

1 This is a post-peer-review, pre-copyedit version of an article published in Aquatic Sciences.

2 The final authenticated version is available online at: <https://doi.org/10.1007/s00027-021->

3 00779-7

4 This document is the accepted manuscript version of the following article: Siebers, A.R.,

5 Paillex, A. & Robinson, C.T. Riparian hunting spiders do not rely on aquatic subsidies from

6 intermittent alpine streams. *Aquat Sci* 83, 25 (2021).

ACCEPTED

Title: **Riparian hunting spiders do not rely on aquatic subsidies from intermittent alpine streams**

Andre R. Siebers<sup>1,3,†</sup>, Amael Paillex<sup>1,4</sup>, Christopher T. Robinson<sup>1,2</sup>

<sup>1</sup>Department of Aquatic Ecology, Eawag, Swiss Federal Institute of Aquatic Sciences and Technology, 8600 Dübendorf, Switzerland

<sup>2</sup>Institute of Integrative Biology, ETH Zürich, 8092 Zürich, Switzerland

<sup>3</sup>Current address: Centre for Freshwater Ecosystems, La Trobe University, Wodonga, VIC 3690, Australia

<sup>4</sup>Current address: ECOTEC Environment SA, 1203 Geneva, Switzerland

Email addresses: andre.siebers@outlook.com, amael.paillex@hispeed.ch, robinson@eawag.ch

ORCIDs:

A. R. Siebers: 0000-0002-8326-1649. A. Paillex: 0000-0001-9255-3662.

<sup>†</sup>Author for correspondence: andre.siebers@outlook.com

## Abstract

Drying in alpine streams might decrease aquatic-terrestrial trophic linkages by reducing terrestrial predation on aquatic prey. We tested this hypothesis by investigating whether a common riparian predator (hunting spiders) in alpine environments assimilated a lower proportion of aquatic prey with increasing stream intermittency. We used high temporal-resolution data from electrical resistance sensors to map patterns of naturally-occurring flow intermittency across 30 headwater streams of Val Roseg, a glacierized catchment in the Swiss Alps. We collected riparian hunting spiders, as well as potential terrestrial and aquatic macroinvertebrate prey, from streams and their associated riparian zones across two seasons (Alpine spring and summer). We estimated aquatic contributions to spider diets ( $p_A$ ) using (i) a gradient approach with aquatic invertebrate and spider carbon stable isotope ratio values ( $\delta^{13}\text{C}$ ), and (ii) Bayesian carbon and nitrogen ( $\delta^{15}\text{N}$ ) isotope mixing models. Spider  $p_A$  from the gradient method were not statistically different from zero in spring ( $0.08 \pm 0.10$ ) and low in summer ( $0.16 \pm 0.04$ ). Mixing models also estimated low dependence on aquatic prey in both seasons, although with potentially higher contributions in summer. Spider diet did not vary with increasing flow intermittency in either season. Our results suggested that alpine hunting spiders obtain most of their carbon from terrestrial prey. The slight increase in spider  $p_A$  during summer may correlate with peak emergence periods for aquatic insects, indicating opportunistic feeding by this riparian predator.

**Keywords:** Lycosidae, trophic linkages, stable isotopes, macroinvertebrates

## 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 Benjamin Misteli, Marion Caduff, Larissa Schädler, Jorrit Mesman, Christian Ebi, and Christa Jolidon for assistance in the field and laboratory. We further thank Christian Ebi for development of the electrical resistance loggers and assistance in the field. We thank Serge Robert for analysis of EA-IRMS samples. 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. We thank the editor and two anonymous reviewers, who provided comments that improved the quality of this article.

## 58    **Introduction**

59    Stream and riparian ecosystems are strongly coupled by cross-transfer energy flows. In terms  
60    of mass, inputs of organic matter from terrestrial to aquatic environments predominate (Tank  
61    et al. 2010). However, reciprocal flows from aquatic to terrestrial environments are often of  
62    high energy and nutrient quality (Schindler and Smits 2017). In particular, emergence of  
63    aquatic insects represents a major flux of nutrients into riparian zones (Bartrons et al. 2013).  
64    Emergent aquatic insects can contribute substantially to the diets of birds, lizards, bats, and  
65    predatory arthropods in riparian areas (Baxter et al. 2005).

66            In alpine catchments, riparian zones are often small, yet show distinct ecological  
67    differences from the surrounding terrestrial environment (McKernan et al. 2018). Benthic  
68    invertebrates in alpine streams often change their diets in response to changing quality and  
69    quantity of terrestrial organic matter inputs (Füreder et al. 2003; Niedrist and Füreder 2017;  
70    Siebers et al. 2019). However, relatively little is known of how emergent aquatic insects  
71    might subsidize alpine riparian ecosystems. Terrestrial productivity in most alpine  
72    catchments is low (ca.  $< 400 \text{ g C m}^{-2} \text{ y}^{-1}$ ) (Körner 1999, Jansson et al. 2008). Emergent  
73    aquatic insects thus may provide an important subsidy to riparian predators in alpine  
74    catchments (Marczak et al. 2007). However, alpine headwater streams are often narrow, cold,  
75    and high gradient, resulting in low surface area to edge ratios and low aquatic secondary  
76    productivity that might limit aquatic-terrestrial trophic links overall (Ward 1997; Schindler &  
77    Smits 2017). Yet, the little information available on riparian predator diets in alpine  
78    catchments is contradictory: Alp et al. (2013) estimated low contributions of aquatic  
79    emergents to hunting spider diets at two forested, mountain headwater streams, while  
80    Robinson et al. (2015) suggested that spiders were active predators of emergent aquatic  
81    insects at 6 headwater streams in a glacierized alpine catchment. Lastly, alpine ecosystems  
82    are also seasonal, with short “windows of opportunity” for emergent insects to experience

favourable conditions on land (Füreder et al. 2005). Predation on emergent aquatic insects is often seasonal in other fluvial networks, with peak assimilation of aquatic prey during peak emergence periods (Paetzold et al. 2005). Consequently, predation on emergent aquatic insects may be seasonally-dependent in alpine catchments.

Alpine streams exist along other strong environmental gradients that may affect the proportional contribution of emergent aquatic insects to riparian predators. For instance, alpine headwater streams experience wide variation in the occurrence of drying events, both from winter freezing and summer or autumn drought (Robinson et al. 2016a; Gabbud et al. 2019; Paillex et al. 2020). While drying events in intermittent rivers or streams can cause pulses of aquatic prey availability to riparian predators, these pulses are often short-lived (Steward et al. 2017). In addition, aquatic macroinvertebrates in alpine streams likely use the hyporheic zone as a refuge (Malard et al. 2003a; b) and may not be accessible to riparian predators during drying events. Overall, flow reductions and increased occurrence of drying are more likely to limit the density of aquatic insects, thereby reducing emergence rates and predation by riparian animals (e.g., Greenwood and McIntosh 2010). Yet, a recent field experiment in an alpine stream suggests that hunting spiders may increase their consumption of emergent aquatic insects following drying events, despite a reduction in the density of emergent aquatic taxa (Siebers et al. 2020a). Further information at the catchment scale is thus necessary to determine (i) the general extent of aquatic subsidies to riparian predators along alpine streams, and (ii) the extent to which flow intermittency influences predation on emergent aquatic insects.

In this study, we tested whether aquatic macroinvertebrates across a natural gradient of alpine headwater flow intermittency contribute more greatly to the diet of riparian hunting spiders when streamflow is less intermittent. Our first objective (i) was to identify the extent to which riparian spiders rely on aquatic macroinvertebrate prey, and whether this differs

across seasons. We predicted that assimilation of aquatic macroinvertebrates would be greater in summer (peak emergence period) than spring. Our second objective (ii) was to determine if spiders assimilate a greater proportion of aquatic macroinvertebrates as streamflow becomes less intermittent. We predicted that assimilation of aquatic macroinvertebrates would increase along a gradient of flow intermittency from high (periodically intermittent throughout the year) to low (perennial flow), with a more pronounced effect in summer than spring.

## Materials and Methods

### *Site description and sampling design*

We undertook this study in Val Roseg, a glacierized river valley located within the Bernina Massif in southeast Switzerland (Fig. 1). In Val Roseg, seasonality in contributions of glacial meltwater, snowmelt, and rainfall drive marked expansion and contraction dynamics in the fluvial network (Malard et al. 2006). In particular, varying contributions of different water sources contribute to high levels of flow intermittency across headwater streams (up to 90% of the headwater network) (Robinson et al. 2016a; Paillex et al. 2020).

We sampled 30 headwater streams and their associated riparian zones across Val Roseg. The streams were chosen as part of a long-term monitoring project that examined patterns of flow intermittency and its ecological effects across Val Roseg (Siebers et al. 2019, Paillex et al. 2020). The 30 streams exist along a gradient from the head of the catchment (South) to the valley outlet (North) (Fig. 1), essentially incorporating the entire headwater network. The streams are typically small and high gradient (ca. 1-3 m wide, mean 13° slope; Paillex et al. 2020). Streambeds are generally composed of cobbles and coarse gravels. The streams occur across a range of elevations (2002-2181 m a.s.l.) and riparian tree cover (0-53% in 100-m radius around sampling sites); from lower channels passing through European larch (*Larix decidua*) and stone pine (*Pinus cembra*) forests, to mixed shrubs (*Juniperus*

*communis* subsp. *alpina*, *Rhododendron ferrugineum*) and small trees (*Alnus viridis*, *Salix* spp.), to alpine herbs, grasses and sedges (*Festuca* spp., *Carex* spp.) (Siebers et al. 2019).

Approximately 40% of the streams experienced drying events (i.e., absence of surface water) through summer/autumn of 2017, while 80% of the streams experienced drying events in winter of 2017/18 (Paillex et al. 2020). Snowmelt occurred in early May 2018, inducing flow in all streams (Paillex et al. 2020). Previous samplings of the catchment identified wide variation in flow intermittency and the trophic base of aquatic macroinvertebrates in autumn (September) 2017 (Siebers et al. 2019): the strategy for this study was thus to expand on these results by also sampling riparian communities, across more seasons, in 2018. We undertook sampling in the alpine spring (June) and summer (August) of 2018 aiming to capture (i) the spring period in which most streams were either flowing or were only experiencing infrequent, isolated drying events, and (ii) the summer period of greatest differences in streamflow intermittency, aquatic macroinvertebrate density, and aquatic insect emergence.

#### *Flow intermittency – measurement*

We measured the presence or absence of surface water in each stream using HOBO Pendant Temperature/Light 64K data loggers (Onset Computer Corporation, Bourne, MA, USA) modified as per Chapin et al. (2014) to measure electrical resistance and temperature. As detailed in Paillex et al. (2020), the original light sensors were modified to record electrical resistance via connection to external electrodes, resulting in measurements given in the original measurement units (Lux), which are nonetheless linearly correlated with electrical conductivity (Chapin et al. 2014). Zero readings thus indicate the absence of liquid water and, when installed at the streambed, indicate drying events in the surface channel (Paillex et al. 2020). Loggers were installed in the thalweg of each stream in July 2017 and set to record



hourly. Loggers were removed briefly for maintenance in November 2017 and May 2018, then downloaded in October 2018. One logger (site “12”) malfunctioned in December 2017. In this case, we replaced the logger following maintenance in May 2018, and were able to use visual observations of the dry stream and data from a nearby logger to substitute for the missing winter freeze period. Another logger (site “20”) was buried for 1.5 months after a landslide in 2017. For this site, this period was removed from the data before analysis. Otherwise, we used electrical resistance data for the entire July 2017 to October 2018 measurement period, resulting in ca. 4 months additional data beyond that presented in Paillex et al. (2020).

#### *Sample collection and laboratory analysis*

Spiders were collected along a 20-30 m stream reach where loggers were installed. We conducted consistent, systematic searches for spiders on the ground within 2 m of the stream edge (15 min total, at least one inspection of each side of the total reach), manually caught specimens when found, and stored collected spiders within dry 50 mL plastic vials. The main potential predators of emergent stream invertebrates in the Alps are likely to be hunting (e.g., Lycosidae, Gnaphosidae) rather than web-spinning spiders (Thaler 2003). For example, Lycosidae made up 48-74 % of the spiders collected by Zingerle (1999) in alpine grasslands of the Italian Dolomites. We thus attempted to consistently collect Lycosidae across sites to reduce potential variability in diets across taxa. However, where Lycosidae were unable to be found ( $n = 6$ ), we collected other hunting spider taxa (Gnaphosidae). We attempted to collect similarly-sized individuals to avoid differences in feeding habits with age (e.g., Oelbermann & Scheu 2002). Web-building spiders were observed in low numbers and thus not collected. In spring, we were unable to find any spiders at three of the sites. In summer, we were also unable to find any spiders at three sites (different from spring). At the other 27 sites, in each

season, we collected 1-3 specimens depending on availability. Spiders were stored at ambient temperature for ~8 hours to void stomach contents and then stored at -20 °C until analysis.

As an indicator of macroinvertebrates feeding largely on terrestrial sources, we collected ants (Formicidae) using the same procedure as outlined above for spider collection (i.e., 15 min sweeps of the ground in riparian zones). Ants are usually predated on by specialist spider taxa rather than generalists like Lycosidae (Nentwig 1987), but while other potential prey were present (e.g., Coleoptera) they were not as abundant. In addition, previous surveys of riparian invertebrates within Val Roseg (Siebers et al. 2020a) have shown that Coleoptera have similar  $\delta^{13}\text{C}$  values (ca. -27 ‰, unpub. data) to other terrestrial invertebrates and organic matter (e.g., Siebers et al. 2019). We thus used ants as an indicator of the  $\delta^{13}\text{C}$  values of invertebrates feeding largely on terrestrial organic matter rather than a potential source of C for spiders *per se* (See: Stable isotope data analysis). We collected ants at 21 sites in spring, and 22 sites in summer. Again, we collected 1-3 specimens depending on availability. Ants were stored at ambient temperature for ~8 hours to void stomach contents and then stored at -20 °C until analysis.

As potential aquatic prey, aquatic macroinvertebrates were collected for stable isotope analysis by repetitive kick-sampling (mesh size: 250  $\mu\text{m}$ ) within each 20-30m stream reach. We conducted a qualitative sweep including all substrate types present (e.g., cobbles, woody debris, mosses) and using three kick-sample sweeps per stream. We conducted additional, targeted sweeps if it was deemed that rare or smaller species required more biomass to conduct isotope analysis ( $\sim \geq 0.5$  mg). Sweeps were not conducted if the channel was dry (n = 6, all in summer). Aquatic macroinvertebrates were hand-picked from kick-net samples and immediately sorted into 50 mL plastic vials containing stream water. Predatory species were stored separately. Aquatic macroinvertebrates were stored at ambient temperature in stream water for ~8 hours to void stomach contents, and then stored at -20 °C until analysis.

Where present, we also collected periphyton and filamentous algae from streams to characterise in-stream basal resources. Periphyton samples were collected by scrubbing the top of 5 randomly collected cobbles from each study reach with a wire brush and rinsing with stream water. Filamentous algae samples were detached by hand from 3 separate colonies within each stream. All periphyton and filamentous algae samples were stored at -20 °C until analysis.

All invertebrate, periphyton and algae samples were freeze-dried for 48 hours in a Lyovac GT 2-E lyophilizer (STERIS GmbH, Hürth, Germany). Invertebrate samples were finely chopped after drying and we individually weighed ~ 0.8 mg of each sample. For spiders, we selected leg and cephalothorax material wherever possible (e.g., Collier et al. 2002). We individually weighed ~ 1.2 mg of each periphyton and algae sample. Samples were combusted 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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are presented in permille (‰) after normalisation to reference material (NBS 19, L-SVEC, IAEA-N-1 and IAEA-N-2, provided by Biogeochemical Laboratories, Indiana University). Analytical uncertainty was 0.1‰ for  $\delta^{13}\text{C}$  and 0.2 ‰ for  $\delta^{15}\text{N}$ .

#### *Flow intermittency data and spatial analysis*

In Val Roseg, most of the variation in flow intermittency between sites is driven by the total duration and frequency of drying events (Robinson et al. 2016a; Siebers et al. 2019; Paillex et al. 2020). Consequently, we used an integrative measure of overall flow intermittency (FI) across sites using metrics based on these flow properties. First, we calculated the average percentage each stream was dry over a year. Second, we calculated the average number of drying events per year. Next, we standardised each metric to account for differences in their

units (i.e., converted to  $Z$  scores). Finally, we calculated the Euclidean distance between metric values for each site and values for known perennial sites (i.e., corresponding  $Z$  scores for 0% dry  $\text{yr}^{-1}$  and 0 drying events  $\text{yr}^{-1}$ ) to obtain the final FI value. FI values were strongly, linearly correlated with previous metrics used as estimates of overall flow intermittency (based on PCA) from Paillex et al. (2020) ( $a = 1.04$ ,  $R^2 = 0.67$ ) and Siebers et al. (2019) ( $a = 1.00$ ,  $R^2 = 0.76$ ) to validate this approach. We then grouped sites into flow intermittency classes of perennial/low intermittency ( $< 5\%$  dry  $\text{yr}^{-1}$ ,  $< 5$  events  $\text{yr}^{-1}$ ), moderate (5-40% dry  $\text{yr}^{-1}$ , 1-55 events  $\text{yr}^{-1}$ ), and high intermittency ( $> 35\%$  dry  $\text{yr}^{-1}$ ,  $> 10$  events  $\text{yr}^{-1}$ ).

Spatial data on site locations were imported from field GPS measurements into the Geographic Information System ArcGIS (v. 10.7) together with FI values. We then mapped sites, and the gradient in FI across sites, on top of the fluvial network for Val Roseg (swisstopo 2018, swissTLM3D & swissALTI3D, Bundesamt für Landestopographie (Art.30 Geo IV): 5704 000).

#### *Stable isotope data analysis*

We used a gradient approach to estimate spider diets, with the linear regression slope of spider  $\delta^{13}\text{C}$  versus aquatic macroinvertebrate  $\delta^{13}\text{C}$  as an indicator of reliance on aquatic prey across all sites (Rasmussen 2010). The gradient approach provides an alternative to mixing model analysis when overlap in source isotope values might lead to high uncertainty in model results, yet there are distinct spatial patterns in the isotope values of contrasting sources (Rasmussen 2010). We did not use  $\delta^{15}\text{N}$  values for gradient analysis (e.g., Bunn et al. 2013) as terrestrial and aquatic sources varied similarly across environmental gradients in Val Roseg (Fig. A.1). While the small sample sizes for spiders (1-3 per site) limit our ability to estimate variation in spider isotope signatures within sites, a previous study of hunting spiders in Val Roseg showed low coefficients of variation in hunting spider  $\delta^{13}\text{C}$  (0.7-2.1 %)

within two sites across a seven-month period (Siebers et al. 2020a). We used a subset of  
 aquatic macroinvertebrate  $\delta^{13}\text{C}$  values incorporating only hemimetabolous taxa  
 (Ephemeroptera, Plecoptera); i.e., those that emerge at the stream edge and might thus be  
 potential prey for hunting spiders. At 2 sites (site 11 and 25) there were no Ephemeroptera  
 and Plecoptera found, and we excluded these sites from the following analyses. Mean aquatic  
 macroinvertebrate  $\delta^{13}\text{C}$  values for each site were strongly, linearly correlated across seasons  
 ( $a = 1.16$ ,  $R^2 = 0.75$ ). Consequently, we set aquatic macroinvertebrate  $\delta^{13}\text{C}$  values as means  
 for each site across both seasons. We analysed a subset of 20 ant samples (chosen non-  
 randomly to represent the range of FI values across sites, from both seasons) to provide an  
 indicator of terrestrial invertebrate  $\delta^{13}\text{C}$  ranges. Terrestrial invertebrate (ant)  $\delta^{13}\text{C}$  values had  
 low variation across sites and seasons (mean  $\pm$  sd,  $-25.9 \pm 1.2$  ‰). We therefore used 95 %  
 confidence intervals of mean  $\delta^{13}\text{C}$  as an indicator of  $\delta^{13}\text{C}$  values for spiders feeding solely, or  
 predominantly, on terrestrial prey. We also used mean  $\pm$  sd  $\delta^{13}\text{C}$  values of terrestrial plants  
 from Siebers et al. (2019) (from Sept 2017,  $n = 45$ ) as a further indicator of basal terrestrial  
 OM source signatures. Given that adequately characterising periphyton and algae  $\delta^{13}\text{C}$  values  
 requires detailed sampling of their spatial and temporal variability (Jardine et al. 2014), we  
 calculated mean periphyton and algal isotope values from the samplings both here and in  
 Siebers et al. (2019) (from Sept 2017,  $n = 24$ ). We corrected aquatic macroinvertebrate  $\delta^{13}\text{C}$   
 values for trophic enrichment factors (TEFs) following Caut et al. (2009) as  $\delta^{13}\text{C}_{(\text{TEF-corrected})} =$   
 $-0.113 * \delta^{13}\text{C} - 1.916$ . Calculated TEFs averaged  $1.1 \pm 0.3$  ‰, similar to experimentally-  
 determined values for lycosid spiders (Oelbermann and Scheu 2002).

We used generalised linear models (GLMs) to model the response of spider  $\delta^{13}\text{C}$   
 values to prey  $\delta^{13}\text{C}$  across sites. If there was little evidence that the regression slope differed  
 from zero ( $\alpha = 0.05$ ), we considered spiders as completely dependent on terrestrial prey  
 across sites. If there was evidence that slope differed from zero, we considered the slope of

the regression (i.e.,  $a$ ) as an estimate of the proportion of aquatic prey ( $p_A$ ) assimilated by spiders (Rasmussen 2010). Before GLMs were constructed, we also used Moran's I tests ( $\alpha = 0.05$ ) to ensure there was no spatial autocorrelation in FI ( $p = 0.88$ ) or aquatic macroinvertebrate  $\delta^{13}\text{C}$  values ( $p = 0.88$ ). Spider  $\delta^{13}\text{C}$  values in summer are also unlikely to reflect spring feeding habits, as tissue turnover in small invertebrates is usually on the order of days (Vander Zanden et al. 2015). To identify potential differences in  $p_A$  between seasons (i), we constructed a GLM with TEF-corrected aquatic macroinvertebrate  $\delta^{13}\text{C}$ , season, and the interaction between the two as fixed predictor variables. To identify potential variation in spider  $\delta^{13}\text{C}$  values with flow intermittency (ii), we first split the data by season (spring vs. summer). We then constructed GLMs for each season with TEF-corrected aquatic macroinvertebrate  $\delta^{13}\text{C}$ , flow intermittency PCA values, and the interaction between the two as fixed predictor variables. All GLM analyses were conducted using core packages in R (v. 3.6.1). Moran's I was calculated using the ape package (v. 5.3) in R (v. 3.6.1).

As an additional estimate of the proportion of aquatic prey assimilated by spiders, we used the Bayesian mixing model MixSIAR (Stock et al. 2018) to produce probability distributions of the dietary contributions of aquatic and terrestrial food sources to riparian hunting spiders. Terrestrial plants and hemimetabolous aquatic macroinvertebrates were included as potential sources in the models. Terrestrial plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were set as averages  $\pm$  standard deviation for each site in September 2017 (Siebers et al. 2019). Where terrestrial plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were unavailable ( $n = 11$ ), we used coarse particulate organic matter (CPOM) isotope values from Siebers et al. (2019) as a surrogate for terrestrial plants. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for aquatic macroinvertebrates were set as averages  $\pm$  standard deviation for each site across both seasons. Trophic enrichment factors for emergent aquatic insects were set at  $1.1 \pm 0.3$  ‰ for  $\delta^{13}\text{C}$  (as above) and  $3.4 \pm 1.1$  ‰ for  $\delta^{15}\text{N}$  (Oelbermann & Scheu 2002). For terrestrial sources, we set trophic enrichment factors at 2.2

$\pm 0.6$  ‰ for  $\delta^{13}\text{C}$  (as above) and  $6.8 \pm 2.2$  ‰ for  $\delta^{15}\text{N}$ ; i.e., accounting for both the trophic step between spiders and prey taxa and the step between prey taxa and terrestrial organic matter (Semenina & Tiunov 2011). Generalist priors were used. Each model was set to run 100,000 times with the first 50,000 runs discarded. We ran the models separately for each site in each season. All models were created using the MixSIAR package in R (v. 3.6.1). Model results for each site were grouped *post hoc* into flow intermittency classes (perennial/low, moderate, high) for each season and summarised by kernel density and median/quartile analysis in R (v. 3.6.1). As the models only estimated proportions of two sources (i.e., each estimated source proportion is 1 minus the other source estimate), we present here again results for estimates of aquatic macroinvertebrate assimilation by spiders ( $p_A$ ).

### **Data availability**

The datasets analysed during the current study are available in the Dryad repository <<http://doi.org/10.5061/dryad.gxd2547jp>> (Siebers et al., 2020b).

## **Results**

### *Flow intermittency*

Six of the streams we monitored within Val Roseg were perennial (FI = 0). Of the 24 other, intermittent streams, there was wide variation in the percentage of year spent dry (1.3 to 59.4 %) and average number of drying events per year (0.8 to 67.2 yr<sup>-1</sup>) (Fig. 2), although the two metrics were not linearly correlated ( $p = 0.1$ ,  $R^2 = 0.06$ ). As with previous analyses of flow intermittency within Val Roseg (Siebers et al. 2019; Paillex et al. 2020), FI values were not clustered spatially within the catchment (Fig. 1).

### *Stable isotope data and gradient analysis*

Mean aquatic macroinvertebrate  $\delta^{13}\text{C}$  values varied from -34.2 to -21.8 ‰ across sites, while mean periphyton and algae  $\delta^{13}\text{C}$  values varied from -33.6 to -17.4 ‰ (although with very high variability within sites; standard deviations ranged from 0.1 to 6.4 ‰) (Fig. 3). Spider  $\delta^{13}\text{C}$  values differed less across sites in both seasons (range: -28.2 to -24.4 ‰), and ant  $\delta^{13}\text{C}$  values differed similarly to spiders (-29.4 to -24.5 ‰) (Fig. 3). Mean aquatic macroinvertebrate  $\delta^{15}\text{N}$  values also differed slightly more across sites (-4.4 to 3.2 ‰) than those of spiders (0.6 to 7.4 ‰) or ants (-0.1 to 5.7 ‰) (Fig. 3).

Gradient analysis indicated that spiders likely assimilated C from both aquatic and terrestrial prey (Fig. 4). In spring, spiders likely obtained all of their C from terrestrial prey (Fig. 4a). In summer, spiders likely assimilated more aquatic C than in spring, with approximately 16 % ( $pA = 0.16$ ) of their C input from aquatic prey (Fig. 4b). Spider  $\delta^{13}\text{C}$  values did not vary across the gradient in stream flow intermittency in either spring ( $p = 0.69$ ) or summer ( $p = 0.14$ ) (Fig. A.2). Further, there was no evidence of an interaction effect in either spring ( $p = 0.72$ ) or summer ( $p = 0.19$ ).

Bayesian mixing model analysis indicated that spiders assimilated C and N from both aquatic and terrestrial prey (Fig. 5). Median estimates of  $pA$  from mixing models were lower in spring (0.04 to 0.05) than summer (0.36 to 0.37) and did not vary greatly across flow intermittency classes (Fig. 5). However, the density distribution of mixing model results for  $pA$  in summer were much more diffuse than those for spring (Fig. 5).

## Discussion

As in previous studies of Val Roseg, we observed wide variation in flow intermittency and the carbon isotope values ( $\delta^{13}\text{C}$ ) of aquatic macroinvertebrates across streams (Siebers et al. 2019; Paillex et al. 2020). However, there was little variation in the  $\delta^{13}\text{C}$  of riparian hunting spiders across sites. Spiders likely assimilated more C from aquatic prey in summer than



spring, although the difference was minor (c. 16% of diet). Spiders also likely assimilated more N from aquatic prey in summer, although mixing model results were too diffuse for precise estimations. Gradients or groupings of streamflow intermittency had no correlation with the estimated assimilation of aquatic prey. Consequently, it appears likely that riparian hunting spiders in this alpine catchment rarely predate on emergent aquatic insects.

We estimated that riparian hunting spiders assimilated a low proportion (ca. 0 to 16%) of their C from emergent aquatic prey, similar to the estimates of Alp et al. (2013) at two forested mountains streams. In non-alpine environments, estimates for assimilation of emergent aquatic insects by riparian Lycosidae can be much higher. Lycosid spiders in relatively open or agricultural habitats can obtain a high proportion (ca. 50-72%) of their C from emergent aquatic insects when compared with Lycosidae along forested streams (ca. 5-20%) (Collier et al. 2002; Sanzone et al. 2003; Paetzold et al. 2005, Krell et al. 2015). These studies point to riparian vegetation cover as a potential driver for hunting spider diets. Yet, the headwater streams we sampled had relatively low tree cover (0-53% within 100m-radius of sites; Siebers et al. 2019). Further, Stenroth et al. (2015) found that lycosid spiders at forested sites assimilated ca. 43% of their diet from aquatic sources, and attributed these patterns to high availability of relatively large emergent insects (Plecoptera) at these streams.

Larger-bodied emergent aquatic insect taxa (e.g., Ephemeroptera, Plecoptera) are present across most headwater streams in Val Roseg (Siebers et al. 2019). Further, summer is the peak emergence period for alpine streams (Füreder et al. 2005), particularly for larger-bodied taxa (Robinson et al. 2010). Different Ephemeroptera and Plecoptera genera can show more diffuse or later patterns of emergence in some alpine catchments, but emergence is still generally very low in spring (e.g., Finn & Poff 2008). Our results suggest that peak emergence periods in Val Roseg correlate with increased spider predation on emergent aquatic insects, but that terrestrial prey may still comprise the majority of spider diets at these

times. Relatively high-quality terrestrial prey such as Collembola (Toft 1999) might be abundant along streams in Val Roseg (Misteli 2019), potentially reducing the need for reliance on aquatic subsidies (e.g., Krell et al. 2015). Hunting spiders along alpine streams may therefore only prey upon emergent aquatic insects to obtain essential dietary elements, such as fatty acids (Martin-Creuzburg et al. 2017). Further, emergent aquatic insect densities in alpine headwaters are generally lower than those in low elevation headwaters (Ward 1994). As mentioned previously, the headwaters of Val Roseg are small, high gradient (ca. 1-3 m wide, mean 13° slope; Paillex et al. 2020), and have low productivity (Logue et al. 2004). Riparian zones in Val Roseg, and along alpine headwaters more generally, may thus experience relatively low magnitudes of aquatic subsidies in a global context (Ballinger and Lake 2006; Muehlbauer et al. 2014; Schindler and Smits 2017).

We found no correlation between estimated spider diets and streamflow intermittency. In addition, our estimates of spider predation on emergent aquatic insects are much lower than those from other intermittent fluvial systems (ca. 48-68% of diet) (Sanzone et al. 2003; Paetzold et al. 2005). Much of this difference is likely because of the specific environmental limitations of alpine headwater streams, as outlined above. In contrast to the small headwater streams we studied, Sanzone et al. (2003) sampled spiders along a desert stream with a high productivity gradient between the aquatic and riparian environments, while Paetzold et al. (2005) sampled spiders along a braided floodplain river with wide, low productivity riparian areas (i.e., gravel bars) that represent relatively open aquatic-terrestrial boundaries. The higher assimilation estimates from these other intermittent systems thus likely reflect local environmental differences, rather than an effect of flow intermittency itself. Yet, our results contradict that of an earlier experimental study in Val Roseg, which showed increases in estimated assimilation of emergent aquatic insects by hunting spiders with the onset of drying events (Siebers et al. 2020a). Our results here suggest that this earlier study may have

captured a pulse of aquatic prey accessibility, perhaps attributable to the unpredictability of the induced drying events that represented a unique but short-lived opportunity for lycosid predators (Steward et al. 2017). At a catchment scale, longer-term patterns of flow intermittency may already contribute to lowered densities of emergent aquatic insects (e.g., Drummond et al. 2015) and further drying events might therefore not create pulses of prey availability for riparian predators.

Hunting spiders such as Lycosidae may be opportunistic feeders and thus less reliant on aquatic resources than other spider taxa, which can be more highly specialised for predation on aquatic or emergent invertebrates (Nentwig 1987). For example, semi-aquatic fishing or water spiders and long-jawed orb weavers, which build webs on overhanging riparian vegetation, can show a high reliance on aquatic prey (Burdon and Harding 2008; Greenwood and McIntosh 2010; Krell et al. 2015). Web-weaving spiders in general may be more efficient at catching emerging invertebrates than hunting spiders, and thus obtain a greater proportion of their diet from aquatic sources (Collier et al. 2002; Sanzone et al. 2003). Where they occur, web-building spiders in alpine catchments may indeed obtain a majority of their diet from aquatic sources (Robinson et al. 2015). Further, despite their low abundance, other invertebrate predators in alpine riparian zones (e.g., Coleoptera) may rely more on emergent aquatic insects than spiders (Hering and Plachter 1997). Highly mobile taxa such as birds are also able to seasonally migrate to take advantage of peak emergence periods (e.g., Epanchin et al. 2010). In addition, Chironomidae are typically the most abundant taxa in alpine streams (Robinson et al. 2016b; Alther et al. 2019), including Val Roseg (Burgherr et al. 2002). As discussed above, when they do predate on emergent macroinvertebrates, hunting spiders likely rely on larger taxa such as stoneflies (Briers et al. 2005; Stenroth et al. 2015). Smaller aquatic invertebrates such as Chironomidae also often disperse farther from streams than larger taxa (Muehlbauer et al. 2014). In alpine areas, Chironomidae may

disperse across relatively open riparian zones to forested areas, where small sheet-weaving spiders (Linyphiidae) can be abundant (Zingerle 1999; Thaler 2003). While hunting spiders may usually be the most abundant predatory taxa in alpine catchments, energetic fluxes from aquatic-terrestrial ecosystems might instead be concentrated along less-abundant linkages that we did not sample.

There are several limitations to the conclusions we can draw from both analyses (gradient and Bayesian mixing models) due to the nature of the underlying data. First, the isotope signatures of terrestrial and aquatic sources overlapped for several sites, likely resulting in diffuse mixing model results due to insufficient distinction in isotopic source values (Phillips et al. 2014). The large range and variability in periphyton and algae isotope signatures, possibly driven by high variation in productivity (Uehlinger et al. 1998) and inorganic carbon sources (Burrows et al. 2018) both across and within sites, likely contributes to the lack of distinction in aquatic macroinvertebrate isotope values. Second, terrestrial plant and aquatic macroinvertebrate  $\delta^{15}\text{N}$  values varied similarly across environmental gradients in Val Roseg (Fig. A.1). In particular, variation across elevation gradients may reflect differences in the sources and availability of inorganic nitrogen corresponding with age since glaciation (e.g., Hobbie et al. 1998). Variation in  $\delta^{15}\text{N}$  of spiders thus likely also reflects this gradient in inorganic N sources rather than differences in spider diet. Regardless, similar variation across basal N sources both rules out gradient analysis of  $\delta^{15}\text{N}$  and complicates distinction between sources for mixing model analysis. Lastly, variation largely occurred in the  $\delta^{15}\text{N}$  values of spiders rather than  $\delta^{13}\text{C}$ . Choice of mixing model parameters (trophic enrichment factors) can thus highly influence the estimated source proportions (Alp et al. 2013). While we adopted a widely used trophic enrichment factor ( $\Delta^{15}\text{N}$ ) of 3.4 ‰ (Post 2002),  $\Delta^{15}\text{N}$  can vary widely across taxa and with consumer age, source isotope signatures, or food quality (e.g., Oelbermann & Scheu 2002, Caut et al. 2009, Semenina & Tiunov 2011).

Further, consumer age might also affect the ability of spiders to capture emergent aquatic insects. The additional trophic step we considered adds a further element of error, as potential spider prey which feed on terrestrial plant-derived organic matter can show wide variation in  $\Delta^{15}\text{N}$  themselves (e.g., Semenina & Tiunov 2011). Ultimately, we lack experimentally-determined  $\Delta^{15}\text{N}$  values for taxa within Val Roseg. We therefore emphasise that results derived from  $\delta^{15}\text{N}$  values here are of lower confidence than those derived from  $\delta^{13}\text{C}$ .

In this study, we provide the first estimates of aquatic-terrestrial trophic linkages along alpine headwater streams. Further, our study represents the first test of potential variation in aquatic-terrestrial linkages along a gradient of streamflow intermittency. Our results suggest that riparian hunting spiders in this alpine catchment rely little on aquatic subsidies and that streamflow intermittency has no effect on the availability of aquatic prey. Yet, these patterns likely reflect specific ecological attributes of alpine headwater streams and associated riparian zones. In particular, the steep, channelized geomorphology and low productivity of alpine headwater streams likely restrict predation on emergent aquatic insects overall. Taxa-specific predation behaviours also likely affected our results, as the most abundant potential predators were probably opportunistic generalists (Nentwig 1987) rather than riparian specialists. We suggest that other, less common predatory taxa in alpine catchments may still take advantage of “pulses” in emergent aquatic insects, for example, web-building spiders in both riparian and forested habitats. Flow intermittency and drying can have strongly pulsed effects on aquatic-terrestrial trophic linkages (Steward et al. 2017). However, the flow of energy and nutrients between aquatic and terrestrial ecosystems may be more dependent on other drivers of riparian assemblage structure and trophic diversity, such as the productivity, size, or lateral connectivity of channels (Schindler and Smits 2017). Our results suggest the magnitude of aquatic-terrestrial trophic linkages in alpine catchments will

be highly dependent on local environmental conditions and the specific life-history strategies of the taxa involved, across both perennial and intermittent rivers and streams.

#### **Conflicts of interest**

The authors declare they have no competing financial interests.

#### **References**

- Alp M, Peckarsky BL, Bernasconi SM, Robinson CT (2013) Shifts in isotopic signatures of animals with complex life-cycles can complicate conclusions on cross-boundary trophic links. *Aquat Sci* 75:595-606
- Alther R, Thompson C, Lods-Crozet B, Robinson CT (2019) Macroinvertebrate diversity and rarity in non-glacial Alpine streams. *Aquat Sci* 81:42
- Ballinger A, Lake PS (2006) Energy and nutrient fluxes from rivers and streams into terrestrial food webs. *Mar Freshwater Res* 57:15-28
- Bartrons M, Papeş M, Diebel MW, Gratton C, Vander Zanden MJ (2013) Regional-level inputs of emergent aquatic insects from water to land. *Ecosystems* 16:1353-1363
- Baxter CV, Fausch KD, Carl Saunders W (2005) Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshw Bio* 50:201-220
- Briers RA, Cariss HM, Geoghegan R, Gee JH (2005) The lateral extent of the subsidy from an upland stream to riparian lycosid spiders. *Ecography* 28:165-170
- Bunn SE, Leigh C, Jardine TD (2013) Diet-tissue fractionation of  $\delta^{15}\text{N}$  by consumers from streams and rivers. *Limnol Oceanogr* 58: 765-773
- Burdon FJ, Harding JS (2008) The linkage between riparian predators and aquatic insects across a stream-resource spectrum. *Freshw Bio* 53:330-346

495 Burgherr P, Ward J, Robinson C (2002) Seasonal variation in zoobenthos across habitat  
 496 gradients in an alpine glacial floodplain (Val Roseg, Swiss Alps). *J N Am Benthol*  
 497 *Soc* 21:561-575

498 Burrows RM, Rutledge H, Valdez DG, Venarsky M, Bond NR, Andersen MS, Fry B,  
 499 Eberhard SM, Kennard MJ (2018) Groundwater supports intermittent-stream food  
 500 webs. *Freshw Sci* 37: 42-53

501 Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ( $\Delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$ ):  
 502 the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol*  
 503 46:443-453

504 Chapin TP, Todd AS, Zeigler MP (2014) Robust, low-cost data loggers for stream  
 505 temperature, flow intermittency, and relative conductivity monitoring. *Water Resour*  
 506 *Res* 50:6542-6548

507 Collier KJ, Bury S, Gibbs M (2002) A stable isotope study of linkages between stream and  
 508 terrestrial food webs through spider predation. *Freshw Bio* 47:1651-1659

509 Drummond LR, McIntosh AR, Larned ST (2015) Invertebrate community dynamics and  
 510 insect emergence in response to pool drying in a temporary river. *Freshw Bio*  
 511 60:1596-1612

512 Epanchin PN, Knapp RA, Lawler SP (2010) Nonnative trout impact an alpine-nesting bird by  
 513 altering aquatic-insect subsidies. *Ecology* 91:2406-2415

514 Finn DS, Poff NL (2008) Emergence and flight activity of alpine stream insects in two years  
 515 with contrasting winter snowpack. *Arct Antarct Alp Res* 40: 638-646.

516 Füreder L, Wallinger M, Burger R (2005) Longitudinal and seasonal pattern of insect  
 517 emergence in alpine streams. *Aquatic Ecol* 39:67-78

518 Füreder L, Welter C, Jackson JK (2003) Dietary and stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) analyses in  
 519 alpine stream insects. *Int Rev Hydrobiol* 88:314-331

520 Gabbud C, Robinson CT, Lane SN (2019) Sub-basin and temporal variability of  
521 macroinvertebrate assemblages in Alpine streams: when and where to sample?  
522 *Hydrobiologia* 830:179-200

523 Greenwood MJ, McIntosh AR (2010) Low river flow alters the biomass and population  
524 structure of a riparian predatory invertebrate. *Freshw Bio* 55:2062-2076

525 Hering D, Plachter H (1997) Riparian ground beetles (Coeloptera, Carabidae) preying on  
526 aquatic invertebrates: a feeding strategy in alpine floodplains. *Oecologia* 111:261-270

527 Hobbie EA, Macko SA, Shugart HH (1998) Patterns in N dynamics and N isotopes during  
528 primary succession in Glacier Bay, Alaska. *Chem Geol* 152: 3-11

529 Jansson M, Hickler T, Jonsson A, Karlsson J (2008) Links between terrestrial primary  
530 production and bacterial production and respiration in lakes in a climate gradient in  
531 subarctic Sweden. *Ecosystems* 11:367-376

532 Jardine TD, Hadwen WL, Hamilton SK, Hladyz S, Mitrovic SM, Kidd KA, Tsoi WY, Spears  
533 M, Westhorpe DP, Fry VM, Sheldon F (2014) Understanding and overcoming  
534 baseline isotopic variability in running waters. *River Res Appl* 30:155–165.

535 Körner C (1999) Alpine plant life: functional plant ecology of high mountain ecosystems.  
536 Springer

537 Krell B, Röder N, Link M, Gergs R, Entling MH, Schäfer RB (2015) Aquatic prey subsidies  
538 to riparian spiders in a stream with different land use types. *Limnologica* 51:1-7

539 Logue JB, Robinson CT, Meier C, der Meer JRV (2004) Relationship between sediment  
540 organic matter, bacteria composition, and the ecosystem metabolism of alpine  
541 streams. *Limnol Oceanogr* 49:2001-2010

542 Malard F, Ferreira D, Dolédec S, Ward JV (2003a) Influence of groundwater upwelling on  
543 the distribution of the hyporheos in a headwater river flood plain. *Arch Hydrobiol*  
544 157: 89-116



545 Malard F, Galassi D, Lafont M, Doleddec S, Ward JV (2003b) Longitudinal patterns of  
546 invertebrates in the hyporheic zone of a glacial river. *Freshw Bio* 48: 1709-1725

547 Malard F, Uehlinger U, Zah R, Tockner K (2006) Flood-pulse and riverscape dynamics in a  
548 braided glacial river. *Ecology* 87:704-716

549 Marczak LB, Thompson RM, Richardson JS (2007) Meta-analysis: trophic level, habitat, and  
550 productivity shape the food web effects of resource subsidies. *Ecology* 88:140-148

551 Martin-Creuzburg D, Kowarik C, Straile D (2017) Cross-ecosystem fluxes: Export of  
552 polyunsaturated fatty acids from aquatic to terrestrial ecosystems via emerging  
553 insects. *Sci Total Environ* 577:174-182

554 McKernan C, Cooper DJ, Schweiger EW (2018) Glacial loss and its effect on riparian  
555 vegetation of alpine streams. *Freshw Bio* 63:518-529

556 Misteli B (2019) Flow intermittency relations on biodiversity in an alpine fluvial network.  
557 Unpublished master's thesis, Swiss Federal Institute of Technology, Zürich

558 Muehlbauer JD, Collins SF, Doyle MW, Tockner K (2014) How wide is a stream? Spatial  
559 extent of the potential “stream signature” in terrestrial food webs using meta-analysis.  
560 *Ecology* 95:44-55

561 Nentwig W (1987) The prey of spiders. In: *Ecophysiology of spiders*. Springer, pp. 249-263

562 Niedrist GH, Füreder L (2017) Trophic ecology of alpine stream invertebrates: current status  
563 and future research needs. *Freshw Sci* 36:466-478

564 Oelbermann K, Scheu S (2002) Stable isotope enrichment ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) in a generalist  
565 predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia*  
566 130:337-344

567 Paetzold A, Schubert CJ, Tockner K (2005) Aquatic terrestrial linkages along a braided-river:  
568 riparian arthropods feeding on aquatic insects. *Ecosystems* 8:748-759

- Paillex A, Siebers AR, Ebi C, Mesman J, Robinson CT (2020) High stream intermittency in an alpine fluvial network: Val Roseg, Switzerland. *Limnol Oceanogr* 65:557–568
- Phillips DL et al. (2014) Best practices for use of stable isotope mixing models in food-web studies. *Can J Zool* 92:823-835
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703-718
- Rasmussen JB (2010) Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for  $\delta^{13}\text{C}$ . *J Anim Ecol* 79:393-402
- Robinson C, Kawecka B, Füreder L, Peter A (2010) Biodiversity of flora and fauna in alpine waters. In: *Alpine Waters*. Springer, pp 193-223
- Robinson CT, Alther R, Leys M, Moran S, Thompson C (2015) A note on the trophic structure of alpine streams in the Wind River Mountains, Wyoming, USA. *Fund Appl Limnol* 187:43-54
- Robinson C, Tonolla D, Imhof B, Vukelic R, Uehlinger U (2016a) Flow intermittency, physico-chemistry and function of headwater streams in an Alpine glacial catchment. *Aquat Sci* 78:327-341
- Robinson CT, Thompson C, Lods-Crozet B, Alther R (2016b) Chironomidae diversity in high elevation streams in the Swiss Alps. *Fund Appl Limnol* 188:201-213
- Sanzone D, Meyer J, Martí E, Gardiner E, Tank J, Grimm N (2003) Carbon and nitrogen transfer from a desert stream to riparian predators. *Oecologia* 134:238-250
- Schindler DE, Smits AP (2017) Subsidies of aquatic resources in terrestrial ecosystems. *Ecosystems* 20:78-93
- Semenina EE, Tiunov AV (2011) Trophic fractionation ( $\Delta^{15}\text{N}$ ) in Collembola depends on nutritional status: A laboratory experiment and mini-review. *Pedobiologia* 54:101-109

- Siebers AR, Paillex A, Robinson CT (2019) Flow intermittency influences the trophic base, but not the overall diversity of alpine stream food webs. *Ecography* 42:1523-1535
- Siebers AR, Paillex A, Misteli B, Robinson CT (2020a) Effects of an experimental increase in flow intermittency on an alpine stream. *Hydrobiologia* 847:3453–3470
- Siebers AR, Paillex A, Robinson CT (2020b) Data for: Riparian hunting spiders do not rely on aquatic subsidies from intermittent alpine streams. Dryad, <<http://doi.org/10.5061/dryad.gxd2547jp>>
- Stenroth K, Polvi LE, Fältström E, Jonsson M (2015) Land-use effects on terrestrial consumers through changed size structure of aquatic insects. *Freshw Bio* 60:136-149
- Steward AL, Langhans SD, Corti R, Datry T (2017) The biota of intermittent rivers and ephemeral streams: terrestrial and semiaquatic invertebrates. In: *Intermittent Rivers and Ephemeral Streams*. Elsevier, pp 245-271
- Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX (2018) Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* 6: e5096
- Tank JL, Rosi-Marshall EJ, Griffiths NA, Entrekin SA, Stephen ML (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *J N Am Benthol Soc* 29:118-146
- Thaler K (2003) The diversity of high altitude arachnids (Araneae, Opiliones, Pseudoscorpiones) in the Alps. In: *Alpine biodiversity in Europe*. Springer, pp 281-296
- Toft S (1999) Prey choice and spider fitness. *J Arachnol* 27:301-307
- Uehlinger U, Zah R, Burgi H (1998) The Val Roseg project: temporal and spatial patterns of benthic algae in an Alpine stream ecosystem influenced by glacial runoff. *IAHS Publ* 248: 419-434

618 Vander Zanden MJ, Clayton MK, Moody EK, Solomon C., Weidel BC (2015) Stable isotope  
619 turnover and half-life in animal tissues: a literature synthesis. PloS one 10: e0116182.  
620 Ward J (1994) Ecology of alpine streams. Freshw Bio 32:277-294  
621 Zingerle V (1999) Spider and harvestman communities along a glaciation transect in the  
622 Italian Dolomites. J Arachnol 27:222-228

ACCEPTED

## Figure Captions

Figure 1. Maps of Val Roseg showing: (a) location of the catchment (black circle) within outline map of Switzerland; and (b) location of sensor and invertebrate collection sites (circles) within the stream network (black lines) of Val Roseg with differences in flow intermittency (FI-values) across sites (grey-scale, see legend), derived from electrical resistance sensor data. Glacial lake is coloured white in (b). Hillshade in (b) is derived from altitude data from 25 m DEM model of Switzerland (swisstopo 2018, swissTLM3D & swissALTI3D, Bundesamt für Landestopographie (Art.30 Geo IV): 5704 000).

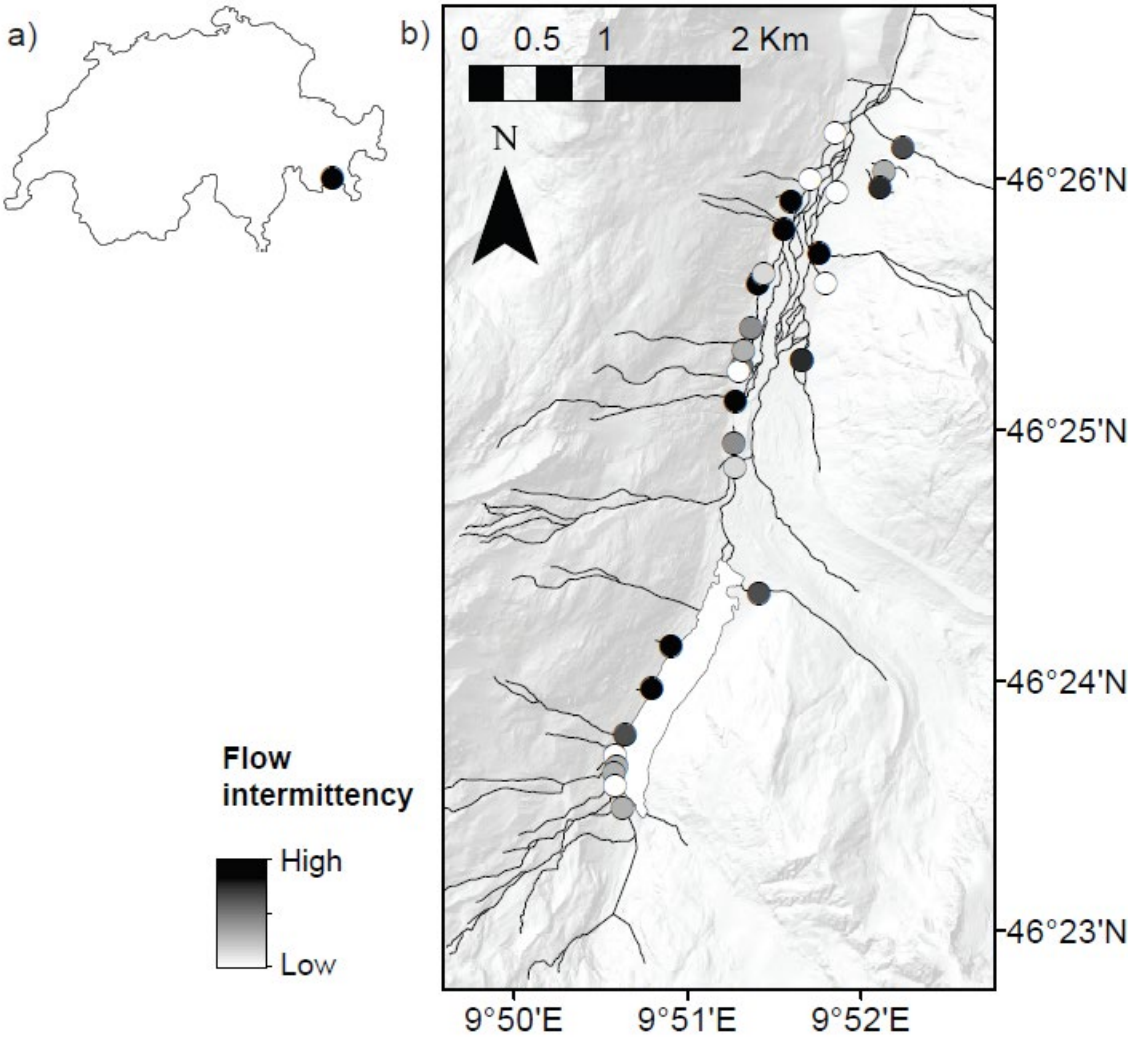
Figure 2. Relationship between flow intermittency metrics (average number of drying events per year, percentage of year channel was dry) across the 30 headwater study streams. Data are derived from electrical resistance sensors measuring presence/absence of surface water, installed from July 2017 to October 2018. Colours indicate grouping of sites into flow intermittency classes (perennial/low – high).

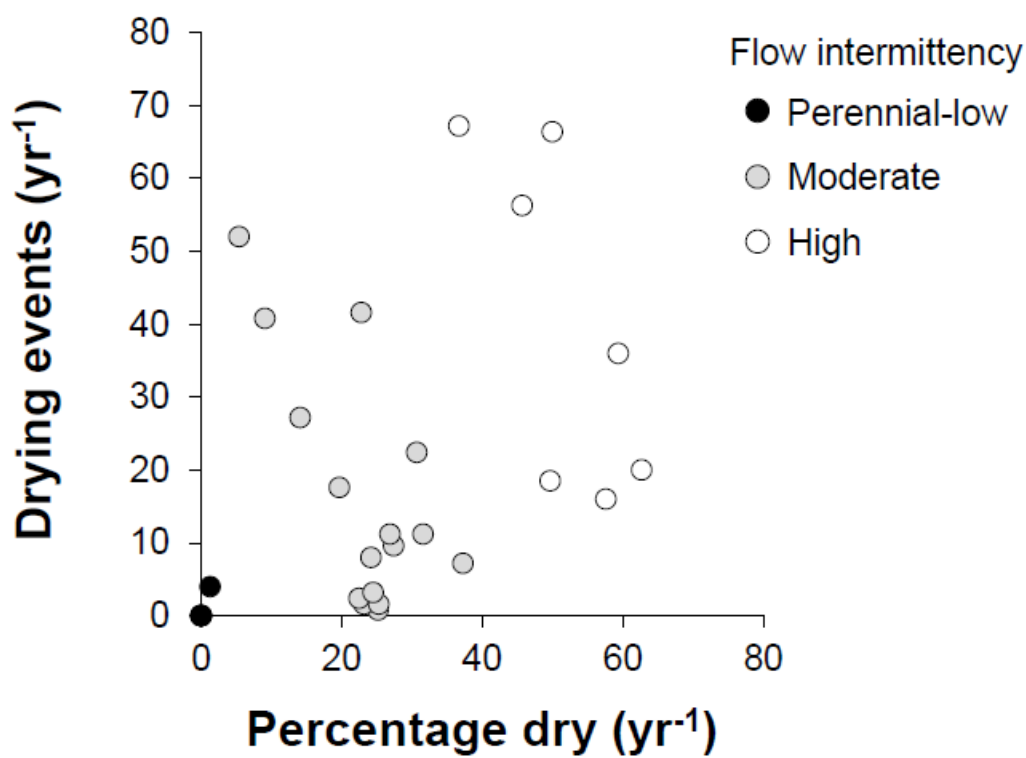
Figure 3. Biplot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for riparian hunting spiders, ants, aquatic Ephemeroptera, and aquatic Plecoptera from (a) spring (June 2018) and (b) summer (August 2018) samplings. Aquatic macroinvertebrate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are shown as site mean  $\pm$  standard deviation. Terrestrial plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (mean  $\pm$  sd) are given from Siebers et al. (2019) as reference. Periphyton and filamentous algae  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (site mean) are calculated from spring and summer samplings as well as Siebers et al. (2019). Values are not corrected for trophic enrichment. Note: standard deviation for periphyton and filamentous algae  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values within sites (not shown for visual clarity) range from 0.1 to 6.4 ‰ for  $\delta^{13}\text{C}$  and 0.1 to 3.0 ‰ for  $\delta^{15}\text{N}$ .

Figure 4. Site-specific  $\delta^{13}\text{C}$  of riparian hunting spiders vs. mean  $\delta^{13}\text{C}$  of aquatic macroinvertebrates from (a) spring (June 2018) and (b) summer (August 2018) samplings.

Aquatic macroinvertebrate  $\delta^{13}\text{C}$  values are corrected for trophic enrichment factors (TEF).  
Dashed line indicates 1:1 line, shaded area indicates 95 % confidence bounds for terrestrial  
prey  $\delta^{13}\text{C}$  (corrected for TEF). Solid line indicates line of best fit significantly different from  
zero (at  $p = 0.05$ ).

Figure 5. Estimated dietary contribution (as proportions of total) of emergent aquatic insects  
to riparian hunting spiders across flow intermittency classes and seasons. Estimated  
contributions are shown as (i) medians, 1<sup>st</sup> and 3<sup>rd</sup> quartiles (boxplots), and (ii) density  
distributions (violin plots) of Bayesian mixing model results. Spring – white; summer – grey.

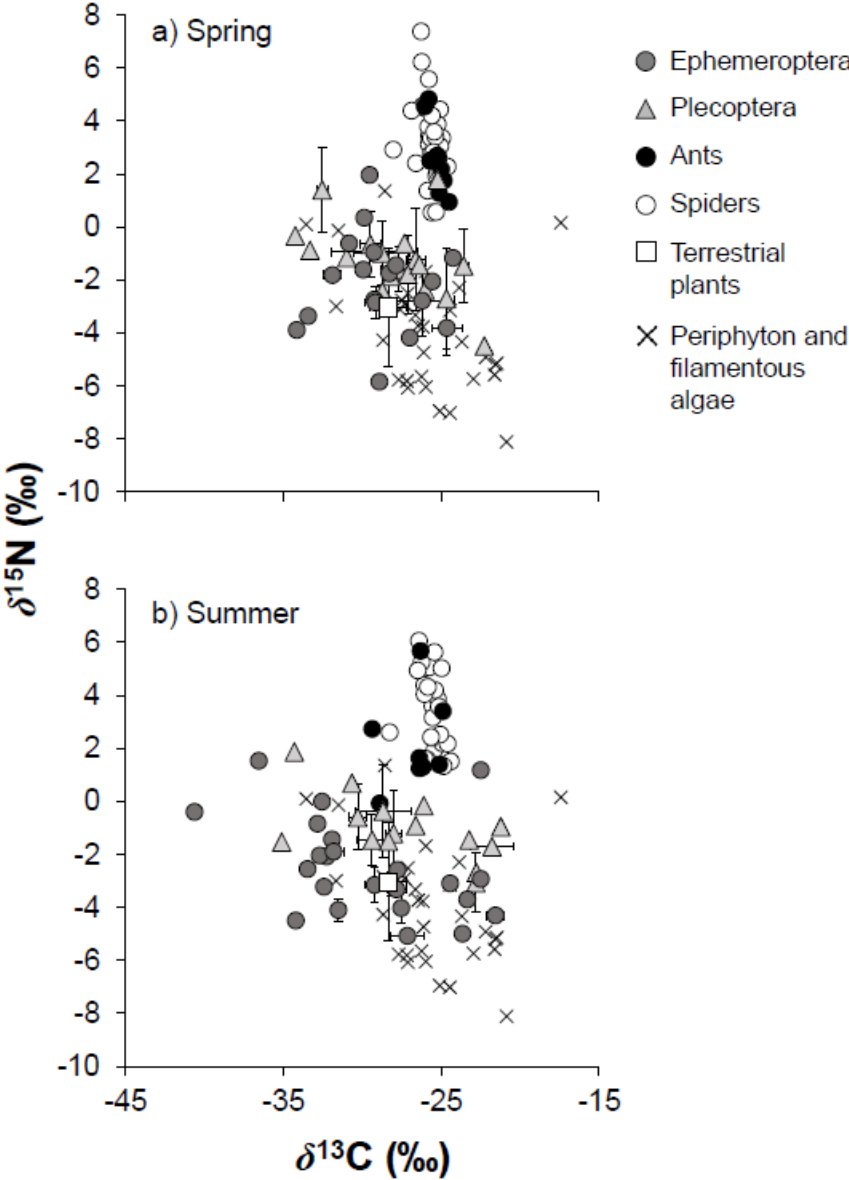




660

661

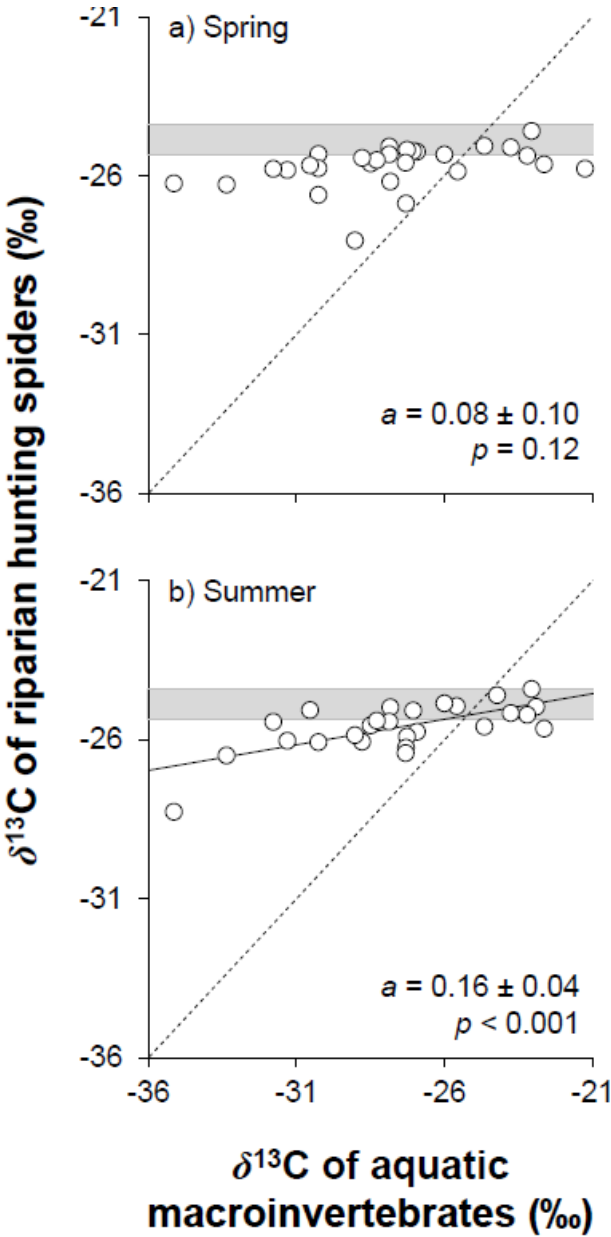




663

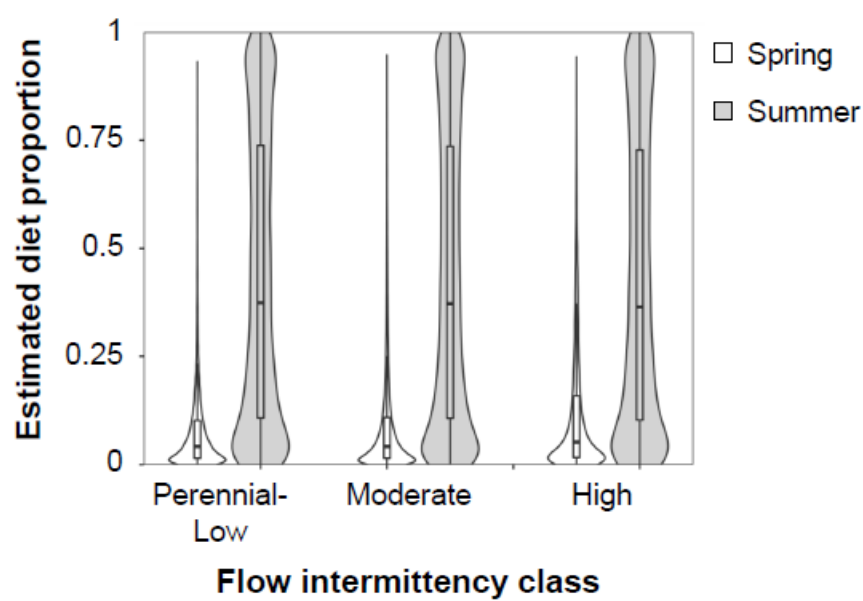
664

665 Figure 4



666

667

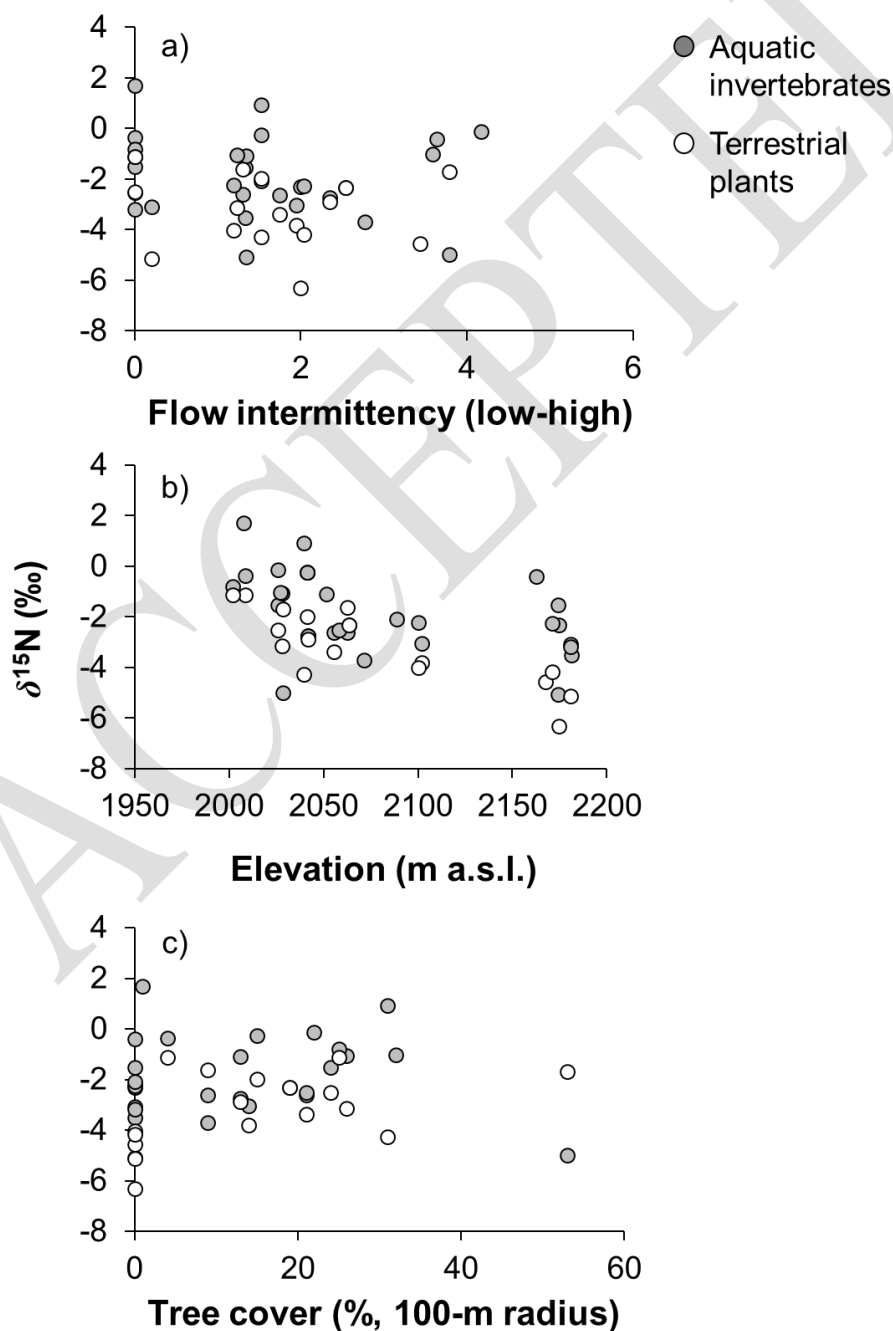


669

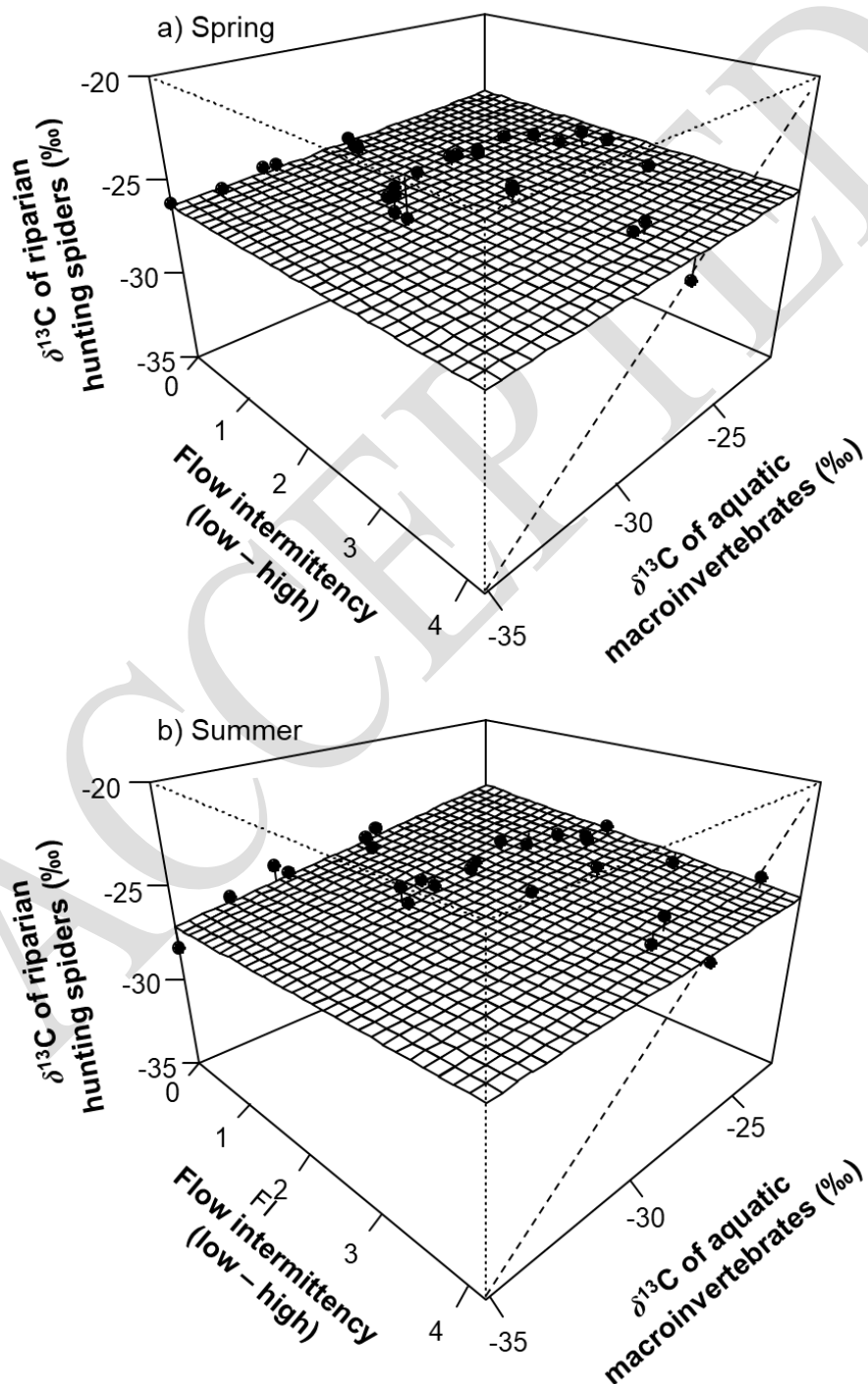
670

## Supplementary material

Figure A.1: Variation in  $\delta^{15}\text{N}$  values of hemimetabolous aquatic macroinvertebrates and terrestrial plants across gradients in (a) flow intermittency (FI) values, (b) site elevation, and (c) tree cover within a 100-m radius of sites. FI values are derived from electrical resistance sensors measuring presence/absence of surface water, installed from July 2017 to October 2018. Terrestrial plant  $\delta^{15}\text{N}$  and site tree cover estimates are taken from Siebers et al. (2019).



678 Figure A.2: Site-specific  $\delta^{13}\text{C}$  of riparian hunting spiders vs.  $\delta^{13}\text{C}$  of aquatic  
679 macroinvertebrates from (a) spring (June 2018) and (b) summer (August 2018) samplings,  
680 with respect to the gradient in site streamflow intermittency (low to high FI). Response  
681 surface shows fitted function from generalised linear model (GLM). Original data points and  
682 residuals relative to GLM fitted values are also shown.



684 **References**

685 Siebers AR, Paillex A, Robinson CT (2019) Flow intermittency influences the trophic base,  
686 but not the overall diversity of alpine stream food webs. *Ecography* 42:1523-1535

687

ACCEPTED