

**Effects of an experimental increase in flow intermittency on an alpine stream**

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This document is the accepted manuscript version of the following article:

Siebers, A. R., Paillex, A., Misteli, B., & Robinson, C. T. (2020). Effects of an experimental increase in flow intermittency on an alpine stream. *Hydrobiologia*.  
<https://doi.org/10.1007/s10750-020-04350-7>

## Abstract

Flow intermittency occurs naturally in alpine streams. However, changing rainfall patterns and glacier retreat are predicted to increase the occurrence of flow intermittency in alpine catchments, with largely unknown effects on ecosystem structure and function. We conducted a flow manipulation experiment within a headwater stream of Val Roseg, a glacierized alpine catchment, to determine the effects of increased flow intermittency on aquatic macroinvertebrates, periphyton, benthic organic matter, and trophic structure. Compared to an adjacent reference channel, an increase in flow intermittency reduced macroinvertebrate density, taxa richness, and the proportion of rheophilic taxa. Density and richness remained low in the manipulated channel after resumption of natural flow. Flow intermittency did not affect organic matter standing stocks, but increased assimilation of periphyton by aquatic macroinvertebrates. Predation on aquatic invertebrates by riparian spiders also increased. We attribute many of these patterns to the timing of drying, which likely excluded summer-growing cohorts of rheophilic, aerial dispersers. This study suggests that reductions in summer glacial melt and rainfall events might increase flow intermittency and lead to fundamental changes in diversity and function of alpine fluvial networks.

**Keywords:** glacial, macroinvertebrates, stable isotopes, drift, biofilm, benthic organic matter

## Introduction

Alpine streams are seasonal ecosystems. In particular, the contribution of different water sources to streams varies across both space and time in alpine fluvial networks (Malard et al., 1999; Brown et al., 2003; Robinson et al., 2016a). Relative contributions of snowmelt, glacial melt, groundwater, and rainfall drive wide variation in the physical and chemical environment of alpine streams and, as a consequence, the structure and function of benthic ecosystems (Ward, 1994; Brown et al., 2003). Alpine landscapes also exhibit high gradients, shallow aquifers, and limited sub-surface storage that contribute to flashy flow regimes linked to seasonality in glacial meltwaters and major rain events (Malard et al., 2000; Robinson & Matthaei, 2007; Robinson et al., 2016a). Several recent studies have documented widespread, seasonal flow intermittency among alpine headwaters (Robinson et al., 2016a; Gabbud et al., 2019; Paillex et al., 2020).

Intermittent alpine streams dry most widely during winter freeze periods, but summer and autumn drying periods are also common (possibly 40% of headwater networks: Paillex et al., 2020). However, current climate models predict increased temperatures and decreased rainfall during summer for alpine regions (Horton et al., 2006; Zemp et al., 2006; IPCC, 2014). While increasing summer temperatures might increase glacial melt contributions to streams in the short term, reductions in glacier volume will ultimately decrease this source of flow (Huss & Hock, 2018). Reductions in glacial melt and rainfall likely will increase flow intermittency in streams during summer and early autumn (Paillex et al., 2020). General expectations of glacial melt reductions are well documented for streams in terms of temperature and channel stability, with relatively less consideration of drying events (Brown et al., 2017; Milner et al., 2017). Comparatively, we thus have little understanding of how flow intermittency might affect the ecology of alpine fluvial networks.

60           Reductions in flow associated with decreases in glacial melt initially warm and stabilize  
61 alpine stream flows, which leads to increases in benthic production (Cauvy-Fraunié et al., 2016).  
62 Further, many rheophilic taxa may be replaced by generalist species more tolerant of low flow  
63 conditions (Milner et al., 2009; Cauvy-Fraunié et al., 2016). Indeed, rheophilic species may be  
64 quite sensitive to the onset of drying events (Piano et al., 2019a). Macroinvertebrates inhabiting  
65 alpine streams can be highly resistant to low temperatures, high turbidity, and unstable channels  
66 (Hotaling et al., 2017), but have low resistance to low flows and desiccation (Piano et al., 2019a).  
67 Here, resilience mechanisms such as drift (Doretto et al., 2019) and facultative use of the  
68 hyporheos (Malard et al., 2003a; b) are common. Further, many alpine aquatic insects disperse  
69 aerially as adults (Cauvy-Fraunié et al., 2015; Alther et al., 2019) and can quickly recolonize  
70 intermittent streams upon rewetting (Shama et al., 2011). Drying may thus reduce  
71 macroinvertebrate densities and richness sharply, but recovery after drying could be rapid (Lake,  
72 2003).

73           The function of benthic ecosystems also likely changes after drying. Coarse particulate  
74 organic matter quality is reduced in channels that dry through reduced leaching and breakdown  
75 by microbes and invertebrates (Datry et al., 2018). Alpine macroinvertebrate assemblages consist  
76 of many generalist grazers, gatherers, and omnivorous taxa, and thus exhibit high dietary  
77 flexibility across alpine environmental gradients (Zah et al., 2001; Fell et al., 2017; Piano et al.,  
78 2019b). Benthic macroinvertebrates in increasingly intermittent alpine streams may thus shift  
79 their diets to include a greater proportion of autochthonous resources (Siebers et al., 2019),  
80 thereby reducing the ecological niche of grazers (Piano et al., 2019b). Flow intermittency has  
81 been shown to alter species turnover (Gabbud et al., 2019), population genetic structure (Shama  
82 et al., 2016), and carbon cycling (Harjung et al., 2019) in alpine streams. Although these studies

suggest flow intermittency to be a key driver of ecological processes in alpine streams, more field experiments are required to elucidate the mechanistic factors of influence (e.g., Cauvy-Fraunié et al., 2016; Doretto et al., 2019).

Here, we conducted an experimental flow manipulation in an alpine stream using a before-after-control-impact (BACI) design. Flow was manipulated in a natural stream to simulate the onset of summer drying periods in an otherwise only winter-drying alpine stream (Paillex et al., 2020). Our first objective (i) was to compare differences in the density and composition of benthic macroinvertebrates between the manipulated stream and an adjacent reference channel, in the periods before, during and after the flow manipulation. We predicted that the density and richness of macroinvertebrates would be lower in the manipulated channel during the manipulation, rheophilic species in particular, but that recovery would occur following resumption of natural flow (i.e., within 2 months, Larned et al., 2010). Our second objective (ii) was to compare differences between the channels in benthic organic matter (CPOM and periphyton) density and quality, as well as the estimated dietary proportions of aquatic macroinvertebrates and predatory riparian invertebrates. We predicted that terrestrial organic matter densities would be higher in the manipulated channel during the flow manipulation, but be lower in quality, thus shifting the trophic base of benthic and riparian food webs towards aquatic sources both during and after the flow manipulation. We discuss our results in the context of future climate predictions for alpine regions.

## **Material and Methods**

### *Catchment and study channels*

Val Roseg is a glacierized valley within the Bernina massif of the eastern Swiss Alps (46°42'N, 9°86'E) (Ward & Uehlinger, 2003). Flow regimes are driven by seasonally and topographically complex combinations of glacial meltwater, rainfall, and snowmelt (Malard et al., 2005). Glacial meltwater primarily feeds a second-order braided river with numerous headwater tributaries draining valley side-slopes. Up to 90% of headwater streams in the fluvial network are intermittent due to high gradients, shallow aquifers, and seasonal and spatial variation in water sources (Robinson et al., 2016a; Paillex et al., 2020).

A naturally winter-drying headwater stream was chosen for the experiment based on prior surveys and measurements of flow intermittency (Robinson et al. 2016a, Paillex et al., 2020). The stream originates as a single channel around 650 m upstream of the experimental section where it then splits naturally into two channels for around 350 m before converging back into a single channel again. The study reaches (100 m in length) were located ca. 200 m downstream of where the channel divides. The study reaches averaged 10-15 m apart and were similar in altitude (2041 m a.s.l.), slope (1.2°) and vegetation (scattered small trees – *Larix decidua*, *Pinus cembra*, *Alnus viridis*; shrubs – *Juniperus communis* subsp. *alpina*; and grasses and sedges – *Festuca* spp., *Carex* spp.) (Fig. 1).

#### *Flow manipulation and measurement*

The presence of surface water in each channel was measured using HOBO Pendant Temperature/Light 64K data loggers (Onset Computer Corporation, Bourne, MA, USA) modified to record electrical resistance and temperature (Chapin et al., 2014; Paillex et al., 2020). The loggers were originally designed to record light intensity, but were modified to include external electrodes connected to the light sensor contact pads. Consequently, the sensor records and outputs relative electrical resistance as Lux (original units of light intensity). Lux readings are

linearly correlated with electrical conductivity up to ca. 1000  $\mu\text{S}/\text{cm}$  (Chapin et al., 2014). Zero readings thus indicate absence of liquid water in channels (Paillex et al., 2020). Temperature readings record the temperature of the medium at the time (water, air or ice). Loggers were installed in the thalweg of each stream some months prior to the first sampling occasion, and recorded hourly the presence and absence of surface water as well as temperature. Loggers were removed briefly for maintenance in October 2018, but otherwise recorded data during the entire experimental period.

A temporary flow diversion (dam) was installed on 5 June 2018 upstream of the sampling reaches that diverted flow from entering one of the channels (Fig. 2). The flow manipulation reduced discharge in this ‘manipulation’ channel (MC) directly downstream from the diversion from  $0.01 \text{ m}^2 \text{ s}^{-1}$  pre-manipulation (Fig. 1c) to  $0 \text{ m}^2 \text{ s}^{-1}$  (Fig. 1d). Local bank storage, hyporheic upwellings, and rainfall events continued to provide flow at various points further down the MC (pers. obs., the authors), but the diversion eventually caused an increase in flow intermittency in the channel (Fig. 2d). The ‘reference’ channel (RC) continued flowing both before (Fig. 1a) and during (Fig. 1b) the manipulation. The flow diversion was removed on 20 September 2018, after which the MC quickly resumed surface flow (Fig. 2d). The experiment thus included three distinct measurement periods: 2 months from snowmelt until the flow manipulation (April to June), 3 months of flow manipulation over summer and early autumn (June to September), and 2 months from removal of the manipulation until snow cover (September to November).

#### *Sample collection and analysis*

Sampling was conducted during the snow-free period monthly from May (prior to manipulation) to November 2018 in both RC and MC (Fig. 2). We conducted two additional samplings on 30 August and 20 September to further characterize the flow manipulation period. When surface

water was present, water temperature and electrical conductivity were measured on each site visit with a WTW-3110 portable meter (Weilheim, Germany) for cross-validation with logger readings. Water samples for chemical analysis were collected in triple-rinsed 0.5 L polypropylene bottles without headspace, and stored at 4°C for transport to the laboratory. Analyses were conducted for alkalinity, pH, dissolved organic carbon (DOC), total nitrogen (TN), nitrate (NO<sub>3</sub>-N), total phosphorus (TP), soluble reactive phosphate (SRP; PO<sub>4</sub>-P), and silicate (H<sub>4</sub>SiO<sub>4</sub>) after Tockner et al., (1997).

Benthic macroinvertebrates were collected using a Hess sampler (mesh size: 250 µm; area: 0.0425 m<sup>2</sup>) at 3 to 5 representative locations along the 100 m study reach of each stream. When surface water was flowing, samples were collected by disturbing the loosely consolidated cobbles composing the streambed (10 cm depth) within the Hess sampler for 10 seconds. When the MC lacked surface water, we collected samples by excavating the dry streambed to the same depth of 10 cm, within the same area as a Hess sampler. Samples were elutriated in the field to remove inorganic particles and stored in 80% ethanol until analysis in the laboratory.

In the laboratory, macroinvertebrates were hand-picked from each sample using a dissecting microscope at 10x magnification. Many individuals were too small to identify confidently to genus, and most families were represented by ≤ 3 functionally similar genera (e.g. *Baetis* spp. for Baetidae, *Dictyogenus* spp. and *Isoperla* spp. for Perlodidae). Macroinvertebrates were therefore identified to the lowest common, practical taxonomic level (family for insects, order for non-insects) and counted. Identification followed Tachet et al., (2010) with additional reference for Plecoptera (Lubini et al., 2012), Trichoptera (Waringer & Graf, 2011), Ephemeroptera (Bauernfeind & Humpesch, 2001), Coleoptera (Lucht, 1998) and Diptera (Eiseler, 2010). The remaining material from each benthic sample was air-dried at 60° C,



weighed, combusted at 500° C for 4 h, and reweighed to provide an estimate of coarse (> 250 µm) benthic organic matter (BOM) as ash-free dry mass (AFDM). While we did not measure the finest fraction of BOM (< 250 µm), personal observations suggested that fine particulate matter within both streams was largely inorganic.

Periphyton biomass was collected by randomly collecting 5 cobbles from each study reach on each visit. Periphyton was removed from the top of each stone by scrubbing with a wire brush and rinsing with stream water. The area scrubbed from each stone was measured, and an aliquot of the periphyton suspension was filtered through a pre-combusted glass fiber filter (Whatman GF/F). The filter was air-dried at 60° C, weighed, combusted at 500° C for 4 h, and reweighed to measure AFDM.

Samples for isotope analysis were collected along the 100 m study reach of each stream. Aquatic macroinvertebrates were collected by repetitive kick-sampling (mesh size: 250 µm) at several sites along each reach to include all substrate types present (cobbles, sediment, mosses). Aquatic macroinvertebrates were handpicked from samples and immediately sorted into 50 mL plastic vials containing stream water. When the streambed of the MC was dry, we inspected the top 10cm of streambed for individuals but could not find enough aquatic invertebrate biomass for analysis. Ground-dwelling riparian invertebrates were searched for and manually caught within 2 m of the stream edge at random intervals along the outer side of each reach (i.e., not in the space between the two channels), and stored in dry 50 mL plastic vials. Predatory taxa were stored separately. Macroinvertebrates were left alive for ca. 8 h to void stomach contents, then identified, sorted to family, and stored at -20°C until analysis.

We collected potential basal organic matter (OM) sources as material collected from at least 3 locations along the sampling reach of each stream and then combined into a single sample

(see Jardine et al., 2014). Leaves of riparian plants (separated by species) and aquatic mosses were collected from living plants. Coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM) were collected directly from the stream. Periphyton was collected as epilithon scrubbed with a wire brush and rinsed from cobbles. All OM samples were stored at -20°C until analysis.

All moss and CPOM samples were briefly thawed and then triple-rinsed with distilled water. Terrestrial plant, moss and CPOM samples were air-dried at 60°C for 72 hours and then mechanically ground and homogenized. Periphyton and FPOM samples were centrifuged for 12 min to separate out inorganic matter. Periphyton, FPOM, and invertebrate samples were freeze-dried for 48 hours in a Lyovac GT 2-E lyophilizer (STERIS GmbH, Hürth, Germany). Invertebrate samples were finely chopped and homogenized after drying. We individually weighed ~ 0.5 – 1.5 mg OM, periphyton, and animal samples and combusted them in a Vario PYRO Cube elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) connected to an IsoPrime isotope ratio mass spectrometer (GV Instruments Ltd., Wythenshawe, U.K.) for measurement of total C content, total N content,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Reference materials for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  derived from NBS 19, L-SVEC, IAEA-N-1 and IAEA-N-2 were provided by Biogeochemical Laboratories, Indiana University. Analytical uncertainty was 0.1‰ for  $\delta^{13}\text{C}$  and 0.2 ‰ for  $\delta^{15}\text{N}$ .

#### *Data analysis*

We used a before-after control-impact design (BACI) to test whether macroinvertebrate community composition, diversity and organic matter standing stocks were affected by flow intermittence. The RC was used as the control group. We also sought to characterize the manipulation period itself to determine whether flow intermittence, or the resumption of natural

flow following intermittence, would represent the major perturbation for the benthic ecosystem. We thus split the data into 3 time periods: before the flow manipulation, during the flow manipulation, and after the flow manipulation. Fixed factors for all following analyses were based on the groups imposed by this structure (i.e., the different time periods, separated by site; MC before, RC before, MC during, etc.).

To identify how community composition changed between channels over time, we identified similarities among macroinvertebrate assemblages with non-metric multidimensional scaling (nMDS) using Bray-Curtis dissimilarities. Mean density data were used in the analysis (before  $n = 13$ ; during  $n = 15$ ; after  $n = 6$ ). Taxa were pooled to lowest common taxonomic level (family for insects; order for non-insects) and data were standardized by Wisconsin double standardization. Taxa found in less than 3 samplings were excluded from analysis. We excluded the August 30 sampling of the MC due to extremely low densities (all taxa mean  $< 1$  individuals). We then assessed whether community composition differed between channels and across the different time periods using permutational multivariate analysis of variance (PERMANOVA). We assessed which taxa contributed most to differences across groups using indicator species analysis (ISA). The nMDS and PERMANOVA analysis were conducted using the package *vegan* (Oksanen et al., 2017), and ISA was conducted using the package *indicspecies* (Cáceres & Legendre, 2009) in R 3.4.2 (R core team 2013).

We calculated several biological indices for testing differences in aquatic biodiversity between the two channels over time. We summarized assemblages using total density (individuals  $m^{-2}$ ), density of rheophilic taxa, taxonomic richness ( $s$ ), and proportion of 's' composed of rheophilic taxa (%). Rheophilic taxa were identified using rheophilic/rheobiont current preference following Schmedtje & Colling, (1996) and Buffagni et al., (2018). Indices

summarizing organic matter measurements included density (AFDM per unit area) and C:N ratios (total C content/total N content). We tested whether differences existed across means in biological and organic matter indices of all groups (i.e., before, during, and after time periods in each channel) using analysis of variance (ANOVA). We then compared pairwise differences in the indices between the two channels within the specific before, during, and after manipulation time periods using Tukey's Honest Significant Difference (HSD) tests. All tests were conducted using base packages in R 3.4.2 (R core team 2013).

We also assessed changes in the trophic base of macroinvertebrate food webs over time. Here, we used the Bayesian mixing model SIAR (Parnell et al., 2010) to produce probability distributions of the dietary contributions of basal food resource groups to (i) aquatic macroinvertebrates and (ii) predatory riparian invertebrates. CPOM and periphyton were included as potential sources in the models for aquatic macroinvertebrates. FPOM was excluded as a potential food source due to very low  $\delta^{15}\text{N}$  values relative to aquatic consumers (see Philips et al., 2005; Jardine et al., 2013). Riparian prey (ants; Formicidae) and aquatic prey (aquatic macroinvertebrates) were included as potential sources in the models for riparian predators. We did not include additional prior information on diet proportions because sources in each mixing model were equal to the number of tracers (Fry, 2013; Brett, 2014). We ran the model separately for each time period in each channel (see BACI structure, above). CPOM (Kruskal-Wallis tests;  $p = 0.25$  to  $0.57$ ), periphyton ( $p = 0.28$  to  $0.46$ ), and riparian prey ( $p = 0.21$  to  $0.64$ )  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values did not differ significantly over time and were set as averages for each channel for all sampling times. Aquatic prey  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were set as averages for each time period in each channel. Trophic enrichment factors were set at  $0.4 \pm 1.0$  ‰ for  $\delta^{13}\text{C}$  and  $2.2 \pm 1.2$  ‰ for  $\delta^{15}\text{N}$  (Post, 2002; McCutchan et al., 2003). Each model was set to run 500,000 times, with the first

50,000 iterations discarded. We present the upper and lower quartiles of model results together with the median. We describe results as differing where the median is higher than the upper quartile, or lower than the lower quartile, of another distribution. All models were created using the SIAR package (Parnell et al., 2010) in R 3.4.2 (R Core Team, 2012).

#### *Data Availability Statement*

The datasets analysed during the current study are available in the Dryad repository, <<https://doi.org/10.5061/dryad.xksn02vcr>> (Siebers et al., 2020).

## **Results**

### *Flow intermittency, physical environment, and chemistry*

Both streams began flowing in early April (Fig. 2b, d). The reference channel (RC) then flowed continuously through the sampling period (Fig. 2b). A brief (1 day) first drying event in the manipulation channel (MC) occurred on 10 June, 25 days after installation of the flow diversion (Fig. 2d). More extended drying events (> 1 day in duration) then occurred from 30 June to 19 September in MC (Fig. 2d). During this time, MC dried 8 times for an average of 7.6 days (sd  $\pm 5.5$ ) each. 6 flow events also occurred in MC during the manipulation period, driven by rainfall events greater than  $15 \text{ mm d}^{-1}$  (cross correlation test,  $p > 0.20$  with 2-5 day lag period) (Fig. 2). Electrical resistance readings in RC were largely stable (Dickey-Fuller test,  $p = 0.29$ ) during the manipulation period (Fig. 2), and were not significantly affected by rain events (cross correlation test,  $p < 0.13$ ). After removal of the flow diversion on 20 September, MC resumed flow for the remaining sampling period (Fig. 2d).

Temperature readings in RC (mean  $\pm$ sd;  $4.4 \pm 1.1^\circ \text{C}$ ) remained relatively consistent (Dickey-Fuller test,  $p = 0.20$ ) over the sampling period (Fig. 2c). In MC, temperature readings

were consistent ( $3.4 \pm 2.0$  ° C) before and after the flow manipulation, but increased substantially ( $13.0 \pm 2.7$  ° C) during the manipulation period (Fig. 2e). Dry channel readings (i.e. air temperatures) averaged  $13.9 \pm 2.2$  ° C, but water temperature during the flow manipulation was only slightly lower ( $11.9 \pm 2.9$  ° C). Water chemistry was mostly consistent across both channels and seasons (Table 1). An exception was total phosphorus concentrations, which became higher in MC after the onset of flow intermittency than in RC (Table 1).

### *Biotic responses before/after flow manipulation*

Macroinvertebrates assemblages were differentiated by channel and time period (Fig. 3a). An early seasonal (spring) community characterized by Limoniidae and Simuliidae (ISA  $p = 0.002$ ) was common at both channels before the flow manipulation. Communities in RC diverged from July into a later seasonal assemblage that remained consistent until November, and differentiated from the spring assemblage with higher densities of large predatory dipterans (Muscidae, Empididae) and rheophilic Ephemeroptera (Baetidae) and Plecoptera (Perlodidae, Leuctridae), particularly Nemouridae (ISA  $p = 0.03$ ) (Fig. 3b). Communities in MC diverged from both the spring assemblages and RC following the flow manipulation (Fig. 3a). Communities in MC remained dissimilar from RC following resumption of natural flow (October onwards). Communities in MC during and after the manipulation were differentiated from RC with higher densities of Oligochaeta, Hydracarina, and Pediciidae, although Pediciidae also occurred in RC from October (ISA  $p = 0.008$ ) (Fig. 3b).

Macroinvertebrate densities (ANOVA,  $p < 0.001$ ), densities of rheophilic taxa ( $p < 0.001$ ), taxa richness ( $p < 0.001$ ), and the proportion of rheophilic taxa ( $p < 0.001$ ) all differed similarly across channels and time periods. In spring prior to flow manipulation, macroinvertebrate densities (Tukey's HSD,  $p = 0.99$ ), densities of rheophilic taxa ( $p = 0.94$ ), taxa

richness ( $p = 0.99$ ), and the proportion of rheophilic taxa ( $p = 1$ ) were similar in RC and MC (Fig. 4). During the flow manipulation, macroinvertebrate densities ( $p < 0.001$ ), densities of rheophilic taxa ( $p < 0.001$ ), taxa richness ( $p < 0.001$ ), and the proportion of rheophilic taxa ( $p < 0.001$ ) were lower in MC (Fig. 4). Macroinvertebrate densities ( $p < 0.009$ ), densities of rheophilic taxa ( $p < 0.001$ ), taxa richness ( $p < 0.001$ ), and the proportion of rheophilic taxa ( $p = 0.04$ ) all remained lower in MC after natural flow resumption ( $p = 0.009$ ) (Fig. 4).

#### *Changes in benthic organic matter*

Standing stocks of coarse benthic organic matter (BOM) differed between channels and time periods (ANOVA,  $p = 0.02$ ). Coarse BOM density was higher in RC during spring, prior to the flow manipulation (Tukey's HSD,  $p = 0.03$ ) (Fig. 5a). However, standing stocks of coarse BOM were similar in MC and RC both during ( $p = 0.99$ ) and after the flow manipulation ( $p = 0.99$ ) (Fig. 5a). CPOM C:N ratios also differed between channels and time periods (ANOVA,  $p = 0.02$ ). CPOM C:N ratios were higher in MC than RC prior to the flow manipulation (Tukey's HSD,  $p = 0.04$ ), but were similar in both channels during (0.50) and after the flow manipulation ( $p = 0.99$ ) (Fig. 5b). Standing stocks of periphyton were similar in both channels across all time periods (ANOVA,  $p = 0.16$ ) (Fig. 5c). The C:N ratios of periphyton also were similar in both channels across all time periods (ANOVA,  $p = 0.45$ ) (Fig. 5d).

#### *Trophic base of food webs*

Estimated assimilation of CPOM by macroinvertebrate primary consumers (range of median estimations; 0.47 to 0.73) was generally higher than that of periphyton (0.27 to 0.50) (Fig. 6a, c). Prior to the flow manipulation, estimated assimilation of periphyton was higher in MC (median estimation; 0.40) than RC (0.27) (Fig. 6c). During the flow manipulation, estimated assimilation

of periphyton was similar in RC (0.29) but increased in MC (0.50) (Fig. 6c). After resumption of natural flow, estimated assimilation of periphyton remained higher in MC (0.53) than RC (0.32) (Fig. 6c).

All riparian predators collected were hunting spiders (Lycosidae, Gnaphosidae, Agelenidae). Prior to the flow manipulation, estimated assimilation of aquatic prey was similar in both MC (median estimation; 0.25) and RC (0.34) (Fig. 6d). Estimated assimilation of aquatic prey did not change notably during the flow manipulation, but was higher across both MC (0.48) and RC (0.49) following resumption of natural flow (Fig. 6d), with concurrent reductions in the estimated assimilation of terrestrial prey (Fig. 6b).

## Discussion

### *Intermittency effects on the physical and chemical environment*

Data from the electrical resistance sensors indicated that the reference channel (RC) had a relatively stable flow regime, with continuous summer and autumn flow following spring snowmelt. This flow regime likely reflects a source mix of snowmelt, rainfall and glacial melt (Brown et al., 2003; Paillex et al., 2020). The relatively low variability of electrical resistance readings in RC, combined with a corresponding lack of variability in biological indices in RC, suggest that there were also likely no substantial floods or spates within RC during the year. In contrast, the flow diversion caused discharge in the manipulated channel (MC) to be driven solely by large rainfall events. The lag in response between rain and flow events further indicates that alluvial sediments were not saturated while MC was dry (Malard et al., 1999). The manipulated flow regime was thus analogous to pulsed, ephemeral regimes of many dryland and Mediterranean streams (Costigan et al., 2017). Further, MC had minimal groundwater upwelling



(Paillex et al., 2020) and highly permeable underlying alluvium (Tockner et al., 1997), similar to primary drivers of flow intermittency in other montane catchments (Jaeger & Olden, 2012; Sando & Blasch, 2014).

We observed distinct increases in temperature with increased flow intermittency. In Val Roseg, higher temperatures are characteristic of intermittent channels with rainfall and snowmelt-dominated flow regimes (Paillex et al., 2020). Relatively stable and lower temperatures in RC indicate a possible groundwater or glacial water source, as these streams tend to be colder with more stable thermal regimes (Brown et al., 2003; Paillex et al., 2020). However, both channels had low silicate and alkalinity levels throughout the experiment, indicating that groundwater was not a major water source (Malard et al., 1999; Malard et al., 2006). We conclude that the stream is usually fed by glacial melt via the glacial moraine during summer, with occasional rainfall inputs (see Paillex et al., 2020). Dry channel readings (i.e., air temperature) account for much of the temperature increase in MC. However, low flows, ponding, and drying also may have increased the effect of solar radiation on water and sediment temperatures as the experiment progressed during summer and autumn (Gómez et al., 2017).

In contrast to temperature, the chemical environment of MC was largely unaffected by increased flow intermittency. The exception was higher total phosphorus (P) concentrations in MC than in RC during and after the manipulation period. High total P concentrations are characteristic of glacier-fed streams due to resuspension of glacial flour (Tockner et al., 1997). While organic P also can be released by an increase in organic matter decomposition, we did not observe any change in reactive nutrient forms that might indicate higher biogeochemical processing rates after the flow manipulation (e.g., SRP, NO<sub>3</sub>; von Schiller et al., 2017). Alpine soils and sediments are relatively young in a geological context and thus tend to have low

bioavailable nutrient concentrations (Schlesinger & Bernhardt, 2013). Biogeochemical processes might thus be restricted overall by low nutrient and organic matter concentrations. For example, streams in Val Roseg have relatively low sediment respiration rates compared with non-alpine streams (Logue et al., 2004; Robinson et al., 2016a). Much of the P released through glacial melt is also mineral-bound and not readily available to microbial communities (Hodson et al., 2004). Consequently, it is likely that total P concentrations increased due to increased retention and evapo-concentration of fine particulate material during the flow reduction period.

*Intermittency effects on macroinvertebrate assemblages*

In this study, we observed large reductions in both density and diversity of benthic macroinvertebrates with increased flow intermittency. As above, relatively stable electrical resistance readings and biological indices in RC over time suggest that no substantial perturbations occurred in RC in response to floods or spates. Patterns in MC thus more closely resemble community responses to supra-seasonal drought (i.e., unpredictable in timing and duration) than periodic or seasonal drying (see Lake, 2003). Alpine benthic communities may thus be adapted more toward winter as opposed to summer drying periods (Lytle & Poff, 2004), i.e., towards predictably long rather than shorter but unpredictable drying periods (e.g. Sánchez-Montoya et al. 2018). There was also no increase in density with the initial flow reduction (i.e., after the flow manipulation but before consistent channel drying) (Online resource Fig. S.1) suggesting that there was little concentration of macroinvertebrates into surface water refugia (Acuña et al., 2005; Dewson et al., 2007; Walters & Post, 2011). Streams in Val Roseg are mostly high gradient with limited sub-surface storage, limiting the occurrence and permanence of pools (Malard et al., 2000; Robinson & Matthaei, 2007; Paillex et al., 2020). Most of the benthic taxa observed here also have been recorded in the hyporheos of Val Roseg (Malard et al., 2003a;

b), which may have provided an alternative drying refuge. However, we did not observe rapid recolonization of MC following rewetting, suggesting that hyporheic water tables may have become depleted during intermittency and thereby reduced the value of the hyporheic zone as a refuge (Datry, 2012; Vander Vorste et al., 2016a). We would need to conduct comprehensive hyporheic sampling to confirm this hypothesis, however.

Macroinvertebrate densities in MC did not recover to those observed in RC following resumption of natural flow, yet densities in MC following flow resumption were still higher than those during the end of the flow manipulation. Some level of recolonization via drift (Doretto et al., 2018), emergence from the hyporheic zone (Vander Vorste et al., 2016), and/or oviposition (Cauvie-Fraunié et al., 2015) thus likely occurred. We did not measure these processes directly throughout the study period. However, data on macroinvertebrate drift collected during removal of the flow manipulation (Online resource Fig. S.2) shows that drift patterns differed between MC and RC. In particular, Perlodidae were abundant in MC drift but not RC drift following flow resumption (Online resource Fig. S.2b). However, densities in MC following flow resumption were almost entirely non-rheophilic (Fig. 3). Instead of the hyporheic zone not representing a refuge, as discussed above, we suggest that this pattern reflects emergence from the hyporheic zone directly into drift, and therefore to downstream communities rather than the previously dry channel. In fact, non-rheophilic taxa (e.g., Chironomidae) often have more cohorts during the year when compared with uni- or semi-voltine rheophilic taxa (e.g., Baetidae) (Burgherr & Ward, 2001). We suggest that aerial dispersal therefore represents the most likely source of recolonization at MC (Alther et al., 2019).

The timing of drying (here June-July) coincides with the emergence and egg-laying period of aquatic macroinvertebrates with aerial adult stages (Burgherr & Ward, 2001; Leys et

al., 2017). An increase in densities at this time in RC, particularly for rheophilic taxa (Online resource Fig. S.1), further suggests a summer oviposition peak for taxa with aerial dispersal. The timing of flow resumption (here autumn) thus likely excluded this summer cohort in MC. In addition, adults of rheophilic taxa likely would have avoided MC due to a lack of surface water habitats, even if surface water was present after rain events (Bogan & Boersma, 2012; Doretto et al., 2018). Distribution patterns in alpine streams are often strongly driven by aerial dispersal (Cauvy-Fraunié et al., 2015; Alther et al., 2019). Reductions in both density and diversity in MC are thus likely due to both peaks in emergence immediately prior to drying, and low recolonization from aerial sources while the channel was dry. The lack of a summer-growing cohort for many taxa would also partially explain why density and diversity did not recover in MC following flow resumption.

Despite the high prevalence of flow intermittency in Val Roseg (Robinson et al., 2016a; Paillex et al., 2020), it appears that alpine macroinvertebrates may not be resistant to seasonal cycles of drying (as in, e.g., dryland or Mediterranean rivers; see Bogan et al., 2017). Although many alpine taxa are resistant to low temperatures and low channel stability (Milner et al., 2017), these adaptation mechanisms may not result in co-tolerance to stream drying (see Vander Vorste et al., 2016b). Limited sub-surface storage in alpine headwaters might limit the hyporheic zone as a refuge and other resistance mechanisms during dry periods, as described above. For instance, the low recolonization following flow resumption in MC also suggests that desiccation-resistant taxa are rare in alpine headwaters (Bogan et al., 2017) and that increasing flow intermittency might thus exclude desiccation-sensitive taxa (Arscott et al., 2010; Datry, 2012). In the Swiss Alps, similar genetic diversity in mayfly populations of perennial and intermittent streams (Shama et al., 2011) infers that aerial dispersal and source-sink dynamics can provide strong

resilience to disturbance (Bogan et al., 2017). Our results suggest that the seasonality and timing of flow intermittency is another major determinant of whether this resilience pathway occurs. Our results indicate that resilience mechanisms are intrinsically tied to the geomorphological context of channels and the life-history characteristics of the taxa present, rather than a general trend across intermittent fluvial systems (Robson et al., 2011; Bogan et al., 2017).

#### *Intermittency effects on organic matter and stoichiometry*

We observed no change in periphyton biomass or C:N ratios with experimental flow intermittency. Periphyton biomass in alpine environments is often limited overall by frequent scouring and bed movement, low temperatures, and low inorganic nutrient availability (Uehlinger et al., 2009). In addition, drying of epilithic biofilms might strongly reduce autotrophic biomass and enzymatic activity rather than overall biomass or bulk stoichiometric ratios (Timoner et al., 2012). Thus drying may not have affected bulk periphyton biomass to a measurable extent in MC (e.g., Piano et al., 2019a).

We also observed no change in the quantity or C:N ratios of particulate organic matter with flow manipulation. These patterns contradict our hypothesis that terrestrial organic matter would accumulate in stream channels in the absence of downstream transport (e.g., Acuña et al., 2004) and increase in C:N ratios due to leaching of non-structural compounds and low rates of microbial conditioning (Datry et al., 2018). Litter inputs in Val Roseg show autumnal peaks due to the presence of deciduous trees (Zah & Uehlinger, 2001) and both RC and MC may thus have only received low rates of litterfall during summer, perhaps insufficient to increase coarse BOM standing stocks. During the experimental period, several rainfall events also resulted in pulsed flow events within MC and may have transported benthic organic matter downstream (Larned et al., 2010). Rain events might also rehydrate microbial biofilms (Sabater et al., 2016), thus

potentially compensating for the effects of drying on organic matter stoichiometry. For instance, rates of microbially-mediated biogeochemical processes are often highest in intermittent rivers and streams during rewetting events (Larned et al., 2010; von Schiller et al., 2017). Thus, the consistent quantity and stoichiometry of POM and periphyton here might reflect the averaging of drying and rewetting effects, rather than a true lack of variation.

#### *Intermittency effects on the trophic base of food webs*

Mixing models estimated higher periphyton assimilation in MC than RC after the onset of flow intermittency, matching patterns previously seen in Val Roseg (Siebers et al., 2019). It was posited that decreasing CPOM lability in dry channels was the mechanism driving increased periphyton assimilation (Siebers et al., 2019). As described above, we observed no change in CPOM C:N ratios with experimental flow intermittency. Consequently, macroinvertebrate diets may have instead shifted due to changes in biotic interactions (Holomuzki et al., 2010). Indeed, differences in species composition between channels post-manipulation were largely associated with densities of invertebrate predators (Perlodidae, Empididae, Muscidae), but also grazers (Nemouridae). Reduced predation pressure might increase herbivore activity and/or reduce refuge use, leading to increased grazing on exposed stone surfaces where periphyton is common (Schmitz et al., 2004). In addition, a reduction in grazer densities might reduce intraspecific competition for labile algal resources (e.g., McNeely et al., 2007). An overall decrease in macroinvertebrate density, particularly for obligate grazers such as Baetidae, is likely to reduce resource competition. Drying may also have affected detritivores more strongly than grazers (Corti et al., 2011), although the taxa contributing most to differences between RC and MC post-manipulation did not include any terrestrial OM specialists (e.g. shredders). Thus, it appears

probable that increasing flow intermittency reduced density-dependent interaction pressures upon primary consumers, allowing them to specialize on higher-quality food resources.

Riparian spiders assimilated approximately 25 to 49% of their C and N from aquatic macroinvertebrates, which is similar to estimates from other intermittent running waters (Sanzone et al., 2003; Paetzold et al., 2005). However, the estimated increase of aquatic prey in the diet of riparian spiders at MC after flow manipulation is surprising, given that the density of aquatic macroinvertebrates and rheophilic taxa (i.e., including many taxa here with emergent adult stages) both declined substantially. Aquatic prey availability typically limits both the biomass (Greenwood & McIntosh, 2010) and diversity (McCluney & Sabo, 2012; Corti & Datry 2014) of riparian arthropods. As with the aquatic taxa, competition might thus have decreased if spider densities were lower. Alternatively, aquatic or semi-aquatic prey may have become easier to access with flow reduction or cessation (McIntosh et al., 2017). Spiders also might shift diets to favour aquatic emergents if they cannot obtain enough water from terrestrial prey, i.e. under arid or drought conditions (McCluney & Sabo, 2009). While riparian spiders typically have small home range areas (c. < 20 m from streams; e.g., Briers et al., 2005), we cannot exclude the possibility that spiders fed across both MC and RC, especially given the similar assimilation estimates across both. Consequently, further catchment-scale sampling is necessary to fully clarify how flow intermittency affects riparian spider diets in alpine landscapes.

### *Conclusions*

We conducted our experiment within a single channel, which limits the conclusions we can draw as to how flow intermittency might affect entire alpine fluvial networks. Further, the increase in flow intermittency we induced was artificial and might affect benthic communities differently to natural drying events. For example, the removal of the water associated with the manipulation

might have removed some cues associated with declining flow, e.g., chemical stimuli (Lund et al. 2016). However, it was 25 days between when we began manipulating flow and when drying first occurred. Flow was reduced in the MC during this time but still occurring via discharge from local bank storage, hyporheic upwellings, and rainfall events (pers. obs., the authors). For comparison, flow pulses are often shorter than 25 days in the intermittent headwater streams of Val Roseg (Paillex et al., 2020). In addition, the length of time that we continued sampling after flow resumption (approx. 1.5 months) is usually sufficient time for re-establishment of macroinvertebrate fauna in intermittent systems (Larned et al. 2010). Further, as discussed above for organic matter dynamics, the frequency and duration of rewetting events is an important driver of IRES ecosystem structure and function (e.g., Shumilova et al. 2019). We might thus observe markedly different patterns under different rainfall regimes. Despite this, the changes we saw in density, richness, and composition of macroinvertebrate communities with increased flow intermittency were clear and persisting. We thus conclude that the patterns we saw here are likely to occur in other alpine streams experiencing increased summer flow intermittency. Given that densities and diversity did not recover by the end of the snow-free season, whether the effects of our flow manipulation persist into the following year is likely to be an important, as yet unknown indicator as to the long-term impacts of flow intermittency in alpine streams.

Today, alpine streams are most likely to dry in winter, when water is largely “locked up” in snow and ice reserves, although summer and autumn flow intermittency is also common (Paillex et al., 2020). In the near future, decreasing summer rainfall and loss of glacial melt is predicted to increase summer and autumn drying of streams (IPCC, 2014). Our results suggest that the onset of flow intermittency in summer will result in a loss of habitat for cohorts of rheophilic taxa. In a landscape context, increased flow intermittency may thus favour



multivoltine or overwintering lineages, reducing genetic diversity within catchments (Shama et al., 2011; Leys et al., 2017). High habitat heterogeneity among headwater streams also drives high beta diversity across alpine catchments (Karaus et al., 2013; Robinson et al., 2016b; Alther et al., 2019). Further, reduced densities of benthic macroinvertebrates in MC were more dependent on periphyton. With increased flow intermittency, reduced invertebrate shredding of leaf litter (Corti et al., 2011) might further lead to less bioavailable and more highly pulsed subsidies to downstream reaches (Battin et al., 2008; Larned et al., 2010). An increase in flow intermittency across alpine catchments might thus fundamentally alter both biodiversity and ecosystem function at the landscape scale.

## **Acknowledgements**

Funding for this project was provided through Eawag Discretionary Funds for Research, the Ernst Göhner foundation, Gelbert foundation, and Department of Nature and Environment, Canton Graubünden. We thank Marion Caduff, Larissa Schädler, Jorrit Mesman, and Christa Jolidon for assistance in the field and laboratory. We thank Christian Ebi for development of the electrical resistance loggers. We thank Serge Robert for analysis of EA-IRMS samples, and the AuA Lab at Eawag for analysis of water chemistry. We thank Gemeinde Pontresina for road access to Val Roseg. We are grateful to Lucrezia and Wolfgang Pollak-Thom, and staff of the Hotel Restaurant Roseg Gletscher, for their hospitality. Several anonymous reviewers and the Editor provided suggestions that greatly improved the quality of this article.

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782 **Tables**

783 Table 1: Mean water chemistry of the reference and manipulated channels across time periods.  $< a^\dagger$  indicates that all samples were below  
784 detection limits where  $a$  = detection limit. DOC = dissolved organic carbon. TN = total nitrogen. TP = total phosphorus. SRP = soluble  
785 reactive phosphate.

Channel	Time period	Alkalinity	pH	DOC	TN	NO <sub>3</sub>	TP	SRP	Silicate
	(relative to flow manipulation)								
		mmol L <sup>-1</sup>		mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	µg L <sup>-1</sup>	µg L <sup>-1</sup>	mg L <sup>-1</sup>
Reference	Before (n = 3)	0.5	7.1	0.8	<0.5	0.4	3.9	1.7	8.2
	During (n = 2)	0.5	7.2	0.7	<0.5	0.2	5.6	3.0	8.4
	After (n = 2)	0.7	7.1	0.5	<0.5	0.2	3.8	2.7	8.7
Manipulated	Before (n =3)	0.4	7.1	0.8	<0.5	<0.25	4.7	1.6	7.7
	During (n = 2)	0.5	7.2	1.1	<0.5	0.1	29.4	1.3	7.5
	After (n = 2)	0.6	7.1	0.7	<0.5	0.2	7.0	2.4	8.5

786

## Figure captions

Figure 1: Photos of the two study channels. Shown are the reference channel (a, b) and manipulated channel (c, d) before (June 2018) and during (August 2018) the flow manipulation, respectively.

Figure 2: Scatterplots showing trends over the sampling period (daily means) in (a) rainfall for Val Roseg (data: Piz Corvatsch weather station, MeteoSwiss) as well as (b, d) relative conductivity and (c, e) water temperature in the reference channel (RC) and manipulated channel (MC), respectively. Relative conductivity values of zero indicate no liquid surface water (freezing or drying). Dashed line indicates onset of regular drying periods (30 June) in MC. † indicates dates of sampling. Note log scale in (b) and (d). Break in data (October) indicates logger maintenance period.

Figure 3: Non-metric multidimensional scaling (nMDS) plot of (a) macroinvertebrate assemblages in the reference (green) and manipulated (orange) channels during the course of the study based on standardized abundance data. Grouping of assemblages is based on time period (before the flow manipulation, triangles; during the flow manipulation, circles; after natural flow resumption, squares). (b) Relative influence of different taxa on the ordination. Taxa names are slightly jittered for clarity.

Figure 4: Boxplots showing differences in (a) macroinvertebrate density, (b) density of rheophilic taxa, (c) number of taxa (s), and (d) percentage of total taxa identified as rheophilic in the reference (green) and manipulated (orange) channels. Grouping of data is based on time period (before the flow manipulation, during the flow manipulation, and after natural flow resumption). ind = individuals. R = reference channel. M = manipulated channel. Letters above plots (a, b, c,

d) indicate significantly similar group means (at  $p > 0.05$ ) across channels in all time periods as indicated by Tukey's HSD tests.

Figure 5: Boxplots showing differences in (a) coarse benthic organic matter (BOM), (b) BOM C:N ratios, (c) periphyton biomass, and (d) periphyton C:N ratios in the reference (green) and manipulated (orange) channels. Grouping of data is based on time period (before the flow manipulation, during the flow manipulation, and after natural flow resumption). R = reference channel. M = manipulated channel. Letters above plots (a, b) indicate significantly similar group means (at  $p > 0.05$ ) across channels in all time periods as indicated by Tukey's HSD tests.

Figure 6: Estimated assimilation by invertebrate consumers (as proportions of total) of terrestrial and aquatic basal resources in the reference (green) and manipulated (orange) channels. Estimated contributions are shown as 25th percentile, median and 75th percentile of probability distributions for (a) assimilation of coarse particulate organic matter (CPOM) by aquatic macroinvertebrates, (b) assimilation of terrestrial invertebrates by riparian spiders, (c) assimilation of periphyton by aquatic macroinvertebrates, and (d) assimilation of aquatic macroinvertebrates by riparian spiders. Grouping of data is based on time period (before the flow manipulation, during the flow manipulation, and after natural flow resumption). R = reference channel. M = manipulated channel.



Figure 1





Figure 2

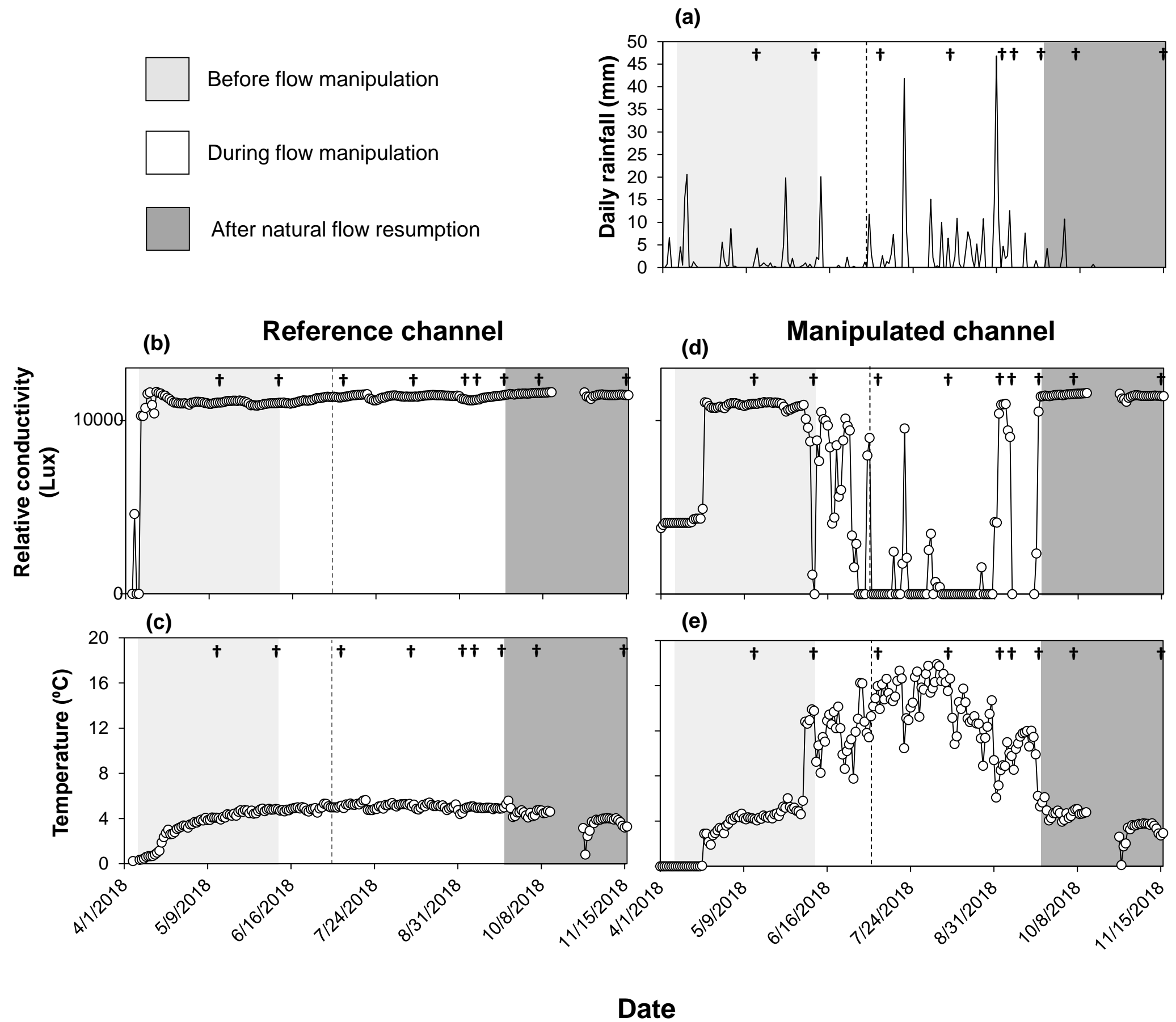


Figure 3

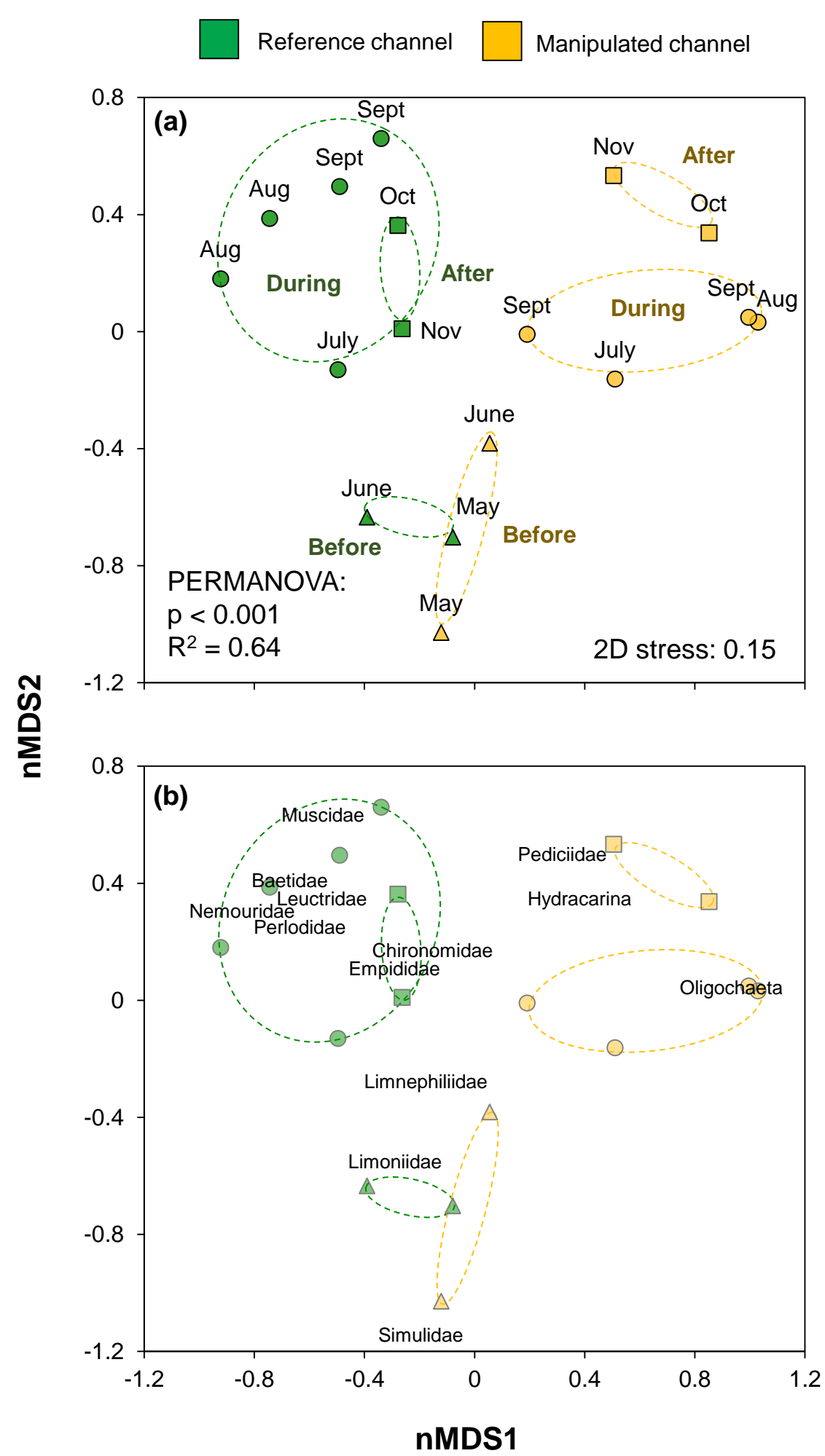


Figure 4

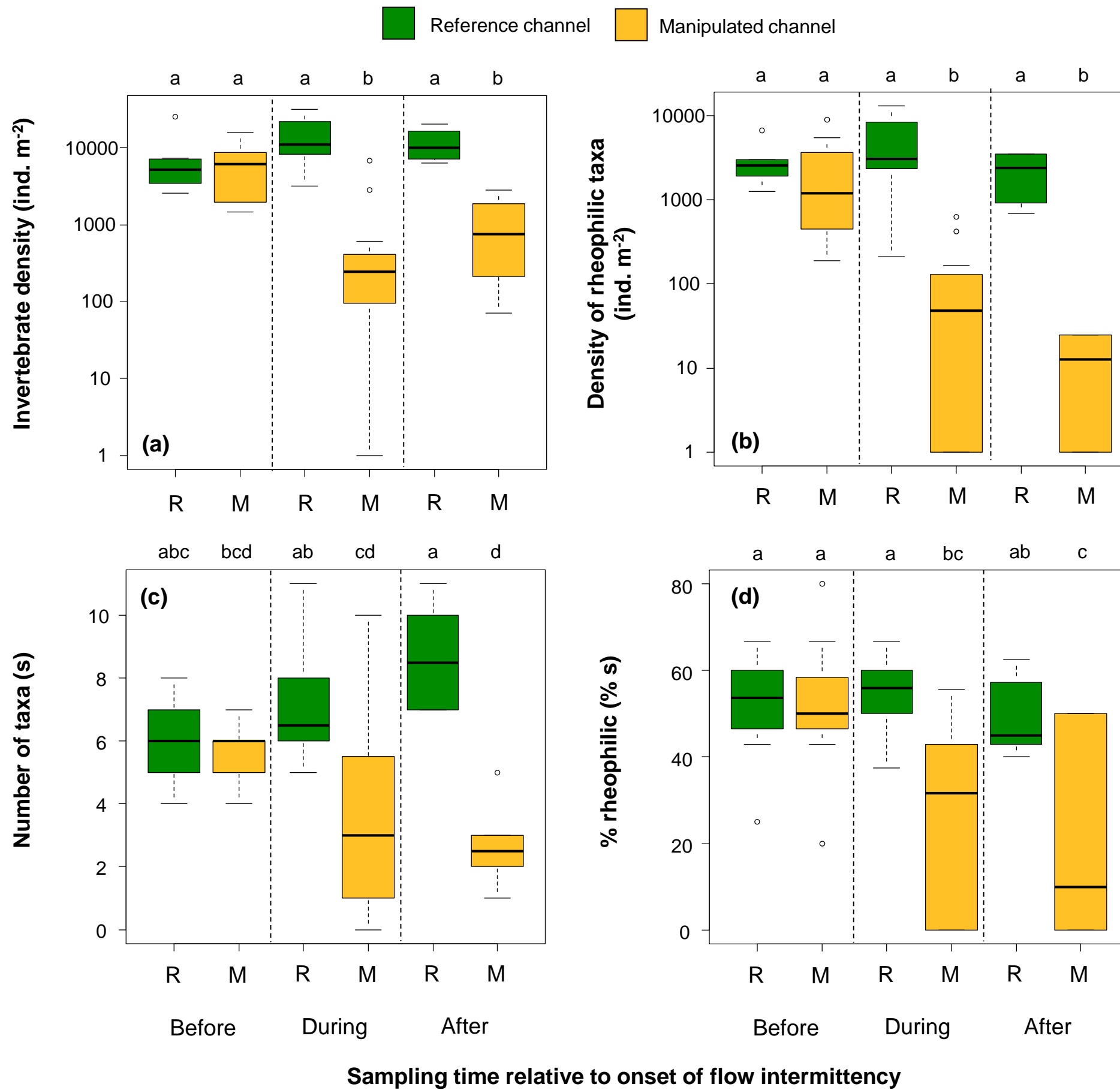


Figure 5

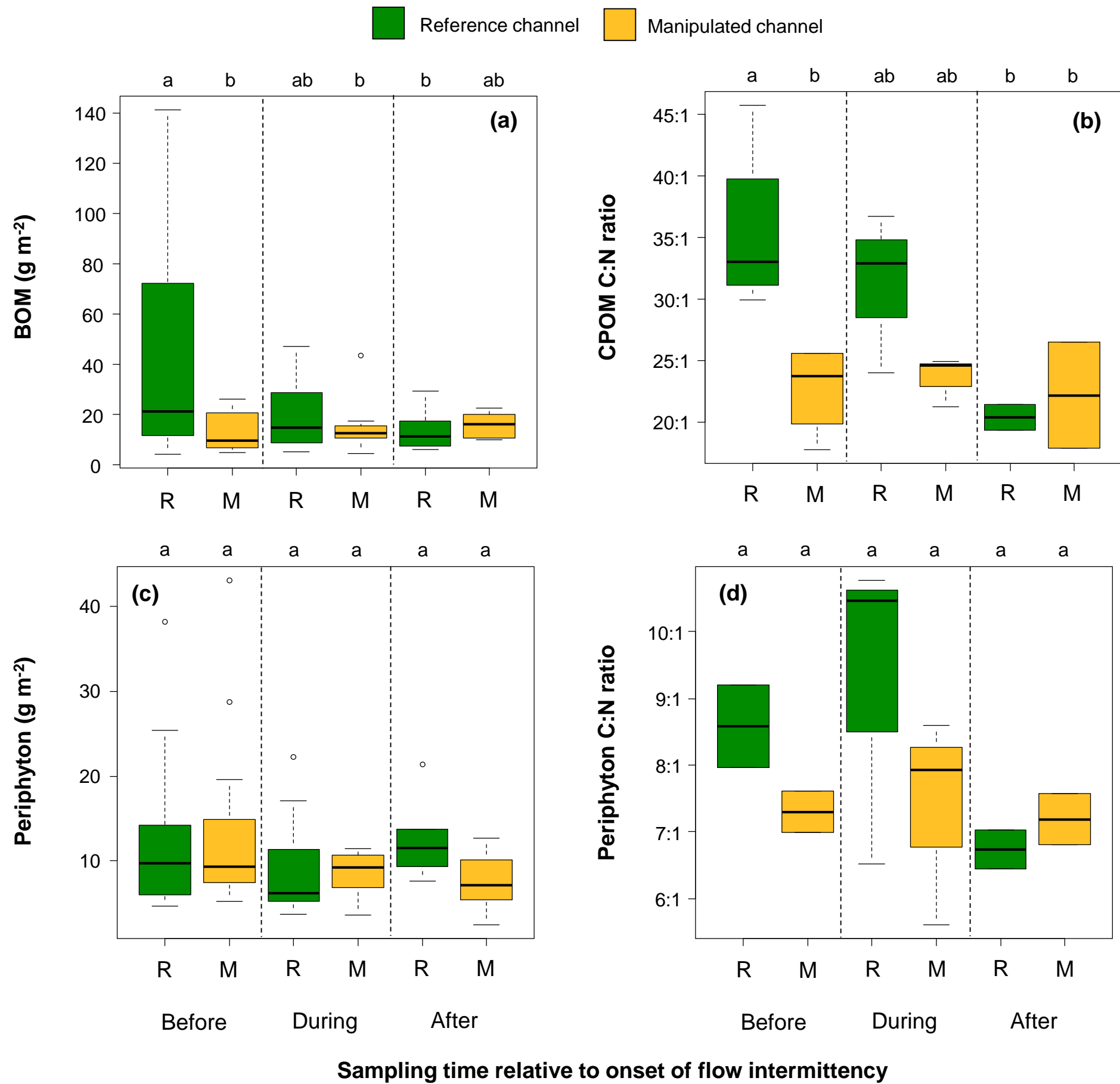


Figure 6

