# Combining hydrologic simulations and stream-network models to reveal flow-ecology relationships in a large Alpine catchment

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## Key points

- The hydrological model HYPERstreamHS was used to simulate natural streamflow series in 100 bio-assessment sites across a large Alpine basin.
- Three flow-regime classes were identified, representing typical nivo-glacial, nivo-pluvial, and pluvial streams.
- Spatial stream-network models identified distinct flow-ecology relationships and relevant spatial autocorrelation across classified regimes, which aid implementing targeted water management schemes.

## Abstract

Flow regimes profoundly influence river organisms and ecosystem functions, but regulatory approaches often lack the scientific basis to support sustainable water allocation. In part, this reflects the challenge of understanding the ecological effects of flow variability over different temporal and spatial domains.

Here, we use a process-based distributed hydrological model to simulate 23 years of natural flow regime in 100 target bioassessment sites across the Adige River network (NE Italy), and to identify typical nivo-glacial, nivo-pluvial, and pluvial reaches. We then applied spatial stream-network models (SSN) to investigate the relationships between hydrologic and macroinvertebrate metrics while accounting for network spatial autocorrelation and local habitat conditions.

Macroinvertebrate metrics correlated most strongly with maximum, minimum and temporal variation in streamflow, but effects varied across flow regime types. For example: i) taxon richness appeared limited by high summer flows and high winter flows in nivo-glacial and pluvial streams, respectively; ii) invertebrate grazers increased proportionally with the annual coefficient of flow variation in nivo-glacial streams but tended to decline with flow variation in pluvial streams. SSN models revealed that most variation in macroinvertebrate metrics was accounted for by spatial autocorrelation, although local land-use and water quality also affected benthic invertebrate communities, particularly at lower elevations.

These findings highlight the importance of developing environmental flow management policies in ways that reflect specific hydro-ecological and land use contexts. Our analyses also illustrate the importance of spatially-explicit approaches that account for auto-correlation when quantifying flow-ecology relationships.

*Keywords: flow-regime classification; flow-ecology relationships; river networks; benthic invertebrates; network spatial patterns; HYPERstreamHS hydrological model.*

## 1. Introduction

The flow regime of streams and rivers has been modified by human activities at the global scale (Grill et al., 2019; Tonkin et al., 2019). As human population continues to grow, the increasing demand for water supply, flood protection and energy production has prompted the widespread adoption of engineering solutions such as the construction of dams, levees and other hydraulic infrastructures (Couto & Olden, 2018; Shumilova et al., 2018; Zarfl et al., 2015). As a result, streams and rivers are under increasing anthropogenic pressure and are among the most threatened ecosystems worldwide, with particularly high rates of species extinctions (Tickner et al., 2020). The ongoing global climate change is expected to further exacerbate this situation by increasing the frequency of extreme hydrologic events such as floods and droughts that act synergistically with other stressors affecting aquatic ecosystems (e.g. Navarro-Ortega et al., 2015). This is of particular concern since freshwater ecosystems support about 10% of all known species (Strayer & Dudgeon, 2010) and are essential for human well-being, providing a wealth of ecosystem services (Green et al., 2015). Understanding and limiting the ecological effects of flow alteration is therefore fundamental for a sustainable use of water resources.

The Natural Flow Regime Paradigm (Poff et al. 1997) is at the heart of environmental flow definition and specifically acknowledges that river biota are adapted to seasonal and interannual variations of river flow. In order to mitigate the ecological impacts associated with human infrastructures while maintaining their functioning, environmental flows (termed *e-flows* hereafter) should mimic the natural streamflow variability in terms of magnitude, frequency, duration, timing and rate of change (Arthington et al., 2018). However, given the limits in the ability to mimic natural regimes in regulated rivers, *e-flows* policy must be informed by a clear understanding of the relation between river ecology and flow characteristics (i.e., flow-ecology relationships), which is, however, hampered by several practical challenges. These include, among others: i) the paucity of stream and river locations for which ecological information can be paired with long term hydrologic records (e.g. Patrick & Yuan, 2017a); ii) the natural variation in flow regime among rivers and sub-

catchments, whereby ecological responses could vary significantly among individual flow regime types (Poff et al., 2010); and iii) the spatial configuration of river ecosystems, which requires statistical approaches able to account for the complex autocorrelation associated with network topology and flow directionality (Peterson et al., 2013).

Several approaches have been used to address these challenges. Matching flow and ecological data is a prerequisite for quantifying flow-ecology relationships, and yet the spatial and temporal overlap between observed hydrologic and biological data is often poor (e.g. Mazor et al., 2018). To mitigate such limitations, either statistical or process-based hydrological models have been used. Statistical hydrological models aim to predict flow metrics at ungauged locations from the observed relation between available streamflow series and catchment characteristics (Booker et al., 2015; Patrick & Yuan, 2017b), or by means of geostatistical interpolation (Skøien et al., 2006). Process-based hydrologic models, on the other hand, directly simulate streamflow time series at specific network locations by integrating the hydrological processes acting within the drainage area: i.e., precipitation, snowmelt, interception, evapotranspiration, infiltration, surface and sub-surface flow, as well as their interactions (see e.g., Beven, 2012).

The second challenge is related to the heterogeneity of river basins where the natural streamflow regime and river biota differ markedly across the network. Therefore, it is necessary to classify flow regimes into distinct and easily interpretable classes in order to define reference flow conditions and implement targeted *e-flows* schemes, while also minimising the effects of other co-varying environmental factors (Belmar et al., 2011; Booker et al., 2015). As a result, *e-flows* can be transferred among similar flow regimes at regional scales. For instance, the identification and classification of reference hydrographs are two key steps (i.e, “Hydrological foundation” and “River classification”) in the assessment of the “Ecological Limits of Hydrologic Alteration” (ELOHA), the holistic framework increasingly adopted to define regional flow standards (Poff et al., 2010).

The third challenge is not strictly associated with flow-ecology research, but it is related to the spatial structure of river networks. The topology of branching river networks implies that classical statistical methods are unable to account for the spatial autocorrelation due to the connectivity and directionality of water flow within the network. Failing to account for such spatial patterns may lead to spurious correlations (Isaak et al., 2014). However, recent advances in the field of fluvial variography (i.e. spatial statistics applied to river networks) have provided the tools to model these spatial dependencies over the Euclidean and

watercourse dimension, while also accounting for flow directionality (Carrara et al., 2012; Ver Hoef & Peterson, 2010; Zimmerman & Ver Hoef, 2017). Such stream-network models have been used to derive spatially-explicit estimates of water quality and population abundance across river basins (Isaak et al., 2017; McGuire et al., 2014), but applications to flow-ecology research are surprisingly scarce (Bruckerhoff et al., 2019).

In this paper we develop and discuss a framework that addresses these challenges using the Adige River basin (northeastern Italy) as case study. In doing so, we aim to contribute to the understanding of flow-ecology relationships at the regional scale and evaluate potential flow-sensitive indicators, since recent works in the Italian Alps showed the poor sensitivity of some of the current Water Framework Directive (WFD) biological indicators to flow parameters (Larsen et al., 2019; Quadroni et al., 2017). Specifically, we focused on benthic macroinvertebrates as model organisms because of their essential role in the functioning of lotic systems, their widespread use as biological indicators and the availability of monitoring data in the region (De Pauw et al., 2006; Friberg, 2014; Larsen et al., 2019). We included both taxonomic and functional (traits-based) metrics, as these provide independent and complementary information that could be valid across biogeographic zones (Heino et al., 2013).

To achieve the above-mentioned goals, first we used the process-based HYPERstreamHS hydrological model (Avesani et al., 2020) to simulate the natural streamflow series of one-hundred stream reaches throughout the Adige River basin where biological information was available. Then, we classified distinct flow regimes representing the natural hydrological conditions of the streams in the basin. Subsequently, we used spatial stream-network models (SSN) to correlate the macroinvertebrate taxonomic and functional metrics with the streamflow characteristics and habitat conditions within each flow regime, while also accounting for spatial autocorrelation.

## 2. Data and Methods

### *2.1 Study area*

The study area is the Adige River basin, an Alpine catchment in northeastern Italy (Fig. 1), closed at 'Vo Destro' gauging station (drainage area 10600 $km^2$). The Adige River is the second longest Italian river, with the typical natural streamflow regime of the Alpine region

showing two seasonal maxima, one occurring in spring-summer due to snow and glacial melt, and the other in autumn triggered by cyclonic storms (Chiogna et al., 2016; Mallucci et al., 2019). Recent analyses of historical hydro-climatic trends revealed that the basin is sensitive to climate change with ongoing reduction of winter snowfall and anticipation of snow-melting season (Diamantini et al., 2018; Lutz et al., 2016; Mallucci et al., 2019), which are likely to alter its flow regime by the second half of 21$^{st}$ century (Majone et al., 2016). Such modifications may have relevant socio-economic consequences in the catchment, where more than 80% of licensed withdrawn water is allocated to large hydropower plants, and about 6% to agriculture (Bellin et al., 2016; Zolezzi et al., 2009). We selected 100 headwater stream reaches (Fig.1) throughout the catchment for which biological information was available and with an almost pristine streamflow regime (i.e. no major in-stream hydraulic infrastructure or impoundments upstream). The selected reaches were mostly 1$^{st}$ and 2$^{nd}$ order streams, with elevation and drainage area ranging from 170 to 1900 m a.s.l. and from 10 to 434 km$^2$, respectively.

### *2.2 Observational datasets*

#### *2.2.1 Flow data*

The regional precipitation and temperature dataset ADIGE (Mallucci et al., 2019) was used as meteorological forcing for hydrological modelling. This dataset provides daily precipitations and temperatures for the time interval 1956-2013 at the spatial resolution of 1 km. The dataset was developed by interpolating the measurements available at the meteorological stations within and nearby the river basin by means of kriging with external drift (Goovaerts, 1997; Mallucci et al., 2019). To comply with the computational grid adopted in the hydrologic modeling, the ADIGE dataset was aggregated to 5-km grid spacing.

Daily streamflow data collected at 9 gauging stations (Fig.1) were provided by the Hydrological Office of the Autonomous Provinces of Trento (www.floods.it) and Bolzano (www.provincia.bz.it/hydro). Stations were selected according to the following criteria: i) observational period including the 1989–2013 time-frame used for calibration and validation of the hydrological model; ii) limited gaps in records; iii) large distance from upstream reservoirs if present; and, iv) broad spatial coverage including the major tributaries of the Adige River. The gauging stations were distributed in sub-catchments of different sizes,

elevation, geology and land-cover, and were therefore representative of the hydrological regimes of the Adige basin.

*2.2.2 Macroinvertebrate data*

Macroinvertebrate data were collected by the Environmental Protection Agencies of the Provinces of Trento and Bolzano as part of their institutional monitoring programmes (Larsen et al., 2019). Sampling was performed according to the multi-habitat sampling approach defined in the AQEM (http://www.aqem.de/) protocol: 10-replicate Surber samples were collected within a 20-50 m reach in proportion to the micro-habitats present (Hering et al., 2004). Samples were collected in the period 2009-2015, and sites were visited several times per year (median = 3), primarily in spring and autumn. Macroinvertebrate densities were averaged over all samples to remove seasonal effects, thereby obtaining a representative community composition of each site.

*2.2.3 Reach-scale environmental data*

Two additional reach-scale environmental variables were included in the analyses besides streamflow regime: the proportion of agricultural land-use ("Agr.landuse"), calculated within 1-km buffer around each sampling location, and the physico-chemical water quality, as expressed by the "LIMeco" index (Livello di Inquinamento da Macrodescrittori per lo stato ecologico), one of the official WFD water quality indicators used to assess the ecological status of running water in Italy (European Commission, 2000). This is a multi-metric indicator assigning quality scores based on threshold levels for concentration of oxygen, ammonia, nitrate and total phosphorus in freshwater (see Azzellino et al., 2015). These environmental descriptors were included as covariates in the quantification of flow-ecology relationship because of their proven influence on the composition of benthic invertebrates in the area (Larsen et al., 2019).

***2.3 Hydrologic simulations***

Hydrological simulations were performed at the daily time scale with the HYPERstreamHS model (Avesani et al., 2020; Laiti et al., 2018), which couples the HYPERstream routing scheme (recently proposed by Piccolroaz et al., 2016) with a continuous Soil Conservation Service (SCS) module for surface flow generation (Michel et al., 2005). Subsurface return flow was modelled by a nonlinear reservoir (Majone et al, 2010). The HYPERstream routing

scheme is specifically designed to couple with climate models and, in general, with gridded meteorological datasets. HYPERstream inherits the computational grid of the climatic model, or of the gridded product providing the meteorological forcing, and preserves geomorphological dispersion due to the structure of the river network (Rinaldo et al., 1991), regardless of grid resolution. In previous studies, the SCS runoff module was successfully applied to two tributaries of the Adige River (Bellin et al., 2016; Piccolroaz et al., 2015). For a detailed description of the hydrologic modelling framework see Laiti et al., (2018) and Avesani et al. (2020).

The hydrological model was calibrated against daily streamflow observations in the time window 1989-2013 using the ADIGE dataset as input meteorological forcing. The parameters space was explored for optimality, according to the Nash-Sutcliffe efficiency index (NSE; Nash & Sutcliffe, 1970), by using the Particle Swarming Optimization algorithm (Kennedy & Eberhart, 1995). NSE was selected because of its effectiveness in assessing the performance of hydrologic models in reproducing observed streamflows. NSE is satisfactory when larger than 0.5 (Moriasi et al., 2007). Because hydrologic modelling was tailored to reproduce streamflow at unimpacted headwater locations (see Sect. Study area), four headwater gauging stations (Vermiglio, Rio Funes, Aurino and Gadera in Fig.1) were calibrated simultaneously (i.e. NSE was defined as the average of individual efficiencies from the four stations). Successively, other five stations distributed across the basin (Saltusio, Vipiteno, Anterselva, Trento and Bronzolo in Fig.1; drainage area ranging from 60 to 9000 $km^2$) were used for validation, in order to assess how the model reproduced streamflow at all the relevant scales. The first two years of the time series, i.e., 1989 and 1990, were used as spin-up for the simulations and therefore were excluded from the computation of NSE. Finally, we used the calibrated hydrologic model to simulate streamflow time series (1991-2013) at the 100 gauged and ungauged locations where biological data were available.

As additional validation of the hydrologic model, we compared patterns of flow-ecology relationships obtained from the observed and simulated streamflow using nine locations for which measured streamflow time series were also available.

### *2.4 Hydrologic classification*

Simulated streamflow time series at the 100 locations were first normalised by their mean annual discharge (MAD) to allow comparison across streams and develop flow-ecology relationships independent of stream size (e.g. Rosenfeld, 2017). In the following step,

streamflow regimes were classified according to their typical seasonality as follows: first, we calculated the mean monthly hydrographs for each location from the MAD-normalised daily streamflow time series (Fig. S1 in SM); then we performed a Principal Component Analysis (PCA) on the resulting hydrographs to synthesize similarities among locations using the first two PC axes. Location scores on the two axes were then weighted by the proportion of variance explained in the PCs and used as synthetic variables in order to cluster the locations based on their flow regime (see e.g. Belmar et al., 2011). A flexible-beta hierarchical clustering approach was used, with the recommended value of beta= - 0.25 (Belmar et al., 2011; Legendre & Legendre, 2012; Mazor et al., 2018), which provides an intermediate solution between chaining obtained via single linkage, and space dilation deriving from complete linkage. To further validate the degree of separation among the classified regimes, we ran a Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2017) based on the Euclidean distance matrix of the weighted PCA scores.

### *2.5 Hydrologic metrics*

We used the 23 years MAD-normalised daily streamflow values to calculate 34 hydrologic metrics following the Indicator of Hydrologic Alteration (IHA; Richter et al., 1997) approach (Tab. 1), implemented in R software with the "IHA" package (R Core Team, 2019). These metrics were averaged over the years to quantify ecologically-relevant components of the long-term flow regime related to magnitude, duration, frequency, timing and rate of change. As an exploratory step, and to visualise and further validate the separation of the hydrologic classes in the multidimensional space defined by the hydrologic metrics, we plotted the streams on the first two PCA axes derived from the correlation matrix of the IHA metrics (Fig. 4). However, our main interest was to quantify flow-ecology relationships within the distinct hydrologic regimes considered as management units. Therefore, we ran additional PCA analyses for each classified regime to identify the most relevant metrics in each group. We then selected a set of non-redundant flow metrics showing high correlation (>0.8) with the 1$^{st}$ or 2$^{nd}$ PC axes (see Tab. S1 and Results), which reflected the key flow components. These flow metrics were subsequently used as predictors in stream-network models for quantifying interpretable flow-ecology relationships.

### *2.6 Data analysis*

We derived a set of taxonomic and functional metrics from the macroinvertebrate community data. Our aim was to examine the sensitivity of different metrics to streamflow conditions to derive valid alternatives to those currently implemented under the WFD. We included metrics related to the diversity of the community, such as taxonomic richness, Shannon diversity (as effective number of species of order q=1; Jost, 2006), Functional Dispersion (FDis, which is minimally influenced by taxonomic richness), as these have been shown to reflect flow alterations elsewhere (e.g. Kennedy et al 2016). We additionally included metrics describing the proportion of different feeding groups (i.e. grazers, shredders, gatherers, filterers and predators) and the proportion of relatively small and large sized (range 0.25-0.5 mm and 20-40 mm, respectively) invertebrates. We focused on feeding traits as they convey information about the functional role of organisms in the ecosystems and on size traits that are a proxy of multiple life-history characteristics like e.g. life cycle duration, longevity (Poff et al., 2006) and may respond to variation in shear stress (e.g. Merigoux & Doledec, 2004). Finally, we also examined how the WFD Star_ICMi index responded to flow characteristics. The Star_ICMi is the official Biological Quality Element used in Italy to classify the status of running water in line with the WFD requirements (Buffagni et al., 2006; Buffagni & Erba, 2007). The index is formulated combining six normalised and weighted metrics, including richness, diversity and taxa sensitivity to organic pollution (Buffagni et al., 2006). Information for functional traits of the taxa was gathered from the online database of freshwater ecology (www.freshwaterecology.info; Schmidt-Kloiber & Hering, 2015). For the calculation of FDis, we included 13 traits (Tab. S2) in order to provide an inclusive measure of functional diversity. Feeding information was available for all taxa included in the analysis, whereas size traits were available for about 50% of the taxa. As such, taxa with no information for a given trait, were not considered in the analyses.

*2.6.1 Spatial stream-network models*

Spatial stream-network models (SSN; Ver Hoef et al., 2014; Ver Hoef & Peterson, 2010) were run separately for each flow regime to quantify the relation between the biotic and hydrologic metrics, while accounting for the autocorrelation structures of the dendritic network. The LIMeco index and Agr.landuse indicator were included as additional covariates in the models. ArcMap 10.5 and the STARS toolset (Peterson & Hoef, 2014) were used to generate the spatial data necessary to analyse stream-network models. The full set of autocovariance functions were used to model spatial autocorrelation, including Euclidean, in-stream flow-connected (locations in which water flows from one to the other) and flow-

unconnected (connected within the network, but not reflecting the directionality of the water flow) functions. This approach allows accounting simultaneously for the along-channel and across-basin (flow-unconnected and Euclidean, respectively) patterns of autocorrelation, while also distinguishing locations linked by direct water flow (i.e. flow-connected). In particular, SSN models take the form:

$$y = X\beta + z_{TU} + z_{TD} + z_{EU} + \varepsilon \quad (1)$$

where $y$ is the response variable (i.e., macroinvertebrate metrics in this study), $X$ is the matrix of predictors (flow metrics, LIMeco, and Agr.landuse) with associated β regression parameters, while $z_{TU} + z_{TD} + z_{EU}$ are zero-mean random variables with autocorrelation structure based on tail-up (TU), tail-down (TD) and Euclidean (EU) functions, respectively, and $\varepsilon$ is the random independent error. The TU and TD functions are moving-averages functions autocorrelated in an upstream and downstream direction, respectively. Tail-up function assigns different weights to locations upstream of a given site according to the catchment area, used here as a proxy of streamflow. In this way, the moving-average autocorrelation is split at confluences so that upstream locations with larger catchments have a stronger influence on downstream communities. The reader can refer to Peterson et al. (2013) and Ver Hoef et al. (2010) for a detailed description of the SSN framework.

Biotic metrics expressed as proportions (i.e. feeding and size traits) were logit-transformed as recommended (Warton & Hui, 2011), and maximum-likelihood was used for parameter estimation in all SSN models. For each biotic metric, the most supported model was selected based on the root mean-square prediction errors (RMSPE), which focus on model predictive power (Ver Hoef et al. 2014). The model was developed in a stepwise fashion, following guidelines provided in Ver Hoef et al. (2014). We first included all predictors (the selected flow metrics, LIMeco, Agr.landuse), and the full set of autocovariance functions (i.e. tail-up, tail-down and Euclidean). Then we manually removed non significant predictors and subsequently refined the spatial components. These can be defined by considering different autocovariance functions, including e.g. exponential, Mariah, spherical, linear-with sill, though spatial stream-network models appear little influenced by their mis-specification (Garreta et al. 2009; Isaak et al. 2014). We compared or removed different functions for the Euclidean, tail-up and tail-down components and selected the final model with the lowest RMSPE. If different models had identical RMPSE values, the most parsimonious solution

was selected based on Akaike Information Criterion (AIC). The spatial autocovariance functions were refined after the selection of the model predictors, since the model accounts for spatial correlation in the error term after the effects of the covariates is removed (Frieden et al. 2014).

The SSN package (Ver Hoef et al. 2014) for R software (R Core Team, 2019) was used to run the stream-network models.

## 3. Results

### *3.1 Hydrologic simulations and classification*

The capability of HYPERstreamHS hydrological model to reproduce the observed daily streamflow time series in the Adige River was validated in the period 1991- 2013 by computing NSE at the 9 gauging stations described in Sect. 2.4 (Fig. 1). The parameters of the hydrologic model were inferred by maximizing the average NSE at Vermiglio, Rio Funes, Aurino and Gadera gauging stations. Calibration produced a satisfactory mean NSE of 0.623 (range: 0.58-0.70; Figs. S2 and S3). At the validation stations, mean NSE was 0.620 (range: 0.48-0.79), with lower values in the smaller subcatchments (Saltusio, Isarco, Anterselva; Fig. S2), and higher values at the larger downstream subcatchments of Trento and Bronzolo (0.74 and 0.79, respectively; Figs. S2 and S3). Remarkably, average value and range of variation of NSE efficiency did not deteriorate from calibration to validation sites, suggesting that the model parameters are representative of the entire river basin. The limited reduction of NSE efficiency at Saltusio, Vipiteno, and Anterselva gauging stations is in line with the general understanding that an accurate reproduction of observed streamflows in small catchments would require accurate and spatially well-resolved precipitation and temperature fields at small spatial scales (e.g., Heistermann & Kneis, 2011). A further validation of the hydrologic model emerged from the consistent relationship between taxon richness and the IHA flow metrics across observed and simulated flow data, for the locations where measured streamflow series were available (Fig. S4).

The calibrated model was subsequently used to simulate flow time series for the period 1991-2013 in the 100 reference locations for which biological data were available, and to perform the flow regime classification. Three hydrologic classes with distinct flow regimes were

identified by the flexible beta-clustering of the first two weighted PC scores (explaining 92% of the variation) derived from the scaled monthly hydrographs (Fig. 2). The first hierarchical division separated typical "pluvial" streams (n=38), with peak flow in autumn, from those with spring and summer peaks. The second division further distinguished streams with "nivo-glacial" regime (n=30) with summer peak flows and winter low flows, from intermediate "nivo-pluvial" streams (n=32), with earlier spring peak flows and relatively higher autumn flows. A PERMANOVA based on Euclidean distances further validated the separation among the three groups with $R^2$ = 0.85. Fig. 1 shows the distribution of the flow regime classes in the Adige River network. The three flow regimes were distributed along an altitudinal gradient, which reflects also the gradient of anthropogenic influence in the catchment (Fig. 3). Indeed, pluvial streams at lower altitude were characterised by more eutrophic (higher LIMeco scores) waters and higher proportion of agricultural land-use in the adjacent area.

The three hydrologic classes identified in the previous step formed three groups in the first PCA factorial plane (i.e., the first two PCs) derived from IHA metrics, explaining about 80% of the total variation (Fig. 4). This analysis provided additional evidence of the separation among the flow regimes, and allowed identifying the metrics that differed the most among them. For instance, as also evident from the annual hydrographs shown in Fig. 2, nivo-glacial streams displayed higher flow maxima during summer months (June, July; Figs. 4 and S5) as well as faster fall and rise rates. Conversely, streams with pluvial regime showed higher flow minima (e.g. 30 Day Min) and Base index, but more frequent low flow events (Fig. S5). Nivo-pluvial streams systematically showed flow metrics that were intermediate between the nivo-glacial and pluvial regimes.

The first two PCA axes extracted separately within each flow regime, accounted for 75%, 81% and 76% of variation in IHA metrics across the nivo-glacial, nivo-pluvial and pluvial regime, respectively. The loadings of the IHA metrics on the PC axes are shown in Tab. S1, and were used for a parsimonious selection of non-redundant flow metrics to include in the SSN models. After removing correlated metrics, the selection included: February and July streamflow, Low pulse number, Fall rate and annual CV (y.CV). These metrics are included in the first, fourth and fifth flow components of the IHA classification, respectively (Tab.1). However, February and July flow magnitude were strongly correlated with minimum and maximum flows as well as with the Base index, and thus represented a proxy for the second flow component of the IHA classification, which is related to the magnitude of extreme

events. Metrics describing the timing of extreme flows (third component) were not included as they were not particularly relevant within each flow regime, but rather differed among regimes.

### *3.2 Flow-ecology relationship*

A total of 64 invertebrate taxa were identified, mostly at family and genus level (see Tab. S3). The SSN models identified several significant relationships between biotic and hydrologic metrics and the covariates related to water quality and land-use (Fig. 5; Tab. S4). The relations differed among the flow regimes both in terms of explained variance and selected covariates. Overall, the influence of water quality (LIMeco index) and agricultural land-use on macroinvertebrate communities was also evident, especially for pluvial streams at lower altitude.

Few response variables responded consistently to the hydrologic metrics across flow regimes, and rather, flow-ecology relationships often displayed divergent patterns (Fig. 6). For instance, while taxon richness declined significantly with February flow magnitude in pluvial streams, it tended to increase in the nivo-glacial streams, where instead it declined significantly with increasing July flow. Similarly, while Shannon diversity increased with the LIMeco index consistently across flow regimes, it showed a positive association with the number of low pulses, but only in the pluvial streams. Trait-based metrics also displayed rather unique patterns for each flow regime. The proportion of grazers displayed different relations with February flows across the three regimes. In addition, grazers increased significantly with annual flow variation (y.CV) only in nivo-glacial streams, while the proportion of filter feeders declined with increasing February flow, but only in the pluvial streams (Fig. 6).

Overall, spatial autocorrelation, considering the flow-connected, flow-unconnected and Euclidean dimensions (i.e. $z_{TU}$ , $z_{TD}$ , $z_{EU}$ in equation 1), explained a larger proportion of variance (respectively 50%, 77% and 44% in the nivo-glacial, nivo-pluvial and pluvial streams) than the model predictors ($X\beta$ in equation 1), which explained 13-26% of the variance (Fig. 7). The tail-up and tail-down components (reflecting autocorrelation along the watercourse dimension) explained more residual variance (mean across flow regime: 35.3%) than the Euclidean spatial component (mean: 21.5%).

## *4.* Discussion

In this study we developed and applied a framework to assess the relationship between river ecology and flow characteristics, while overcoming some of the challenges typically associated with flow-ecology research. By employing the HYPERSstreamHS hydrological model, we were able to reproduce natural streamflow time series at 100 ungauged biological sampling stations throughout the Adige River basin. An important validation of the model stems from the consistent patterns of flow-ecology relationships obtained from the observed and simulated streamflow. This is key when hydrological models are used to investigate ecological responses (Kiesel et al., 2020), as the uncertainty associated with modelled streamflow is a fundamental limitation hampering the study of flow-ecology relationship at the pan-European scale (Vigiak et al., 2018). We subsequently identified three distinct flow regime classes within which flow-ecology relationships were examined using spatially-explicit geostatistical approaches. This allowed us to account for the natural variability of flow regimes, while also controlling for the spatial autocorrelation patterns of dendritic river networks.

The three identified flow regimes represent typical hydrologic patterns of the Alpine region. Low-order streams at higher elevation are fed primarily by glacial melt, snowmelt and associated groundwater flow; streams at intermediate elevations by snowmelt and rain, and those at lower elevation mirror rainfall timing. While realistically representing a gradient of conditions, the three regimes were distinct enough to form separate groups according to both mean annual flow series and flow metrics. As such, they showed distinct flow-ecology relationships, especially dependent on high and low streamflow conditions and interannual variability in discharge.

The three flow regimes were also well-separated along a gradient of anthropogenic influence represented by water quality and riparian land-use. This further highlights how classifying regimes can help minimise the effect of confounding factors in flow-ecology research: as streams with different flow regimes often occupy separate sections of the catchment, systematic difference in elevation, anthropogenic land-use and underlying geology may confound the influence of streamflow characteristics, hindering the robust identification of flow-ecology relationships.

Surprisingly, while the classification of flow regimes is a common endeavour in hydrologic research (e.g. Belmar et al., 2011; Di Prinzio et al. 2011; McManamay et al., 2012; Snelder & Booker, 2013), its applications in flow-ecology studies remains relatively rare.

The few studies that have compared flow-ecology relations across classified flow regimes (e.g. Bruckerhoff et al., 2019; Mims & Olden, 2012), indeed showed that ecological responses can often diverge. The three streamflow typology analyzed here, in fact, are characterized by different levels of environmental harshness, which declines with decreasing glacial influence (Brighenti et al 2020). Had we combined all streams in the same analysis, we would have drawn different conclusions regarding the response of some biotic metrics, such as taxon richness and the proportion of grazers (*cfr* grey dashed line with individual fits in Fig. 6). Richness, for instance, appeared limited by different flow characteristics; it declined with increasing July flows in the nivo-glacial streams (representing the summer peak of snow- and glacial-melt), while in the pluvial regime, richness declined with increasing February flows (representing the winter low rainfall periods). Summer discharge is high in nivo-glacial streams, where increasing flows could cause higher drift rates and thus lower the observed benthic richness (e.g. Brittain & Eikeland, 1988, Naman e al., 2015). It is known that in glacial-fed streams at high elevation, invertebrate abundances can be greatly reduced in summer due to the increased environmental harshness (Robinson et al., 2004; Ilg et al., 2001). Conversely, in temperate streams characterized by pluvial regimes, minimum densities often occur in winter (Brittain & Eikland, 1988), when higher-than-average flows could further decrease benthic richness. In the nivo-pluvial and pluvial streams, in fact, the magnitude of February flows appeared to limit the biotic metrics mainly related to community diversity (i.e. richness, Star_ICMi, Fdis). On the other hand, the number of low flow pulses displayed mainly positive association with both diversity and trait based metrics across flow regimes. Low flow pulses can increase local diversity by preventing excessive drift while also favoring the deposition of fine organic matter, as suggested by the positive response of gatherers. Together with the positive association of both grazers and gatherers with the annual coefficient of variation in nivo-glacial streams, these results indicate how natural flow variation is fundamental for sustaining aquatic biodiversity.

The SSN models revealed how the STAR_ICMi index mostly responded to streams' physico-chemical parameters and riparian land-use. In agreement with recent investigations (Larsen et al., 2019; Quadroni et al., 2017), these results provide additional evidence of the limited sensitivity of WFD biological quality indicators, such as the Star_ICMi index, to stream

hydrologic conditions. As most present bioindicators (Friberg, 2014), the Star_ICMi is designed to reflect organic pollution and habitat degradation, and should be used for hydrologic assessment or guide *e-flows* with great caution. Indeed, one of the aims of the present study was to evaluate alternative metrics that could serve as flow-sensitive indicators to be included in assessment schemes like the one of the European WFD. To this end, species life-history traits can provide the mechanistic link between river biota and flow conditions that could be valid across large spatial scales (Heino et al., 2013; Mims & Olden, 2012). In the Adige River basin, the relative proportion of different feeding habits and body size structure appeared relatively sensitive to hydrologic conditions across flow regimes and thus deserve further investigation. The sensitivity of grazers to flow conditions, for example, can derive from their reliance on attached algae, which can be scoured during high flows or buried by fines during low flows (Buchanan et al., 2013; Doretto et al., 2020; Kennen et al., 2010). Nonetheless, our results suggest that their response to flow conditions can differ substantially across flow regimes. The response of body size structure to the magnitude of high and low flows may reflect the sensitivity of different developmental stages to shear stress, especially for insect larvae (e.g. Merigoux & Doledec, 2004), but detailed information on body size was not available for many taxa, and this response requires further examination.

Overall the SSN modelling revealed rather idiosyncratic responses of the biotic metrics to flow conditions. On the other hand, the effect of water quality and local land-use appeared generally consistent with a positive and negative effect of LIMeco and agricultural land-use, respectively. This has both practical and fundamental implications. From an applied perspective, it implies that ecological responses to flow alterations can be contingent on local eco-hydrologic conditions, a caveat which must be considered when setting regional flow standards. In the Adige Basin, for instance, sustaining invertebrate richness, or specific feeding groups, would require a distinct management of high and low flows among the identified flow regimes.

On a more fundamental level, it indicates that the often observed nonlinear flow-ecology relationships (Rosenfeld, 2017) can reflect the distinct response of multiple flow regimes in the basin. More generally, the “shape” of flow-ecology relationships is likely to be scale-dependent and determined by the range of hydrologic conditions in the region and the size of the species pool included.

591 The three identified flow regimes formed geographically separated clusters along a north-
592 south axis, reflecting the orography of the basin. While this further validated our flow regime
593 classification, it also introduced possible autocorrelation issues. The SSN models revealed
594 that most variation in macroinvertebrate metrics was in fact associated with spatial patterns.
595 This is typical for communities dwelling in complex habitats, such as river networks, whose
596 geometry and flow directionality influence environmental and eco-evolutionary dynamics
597 (Frieden et al., 2014; Isaak et al., 2014; Larsen et al., 2019). For some biotic metrics, the
598 spatial autocorrelation was by far the most important component, explaining more than 80%
599 of the variation (e.g. Shannon diversity and gatherers in both nivo-glacial and nivo-pluvial
600 streams). Not surprisingly, given the dendritic configuration of river networks and the
601 directionality of water flow, autocorrelation along the watercourse dimension (i.e. tail-up and
602 tail-down) appeared stronger than over the isometric Euclidean dimension. However, the
603 influence of autocorrelation differed across regimes, being particularly large in the nivo-
604 pluvial streams, where the tail-up flow-connected component accounted for more than 40%
605 of the residual variance. This could be due to the distribution of multiple sampling sites along
606 the same river segments, as occurred in the eastern section of the basin. However, the
607 Euclidean component, which reflects large scale variability across the basin, also accounted
608 for more than 35% of the variance in nivo-pluvial streams. This may be due to the fact that
609 streams with nivo-pluvial regimes were relatively more widely distributed throughout the
610 basin.
611 These findings are in line with a recent study from the US where SSN models were used to
612 examine variation in fish community traits (Bruckerhoff et al., 2018). However, comparison
613 across studies is challenging as the effects of spatial autocorrelation in a given model depend
614 on the response variable and the covariates included, as well as on the spatial relationship of
615 samples. Therefore, spatial effects may vary substantially across regions, but they are likely
616 relevant for flow-ecology studies when these are paralleled with flow regime classification in
617 which sites might be spatially structured (e.g. Bruckerhoff et al., 2019; Snelder & Booker,
618 2013). Ignoring such spatial dependency could lead to increased Type I error rates ("false
619 positive"; Legendre & Legendre, 2012), with important implications for the success of *e-*
620 *flows* design.
621 An additional issue to consider is that, although we selected streams with no evident
622 alteration of flow regime, the influence of other factors on stream invertebrates was evident.
623 The negative effect of agricultural land-use on multiple biotic metrics was evident (e.g.
624 (richness, Star_ICMi), especially in the nivo-glacial and nivo-pluvial streams, while water

quality had the strongest (positive) influence on the lower gradient pluvial streams (e.g. increasing diversity, shredders, predators). These results are not surprising and in line with a recent study that included a larger sample of locations throughout the basin (Larsen et al., 2019). However, this highlights how defining a baseline flow-ecology relationship under natural conditions might become increasingly difficult as river catchments are modified globally (Tickner et al., 2020). In addition, alteration of flow regimes is often accompanied by changes in water temperature and in-stream habitat structure (e.g. Zolezzi et al., 2011). Therefore, non-hydrologic factors must be incorporated in *e-flow* frameworks to identify circumstances that might limit the desired outcome of flow management (Poff, 2018).

At the management level of *e-flows* setting, the present work represents the first data-driven classification of natural flow regimes in Italy that is paralleled by an ecological assessment. Previous catchment regionalisation schemes were produced at the national scale (Di Prinzio et al., 2011), but they focused primarily on estimating streamflow at ungauged sites. Results demonstrated that flow-ecology relationships can substantially vary among flow regimes, highlighting the importance of developing *e-flows* tailored to specific eco-hydrologic contexts. Moreover, although analyses were conducted within a single river basin, and thus minimized the influence of larger-scale confounding factors, spatial patterns accounted for most of the variance in the data. The importance of using spatially-explicit approaches to model empirical data in river networks is increasingly recognised (Frieden et al., 2014; Isaak et al., 2017; Larsen et al., 2019), and our results further support their application in flow-ecology research (Bruckerhoff et al., 2019).

In conclusion, we addressed three main challenges of flow-ecology research derived from: i) the limited availability of streamflow time series at sampling sites, ii) the natural variability of flow regimes and iii) the spatial autocorrelation unique to dendritic river networks. In doing so we also completed the first two steps of the ELOHA framework (Poff et al., 2010), namely developing the hydrological foundation and classifying natural flow regimes across the catchment; to our best knowledge, it is the first time that this is applied to a very heterogeneous Alpine river catchment.

Future developments should address the challenge of incorporating hydrologic variability when setting environmental flows, and of assessing the ecological effects of specific flow events or sequence of events without relying on stationary long-term flow records as baseline reference (Horne et al., 2019; Poff, 2018). The framework presented in this paper could thus be extended to include future climate scenarios to feed the hydrologic model. Simulated

projections of streamflow could then be used to estimate future ecological responses to flow alteration.

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# Tables

**Table 1**- List of the IHA flow metrics computed from the 23 years simulated streamflow time series. Metrics in bold were selected for the examination of flow-ecology relationship in the SSN models.

| Flow component | IHA flow metric name | Description |
|---|---|---|
| *1 - Magnitude of monthly flow conditions (12 parameters)* | January, February, March, April, May June, July, August, September, October, November, December | Mean flow for January, …, December |
| *2 - Magnitude and duration of extreme conditions (11 parameters)* | 1, 3, 7, 30, 90 Day Min | Minimum flow, 1, 3, 7, 30, 90 day mean |
| | 1, 3, 7, 30, 90 Day Max | Maximum flow, 1, 3, 7, 30, 90 day mean |
| | Base index | 7 days minimum / mean flow |
| *3 - Timing of extreme flow conditions (2 parameters)* | min Julian | Mean Julian data of annual 1-day maximum |
| | max Julian | Mean Julian data of annual 1-day minimum |
| *4 - Frequency and duration of high and low pulses (4 parameters)* | Low pulse number | Number of flow events below 25th percentile |
| | High pulse number | Number of flow events above 75th percentile |
| | Low pulse length | Number of days below 25th percentile |
| | High pulse length | Number of days above 75th percentile |
| *5 – Rate of change and variation (5 parameters)* | Rise rate | Median of all positive differences between consecutive values |
| | Fall rate | Median of all negative differences between consecutive values |
| | Reversals | Number of times flow period switches from rising to falling and vice-versa |
| | y.CV | Average annual coefficient of variation (SD/mean) |
| | m.CV | Average monthly coefficient of variation (SD/mean) |

## Figures captions

**Figure 1** - Map of the Adige River network (northeast Italy) showing the locations of the 100 biological monitoring sites for which 25 years of natural streamflow time series were simulated. Colours define the distribution of the three identified flow regime classes (see Sect. 2.4). Location of the gauging stations providing observed streamflow series are also shown, including those used for calibration (c) and validation (d) of the hydrological model.

**Figure 2** - Dendrogram of the study reaches based on flexible beta-clustering of the first two weighted Principal Components (explaining 92% of variation) of the MAD-normalised monthly hydrography (expressed as proportion of mean annual discharge). Lower panels show the mean across sites (± SD) of MAD-normalised hydrographs for each identified streamflow regime.

**Figure 3**- Boxplot of selected environmental descriptors for each identified streamflow regime. The following boxplot representation is adopted: horizontal line for median; box for the inter-quartile range; whiskers for 1.5 times the inter-quartile range; dots for outliers. The LIMeco index and the percentage of local agricultural land-use (Agr.landuse) were included as covariates in the SSN models.

**Figure 4** - Biplot of the PCA based on 34 IHA flow metrics (direction and loading indicated by the blue arrows) derived from the 23 years streamflow series for the 100 investigated study sites. The sites are grouped according to the streamflow regime previously determined by the flexible beta-clustering approach.

**Figure 5 –** Heatmap showing the standardized SSN coefficients ($\beta$) of the covariates included in the most supported models for each biotic metric in the three flow regimes, according to the RMSPE. '*NS*' indicates a non-significant predictor ($p > 0.05$).

**Figure 6** – Scatterplot of selected flow-ecology relationships within each flow regime. Continuous and dashed fit lines respectively indicate significant and non-significant relationships according to the SSN models. Grey dashed line indicates the overall relationship observed combining all three flow regimes.

**Figure 7** – Proportion of variance of each biotic metric explained by the covariates (i.e. flow metrics, water quality and local land-use) and by the spatial components modelled with tail-up, tail-down and Euclidean autocovariance functions across the three flow regimes. The nugget represents the residual variance.

**Figure 1.**

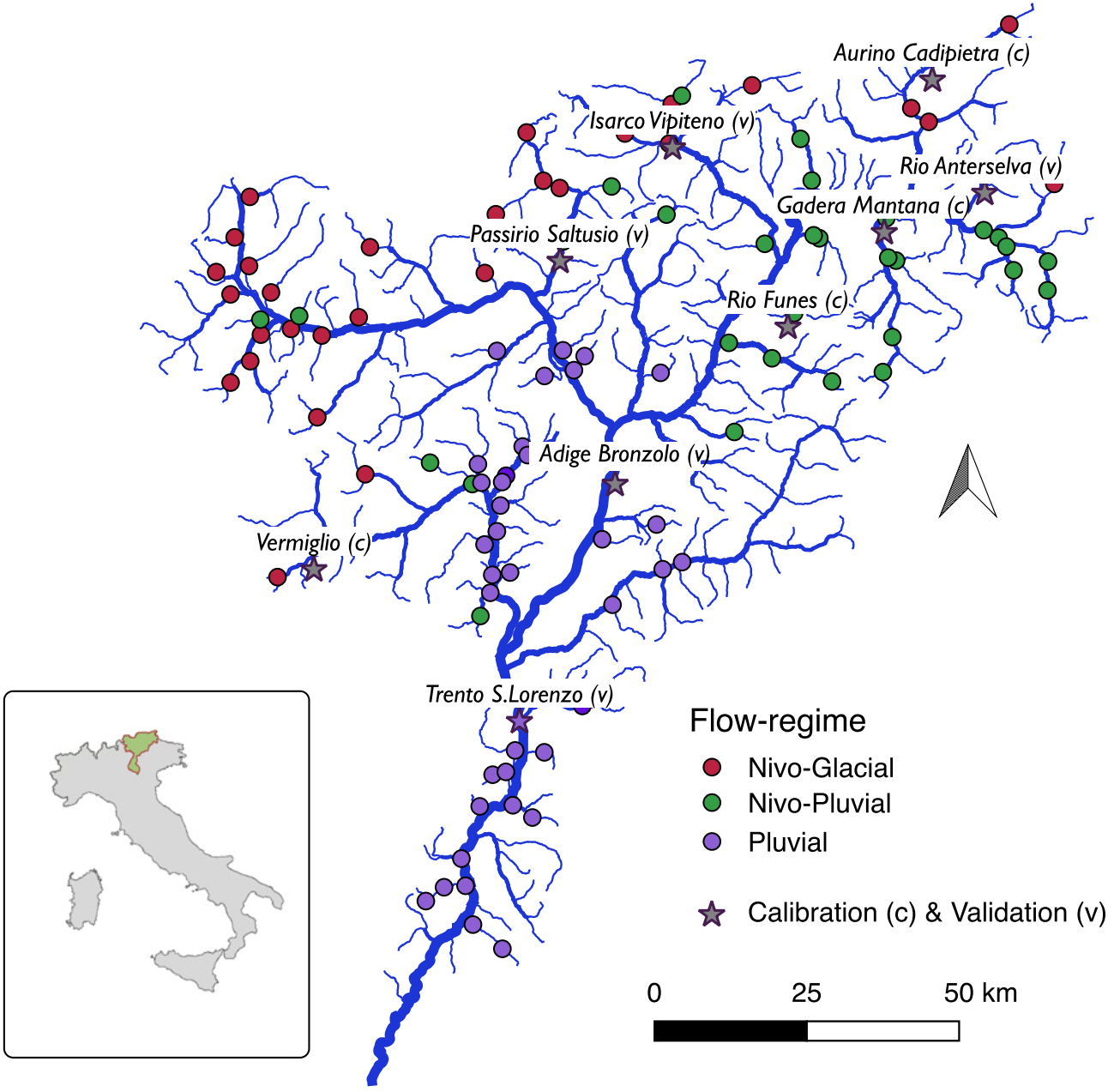

Aurino Cadipietra (c)
Isarco Vipiteno (v)
Rio Anterselva (v)
Gadera Mantana (c)
Passirio Saltusio (v)
Rio Funes (c)
Adige Bronzolo (v)
Vermiglio (c)
Trento S.Lorenzo (v)
Flow-regime
Nivo-Glacial
Nivo-Pluvial
Pluvial
Calibration (c) & Validation (v)
0
25
50 km

**Figure 2.**

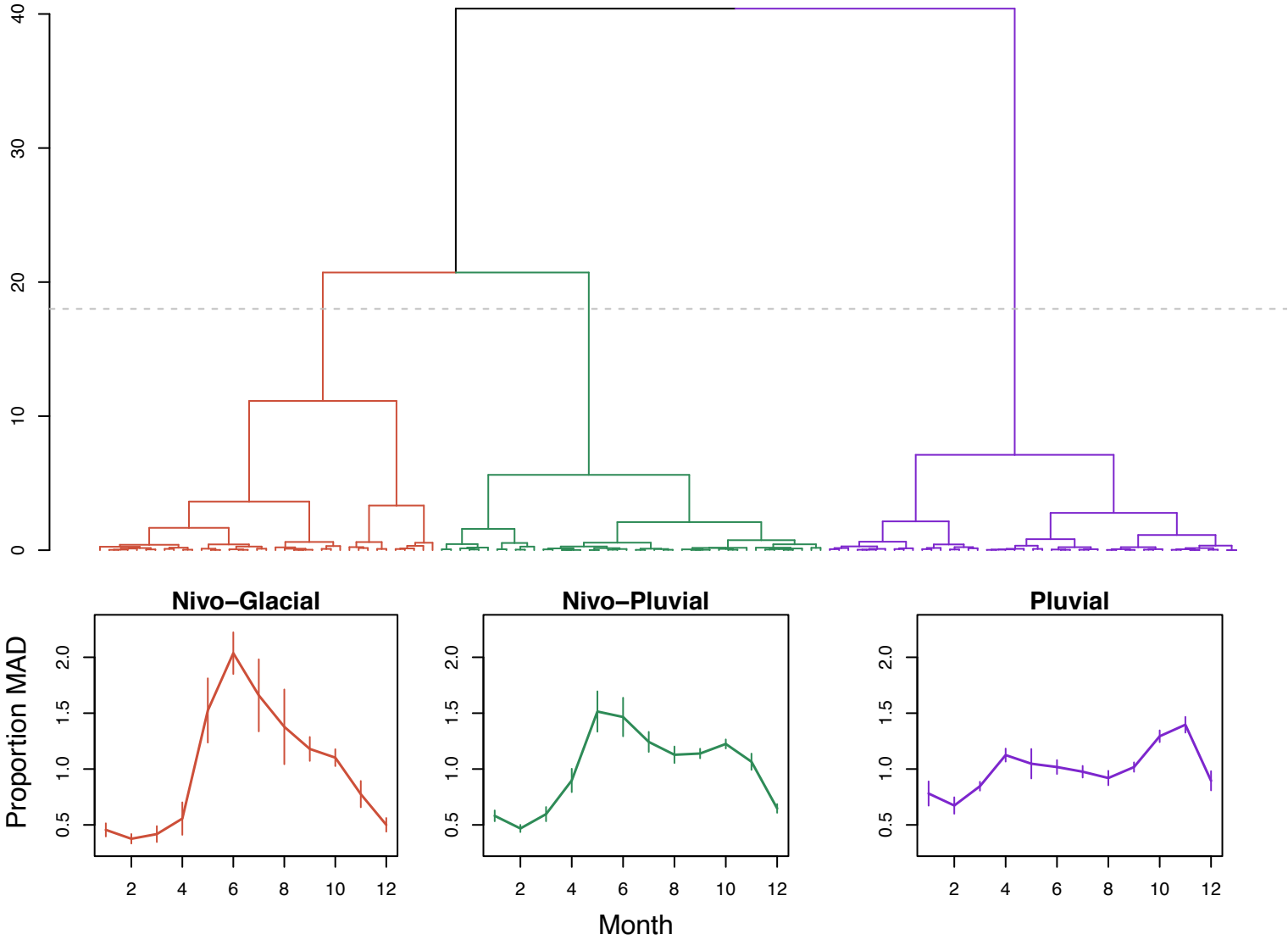

0
10
20
30
40
Nivo–Glacial
Nivo–Pluvial
Pluvial
Proportion MAD
0.5
1.0
1.5
2.0
2
4
6
8
10
12
Month

**Figure 3.**

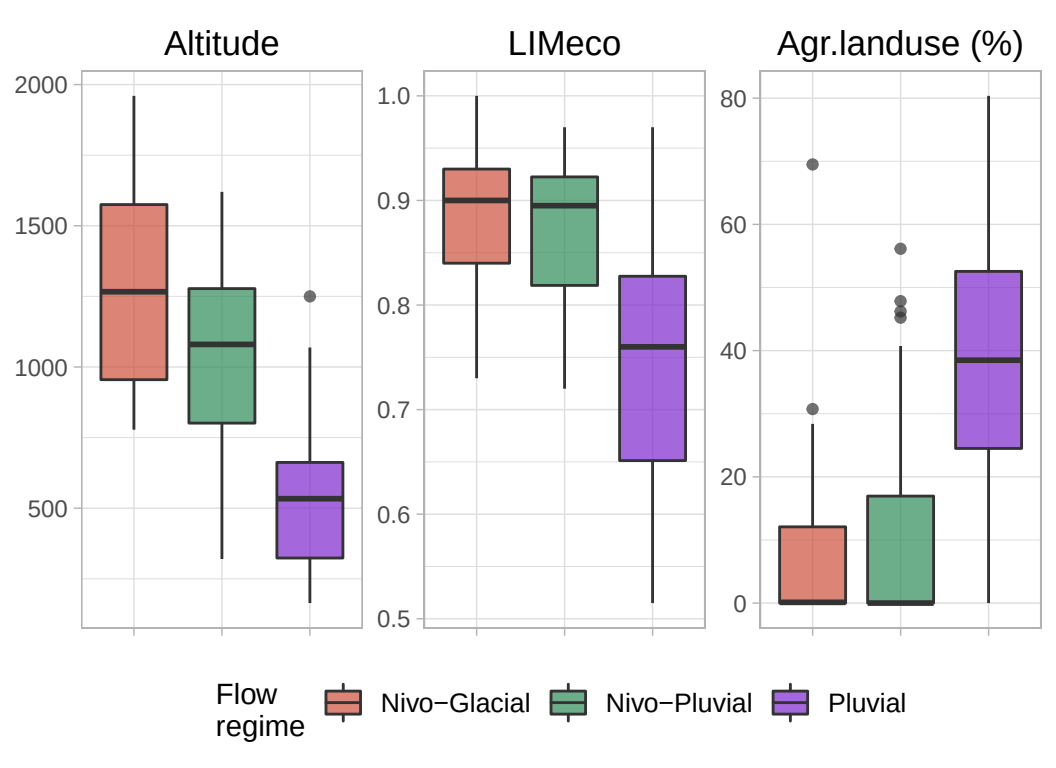

Altitude
LIMeco
Agr.landuse (%)
2000
1500
1000
500
1.0
0.9
0.8
0.7
0.6
0.5
80
60
40
20
0
Flow regime
Nivo−Glacial
Nivo−Pluvial
Pluvial

**Figure 4.**

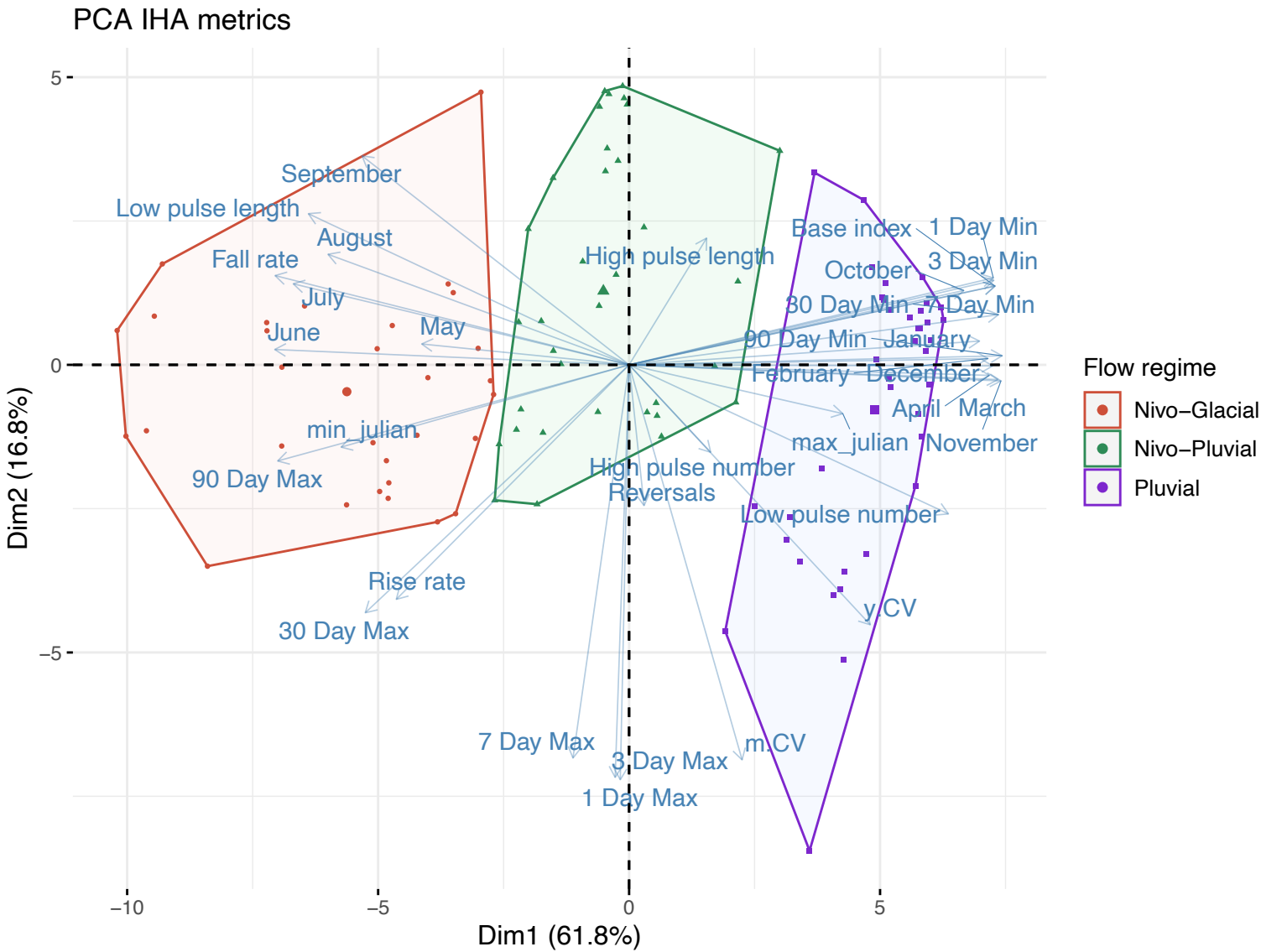

PCA IHA metrics
Dim2 (16.8%)
Dim1 (61.8%)
5
0
−5
−10
−5
0
5
September
Low pulse length
August
Fall rate
July
June
May
min_julian
90 Day Max
Rise rate
30 Day Max
High pulse length
High pulse number
Reversals
7 Day Max
3 Day Max
1 Day Max
Base index
1 Day Min
October
3 Day Min
30 Day Min
7 Day Min
90 Day Min
January
February
December
April
March
max_julian
November
Low pulse number
y.CV
m.CV
Flow regime
Nivo−Glacial
Nivo−Pluvial
Pluvial

**Figure 5.**

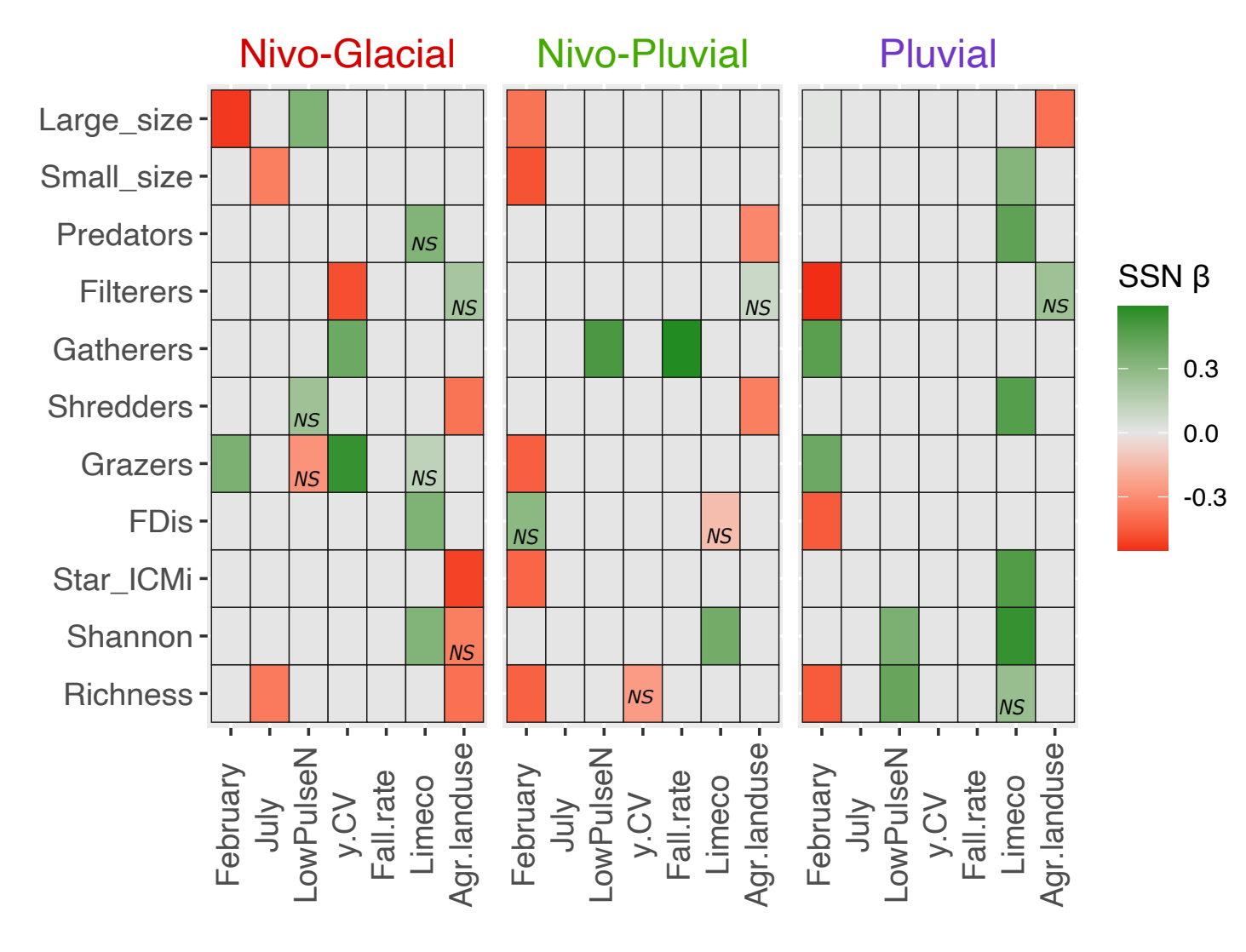

Nivo-Glacial
Nivo-Pluvial
Pluvial
Large_size
Small_size
Predators
Filterers
Gatherers
Shredders
Grazers
FDis
Star_ICMi
Shannon
Richness
NS
February
July
LowPulseN
y.CV
Fall.rate
Limeco
Agr.landuse
SSN β
0.3
0.0
-0.3

**Figure 6.**

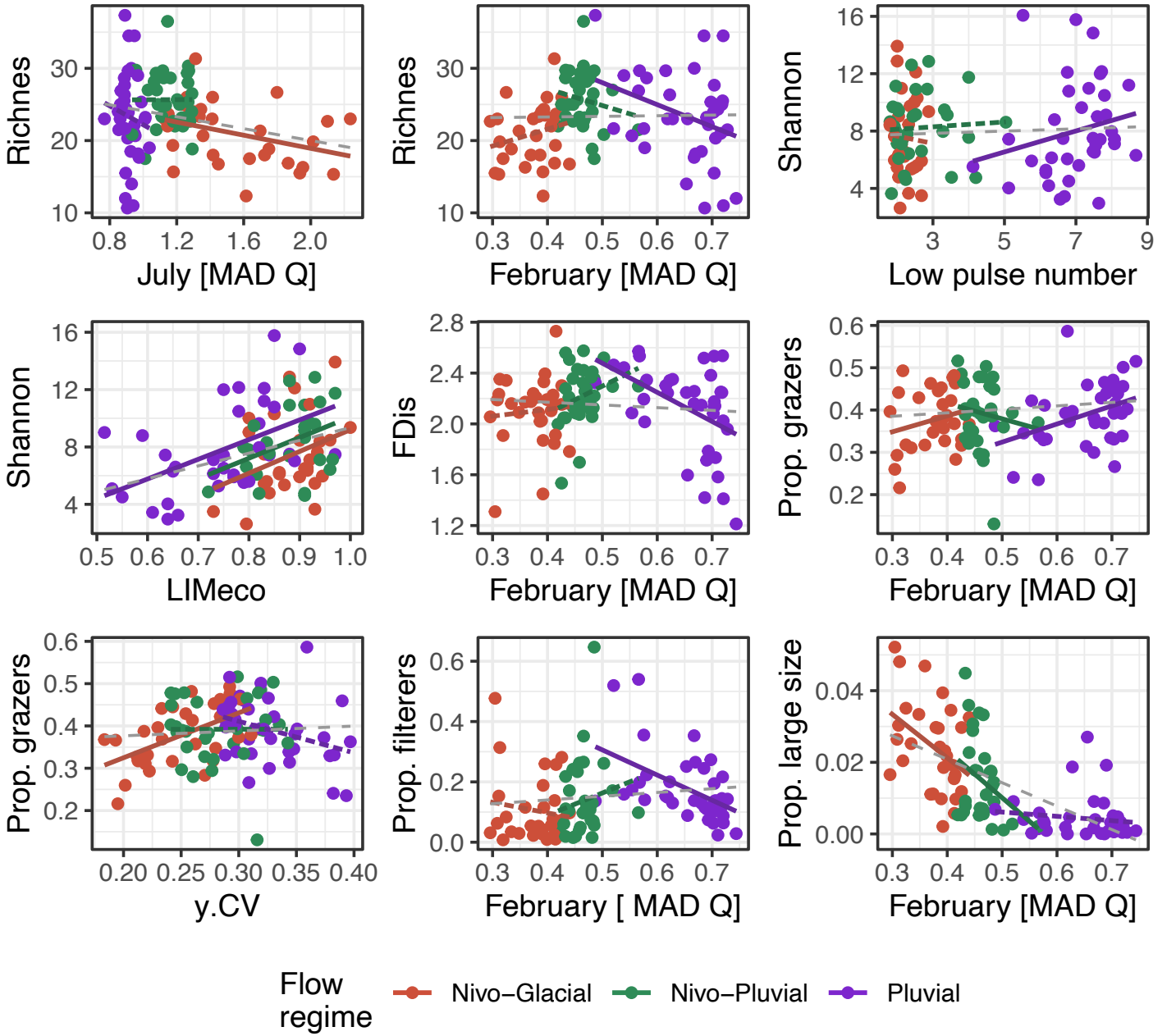
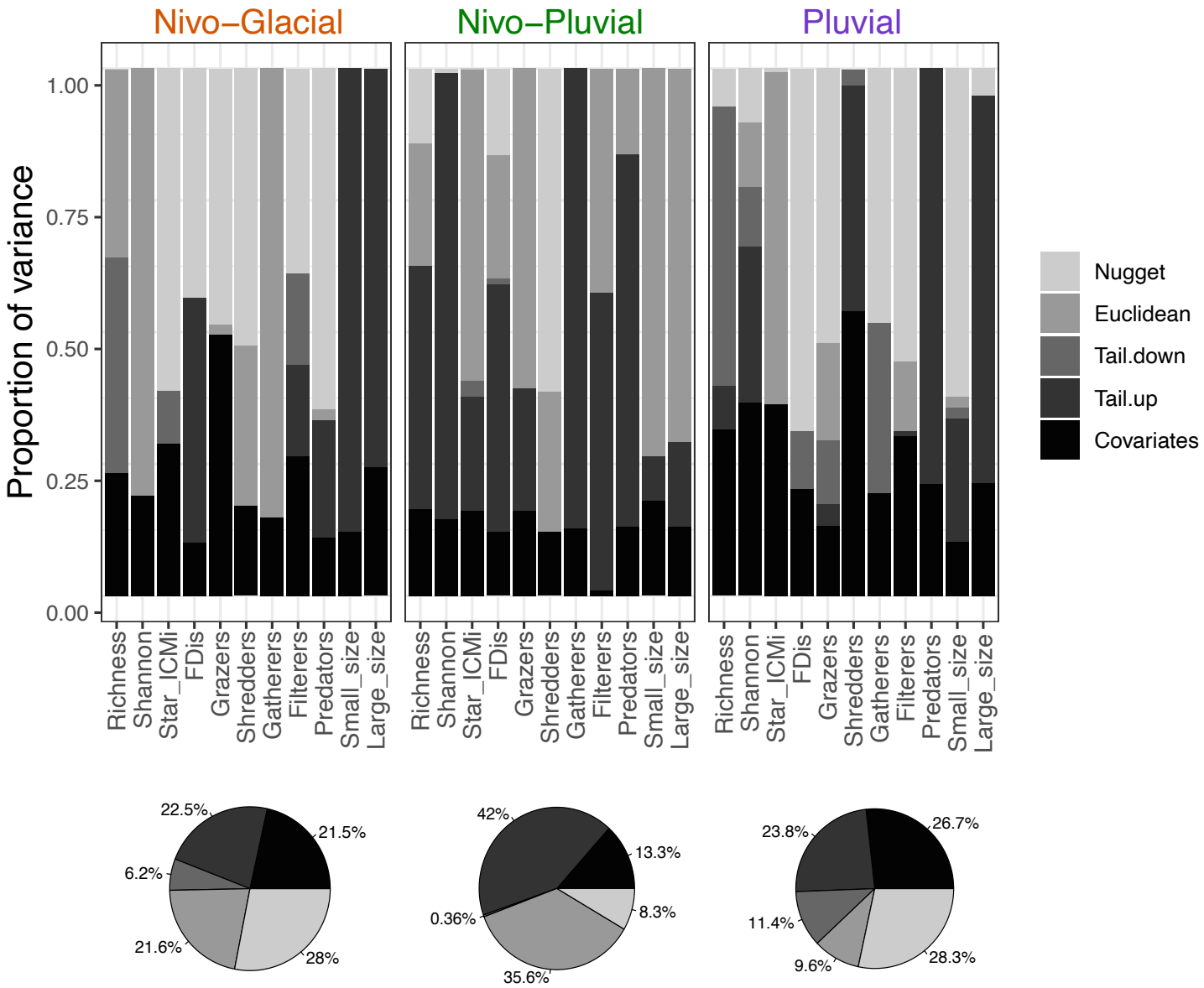

Richnes
July [MAD Q]
Richnes
February [MAD Q]
Shannon
Low pulse number
Shannon
LIMeco
FDis
February [MAD Q]
Prop. grazers
February [MAD Q]
Prop. grazers
y.CV
Prop. filterers
February [ MAD Q]
Prop. large size
February [MAD Q]
Flow regime
Nivo−Glacial
Nivo−Pluvial
Pluvial

**Figure 7.**


Nivo−Glacial
Nivo−Pluvial
Pluvial
Proportion of variance
1.00
0.75
0.50
0.25
0.00
Richness
Shannon
Star_ICMi
FDis
Grazers
Shredders
Gatherers
Filterers
Predators
Small_size
Large_size
Nugget
Euclidean
Tail.down
Tail.up
Covariates
22.5%
21.5%
6.2%
21.6%
28%
42%
13.3%
0.36%
8.3%
35.6%
23.8%
26.7%
11.4%
9.6%
28.3%