>Precipitation (mm)</b>                       | 1.15–3.25              | 1.02–4.14      | 0.83–2.40       |
| <b>Temperature (°C)</b>                         | 7.36–10.77             | 6.53           | 7.53–12.50      |
| <b>Strahler order</b>                           | 1–7                    | 1–8            | 1–10            |
| <b>Elevation (m)</b>                            | 2.60–68.2              | 1.5–356.3      | 76.4–194.1      |
| <b>Native species abundance</b>                 | 10–15060               | 7–12977        | 0–4354          |
| <b>Native species richness</b>                  | 9–85                   | 2–56           | 1–59            |

**Table S4:** Table summarizing the biological traits modalities

| <b>Biological trait</b>            | <b>Modalities</b>    |
|------------------------------------|----------------------|
| Aquatic stages according to Tachet | Egg                  |
|                                    | Larva                |
|                                    | Nymph                |
|                                    | Adult                |
| Dispersal according to Tachet      | Aquatic passive      |
|                                    | Aquatic active       |
|                                    | Aerial passive       |
|                                    | Aerial active        |
| Feeding habits according to Tachet | Absorber             |
|                                    | Deposit feeder       |
|                                    | Shredder             |
|                                    | Scraper              |
|                                    | Filter-feeder        |
|                                    | Piercer              |
|                                    | Predator             |
|                                    | Parasite             |
| Feeding type                       | Grazers and scrapers |
|                                    | Miners               |
|                                    | Xylophagous          |

|   |                           |
|---|---------------------------|
|   | Shredders                 |
|   | Gatherers/collectors      |
|   | Active filter feeders     |
|   | Passive filter feeders    |
|   | Predators                 |
|   | Parasites                 |
|   | Other feeding types       |
| Food according to Tachet                              | Microorganisms            |
|   | Detritus < 1mm            |
|   | Dead plant $\geq 1$ mm    |
|   | Living microphytes        |
|   | Living macrophytes        |
|   | Dead animal $\geq 1$ mm   |
|   | Living microinvertebrates |
|   | Living macroinvertebrates |
|   | Vertebrates               |
| Life cycle duration according to Tachet               | $\leq 1$ year             |
|   | > 1 year                  |
| Locomotion and substrate relation according to Tachet | Flier                     |
|   | Surface swimmer           |
|   | Full water swimmer        |

|   |                        |
|---|------------------------|
|   | Crawler                |
|   | Burrower               |
|   | Interstitial           |
|   | Temporarily attached   |
|   | Permanently attached   |
| Maximal potential size according to Tachet              | $\leq 0.25$ cm         |
|   | $> 0.25 - 0.5$ cm      |
|   | $> 0.5 - 1$ cm         |
|   | $> 1 - 2$ cm           |
|   | $> 2 - 4$ cm           |
|   | $> 4 - 8$ cm           |
|   | $> 8$ cm               |
| Reproduction according to Tachet                        | Ovovivipar             |
|   | Free isolated eggs     |
|   | Cemented isolated eggs |
|   | Fixed clutches         |
|   | Free clutches          |
|   | Clutches in vegetation |
|   | Terrestrial clutches   |
|   | Asexual                |
| Potential number of cycles per year according to Tachet | $< 1$                  |

|                                 |                     |
|---------------------------------|---------------------|
|                                 | 1                   |
|                                 | > 1                 |
| Respiration according to Tachet | Tegument            |
|                                 | Gill                |
|                                 | Plastron            |
|                                 | Spiracle            |
|                                 | Hydrostatic vesicle |



**Table S5:** Table summarizing the ecological traits modalities

| Ecological traits                                  | Modalities           |
|--|----------------------|
| Altitude according to Tachet                       | Lowlands             |
|  | Piedmont level       |
|  | Alpine Level         |
| Current velocity (preferendum) according to Tachet | Null                 |
|  | Slow                 |
|  | Medium               |
|  | Fast                 |
| Longitudinal distribution according to Tachet      | Crenon               |
|  | Epirithron           |
|  | Metarithron          |
|  | Hyporithron          |
|  | Epipotamon           |
|  | Metapotamon          |
|  | Estuary              |
|  | Outside river system |
| pH (preferendum) according to Tachet               | $\leq 4$             |
|  | $> 4 - 4.5$          |
|  | $> 4.5 - 5$          |
|  | $> 5 - 5.5$          |

|  |                                      |
|--|--------------------------------------|
|  | > 5.5 - 6                            |
|  | > 6                                  |
| Salinity (preferendum) according to Tachet   | Fresh water                          |
|  | Brackish water                       |
| Substrate (preferendum) according to Tachet  | Flags/boulders/cobbles/pebbles       |
|  | Gravel                               |
|  | Sand                                 |
|  | Slit                                 |
|  | Macrophytes                          |
|  | Microphytes                          |
|  | Twigs/roots                          |
|  | Organic detritus/litter              |
|  | Mud                                  |
| Temperature according to Tachet              | Psychrophilic                        |
|  | Thermophilic                         |
|  | Eurythermic                          |
| Transversal distribution according to Tachet | River channel                        |
|  | Banks, connected side-arms           |
|  | Ponds, pools, disconnected side-arms |
|  | Marshes, peat bogs                   |
|  | Temporary waters                     |

|  |              |
|--|--------------|
|  | Lake         |
|  | Groundwaters |
| Trophic status (preferendum) according to Tachet | Oligotrophic |
|  | Mesotrophic  |
|  | Eutrophic    |

**Table S6:** Table summarizing the VIF (Variance Inflation Factor) values of the  $\alpha$  and  $\beta$  diversity models for each cluster and all cluster combined

| Explicatives variables                   | the Netherlands |         | England  |         | Hungary  |         |
|--|-----------------|---------|----------|---------|----------|---------|
|  | $\alpha$        | $\beta$ | $\alpha$ | $\beta$ | $\alpha$ | $\beta$ |
| Year                                     | 1.40            | 1.19    | 1.16     | 1.22    | 1.07     | 1.07    |
| Slope (°)                                | 1.95            | 1.95    | 1.12     | 1.12    | 1.09     | 1.08    |
| Distance to outlet (m)                   | 3.19            | 3.05    | 1.48     | 1.46    | 2.19     | 2.18    |
| %-Urban areas                            | 1.25            | 1.25    | 1.16     | 1.17    | 1.27     | 1.21    |
| %-Water bodies                           | 1.98            | 1.98    | 1.13     | 1.09    | 1.29     | 1.26    |
| Precipitation (mm)                       | 1.12            | 1.12    | 2.10     | 2.10    | 1.27     | 1.26    |
| Temperature (°C)                         | 1.08            | 1.10    | 2.74     | 2.76    | 1.68     | 1.59    |
| Strahler order                           | 2.00            | 1.86    | 1.18     | 1.18    | 1.37     | 1.33    |
| Elevation (m)                            | 4.64            | 4.35    | 2.86     | 2.84    | 2.58     | 2.50    |
| Distance to the next upstream barrier(m) | 1.56            | 1.39    | 1.17     | 1.17    | 1.74     | 1.30    |
| Native species abundance                 | 1.08            | 1.09    | 1.17     | 1.14    | 1.38     | 1.33    |
| Native species richness                  | 1.51            | 1.38    | 1.25     | 1.25    | 1.53     | 1.44    |

**Table S7:** Table summarizing the results of the  $\alpha$  diversity models for each country. Edf corresponds to the “Effective Degrees of Freedom” and Ref.df corresponds to the “Reference Degrees of Freedom”.

| Explicatives variables                          | the Netherlands                   |        |         | England                           |        |         | Hungary                           |        |         |
|---|-----------------------------------|--------|---------|-----------------------------------|--------|---------|-----------------------------------|--------|---------|
|   | Edf                               | Ref.df | p-value | Edf                               | Ref.df | p-value | Edf                               | Ref.df | p-value |
| <b>Year</b>                                     | 1.00                              | 1.00   | < 0.01  | 4.67                              | 5.37   | < 0.01  | 5.16                              | 5.73   | < 0.01  |
| <b>Slope (°)</b>                                | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| <b>Distance to outlet (m)</b>                   | Not selected by the random forest |        |         | 1.00                              | 1.00   | 0.81    | 1.00                              | 1.00   | 0.86    |
| <b>%-Urban areas</b>                            | Not selected by the random forest |        |         | Not selected by the random forest |        |         | 1.29                              | 1.31   | < 0.01  |
| <b>%-Water bodies</b>                           | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| <b>Precipitation (mm)</b>                       | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| <b>Temperature (°C)</b>                         | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| <b>Strahler order</b>                           | 1.00                              | 1.00   | 0.88    | 1.00                              | 1.00   | 0.99    | Not selected by the random forest |        |         |
| <b>Elevation (m)</b>                            | Not selected by the random forest |        |         | 1.00                              | 1.00   | 0.88    | 1.00                              | 1.00   | 0.35    |
| <b>Distance to the next upstream barrier(m)</b> | 1.00                              | 1.00   | 0.37    | 1.00                              | 1.00   | 0.98    | 2.30                              | 2.33   | < 0.05  |
| <b>Native species abundance</b>                 | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| <b>Native species richness</b>                  | 3.69                              | 4.53   | < 0.01  | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| <b>Site</b>                                     | 25.62                             | 26.00  | < 0.01  | 166.42                            | 167.00 | < 0.01  | 51.46                             | 56.00  | < 0.01  |

**Table S8:** Table summarizing the results of the  $\beta$  diversity models for each country. Edf corresponds to the “Effective Degrees of Freedom” and Ref.df corresponds to the “Reference Degrees of Freedom”.

|  | the Netherlands                   |        |         | England                           |        |         | Hungary                           |        |         |
|--|-----------------------------------|--------|---------|-----------------------------------|--------|---------|-----------------------------------|--------|---------|
| Explicatives variables                   | Edf                               | Ref.df | p-value | Edf                               | Ref.df | p-value | Edf                               | Ref.df | p-value |
| Year                                     | 4.31                              | 5.06   | < 0.01  | 4.00                              | 4.00   | < 0.01  | 2.12                              | 2.62   | <0.05   |
| Slope (°)                                | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| Distance to outlet (m)                   | 1.00                              | 1.00   | 0.91    | 1.00                              | 1.00   | 0.68    | Not selected by the random forest |        |         |
| %-Urban areas                            | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| %-Water bodies                           | Not selected by the random forest |        |         | 1.00                              | 1.00   | 0.86    | 1.00                              | 1.00   | 0.85    |
| Precipitation (mm)                       | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| Temperature (°C)                         | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| Strahler order                           | Not selected by the random forest |        |         | 1.00                              | 1.00   | 0.89    | Not selected by the random forest |        |         |
| Elevation (m)                            | Not selected by the random forest |        |         | Not selected by the random forest |        |         | 1.00                              | 1.00   | 0.98    |
| Distance to the next upstream barrier(m) | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| Native species abundance                 | Not selected by the random forest |        |         | Not selected by the random forest |        |         | Not selected by the random forest |        |         |
| Native species richness                  | 1.00                              | 1.00   | 0.65    | 1.00                              | 1.00   | 0.97    | 1.00                              | 1.00   | 0.99    |
| Site                                     | 25.66                             | 26.00  | < 0.01  | 167.00                            | 168.00 | < 0.01  | 55.65                             | 57.00  | < 0.01  |

**Table S9:** Table summarizing the results of the  $\gamma$  diversity models for each country. Edf corresponds to the “Effective Degrees of Freedom” and Ref.df corresponds to the “Reference Degrees of Freedom”.

|                      | the Netherlands |        |         | England |        |         | Hungary |        |         |
|----------------------|-----------------|--------|---------|---------|--------|---------|---------|--------|---------|
| Explicative variable | Edf             | Ref.df | p-value | Edf     | Ref.df | p-value | Edf     | Ref.df | p-value |
| Year                 | 2.63            | 3.24   | <0.01   | 1.00    | 1.00   | <0.01   | 3.54    | 4.28   | <0.01   |

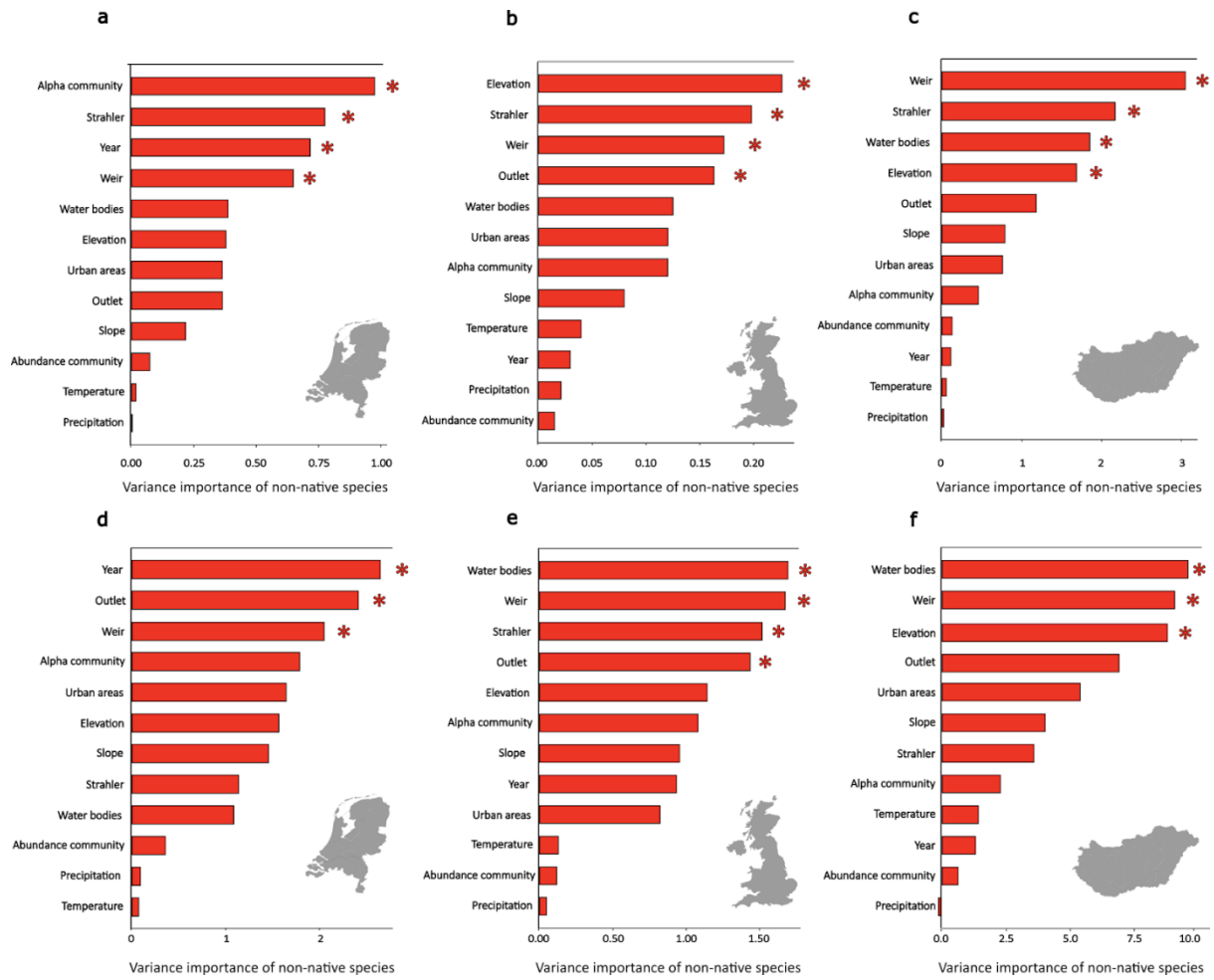
**Table S10:** Table summarizing the results of the models on the trait metrics ( $T_{Dis}$  = trait dispersion,  $T_{Eve}$  = trait evenness,  $T_{Ric}$  = trait richness,  $T_{Div}$  = trait divergence) for each country in function of the time. Edf corresponds to the “Effective Degrees of Freedom” and Ref.df corresponds to the “Reference Degrees of Freedom”.

|                       | the Netherlands |        |               | England |        |              | Hungary |        |              |
|-----------------------|-----------------|--------|---------------|---------|--------|--------------|---------|--------|--------------|
| Explicative variables | Edf             | Ref.df | p-value       | Edf     | Ref.df | p-value      | Edf     | Ref.df | p-value      |
| $T_{Dis}$             |                 |        |               |         |        |              |         |        |              |
| Year                  | 6.557           | 7.66   | <b>0.0244</b> | 1       | 1      | 0.881        | 1       | 1      | 0.695        |
| $T_{Eve}$             |                 |        |               |         |        |              |         |        |              |
| Year                  | 1               | 1      | 0.0798        | 8.49.3  | 8.922  | <b>0.048</b> | 2.585   | 3.209  | <b>0.035</b> |
| $T_{Ric}$             |                 |        |               |         |        |              |         |        |              |
| Year                  | 5.397           | 6.519  | 0.13          | 5.102   | 6.18   | 0.107        | 8.04    | 8.723  | 0.146        |
| $T_{Div}$             |                 |        |               |         |        |              |         |        |              |
| Year                  | 3.783           | 4.664  | 0.146         | 1.52    | 1.87   | 0.573        | 1.613   | 1.998  | 0.162        |



**Table S11:** Table summarizing the results of the models on the functional metrics ( $F_{Dis}$  = functional dispersion,  $F_{Eve}$  = functional evenness,  $F_{Ric}$  = functional richness,  $F_{Div}$  = functional divergence) for each country in function of the time. Edf corresponds to the “Effective Degrees of Freedom” and Ref.df corresponds to the “Reference Degrees of Freedom”.

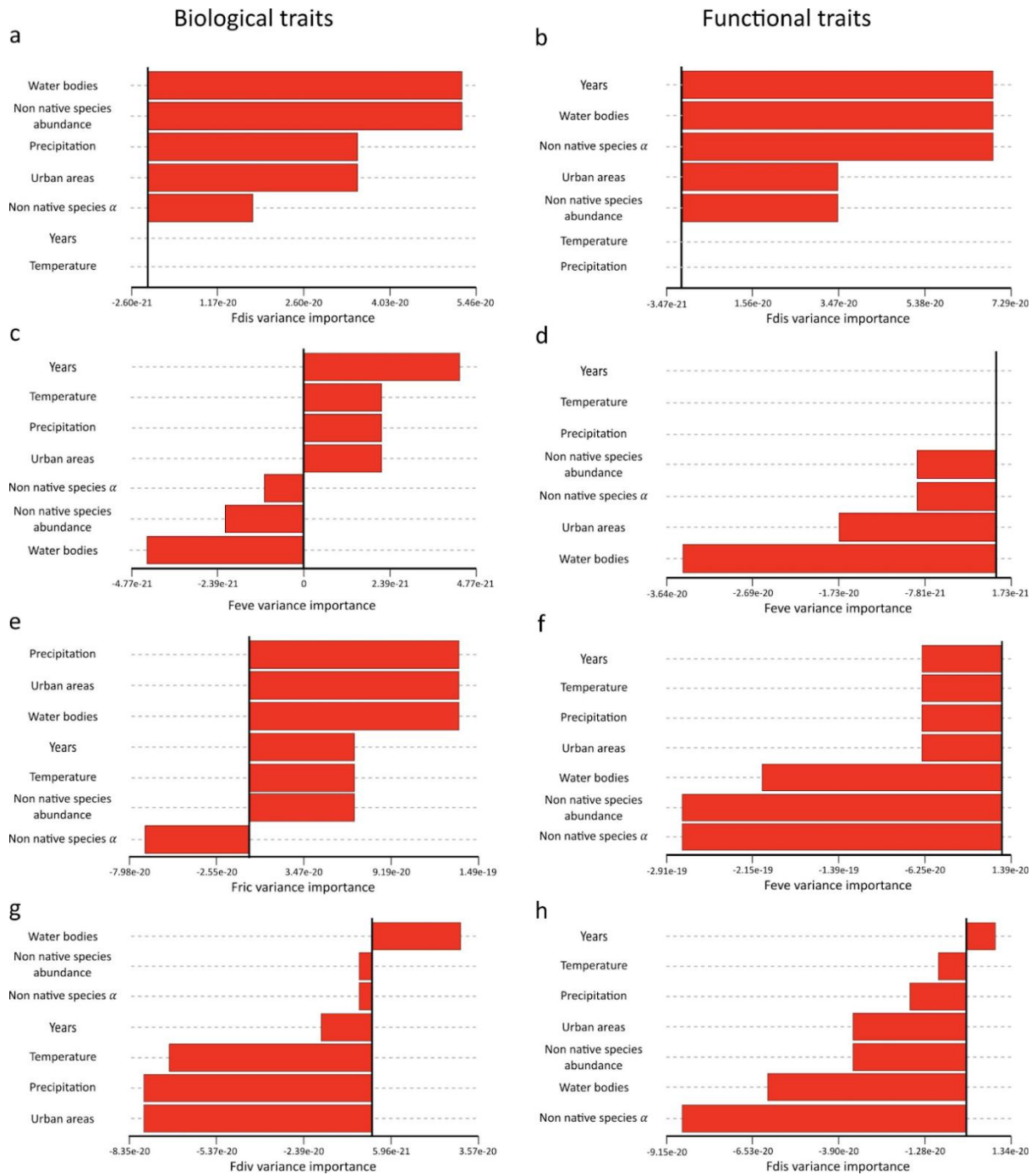
|                        | the Netherlands |        |         | England |        |         | Hungary |        |         |
|------------------------|-----------------|--------|---------|---------|--------|---------|---------|--------|---------|
| Explicatives variables | Edf             | Ref.df | p-value | Edf     | Ref.df | p-value | Edf     | Ref.df | p-value |
| $F_{Dis}$              |                 |        |         |         |        |         |         |        |         |
| Year                   | 2.861           | 3.551  | 0.36    | 1.218   | 1.403  | 0.564   | 8.749   | 8.978  | 0.229   |
| $F_{Eve}$              |                 |        |         |         |        |         |         |        |         |
| Year                   | 1.961           | 2.442  | 0.191   | 7.611   | 8.486  | 0.122   | 7.684   | 8.519  | 0.269   |
| $F_{Ric}$              |                 |        |         |         |        |         |         |        |         |
| Year                   | 1               | 1      | 0.0151  | 3.954   | 4.872  | 0.064   | 1       | 1      | 0.24    |
| $F_{Div}$              |                 |        |         |         |        |         |         |        |         |
| Year                   | 1               | 1      | 0.85    | 1.78    | 2.216  | 0.46    | 1       | 1      | 0.0876  |



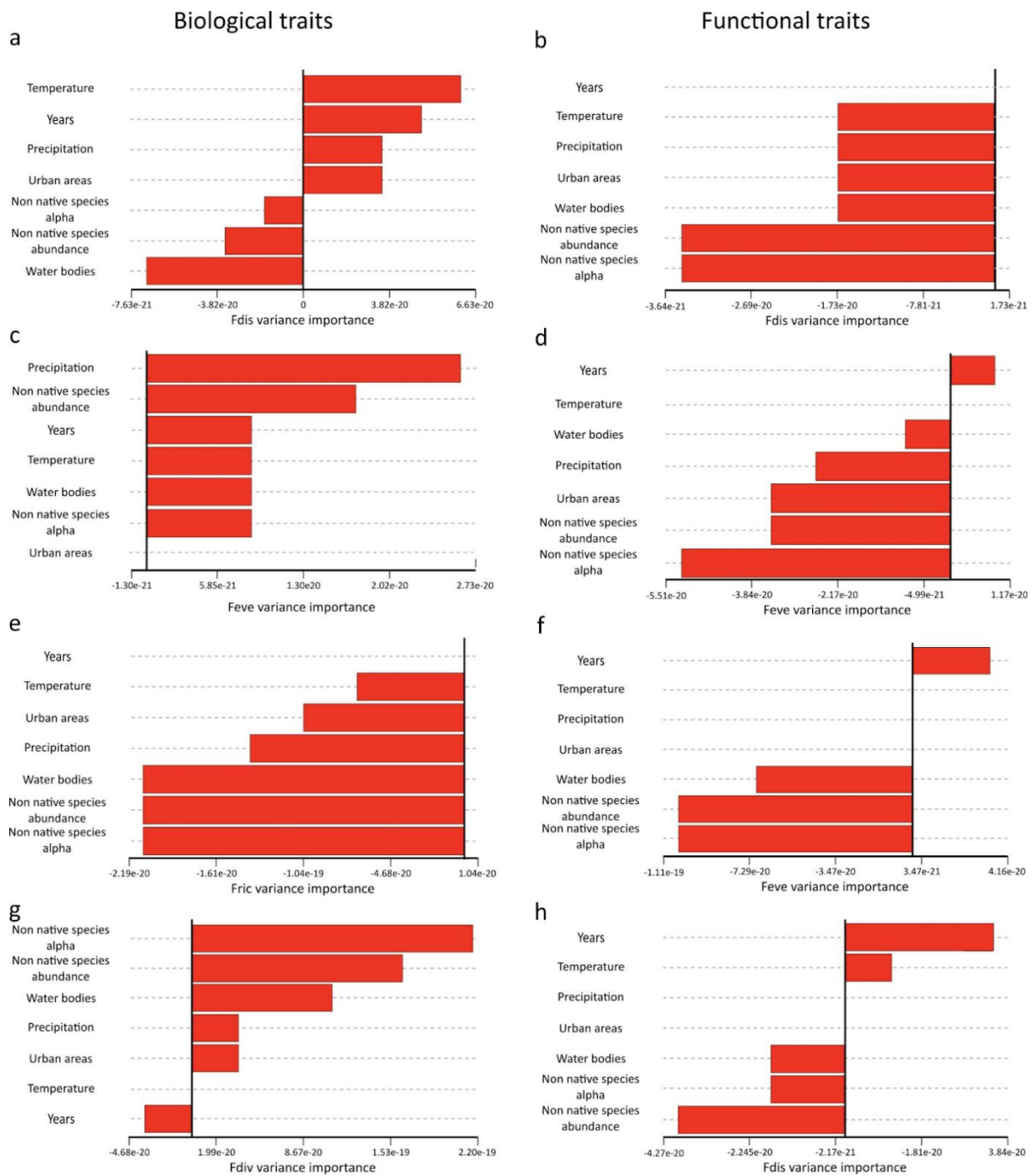
**Figure S1:** The outputs of random forest analyses for  $\alpha$  diversity (a-c) and  $\beta$  diversity (d-f) for the Netherlands (a and d), England (b and e) and Hungary (c and f). The bars represent the proportion of variance for each variable used in the statistical models. Variables marked with an asterisk ‘\*’ indicate their inclusion in the final model.

**Supplement 1:**

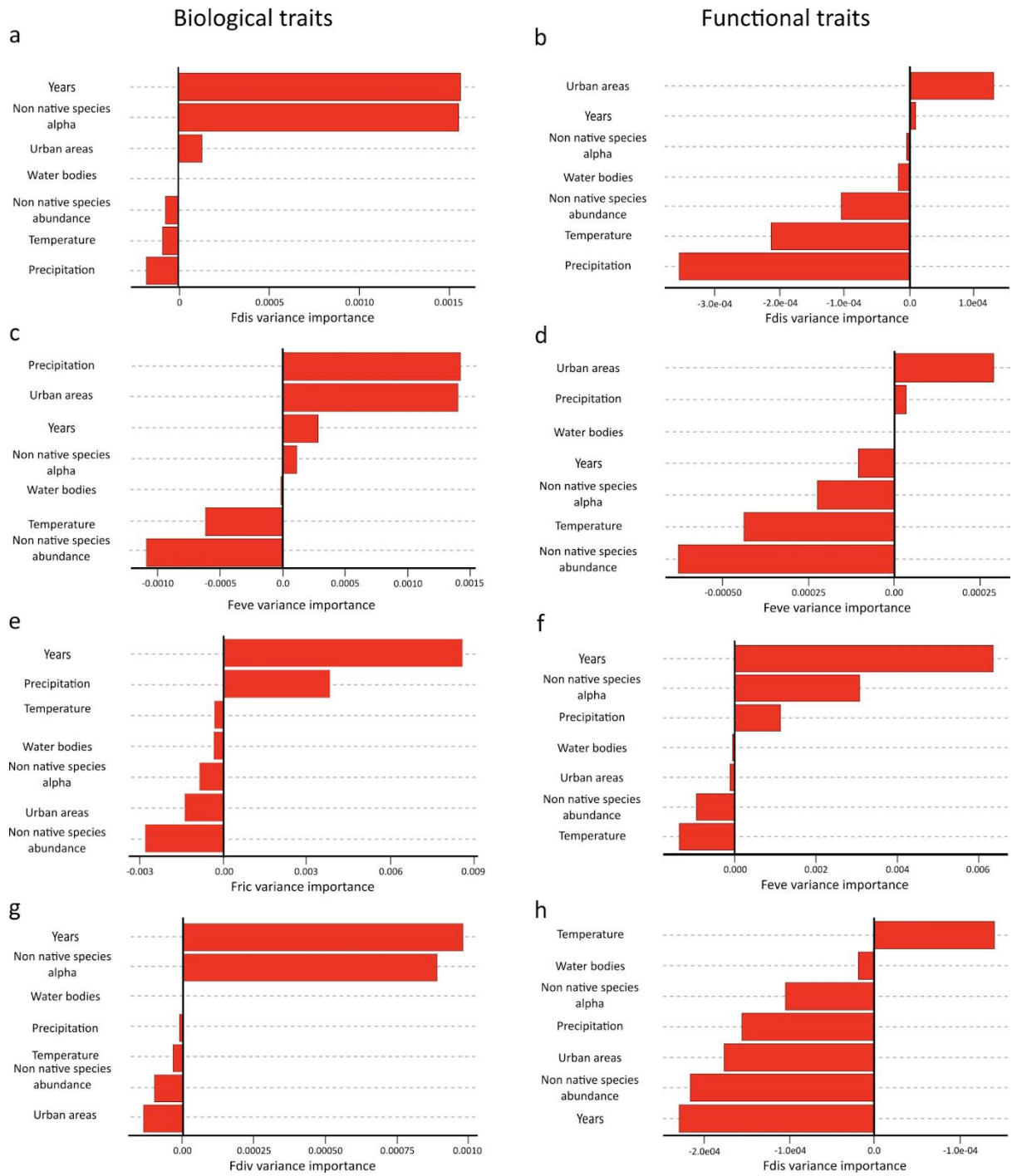
The importance of trait and functional metrics varied across different regions. In England, the most influential variables for all trait and functional metrics were %-waterbodies, year, and non-native species  $\alpha$  diversity (Figure S2). In Hungary, the trait and functional metrics were primarily influenced by  $\alpha$  diversity and ‘abundance of non-native species’ (Figure S3). In the Netherlands, the variance of each trait and functional metric was influenced by different variables, with the year and non-native species abundance being the most recurrent ones (Figure S4).



**Figure S2 :** Random forest analyses for non-native species trait (a, c, e, g) and functional (b, d, f, h) metrics: dispersion (a-b), evenness (c-d), richness (e-f) and divergence (g-h) in England. The bars represent the proportion of variance for each variable used in the statistical models.



**Figure S3 :** Random forest analyses for non-native species trait (a, c, e, g) and functional (b, d, f, h) metrics: dispersion (a-b), evenness (c-d), richness (e-f) and divergence (g-h) in Hungary. The bars represent the proportion of variance for each variable used in the statistical models.



**Figure S4** : Random forest analyses for non-native species trait (a, c, e, g) and functional (b, d, f, h) metrics: dispersion (a-b), evenness (c-d), richness (e-f) and divergence (g-h) in the Netherlands. The bars represent the proportion of variance for each variable used in the statistical models.