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Sub-basin and temporal variability of macroinvertebrate assemblages in Alpine streams: when and where to sample?

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ABSTRACT

The seasonal dynamics and spatial variability of macroinvertebrate assemblages in Alpine streams are becoming better understood. However, the implications of this knowledge for indices used in monitoring programs have yet to be fully considered. Establishing the ecological status of such streams using macroinvertebrates may then be difficult if what is expected to be found, and where, varies across small distances between streams at a given altitude, or between years in response to different climatic characteristics. In this paper, we evaluated the degree of spatial variability in macroinvertebrate abundance and diversity for 14 tributaries at similar altitude that feed a 6-km reach of an Alpine stream, and how this spatial variability differs within and between years. Environmental variables and macroinvertebrates were sampled on 10 dates for assessment. The data showed that differences in environmental characteristics over relatively short distances lead to between tributary differences in the "windows of opportunity" and hence when a tributary should be sampled for monitoring purposes. Superimposed on this spatial variability was inter-annual variability linked to climate differences that shifted this "window of opportunity" in time, and thus altered when monitoring was optimal for any one tributary in any one year. If biological indices are not rendered sensitivity to these variations, the results obtained may reflect more natural variability than possible human impacts.

Aquatic ecology; climate change; spatial variability; bioassessment; ecological index, intermittent streams

INTRODUCTION

It is well established that human activities have modified the instream habitats of many rivers and streams through changing hydrology (Poff et al., 1997), water quality (e.g. temperature, organic matter availability) and river morphology. Such modifications may lead to the degradation of ecological quality (Poff et al., 1997; Hart & Finelli, 1999; Bunn & Arthington, 2002; Poff & Zimmerman, 2010). For this reason, substantial attention has been given to quantifying the extent of stream degradation: (1) to prioritise mitigation measures and improve ecological status; and (2), although less common (Wohl et al., 2015), to determine the success of those measures. For decades, ecological degradation has been expressed in terms of changes in the biological status of streams, and measured using freshwater flora, macrozoobenthos and fish. Benthic macroinvertebrates have been a particular focus (Sandin & Hering 2004; Dohet et al., 2008; Bo et al., 2016). As a result of natural, between-stream variability in flora and fauna, measured biological status also must be compared to a reference state, the latter a measure of what might be expected in a non-, or partially-degraded state. For this reason, stream typologies have been developed that describe what would be expected in a given stream based on reference conditions (e.g. Wright, 1995; Reynoldson et al., 1997; Brown et al., 2003; Clarke et al., 2003; Adriaenssens et al., 2007; Brown et al., 2009; Feio & Poquet, 2011).

Reference conditions may be complicated by natural variability in space or time (e.g. Ghetti & Bonazzi, 1977; Rico et al., 1992; Charvet et al., 1998; Sandin & Johnson, 2000; Pinto et al., 2004; Sandin & Hering, 2004; Aroviita et al., 2010). How streams are sampled spatially is important because habitat and associated macroinvertebrate communities can vary within a stream over quite small spatial scales (meters or 10s of meters) (Keizer-Vlek et al., 2012; Leitão et al., 2014; Burgazzi et al., 2017). If characterisation of ecological status is required over larger scales, then sampling protocols should be adapted to the range of stream habitats present in the landscape; e.g., inter-tributary differences in environmental characteristics within a basin (Li et al., 2001). Similarly, the time when samples are obtained can be important. For instance, in some cases (e.g. Lorenz & Clarke, 2006, but see Sánchez-Montoya et al., 2009) season can have a major influence on macroinvertebrate community composition (Armitage et al., 1995; Clarke et al., 2002).

These points emphasise that careful attention must be given to the definition of the reference condition, something that may have a major impact on whether or not ecological status is defined as degraded (Raven et al., 2002). In Alpine streams, these issues may be more complicated because of strong environmental gradients that influence macroinvertebrate assemblages. For instance, the balance between kryal, nival and krenal contributions to flow and their variation through time can differ greatly between nearby streams within a basin and macroinvertebrate assemblages may reflect this variability (Milner & Petts, 1994; Ward, 1994; Füreder, 1999; Malard et al., 1999; Ward et al., 1999; Füreder et al., 2001; Brown et al., 2003). This balance has been conceptualized as producing “windows of opportunity”, periods when conditions are most optimal for flora and fauna (see Uehlinger et al., 2010) and which may reflect inter-species differences in adaptation to cold environments (Robinson et al., 2001).

Environmental conditions can vary inter-annually, altering the timing of these “windows of opportunity” among streams as a result of climatic variability, and thus when sampling should occur. There are few systematic studies of the temporal variation (seasonality) of Alpine stream fauna (e.g. Brown et al., 2006) and little guidance as to when to sample. Annex 1, for example, shows when researchers have typically sampled Alpine streams. There is surprising inconsistency. There are very few studies that have included any winter (December through February) or spring (March through May) sampling (exceptions include Robinson et al., 2001 and Zah et al., 2001). The most popular sampling month is September (e.g. Lods-Crozet et al., 2001; Robinson et al., 2001; Malard et al., 2003; Ilg & Castella, 2006; Brown et al., 2007; Lencioni et al., 2007; Bizzotto et al., 2009; Maiolini et al., 2011). Further, very few studies have measured the same stream in the same month for more than one year (e.g. Lodz-Crozet et al., 2001; Snook and Milner, 2001; Brown et al., 2007).

The above discussion indicates the need to think carefully about how to sample (where and when) Alpine stream macroinvertebrates. Such sampling should be the basis of identifying more precisely the reference conditions for such streams; an aspect necessary to evaluate human impacts on mountain stream ecosystems (e.g. flow regulation for hydropower). This study aims to fill this gap by testing two hypotheses. (1) There is substantial variability in macroinvertebrate composition between similar altitude sites in tributaries of the main stem of an Alpine stream, and that this variability is related to tributary characteristics. (2) _There is inter-annual variability in macroinvertebrate composition at the same site as a result of inter-annual climatic variability. The hypotheses were addressed by (1) quantifying the extent of macroinvertebrate community differences between tributaries with different physical characteristics; and (2) by sampling tributaries across all seasons of the year, repeated at least twice in different years at each sample site, to evaluate how community composition changes as a function of time.

MATERIALS AND METHODS

STUDY SITE

The study was conducted in tributaries of a glacier-fed stream, the Borgne d'Arolla in southwest Switzerland. The Borgne flows in a glacial valley and is fed by a series of both glacial and nival tributaries (Figure 1a). The climate in the area is temperate, with cold, fairly wet winters (Arnold, 2005) and warm, relatively dry summers, mainly due to the effects of the southern Alpine divide as a topographic barrier against precipitation from the south and west (Micheletti et al., 2015). Annual precipitation is generally between 900 and 1300 mm and mean annual air temperature at 2000 m was 3.4°C for the period 1991 to 2014 (Micheletti et al., 2015, based on Meteosuisse, 2014). The average monthly temperature and the total monthly precipitation are shown for Evolène (Meteosuisse, 2018), at about the same altitude (1800 m) as the study area and very close (about 8 km) (Figure 1b). Glacier-melt, snowmelt water, groundwater and direct precipitation contribute to runoff according to the tributary under consideration (Petts & Bickerton, 1994). The main tributaries (notably those of the Haut Glacier d'Arolla, and Glaciers of Tsijiore Nouve, Pièce, Aiguilles Rouge, Bertol and Douves Blanches) are heavily modified by flow abstraction for hydroelectric power production. Thus, in this study, we focused on those tributaries unaffected by flow abstraction, with only one abstraction-influenced tributary retained.

Geologically, the study area is part of the Dent Blanche nappe, gneiss and granite series (Stampfli, 2015). The Borgne flows in a glacially-incised valley, fed by steep tributaries that cross morainic and related glacial deposits and rockfalls. Some of the tributaries cross alluvial fans before they enter the Borgne. Vegetation is generally pioneer or secondary successional species (shrubs, larch (*Larex* sp.), Alpine grasses, etc.) (Stampfli, 2015). In this region of the European Alps, the current tree line is between c. 2000 and 2100 m. Above this altitude, to about 2400 m, land use is almost entirely extensive but low intensity summer pasture. Above 2400 m, land cover is mainly weathered debris, with occasional permanent snow packs, rock glaciers or small glaciers. The main differences between the tributaries relate to (1) their altitudinal distribution, (2) geomorphic differences (e.g. in slope), (3) geological differences, and (4) extent of glacial influence. The altitudinal range within the basin contributing to each tributary controls the amount of precipitation, the tributary-integrated depth of accumulated snow and the timing of snow melt, stream temperature and the percentage of each tributary below and at tree line, and hence organic matter production.

DATA COLLECTION

A total of 14 tributaries were considered (Figure 1), with a maximum longitudinal distance of 1.5 km between them. Each tributary was sampled at the same altitude, between 1800 and 2000 m. After a winter field visit, we identified 7 tributaries that were permanent (T05, T08, T09, T10, T11, T12, T13 in dark blue) and 7 that were temporary (T01, T02, T03, T06, T07, T14, T15 in purple) (Figure 1) and either completely frozen or dry during winter. T01, T02, T06, T07 and T15 are glacial streams (fed by rock glaciers or small glaciers). None of the tributaries has a gauging station along its stream except T04, which has most of its flow extracted for hydropower flow generation and hence no water or instream life; it was not considered in the current study.

Sampling stations were located as close as possible to the tributary junction with the Borgne without being influenced by flow and/or sediment transport in the Borgne. A sampling station was defined as corresponding to a representative section of the river whose length is 10 times the mean width of the wetted bed of the stream (OFEV, 2010). Except for periphyton, where samples were taken only on 9-10 August 2016, and parameters that do not vary as a function of time (e.g. altitude and slope), all other parameters were measured on the dates shown in Table 1, thereby providing a comparatively high frequency data set. To help ease of reading, only the months are used to describe sample dates , for example “May” means “data collected on 12-13 May”.

PHYSICAL MEASURES AND PERIPHYTON SAMPLING

Velocity (m s^{-1}), temperature (°C), electrical conductivity (µS cm^{-1}), wetted width (m) and water depth (m) were measured inside a predetermined representative reach of each stream. Temperature was measured in the middle of a profile and conductivity was measured as an indicator of dissolved load and hence captures geological differences between tributaries. These parameters were measured using a ProfiLine Cond 3110 multi-parameter sampler. Velocity was measured with an electromagnetic Valeport BFM001 & 002 Open Channel Flow Meter in the middle of each stream at 40% of the flow depth, corresponding to the approximate mean velocity within a water column (Richards, 1982). Depth and velocity were measured at 10 equally defined intervals within a study reach. The percentages of coarse (> 64 mm) and fine (< 2 mm) sediment were classified using a standard protocol (OFEV, 2010).

Mean slope (%) of each stream was estimated from a SwissTopo© altimetric Digital Elevation Model (DEM) (0.5 m resolution). The altitude of the measuring station and mean altitude of each stream were determined from the DEM. The length of each stream was estimated from the origin of flow (considered as the extremity of the glacier for glacial streams and as the first detectable water source according to the SwissTopo© Swiss Map (1:10'000)) until its junction with the Borgne. With the support of the SwissTopo© Swiss Map (1:10'000), the percentage of forest cover directly connected to each tributary also was calculated. All information was obtained using the GIS software ArcGIS.

Periphyton was sampled on 9-10 August 2016 because summer conditions are thought to be less influenced by disturbance in Alpine tributaries (Rott et al., 2006). Collection was based upon quantitative sampling of a predefined area (3.5 cm diameter cylinder) on the upper surface of a stone (Robinson et al., 2001). Five stones in each tributary were selected, chosen to be representative of the heterogeneity of the system. Samples were stored in anti-UV bags on ice. Half of each filter was used for determination of chlorophyll-a by HPLC (Uehlinger, 1991), and the other half was used for determination of periphyton AFDM (Uehlinger, 1991). Lastly, the autotrophic index (AI) was expressed as the ratio of heterotrophic to autotrophic production, based on dividing the AFDW (mg cm^{-2}) values by the CHL (mg cm^{-2}) values (Wu, 2017).

MACROINVERTEBRATE SAMPLING

Macroinvertebrate sampling followed Swiss IBCH guidelines (see OFEV, 2010). The IBCH recognises that altitude will determine when a stream is likely to be suitable for life. It sets temporal windows according to broad altitudinal bands based on the lag of temperature with elevation (0.6°C/100m) and corresponding theoretically to macroinvertebrate life stages. Insect emergence, egg laying and early development typically occur during the summer melt period in non-glacial streams, while glacial stream populations must adapt life cycles to peak flows, high suspended sediment delivery and large temperature variations (Füreder et al., 2001; Saltveit et al., 2001; Brown et al., 2015). A single sample per year is compulsory with an optional window for sampling also proposed (Figure 2), depending on study aims. Hydrologically stable periods must be used and buffer periods are imposed to allow for early or late onset of suitable living conditions due to climatic variability.

After sampling a section of river, the organisms, classified by families, are divided into 9 indicator groups (GIs) according to their sensitivity to water quality, where class 9 represents the more sensitive taxa (OFEV, 2010). Based on the indicator group and taxonomic diversity, the index is subsequently projected on a scale from 1 to 20, representing 5 levels (bad, poor, average, good, very good) where 20 indicates the best quality of a stream (OFEV, 2010). Macroinvertebrates were collected using a kick-sampling method with a 25x25 cm kicknet and a net mesh size of 1 mm. The acquisition method depended on the type of substrate. The Swiss IBCH recognises 11 substrates listed in descending order of habitat suitability, where 8 must be selected to allow the collection of almost 95% of the taxa present at a station (Verneaux, 1982; Agence de l'eau, 2000).

Because of particular conditions related to the altitude of sample sites, only some habitats were present in the field, with only three substrate types commonly present at > 5% (minimal cover to be considered as representative) and thus selected: large stones (> 250 mm), mixed pebbles (250 mm > Ø > 25 mm) and finer sands (Ø < 2.5 mm), with or without organic matter (according to season). For each substrate category, sampling was conducted for the velocity class where the substrate was most represented, remembering that the higher the width and depth of water, the higher the diversity and surface area of habitats, and the likely the richer the system (Newson & Newson, 2000). A current speed between 0.25 to 0.75 ms^{-1} is an important habitat characteristic as it allows good oxygenation of the environment without transporting material downstream (OFEV, 2010). This methodology was validated by a cantonal specialist in macroinvertebrate surveys. The collected macroinvertebrates of the three samples were combined and roughly sorted into: (1) stoneflies, Plecoptera; (2) caddis flies – Trichoptera; (3) mayflies – Ephemeroptera; (4) Diptera; and (5) others, and preserved in 97% ETOH. This produced 140 replicate samples (14 tributaries (with 3 undifferentiated/mixed substrates) and 10 months of sampling). The collected animals were identified using a binocular microscope and determined using Tachet et al. (2010) to at least family level.

DATA ANALYSIS

Statistical analyses were completed within the *R* system (R Development Core Team, 2017) *ade4* and with the library *ade4TkGUI* (see http://pbil.univ-lyon1.fr/ade4/ and Thioulouse et al., 1997).

First, a Principal Component Analysis (PCA) (Eklöv et al., 1999) was performed for the data from August 2016, as this month included periphyton measures as well, to sort tributaries along a gradient of (dis)similarity and to bring out the dominant drivers explaining this gradient (Dray et al., 2014). The method identifies the components that maximise variance (Principal Components or PC). Eigenvalues (variance among the scores) over 1 were kept (Eklöv et al., 1999). Each original variable was correlated with the retained principal components. A *critical r* threshold was calculated to determine significant correlations based on a two-tailed Pearson correlation to obtain 95% significance or confidence in the variables used to explain retained components. This means that each PC (or axis) was defined by the combination of the physical drivers that were significantly brought out by the PCA and which explained the spatial repartition of the tributaries along the gradient in question.

Second, the biotic measures were standardised. Macroinvertebrates were listed at the family/taxa level to insure comparison at the lowest common level. Following standard protocols in Switzerland (OFEV, 2010), the faunistic indicator group must be represented by at least 3 individuals, and 10 for the taxa of Limnephilidae, Ephemerellidae, Baetidae, Caenidae, Elmidae, Gammaridae, Chironomidae, Asellidae, and Oligochaeta, to take into account the influence of organism drift (OFEV, 2010). If this number is not reached, the cell is entered as null, i.e. as containing zero individuals. Respective abundances of each taxon by tributary were then transformed by log(x+1) to avoid extreme spreading and null values. A NMDS (Non-metric MultiDimensional Scaling) was performed to sort the tributaries along a gradient of (dis)similarity according to taxa composition and abundance, i.e. diversity and richness.

Third, an ordination was conducted. Here, the values of each tributary along the respective dominant PCA axis are positioned in one, reduced dimension along each principal component; i.e. a coordinate is given to each site along each axis and arranged by similar sites in terms of physical drivers and biotic communities (Goodall, 1954; Pielou, 1984; Ter Braak, 1987; Dray et al., 2014). A coefficient of determination, R^2, was determined between the two ordinated axes (ordination of PCA vs ordination of NMDS) to show the relationship between taxa abundance and physical measures among sites (Digby & Kempton, 1987; Dray et al., 2014; Bailly & Carrère, 2015). These analyses provided a general overview of the spatial variability of tributaries within the catchment.

Fourth, to consider macroinvertebrate community change over time, the Bray-Curtis Dissimilarity Index was calculated from log(x+1) abundances, and results represented by a dendrogram (Bray & Curtis, 1957; Thioulouse et al., 1997; Legendre & Legendre, 1998). A value of 0 indicates a perfect similarity between two stations (same taxa and abundances), while a value of 1 indicates a null similarity. This index was used to define population patches; i.e. taxa sharing the same habitats present in different tributaries and to compare biological dynamics over time according to:

$$BCjk = 1 - \frac{2\sum_{i=1}^{p} \min(N_{ij}, N_{ik})}{\sum_{i=1}^{p} (N_{ij} + N_{ik})} \quad [1]$$

With N_{ij} = abundance of a taxa *i* in the sample *j* and N_{ik} = abundance of a taxa *i* in the sample *k* (Bray & Curtis, 1957). Taxa of each tributary in each month were compared from May to October 2016. In addition, a comparison of the data for August and October was performed for 2015/2016. Samples without individuals were deleted to avoid the group of similarity equal to zero. The node level of 6 in the dendrogram was chosen as a relevant level to obtain broadly representative groups/patches of trends displaying group similarities. While all tributary values were compared, a graphical distinction was established to highlight differences between intermittent and permanent streams.

Lastly, the Shannon index (H) was calculated:

$$H = -\sum_{i=1}^{s} p_i \ln p_i \qquad [2]$$

where p_i is the proportion of individuals of one particular taxon (n) divided by the total number of individuals (N) and s the number of taxa of the sample. By taking into account the number of individuals per taxa, it allowed comparison of sites that included wide variability in taxa and numbers of individuals (Shannon & Weaver, 1949; Peet, 1974). Maximum diversity occurs when the proportion of individuals is the same for all taxa. Taxa with fewer than 3 to 10 individuals were excluded, which represents between 2% and 8% of the initial sample and the elimination of Empididae, Thaumaleidae and Tipulidae taxa. Boxplots (quartiles, median, mean) were used to display biodiversity results.

To test the significance of comparing independently the tributaries as two groups (temporary and permanent), a two-way ANOVA (analysis of variance) test was performed. This test allowed comparison of the mean differences between groups along two independent factors (McDonald, 2014). The two-way ANOVA test was applied with replication to assess whether there is an interaction between the two independent variables (the groups of tributaries) (McDonald, 2014). If the F statistics (test of the variance of means) is higher that its critical value the null hypothesis can be rejected and the independence of the two groups can be confirmed (see McDonald, 2014 for methodological details).

RESULTS

RELATIONSHIPS BETWEEN PHYSICAL PARAMETERS AND SPECIES ABUNDANCE IN AUGUST 2016

Annex 2 details the measured physical parameters in August 2016 that were used as basis for the PCA. All tributaries had a mean velocity <0.8 ms^{-1}, ranging between 0.3 and 0.6 ms^{-1}, and linked to local slopes between 2 and 18%, except T07 and T09 where cascades were present. T01, T02 and T03 had a mean temperature >9°C, whilst all others were between 5.0 and 7.3°C. Mean basin altitude was between 2020 (T13) and 2410 m (T03). Although sites were all within a 200 m altitude range; there was some association between altitude and parameters such as conductivity. This pattern was because of a weak negative trend in site altitude from upstream to downstream along the 6 km main stem of the stream, corresponding to a small temperature gradient (about 1.3^{o}C), but which was correlated with other variables that have systematic spatial variability such as geology, soil composition, periphyton (autotrophic index) and water origin. Forest cover varied from 0 to 64%, with higher cover found in the downstream part of the catchment, regardless of stream length. Permanent streams were predominantly at lower altitude than temporary ones, except for T14 and T15 (glacier-fed tributaries).

The PCA is illustrated in Figure 3. The spatial repartition of the original values (here as vectors) defined axes by the dominant physical parameters that explained them. The three first axes or Principal Components (PC) with eigenvalues >1 were kept, explaining in total almost 70% of the variation in the original data. Table 2 shows the correlations between the original variable and each PC, marked in bold where the scores were above the threshold r value required for $p<0.05$ with two tails. Axis 1 was positively associated with higher velocity and width, whereas coarser bed sediment and lower temperature had high values on this axis. The combination of these characteristics was used as an index of *torrentiality.* Axis 2 was associated with altitude and periphyton characteristics, being negatively correlated with altitude (measuring station and mean) and positively with autotrophic index and forest coverage; it was used as an index of *organic matter availability*. Axis 3 represented scale effects, showing the prominence of catchment size and distance from water origin; the term *catchment* was used for this axis. These three axes represent three gradients of (dis)similarity along which the tributaries were positioned in PCA space (Figure 3) and they bring out the dominant physical parameters that distinguish the tributaries.

To better visualise these gradients in relation to whether tributaries were temporary or permanent, a coordinate was given to each site along each axis based on the ordinations and displayed along a one-dimensional gradient in Figure 4. Tributaries were quite widely distributed along PC1. However, torrentiality clearly showed association with flow temporality, with more torrential streams likely to be permanent. Distribution of tributaries is more concentrated along PC2. The categorisation temporary/permanent separated organic matter availability less clearly and did not separate the catchment component values. The values in catchment showed that temporary tributaries are of medium length while permanent ones are more variable.

Beyond physical drivers, tributaries were compared in terms of their community composition. Annex 3 shows the abundance of macroinvertebrate families (log(x+1) transformed values, family level) in each tributary in August 2016. The following taxa were present in at least one stream: Planariidae (Platyhelminthes, Turbellaria), Oligochaeta (Annelida, order level), Hydracarina (Arthropoda, Arachnida), Baetidae - Heptageniidae (Insecta Ephemeroptera), Leuctridae - Nemouridae - Perlodidae (Insecta, Plecoptera), Limnephilidae - Rhyacophilidae (Insecta, Trichoptera), Chironomidae - Limoniidae - Simuliidae (Insecta, Diptera). With fewer than 3 to 10 individuals by family, T03 appeared as a stream without macroinvertebrates, while T11 and T12 had, respectively, 11 and 10 taxa of the 13 detected taxa in all samples. Insects largely dominated the other tributaries. In particular, Chironomidae, well-adapted to cold conditions and disturbance, were prevalent in most streams. Baetidae, Nemouridae, Perlodidae and Simuliidae were numerous, and Planariidae were mainly present in permanent streams.

Tributaries were widely distributed along the axis describing the complexity of communities (Figure 5) resulting from the NMDS. This resulting biotic ordination showed a clear separation between permanent and temporary tributaries. Taxa richness and diversity was higher in permanent tributaries than in temporary ones.

Tributaries hence can be distinguished according to biotic (Figure 5) and abiotic (Figure 4) characteristics, or both. Figure 6 shows the three PCAs describing physical variables plotted against community complexity. Torrentiality and community complexity were positively associated (Figure 6a), but the results showed that this positive association reflected stream permanence. Permanent streams were more likely to be torrential and had more complex communities, whereas temporary streams were less likely to be torrential and had less complex communities.

The relation between organic matter availability and biotic communities was less clear (Figure 6b). The higher biotic complexity of permanent streams was evident, but the correlation was less strong. This result indicated a relation between habitat conditions and taxa presence. Some tributaries with low organic matter had a well-developed community complexity, while some tributaries with greater organic matter composition were biologically poor. For example, T11 and T07 were similar in terms of organic matter availability (Figure 4), but had very different community complexity. This result revealed that taxa habitat preferences played a major role among tributaries. Even if geomorphologically relatively similar, T07 possessed only rheophilic taxa (Simuliidae, Planaridae, Nemouridae, Perlodidae and Baetidae), while T11 also had limnophilous taxa (Limnephilidae and Limoniidae).

A higher diversity reflected higher community complexity, and organic matter availability was lower at higher altitudes. In addition, organic matter sources may have a greater effect on assemblages in streams at high mountain altitudes near tree-line than at lower altitudes, reflecting a general resource limitation (Zah et al., 2001). At higher elevations, there were fewer deciduous trees, and fir and larch needles were the predominant source of organic matter. At lower altitudes, the percentage of the basin supplying organic matter increases (Annex 2) and the variety of trees are greater. Autotrophic differences were distinct between lower (heterotrophic) streams with variable food sources and upper streams (except T11, which was completely open canopy) that were generally autotrophic (Annex 2). Thus, organic matter differences were less pronounced for low altitude tributaries than for tributaries higher than 1800 m. Lastly, the diversity and richness of macroinvertebrates were not dependent on catchment size (Figure 6c), showing that the distance from the water source was not a dominant factor in community assembly.

By considering physical characteristics and biotic complexity in combination, we can say that (1) physical factors can be represented along three primary components of torrentiality, organic matter availability and catchment characteristics; (2) how the tributaries map onto these components, notably torrentiality, relates to conditions of stream intermittence with permanent streams more likely to be torrential (high velocity and width, coarser bed sediment and lower temperature); and (3) permanent streams tend to have more complex communities, provided that habitat conditions are favourable throughout the year.

SIMILARITY AND BIOTIC CHANGE THROUGH SEASONS AND YEARS

Temporal variability was assessed using the Bray-Curtis Dissimilarity Index (Equation [1]). Abundance and diversity of macroinvertebrates for the 14 tributaries from May to October 2016 were compared and groups with similar complexity were color-coded. Except for the white group of null data, three main groups were distinguished over the seasons (Figure 7a) and some streams were permanent throughout the period (T01, T07, T08, T09, T10, T11, T12, T13, in blue). The yellow group identified the permanent tributaries T08, T09, T11, T12 and T13 as similar in terms of macroinvertebrate complexity, except for T08 in September and T11 in June. T05, also permanent, contained comparable communities in August and September. Some temporary streams also were classified in this group in spring and autumn: T02 in October, T07 in June and T15 in May. The blue group identified the permanent streams T10, throughout, and T05 in October, and the temporary streams T07 in May and from July to October, T06 in May and September, T14 in August and October, T15 in June and July, and T02 in September and T03 in May. Finally, the red group included the permanent stream T05 from May to July with the temporary streams T01 from June to September, T02 from

May to August, T03 in July and September, T06 in June, July and August, T14 in May, June, July and September and T15 in August, September and October. The color groups remained more consistent throughout the months for temporary tributaries than permanent ones.

The variation between tributaries in space was high, even higher than the variation over time. Spatial variability can be high even between nearby tributaries: the columns of Figure 7a are ordered from upstream to downstream in terms of tributary connection with the main river channel (see Figure 1). Figure 8 shows how group membership changed between years (2015 versus 2016) for the months of August and October. Tributaries in August 2015 were more similar, shown by the dominance of the green group, but this was not the case in 2016. In August, 6 of the 14 tributaries remained in the green group for both 2015 and 2016, showing a certain stability and similarity of communities in summer. Without considering null samples, October displayed a higher similarity between 2015 and 2016 for permanent streams. Temporary streams, by definition, were mainly frozen in 2015 during the transition season due to cold conditions (Figure 1b) that influenced the onset of flow between years. Hence, again, intermittence played a major role in characterising tributary similarity, suggesting temporal variability may be high.

BIODIVERSITY PATTERNS OVER THE SEASONS

The richness, abundance and Shannon Index (Equation [2]) of the macroinvertebrate communities were determined for each tributary from May 2016 to October 2016, plus January and March 2017; the respective mean of each calculation by month was added, plus the means for the temporary and permanent streams (Annex 3). Table 3 gives the mean richness, abundance and Shannon Index by month for all tributaries, but also for tributaries classified as permanent or temporary. To illustrate the monthly patterns, Figure 9 shows boxplots of taxa richness, abundance and the Shannon Index, separately for permanent and temporary streams.

Based on annex 3, Table 4 indicates the two-way ANOVA results. As the F value is greater than its critical value, there is a significant difference between the permanent tributaries and the temporary tributaries and supports that this is meaningful to compare these two groups of tributaries.

Results showed the highest number of families, number of individuals and Shannon Index values were found for permanent streams throughout the year. The highest richness (number of taxa) was found in July and the highest abundance (number of individuals) was found in August, while June and October had the highest mean Shannon Index. Shannon indices for winter were almost as high as summer for permanent tributaries, particularly in March. By focusing only on permanent tributaries, the maximum diversity also occurred in July. Permanent tributaries contained a richer number of families, a higher abundance and a higher Shannon Index throughout the year. Some permanent tributaries (T09, T10, T11, T12 and T13) had a higher diversity than temporary tributaries across all seasons (i.e., a large number of taxa and individuals) (Annex 3). Tributaries with high Shannon indices corresponded to permanent streams at lower altitude (T09, T10, T11, T12 and T13). The variation in Shannon index was greater between tributaries in space than between tributaries over time (Annex 3). Winter values were not that different to other seasons, flagging the importance of this season for community turn-over.

DISCUSSION

Physical variables measured at 14 tributaries of the Arolla catchment could be classified along three principal components. The torrentiality of a stream, linked to velocity, width, coarse bed sediment and low temperature, was the first and most important component (Table 2, Figure 4a). The second main driver was organic matter availability, which correlated with altitude, autotrophic index and forest coverage (Table 2, Figure 4b). This observation underlines the need to consider physical characteristic (dis)similarities when comparing geographically-proximal streams at the catchment scale, especially as the components, notably torrentiality were found to impact community complexity. That said, at least some of this association was due to the fact that component scores were also differentiated by stream intermittence. Indeed, there was a general conclusion from the data that permanent streams behave differently to temporary ones. Permanent streams were predominantly torrential in character (Figure 4a), a hydro-morphological characteristic that reduces the possibility of freezing. These streams thus provide more suitable habitat conditions for macroinvertebrates, especially during relatively more stable periods, in particular during early summer between snow melt and ice melt, and in autumn before the decrease in temperatures (see Schütz et al., 2001; Zah & Uehlinger, 2001). Permanent tributaries had more diverse and abundant taxa (Table 3, Figure 9, Annex 4) and a higher community complexity (Figure 5) over the year. Clearly, categorising streams as permanent or temporary is crucial for comparing the ecological status of Alpine waters (see also Cottenie, 2005).

Macroinvertebrate assemblages were present throughout the year in these relatively harsh high altitude Alpine tributaries, especially in permanent streams. Pioneer families such as Chironomidae were predominant, but pollution- and disturbance-sensitive taxa such as Perlodidae also were common (Annex 3). This result was probably due to the adaptive traits of many high altitudinal taxa that allow them to inhabit the relatively harsher streams found at high elevation (Zbinden et al., 2008), thus underlining the greater diversity observed in summer (Table 3, Annex 3). Rheophilic taxa, able to live in rapidly-flowing waters, were prevalent in the study tributaries (Annex 2). Limnophilous taxa (Oligochaeta, Limneplilidae, Limoniidae), more common in stagnant waters, were present mostly in August in a few tributaries: T05, T10, T11, T12, T13, T14 (Annex 3). This observation emphasizes the need to define the desired target species in order to determine the most significant tributaries to survey.

Macroinvertebrate communities were found to vary in space and time. Some similarities in community complexity were found in mid-summer (June-July, July-August and August-September) (Figure 7). Permanent tributaries were more stable, indicating that they are less influenced by external variability in physical factors than in-stream conditions. Our study also indicated higher taxa richness, abundance and heterogeneity during this period (Annex 4). This contrasts with previous research (e.g. Burgherr & Ward, 2001; Füreder et al., 2001; Robinson et al., 2001; Schütz et al., 2001) that found higher richness and abundance in spring and autumn in high altitude streams. Bray-Curtis dissimilarity indices (Figure 7) suggested that spring and autumn (respectively, May-June and September-October) (Figure 7) had less stable conditions for macroinvertebrates than summer in these tributaries. Hence, inter-annual variability in meteorological conditions (e.g. late onset of spring; early onset of winter) means that either spring or autumn sampling may produce less consistent results among years than summer sampling. This is supported by the greater inter-annual consistency in August than in October (Figure 8).

In addition to temporal diversity, spatial diversity is significant. Spread across a maximum longitudinal distance of 1.5 km, the 14 tributaries were heterogeneous in terms of their community complexity (Figure 7). Elevation also played a role. For instance, one group (poor complexity) comprised tributaries T01 to T06 plus T14 and T15, whereas a second group (high complexity) comprised T08 to T13 (except T10) (Figure 7). This latter group of permanent streams had a well-developed abundance and richness of macroinvertebrates, earlier than higher altitude permanent streams (Annex 4). Slightly lower altitude permanent streams also displayed good stability in macroinvertebrate community composition over the summer (Figure 8), whilst composition varied substantially in slightly higher altitude permanent streams (Figure 7, Annex 3). These findings further emphasise the high sensitivity of Alpine streams to small altitudinal gradients.

Given the high sensitivity of high altitude streams to spatio-temporal variation, it is perhaps surprising given the number of investigations of Alpine stream macroinvertebrates (Annex 1) that the guidance for sampling remains relatively basic. Even within Europe, countries tend to use their own indices and protocols, despite attempts to adopt a European-standardized method. The AQEM (Integrated assessment system for the ecological quality of streams and rivers throughout Europe using benthic macroinvertebrates) method proposes a framework to assess all types of water bodies in Europe (AQEM Consortium, 2002). However, whilst altitudinally-variable sampling windows are defined in the AQEM scheme, all streams above 800 m are combined and classified into a single window as “Alpine”. In Germany, following Schmedtje et al. (2001) and Pottgiesser & Sommerhäuser (2004), ecoregions have been distinguished, but these also have adopted a single window (above 800 m) for Alpine streams. In Italy, the older IBE (Extended Biotic Index) was replaced by the Standardisation of River Classifications – Intercalibration Common Metrics (STAR_ICM) after the Water Framework Directive, but no distinction of high-altitude streams was made, except that they should be considered as particularly sensitive (OCDE, 2002). In France, based on the Biotic Index established by Verneaux and Tuffery (1967), the IBGN (Standardized Global Biological Index) conceived in 2000 does not distinguish specific altitudinal bands. Only a note indicates that scores in high altitude streams must be carefully interpreted as they are areas with basically poor diversity, thus outcomes must be contextualized before being analysed (Agence de l’eau, 2000). Finally, in Switzerland, the IBCH (Swiss Biological Index) (OFEV, 2010) has been adopted as a standardized procedure for the collection and the analysis of macrozoobenthos samples at the regional level. Sites above 1800 m are grouped together into a single category, with sampling targeted for June, possibly late May or early July if conditions require it, and an optional sampling period from mid-September to mid-October (figure 2). Using data in the Swiss Hydrological Atlas (Breinlinger et al., 1992), which classifies Switzerland into small river basins, ca. 30% of these basins (by surface area) have a mean basin altitude greater than 1800 m.

Although it was not possible to confirm that any one month displayed the best representation for macroinvertebrates in the study catchment, summer seemed to be the best period for macroinvertebrate monitoring (Figure 7), even though summer is commonly assumed as the period for flying adults (i.e., less invertebrates at the larvae stage) (Füreder et al., 2005). Importantly, by sampling during the previously established optimal window for life (June), even with the addition of an optional campaign period of late September, it is possible to miss the optimal sampling period in long-term monitoring. This result is probably the effect of altitude, emphasising the need to sample high altitude monitoring sites (> 1800 m in this region) later in summer. When developing large-scale recommendations, using a threshold like 1800 m may not be wise because altitude is not the only effect on local climate. For instance, there is a marked continental gradient from the western Alps to the eastern Alps in Europe, as well as climatic differences between the northern and southern Alps, both partly independent of altitude. Consequently, the required altitudinal threshold for the optimal sampling period is likely to vary with climatic region as well as annual conditions. Many strategies advocate sampling macroinvertebrates in high altitude streams during late Spring (e.g. the Swiss IBCH) on the basis that it is this period that has the highest and most abundance and richness. However, some research has reported significant macroinvertebrate richness and abundance in winter at high altitudes (Burgherr & Ward, 2001; Füreder et al., 2001; Brown et al., 2015). This might appear to be surprising because it doesn’t seem to fit with the observation that colder temperatures negatively impact macroinvertebrate populations (as reflected in population recovery with distance downstream from a glacier margin; Milner and Petts, 1994). However, it can arise in basins which, whether for natural reasons or due to human impacts, summer macroinvertebrate populations are strongly impacted by glacial melt cycles and the associated stream bed disturbance and high suspended sediment loads (Gabbud et al., 2018). High altitude basins may show quite significant differences in the relative importance of kryal, nival and krenal contributions, such as due to the changing relative proportion of glacier melt, over very small distances and sampling needs to be much more sensitive to the physical geography of individual basins and less focused on “simplistic” parameters such as theoretical altitudinal ranges.

The defining characteristic to take into account to answer the question of when and where to sample macroinvertebrates in high elevation streams remains a challenge. Indices such as the Swiss IBCH consider only permanent flows as significant (OFEV, 2010) for sufficient representativeness of macroinvertebrates, and for the most part, this finding is confirmed here (Figure 5, Figure 6a, Figure 9, Annex 4). In the study catchment, all glacial streams (T01, T02, T06, T07 and T15) were temporary and thus should not be considered as reference systems for permanent streams. This result revealed the likely underestimated problem of how to characterise Alpine glacial streams, as central providers of water, sediment and organisms to low elevation waters. Hence, to insure that a stream is permanent, and so to be a candidate as reference to an “undisturbed” state, suitable sensors should be installed for measuring flow presence/absence throughout the year. Regardless, the extent of intermittent streams in Alpine catchments can be high and should not be neglected in understanding the ecology of high elevation drainage networks (Robinson et al. 2015). Shannon Indices also can be quite high in winter in these tributaries (Table 3, Annex 4), showing that despite cold conditions, the winter period allows larval development of macroinvertebrates (Schütz et al., 2001; Sertic Peric et al., 2015). Clearly, a single sampling period is too restrictive to represent the temporal variability of macroinvertebrate assemblages in streams. While the season of sampling certainly matters (Füreder et al., 2005; Callanan et al., 2008), our results showed more differences between streams in space than within a stream through time.

CONCLUSIONS

In this paper, we established the degree of spatial and temporal variability in macroinvertebrate abundance and diversity for 14 tributaries at similar altitude that feed a 6-km reach of an Alpine glacial stream. We showed that biotic assemblages of temporary and permanent streams were clearly different. Hydrologically less prone to freeze during winter (torrential), permanent streams with continuous water running throughout the year provide more suitable conditions for macroinvertebrates, and macroinvertebrate communities that are more diverse and taxa rich. This result supports the conclusion that only permanent streams should be defined as a reference for "undisturbed" streams (as requested by many indices), but establishing them as candidate streams needs confirmation by winter campaigns or deployment of sensors. Further, intermittent streams should not be ignored but perhaps be grouped as a separate stream class for Alpine streams.

Spring and autumn were more likely to vary between years because of climatic conditions, and thus when conditions become suitable for macroinvertebrates also vary inter-annually. This result implies that when to sample likely varies between years to correspond with differences in macroinvertebrate life cycles. Lastly, the variation between adjacent streams at the same altitude (space) was higher than the variation between tributaries over time. Even if sampled at the same time within a year, the reference condition of streams may be very different, thus sampling conditions should focus on sampling more streams in space.

It seems clear that "optimal windows of opportunity" for macroinvertebrates, and hence when a tributary should be sampled, cannot be fixed to a given period of time in the year. We showed that this variability differs both within and between years, leading to a need to shift the "window of opportunity" of any one tributary to either earlier or later. Data analysis suggested that even over very short distances, environmental differences in tributary characteristics lead to different macroinvertebrate assemblages. Altitudinal classification of sites did not fully account for environmental differences among streams and probably underestimates the spatio-temporal variability of high elevation streams, as demonstrated through this field-based research. If this spatio-temporal complexity is not taken into account in Alpine streams, there is a risk that the qualification of ecological status reflects natural geographical and temporal variability and not the true ecological status of the streams being sampled. Alpine streams are notably complex and this complexity must be incorporated into long-term monitoring programs to ensure better understanding and better river protection.

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TABLES

Table 1 : Date of sampling in 2015, 2016 and 2017.

Year	2015	2016	2017
Dates and Month of sampling			30-31 January
			28-29 March
		12-13 May	
		13-14 June	
		12-13 July	
	04-05 August	9-10 August	
		14-15 September	
	12-13 October	11-12 October	

***Table 2** Correlation between the principal components of the PCA and original variables; in bold the scores above critical r = 0.6.*

		PC1	PC2	PC3	PC4
Velocity	(m/s)	**0.91**	-0.18	-0.18	0.12
Temperature	(°C)	**-0.73**	-0.38	-0.39	-0.06
Conductivity	(µS/cm)	0.55	0.36	0.14	**0.67**
Width	(m)	**0.75**	-0.06	-0.18	-0.23
Depth	(m)	0.51	-0.31	0.52	-0.06
Coarse sediment	(% > 64mm)	**0.87**	-0.16	0.06	-0.25
Fine sediment	(% < 2mm)	**-0.66**	0.40	0.28	0.13
Autotrophic index	(-)	-0.15	**0.66**	0.52	-0.36
Slope	(%)	0.51	-0.41	-0.38	-0.18
Measuring station altitude	(m)	-0.50	**-0.64**	-0.29	0.29
Mean altitude	(m)	-0.30	**-0.74**	0.36	0.17
Forest coverage	(%)	0.30	**0.65**	-0.40	0.42
Length	(m)	0.26	-0.54	**0.63**	0.30

***Table 3** Taxa richness (mean number of taxa), abundance (mean number of individuals) and Shannon Index (H mean – Heterogeneity; considered only when H ≠ 0) for each month; (a) for all tributaries together; (b) distinguished between permanent and temporary streams*

TOTAL	Mean nb of families	Mean nb of individuals	Shannon Index (H)
May 2016	5	116	1.51
June 2016	6	135	1.53
July 2016	7	180	1.46
August 2016	7	233	1.38
September 2016	6	189	1.28
Octobrer 2016	5	128	1.56
January 207	2	70	1.44
March 2017	3	69	1.55
PERMANENT			
May 2016	7	176	1.48
June 2016	7	176	1.40
July 2016	8	238	1.75
August 2016	8	350	1.66
September 2016	7	270	1.30
Octobrer 2016	7	207	1.65
January 207	5	140	1.03
March 2017	5	138	1.33
TEMPORARY			
May 2016	4	56	0.90
June 2016	6	95	1.23
July 2016	5	122	0.96
August 2016	5	116	0.91
September 2016	5	108	1.07
Octobrer 2016	3	49	0.80
January 207	0	0	0
March 2017	0	0	0

Table 4 Two-way ANOVA test with F value, P value and F critical value (McDonald, 2014)

Source of variations	*F value*	*P value*	*F critical value*
Sample	22.77	2.22067E-05	4.073
Column	78.30	6.77729E-15	3.22
Interaction	20.983	4.80687E-07	3.21

FIGURE CAPTIONS

Figure 1a *Arolla catchment with study sites indicated*

Figure 1b *Mean monthly temperature and sum of monthly precipitations for Evolène meteorological station (Meteosuisse, 2018) for the period of study*

Figure 2 *Temporal windows according to broad altitudinal bands; above, compulsory campaign periods (F) and buffer periods (T); below, optional campaign periods (modified from OFEV, 2010*

Figure 3 *Resulting PCA from measured physical variables; Axis 1 expresses Torrentiality; Axis 2 expresses Organic matter availability; Axis 3 expresses Catchment; Red triangles for permanent tributaries, blue squares for temporary tributaries.*

Figure 4 *Ordination of the PCA axes: PC1-Torrentiality (top), PC2-Organic matter availability (middle), PC3-Catchment (bottom); Red triangles for permanent and blue squares for temporary tributaries.*

Figure 5 *Ordination of the taxa PCA according to PC1-log abundance; Red triangles for permanent and blue squares for temporary tributaries.*

Figure 6 *Correlation between biotic complexity (taxa richness and diversity) and physical measures of (a) PC1-Torrentiality, (b) PC2-Organic matter availability, and (c) Catchment; Red triangles for permanent and blue squares for temporary tributaries.*

Figure 7 *Similar groups of tributary complexity (same color) from May to October 2016 shown by the Bray-Curtis dissimilarity index; (a) from T01 to T15 from upstream to downstream, (b) according to group similarities; in white are null data (no sampling in May and June in T08; devoid of macroinvertebrates for T01 in May, T03 in June and August, and T06 in October; T01 and T03 were frozen in October). Permanent streams labelled in red and temporary in blue.*

Figure 8 *Groups of tributary complexity (same color) shown by the Bray-Curtis dissimilarity index; (a) August 2015/2016; (b) October 2015/2016; in white are null data (T01 and T03 frozen in October 2015 and 2016; T06 devoid of macroinvertebrates in October 2016; T08 no sampling in October 2015; T15 frozen in October 2015). Permanent streams in red and temporary in blue.*

Figure 9 *Boxplot of (a) Taxa richness (mean number of taxa); (b) Abundance (mean number of individuals); (c) Shannon Index (H mean – Heterogeneity; considered only when H ≠ 0) for each month; Permanent streams in red and temporary in blue; dotted boxplot for winter results*

APPENDIX

***Annex 1** Months and altitudinal range of macroinvertebrate streams in Alpine basins with x =sampled, 2x = sampled during two consecutive years, xx = sampled two times during the same month; the total represents the number of studies effecting samplings at least once during the mentioned month, without the current study (personal communication: Y. Riffault).*

Study	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**Altitudinal range of measurement sites**
Bizzotto et al. 2009						x	x		x	x			2000m
Brown et al. 2007						2x	2xx	2xx	2x				2000m
Burgherr and Ward 2001						x		x		x	x		1760-2150m
Ilg and Castella 2006									x				1755-2610m
Lencioni et al. 2007						x		x	x				1800-3000m
Lods-Crozet et al. 2001						2x		2x	2x				1880-2600m
Maiolini et al. 2011						x		x	x	x	x		(170-)1790-2792m
Malard et al. 2003						x	x	x	x	x			1773-2122m
Petts and Bickerton 1994							x						1800-2500m
Robinson et al. 2001	x	x	x	x	x	x	x	x	2x	x	x	x	1320-2070m
Snook and Milner 2001						x	2x	2x	x				1800-2500m
Zah et al. 2001		x							x				1768-2159m
This study	x		x		x	x	x	xx	x	xx			1800-2000m
Total	2	2	1	1	1	9	6	8	10	5	3	1	

Annex 2 *Physical characteristics of tributaries used as basis of the PCA; August 2016.*

	Velocity (m/s)	Temperature (°C)	Conductivity (µS/cm)	Width (m)	Depth (m)	Coarse sedim. (% >64mm)	Fine sedim. (% <2mm)	Autotrophic Index	Slope (%) (%)	Station altitude (m)	Mean altitude (m)	Forest coverage (%)	Length (m)
	Veloc	Temp	Cond	Width	Depth	Bould64	Fine2	AI	Slope	AltSta	Altmean	Forest	Length
T01	0.343	11.8	51.3	0.7	0.02	15	25	5.298	12	2005	2240	9.4	1117
T02	0.362	10.4	54.2	0.84	0.07	25	40	5.89	5.6	2012	2085	15.6	762
T03	0.332	9.3	79.5	0.9	0.04	40	40	2.942	3.4	2000	2410	0	1420
T05	0.571	6	278	1.44	0.12	60	15	8.911	7.8	1938	2268	13.7	3340
T06	0.382	4.6	168.2	1.3	0.11	50	50	17.858	10	1940	2090	7.6	803
T07	0.493	6.6	85.5	1	0.08	55	25	8.339	27.9	1922	2330	26	2010
T08	0.639	5.6	282	0.85	0.05	75	10	9.106	12	1903	2075	59	1030
T09	0.627	6.3	169.3	2	0.04	85	10	3.717	35.7	1876	2050	44	990
T10	0.464	5.5	220	0.97	0.04	15	60	10.48	3.2	1863	2050	64	721
T11	0.711	5.2	93	1.57	0.15	80	20	6.487	17.9	1854	2235	0	1910
T12	0.292	7.3	135	0.7	0.06	30	60	30.551	3.1	1841	2220	44	1630
T13	0.597	6.5	132	2.3	0.09	60	15	15.438	3.3	1834	2020	49	863
T14	0.361	6.8	128	0.88	0.05	50	30	41.23	2.9	1832	2050	14	1040
T15	0.347	5.8	121	0.75	0.03	65	30	21.912	10	1826	2160	13	1470

Annex 3 *Taxa (families) present in each tributary expressed with log(x+1) transformed values; August 2016.*

	Planariidae	**Oligochaeta**	**Hydracarina**	**Baetidae**	**Heptageniidae**	**Leuctridae**	**Nemouridae**	**Perlodidae**	**Limnephilidae**	**Rhyacophilidae**	**Chironomidae**	**Limoniidae**	**Simuliidae**
T01	0.00	0.00	0.00	1.36	0.00	0.00	0.00	0.00	0.00	0.00	1.49	0.00	0.00
T02	0.00	0.00	0.00	2.39	0.78	0.00	1.58	0.70	0.00	0.00	1.43	0.60	0.60
T03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T05	1.54	0.00	0.00	2.13	1.30	0.00	1.58	1.30	1.34	0.00	2.03	0.78	0.95
T06	0.00	0.00	0.00	1.91	0.60	0.00	0.60	0.70	0.00	0.00	1.36	0.00	0.90
T07	0.70	0.00	0.00	1.23	0.00	0.00	0.70	0.60	0.00	0.00	0.00	0.00	0.60
T08	1.15	0.00	0.00	1.83	0.00	0.00	1.45	1.59	0.00	0.00	1.97	0.90	0.00
T09	1.68	0.00	0.00	1.26	0.00	0.00	1.23	0.70	0.00	0.85	2.17	0.90	1.15
T10	1.20	0.00	0.00	0.00	0.90	0.00	1.46	0.95	1.28	0.00	0.00	0.00	1.20
T11	1.23	0.00	0.00	1.49	1.85	1.30	2.03	1.15	1.20	0.70	2.22	1.04	1.85
T12	1.54	1.53	0.60	1.79	0.00	0.78	0.95	1.04	1.52	0.00	2.11	1.11	0.00
T13	1.28	0.00	0.00	2.24	0.00	0.00	2.08	1.00	0.00	0.00	2.33	0.78	2.02
T14	0.78	1.38	0.00	1.96	0.78	0.78	1.30	0.70	0.00	0.00	0.00	0.00	1.34
T15	0.00	0.00	0.00	1.93	0.00	0.00	1.08	0.95	0.00	0.60	1.04	0.00	0.00

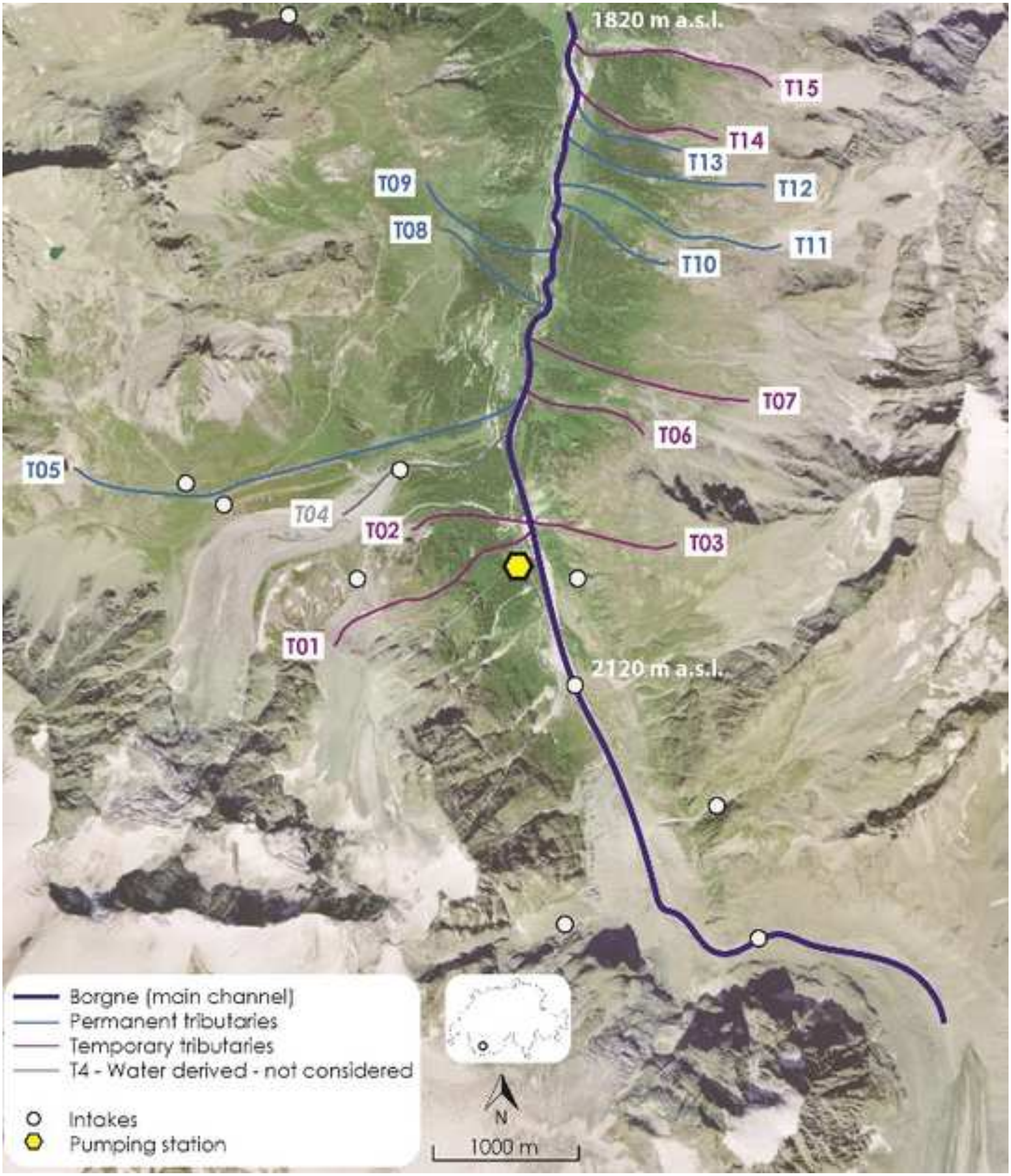
Annex 4 *Taxa richness (mean number of taxa), abundance (mean number of individuals) and Shannon Index (H mean – Heterogeneity; considered only when H ≠ 0) for each tributary by month; Permanent streams in red and temporary in blue.*

	Nb of families	Nb of individuals	Shannon Index (H)
T01_0516	0	0	0.00
T02_0516	4	69	0.90
T03_0516	1	9	0
T05_0516*	6	172	1.32
T06_0516	2	13	0.62
T07_0516	4	40	1.27
T08_0516*	0	0	0
T09_0516*	9	181	1.83
T10_0516*	5	66	1.47
T11_0516*	9	207	1.87
T12_0516*	10	215	1.99
T13_0516*	9	391	1.89
T14_0516	6	135	1.56
T15_0516	8	125	1.93
Mean_Perm0516	**7**	**176**	**1.48**
Mean_Temp0516	**4**	**56**	**0.90**
Mean_Total0516	**5**	**116**	**1.51**
T01_0616	4	64	1.23
T02_0616	7	148	1.34
T03_0616	0	0	0
T05_0616*	8	260	0.98
T06_0616	5	51	0.97
T07_0616	11	173	1.88
T08_0616*	0	0	0
T09_0616*	8	249	1.68
T10_0616*	6	37	1.53
T11_0616*	6	77	1.56
T12_0616*	11	381	2.00
T13_0616*	9	227	2.06
T14_0616	8	115	1.82
T15_0616	7	113	1.33
Mean_Perm0616	**7**	**176**	**1.40**
Mean_Temp0616	**6**	**95**	**1.23**
Mean_Total0616	**6**	**135**	**1.53**
T01_0716	4	39	0.93
T02_0716	8	289	1.12
T03_0716	1	23	0
T05_0716*	6	160	1.37
T06_0716	6	141	1.12
T07_0716	5	65	0.81
T08_0716*	6	263	1.55
T09_0716*	6	90	1.42
T10_0716*	9	93	2.06
T11_0716*	9	154	1.94
T12_0716*	10	259	2.14
T13_0716*	10	650	1.75
T14_0716	7	99	1.68
T15_0716	7	200	1.05
Mean_Perm0716	**8**	**238**	**1.75**
Mean_Temp0716	**5**	**122**	**0.96**
Mean_Total0716	**7**	**180**	**1.46**

	Nb of families	Nb of individuals	Shannon Index (H)
T01_0816	3	52	0.68
T02_0816	7	321	0.87
T03_0816	0	0	0
T05_0816*	9	381	1.76
T06_0816	6	119	1.05
T07_0816	5	30	1.33
T08_0816*	6	243	1.51
T09_0816*	8	257	1.38
T10_0816*	6	91	1.69
T11_0816*	11	516	1.93
T12_0816*	10	325	1.80
T13_0816*	7	638	1.53
T14_0816	8	172	1.50
T15_0816	5	116	0.95
Mean_Perm0816	**8**	**350**	**1.66**
Mean_Temp0816	**5**	**116**	**0.91**
Mean_Total0816	**7**	**233**	**1.38**
T01_0916	5	48	1.38
T02_0916	7	141	1.49
T03_0916	5	343	0.89
T05_0916*	9	492	1.50
T06_0916	1	6	0
T07_0916	2	25	0.67
T08_0916*	5	53	1.39
T09_0916*	7	135	1.27
T10_0916*	4	48	1.01
T11_0916*	12	349	2.10
T12_0916*	6	497	0.86
T13_0916*	5	315	1.00
T14_0916	8	92	1.53
T15_0916	6	98	1.50
Mean_Perm0916	**7**	**270**	**1.30**
Mean_Temp0916	**5**	**108**	**1.07**
Mean_Total0916	**6**	**189**	**1.28**
T01_1016	0	0	0
T02_1016	6	171	1.64
T03_1016	0	0	0
T05_1016*	5	154	1.31
T06_1016	0	0	0
T07_1016	4	39	1.16
T08_1016*	5	310	1.46
T09_1016*	10	109	2.03
T10_1016*	6	128	1.63
T11_1016*	10	364	1.86
T12_1016*	8	196	1.57
T13_1016*	7	186	1.70
T14_1016	6	75	1.46
T15_1016	4	56	1.34
Mean_Perm1016	**7**	**207**	**1.65**
Mean_Temp1016	**3**	**49**	**0.80**
Mean_Total1016	**5**	**128**	**1.56**

	Nb of families	Nb of individuals	Shannon Index (H)
T01_0117	0	0	0
T02_0117	0	0	0
T03_0117	0	0	0
T05_0117*	11	563	1.75
T06_0117	0	0	0
T07_0117	0	0	0
T08_0117*	3	64	0.97
T09_0117*	4	34	1.30
T10_0117*	4	55	1.15
T11_0117*	0	0	0
T12_0117*	10	267	2.05
T13_0117*	0	0	0
T14_0117	0	0	0
T15_0117	0	0	0
Mean_Perm0117	**5**	**140**	**1.03**
Mean_Temp0117	**0**	**0**	**0.00**
Mean_Total0117	**2**	**70**	**1.44**

	Nb of families	Nb of individuals	Shannon Index (H)
T01_0317	0	0	0
T02_0317	0	0	0
T03_0317	0	0	0
T05_0317*	6	259	1.71
T06_0317	0	0	0
T07_0317	0	0	0
T08_0317*	4	62	1.33
T09_0317*	4	39	1.29
T10_0317*	5	65	1.41
T11_0317*	0	0	0
T12_0317*	9	268	1.86
T13_0317*	7	273	1.70
T14_0317	0	0	0
T15_0317	0	0	0
Mean_Perm0317	**5**	**138**	**1.33**
Mean_Temp0317	**0**	**0**	**0.00**
Mean_Total0317	**3**	**69**	**1.55**


1820 m a.s.l.
T15
T14
T13
T12
T09
T08
T11
T10
T07
T06
T05
T04
T02
T03
T01
2120 m a.s.l.
Borgne (main channel)
Permanent tributaries
Temporary tributaries
T4 - Water derived - not considered
Intakes
Pumping station
N
1000 m


Temperature (°C)
20
15
10
5
0
-5
-10
Precipitations (mm)
140
120
100
80
60
40
20
0
07.2015
08.2015
09.2015
10.2015
11.2015
12.2015
01.2016
02.2016
03.2016
04.2016
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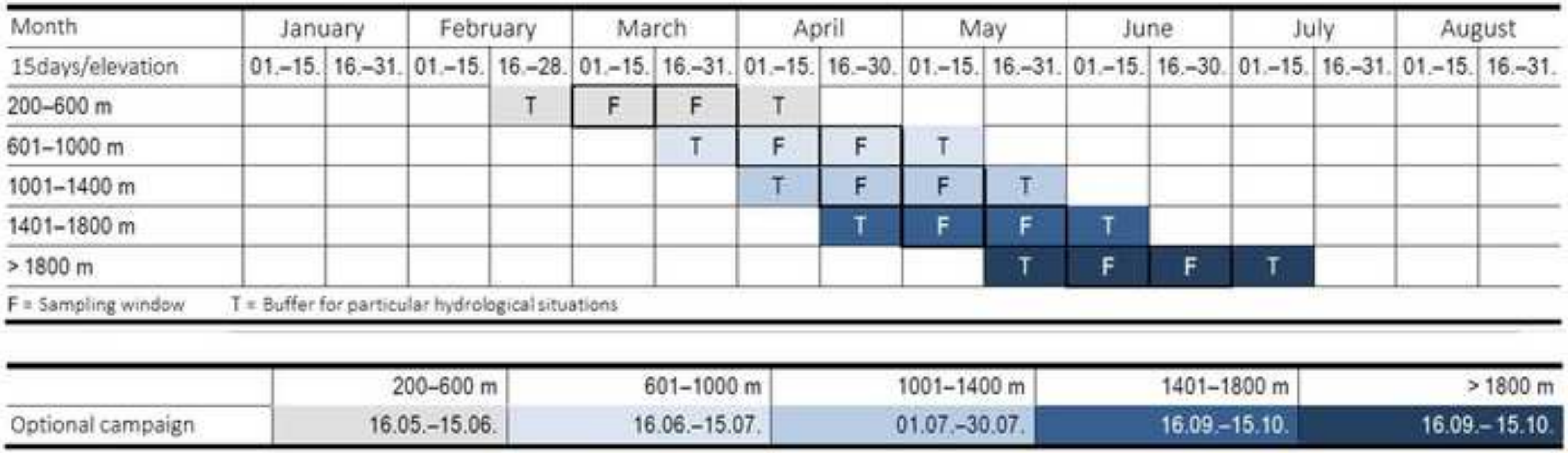
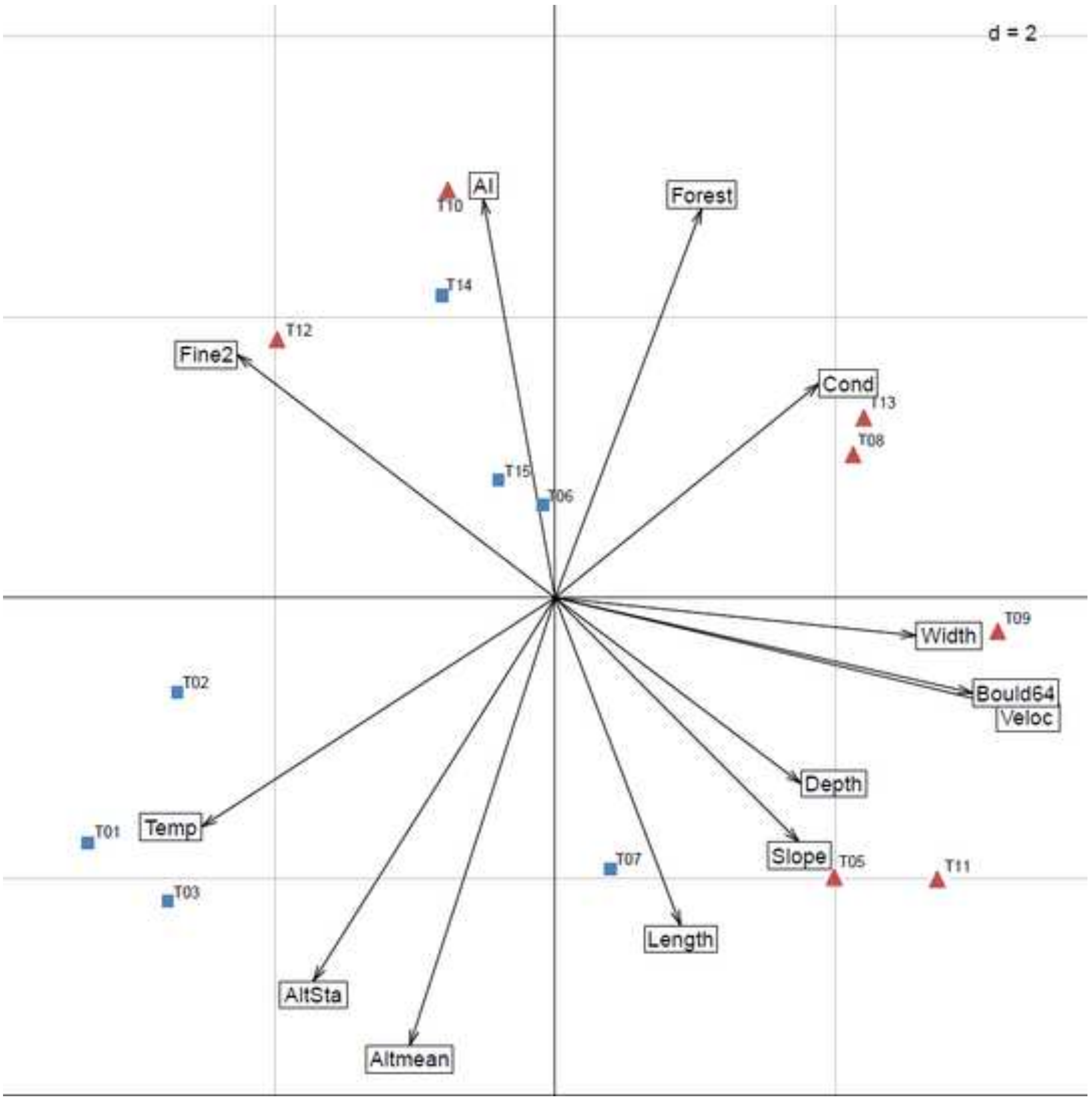


Month	January		February		March		April		May		June		July		August	
15days/elevation	01.–15.	16.–31.	01.–15.	16.–28.	01.–15.	16.–31.	01.–15.	16.–30.	01.–15.	16.–31.	01.–15.	16.–30.	01.–15.	16.–31.	01.–15.	16.–31.
200–600 m				T	F	F	T									
601–1000 m						T	F	F	T							
1001–1400 m							T	F	F	T						
1401–1800 m								T	F	F	T					
> 1800 m										T	F	F	T			

F = Sampling window T = Buffer for particular hydrological situations

	200–600 m	601–1000 m	1001–1400 m	1401–1800 m	> 1800 m
Optional campaign	16.05.–15.06.	16.06.–15.07.	01.07.–30.07.	16.09.–15.10.	16.09.– 15.10.

Figure


d = 2
Al
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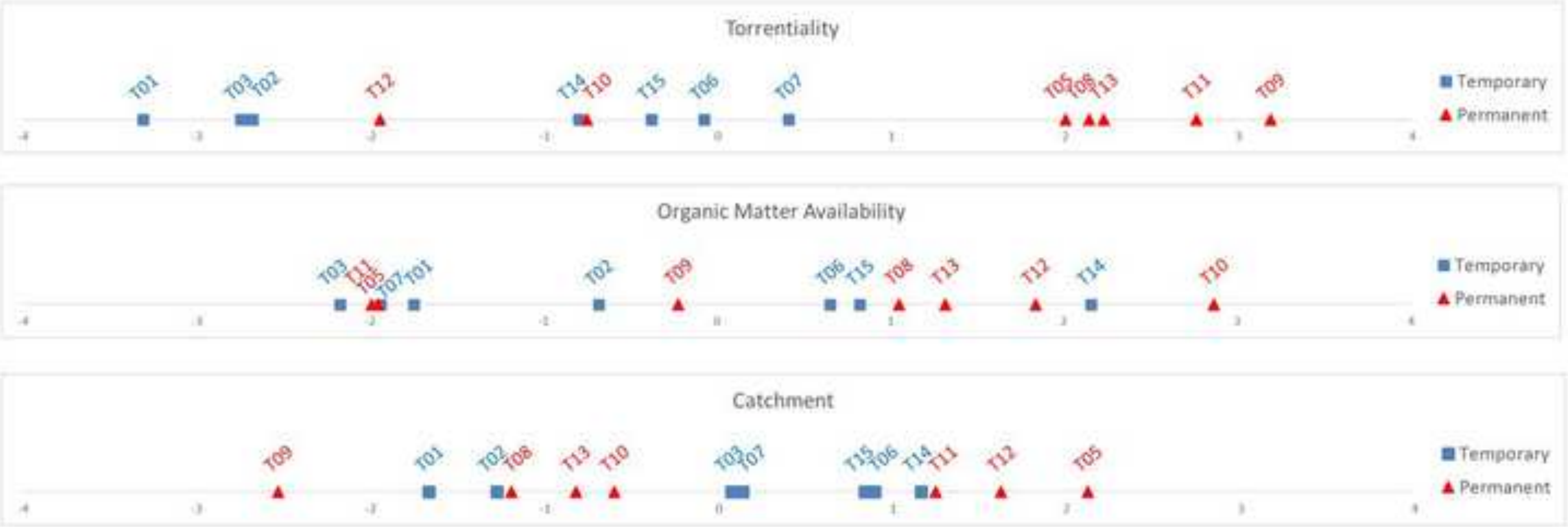
Torrentiality
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T03
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T12
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T09
■ Temporary
▲ Permanent
-4
-3
-2
-1
0
1
2
3
4
Organic Matter Availability
T03
T11
T05
T07
T01
T02
T09
T06
T15
T08
T13
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T14
T10
■ Temporary
▲ Permanent
-4
-3
-2
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4
Catchment
T09
T01
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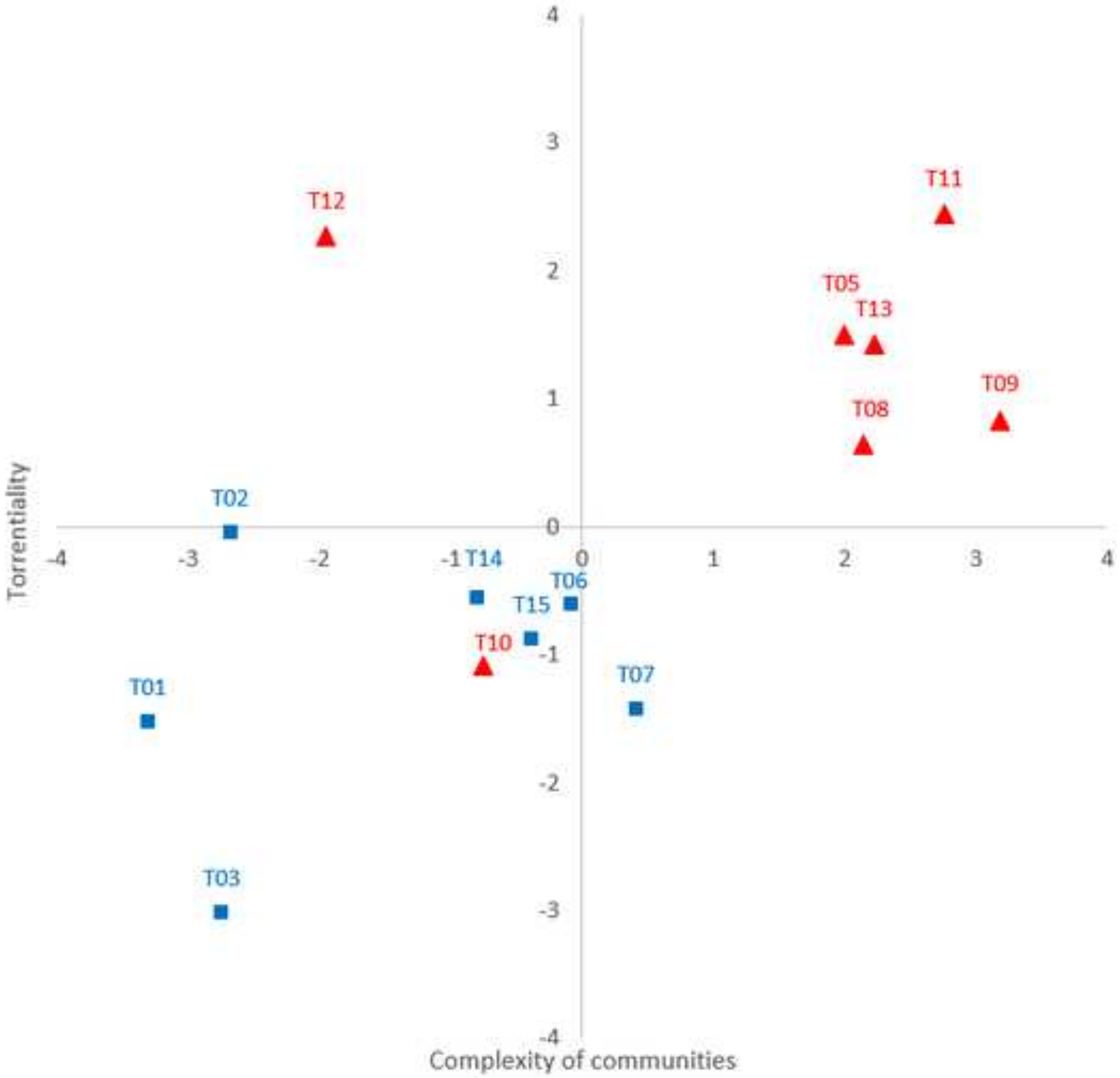
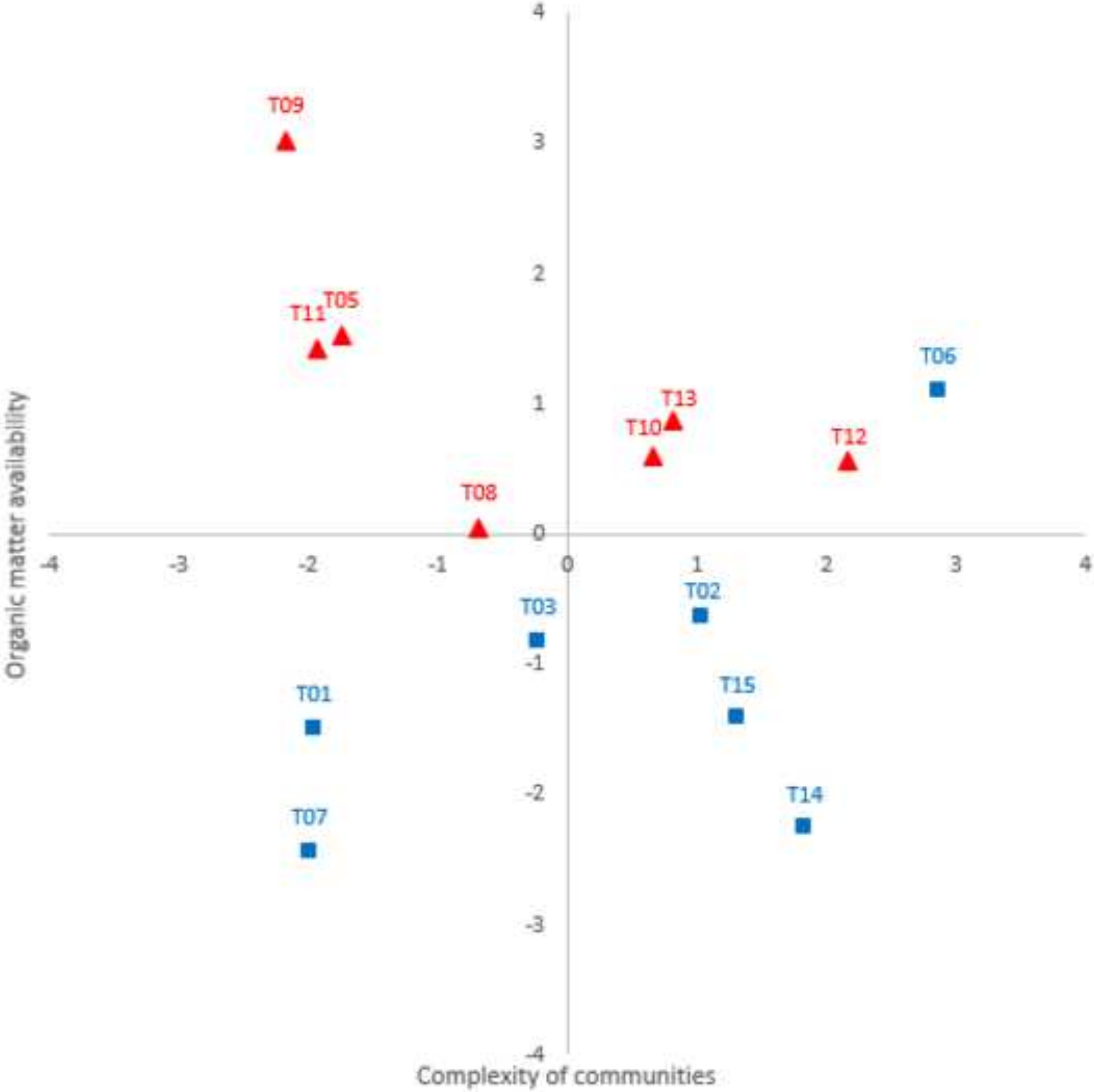
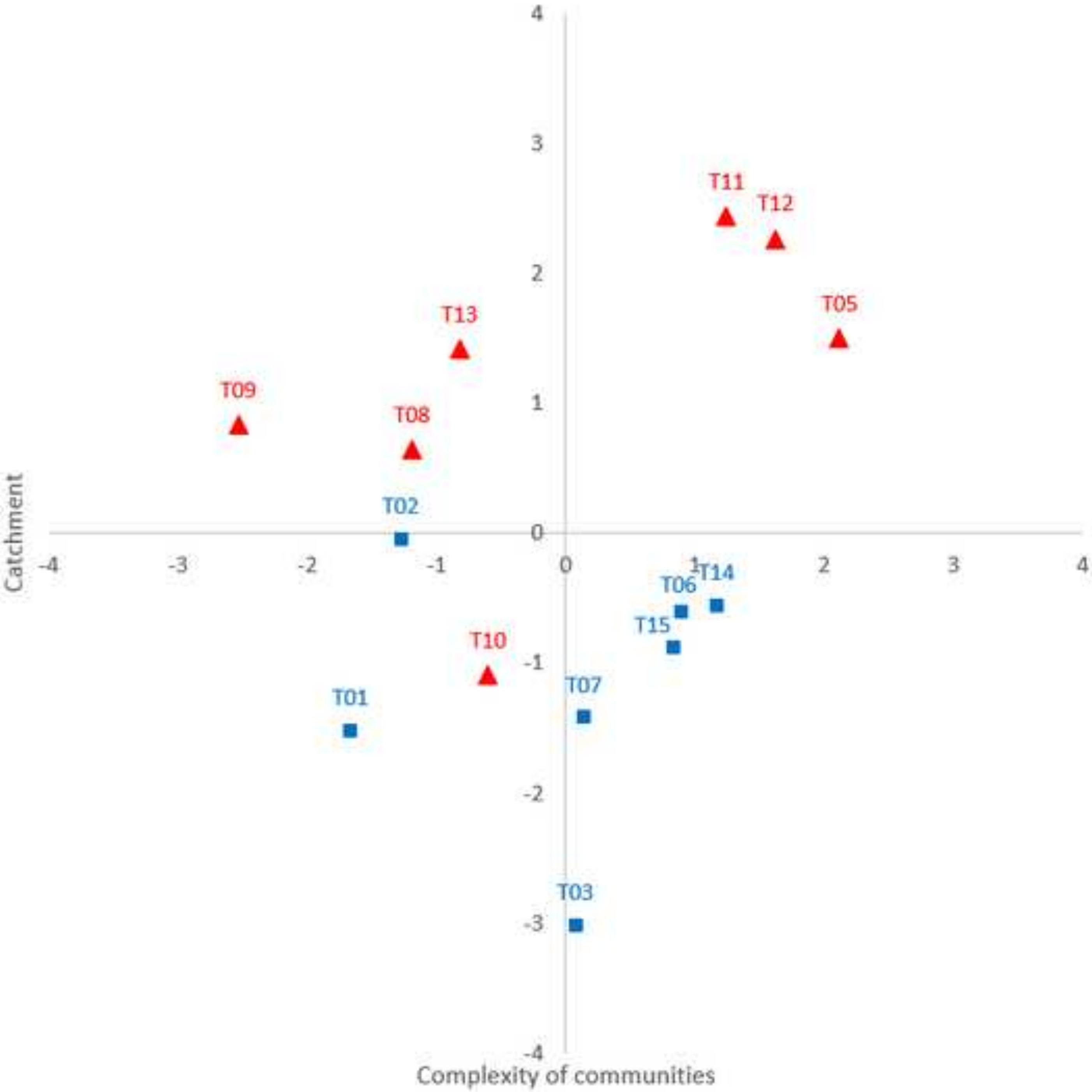
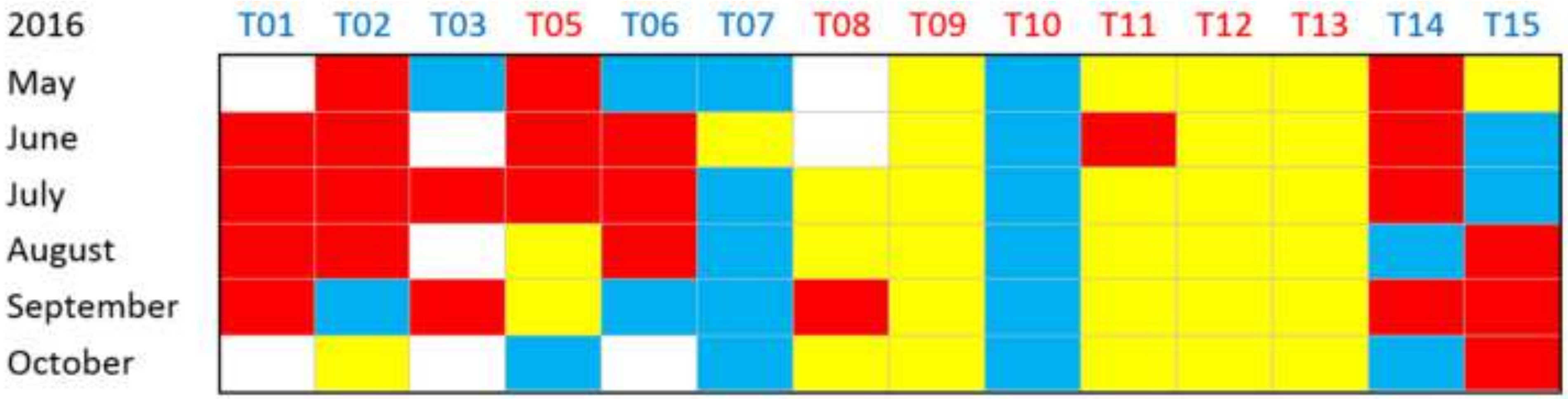
T11
T12
T05
■ Temporary
▲ Permanent
-4
-3
-2
-1
0
1
2
3
4


Complexity of communities
■ Temporary
▲ Permanent
T03
T01
T07
T10
T15
T06
T14
T02
T08
T09
T13
T05
T12
T11
-4
-3
-2
-1
0
1
2
3
4

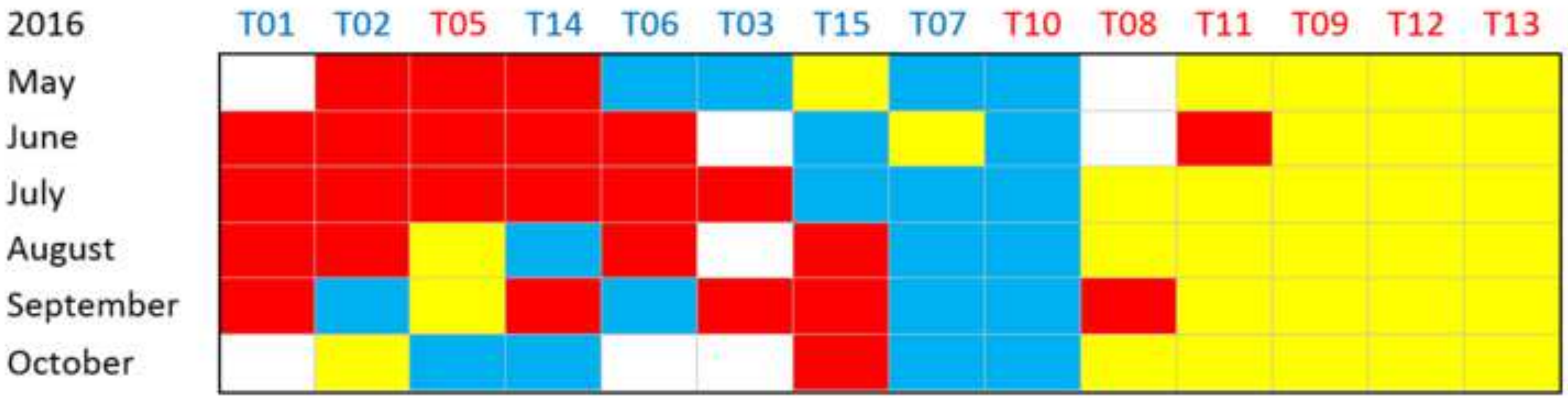

Torrentiality
Complexity of communities
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4
T12
T11
T05
T13
T09
T08
T02
T14
T06
T15
T10
T01
T07
T03


T09
T05
T11
T06
T13
T10
T12
T08
T03
T02
T01
T15
T14
T07
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4
Organic matter availability
Complexity of communities


4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4
Catchment
Complexity of communities
T11
T12
T05
T13
T09
T08
T02
T06
T14
T15
T10
T07
T01
T03



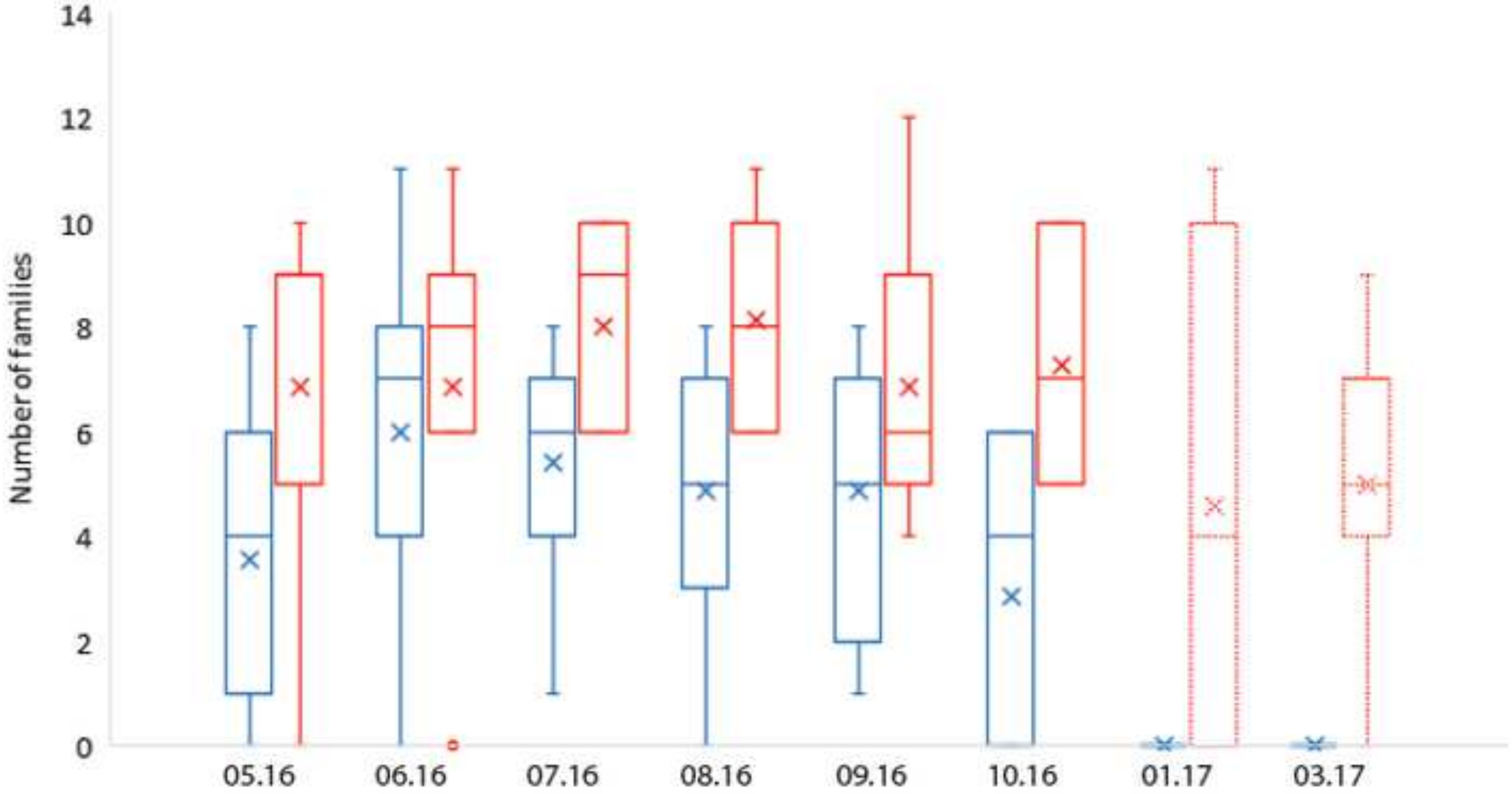
2016	T01	T02	T03	T05	T06	T07	T08	T09	T10	T11	T12	T13	T14	T15
May		🟥	🟦	🟥	🟦	🟦		🟨	🟦	🟨	🟨	🟨	🟥	🟨
June	🟥	🟥		🟥	🟥	🟨		🟨	🟦	🟥	🟨	🟨	🟥	🟦
July	🟥	🟥	🟥	🟥	🟥	🟦	🟨	🟨	🟦	🟨	🟨	🟨	🟥	🟦
August	🟥	🟥		🟨	🟥	🟦	🟨	🟨	🟦	🟨	🟨	🟨	🟦	🟥
September	🟥	🟦	🟥	🟨	🟦	🟦	🟥	🟨	🟦	🟨	🟨	🟨	🟥	🟥
October		🟨		🟦		🟦	🟨	🟨	🟦	🟨	🟨	🟨	🟦	🟥

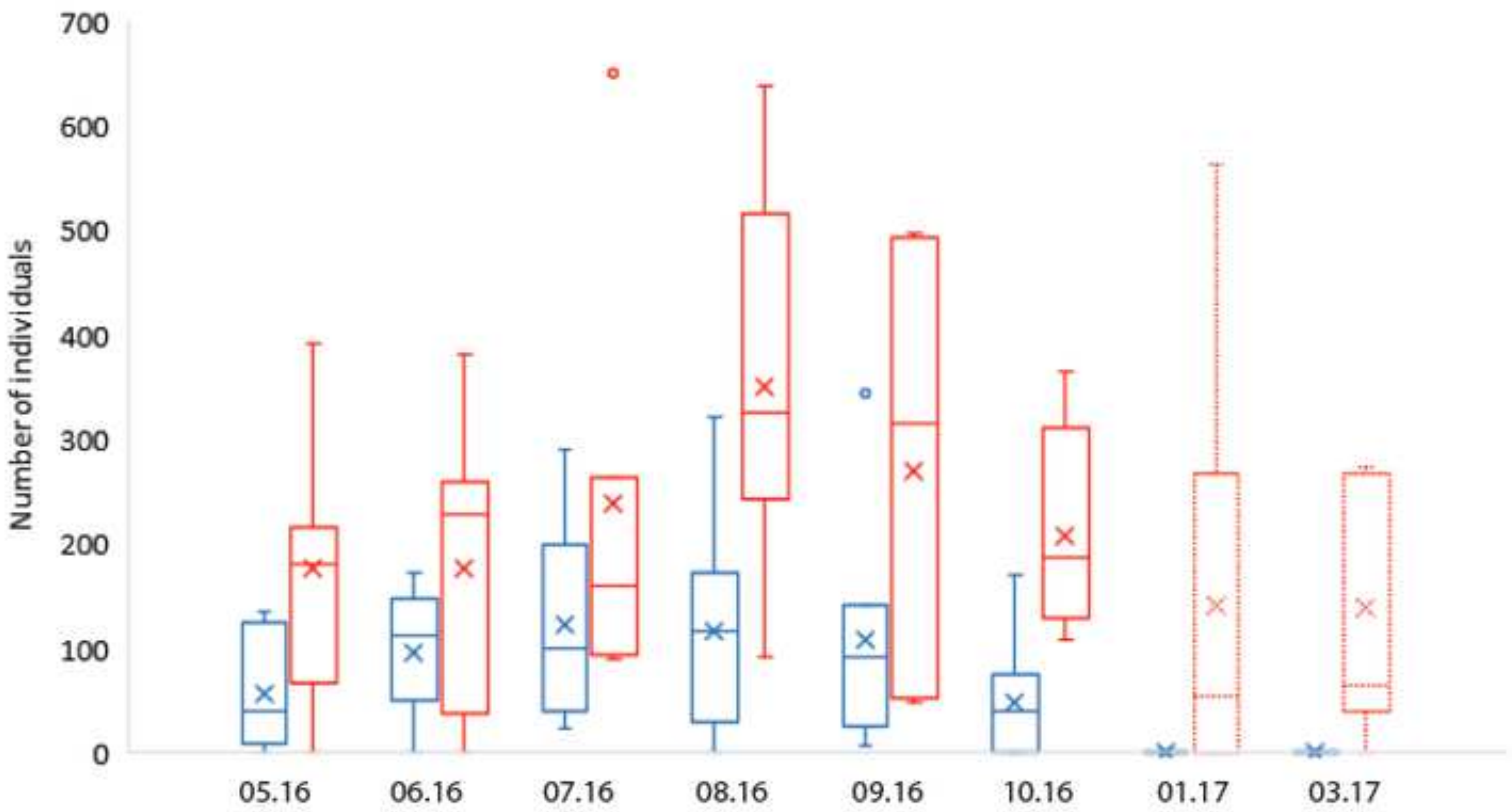


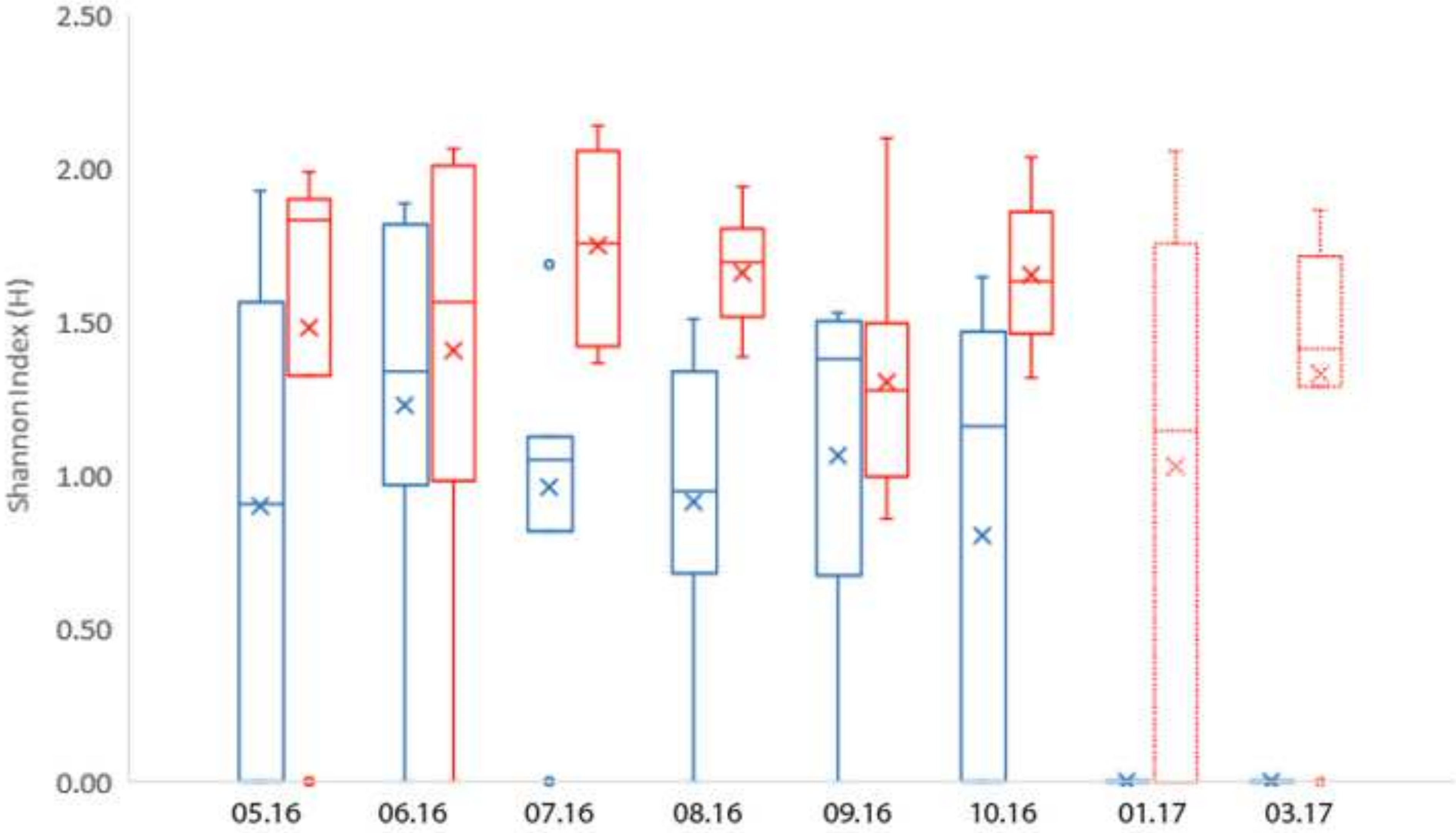
2016	T01	T02	T05	T14	T06	T03	T15	T07	T10	T08	T11	T09	T12	T13
May		🟥	🟥	🟥	🟦	🟦	🟨	🟦	🟦		🟨	🟨	🟨	🟨
June	🟥	🟥	🟥	🟥	🟥		🟦	🟨	🟦		🟥	🟨	🟨	🟨
July	🟥	🟥	🟥	🟥	🟥	🟥	🟦	🟦	🟦	🟨	🟨	🟨	🟨	🟨
August	🟥	🟥	🟨	🟦	🟥		🟥	🟦	🟦	🟨	🟨	🟨	🟨	🟨
September	🟥	🟦	🟨	🟥	🟦	🟥	🟥	🟦	🟦	🟥	🟨	🟨	🟨	🟨
October		🟨	🟦	🟦			🟥	🟦	🟦	🟨	🟨	🟨	🟨	🟨

	T01	T02	T03	T05	T06	T07	T08	T09	T10	T11	T12	T13	T14	T15
August 2015	🟩	🟩	🟩	🟩	🟧	🟩	🟩	▨	🟩	🟪	🟩	▨	🟪	🟩
August 2016	🟩	🟩	🟧	🟪	🟩	🟧	▨	▨	🟧	🟪	🟪	▨	🟩	🟩

	T01	T02	T03	T05	T06	T07	T08	T09	T10	T11	T12	T13	T14	T15
October 2015	⬜	🟦	⬜	⬜	🟦	🟦	⬜	🟥	🟨	⬛	🟥	🟨	🟦	⬜
October 2016	⬜	⬛	⬜	🟦	⬜	🟦	🟥	🟥	🟨	⬛	🟥	⬛	🟦	⬛


14
12
10
8
6
4
2
0
Number of families
05.16
06.16
07.16
08.16
09.16
10.16
01.17
03.17


Number of individuals
700
600
500
400
300
200
100
0
05.16
06.16
07.16
08.16
09.16
10.16
01.17
03.17


Shannon Index (H)
2.50
2.00
1.50
1.00
0.50
0.00
05.16
06.16
07.16
08.16
09.16
10.16
01.17
03.17