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Effects of desiccation on heterotrophic microbial activity in hyporheic sediments

Master Thesis

for obtaining the academic title

Master of Science, MSc

at the University of Natural Resources and Life Sciences, Vienna

by

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Vienna, May 2021

Statutory declaration

I hereby declare that I am the sole author of this work. No assistance other than which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

Acknowledgments

First and foremost I would like to thank my supervisors Gabriele Weigelhofer and Thomas Hein for their unlimited support at every stage of my study and for their patience during my thesis writing. Thank you for your infinite comments and suggestions. This master thesis would not have been successful without your expertise and enthusiasm.

This project was funded by the Klima- und Energiefonds and was carried out within the framework of the "Austrian Climate Research Programme ACRP 10" (ACRP10 – PURIFY – KR17AC0K13643).

Thank you to all the members of WasserCluster Lunz, especially Beate Pitzl, Annette Puritscher and Gertraud Steniczka, for their patience in answering all of my questions, helpful advices and help to expand my laboratory skills.

I would like to thank my colleagues Manuela Waberer and Magdelena Senitza for their infinite support, advices and motivation during the field sampling and the long days and nights in the lab. Thank you for the great time we had together.

Thank you to my family and friends for their endless support, encouragement and belief in me during my studies. I am deeply grateful for having you by my side.

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AMC	7-Amino-4-methylcoumarin
CFA	Continuous-flow analysis
DB	Drosenbach
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DW	Dry weight
EEA	Extracellular enzymatic activity
EPS	Extracellular polymeric substance
FB	Fruttnerbach
GaB	Gatterbach
GB	Glaunigbach
Gluc	β-D-Glucosidase
JB	Joachimsbach
LB	Lieberbach
Leu	Leucin-aminopeptidase
MUF	4-Methyllumbellifery
NDA	No data available
OM	Organic matter
PB	Pfaffenbach
Phos	Phosphatase
POP	Particulate organic phosphorous
RB	Reithschuhlbach
Resp	Microbial respiration
SB	Schwabenbach
SRP	Soluble reactive phosphorus
WB	Wellingbach
WC	Water content
Xyl	β-D-Xylosidase

Abstract

Climate change, as well as water abstractions, have altered the flow regime and led to a reduction of the summer flows in running water systems. These environmental conditions promote flow intermittency and cause many formerly perennial streams to become intermittent. Shortened flow periods and water deficits affect heterotrophic microorganisms living in the sediments. Currently, there is little knowledge about the effects of stream intermittency, especially in temperate climate regions. This study investigates the immediate (during the non-flow period) and long-term (during the flow period) effects of flow intermittency on the activity of microbial communities in temperate streams in Burgenland and Styria, Austria. Hyporheic sediments from both perennial and intermittent streams were sampled during different flow conditions. Extracellular enzymatic activities and microbial respiration were used to determine stress responses to desiccation in heterotrophic organisms. Results show no significant immediate or long-term decline in heterotrophic microbial activities in hyporheic sediments of intermittent streams. During the late non-flow period, intermittent streams showed a significantly higher microbial respiration (0.58 µgC/g/h) and phosphatase activity (214.49 nmol/g/h) than perennial streams (0.43 µgC/g/h and 138.35 nmol/g/h respectively). During the flow period, average microbial respiration and enzymatic activities did not differ significantly. The maintenance of the heterotrophic activity is explained by preserving relatively high moisture content, of around 12%, during the non-flow period in the hyporheic zone. Streambed characteristics such as a high organic matter content and fine sediments favour sufficient wet conditions in sediments. This study highlights the importance of the hyporheic zone as a humid refuge habitat for microbes during the non-flowing period, increasing the resilience against drought events.

Keywords: flow intermittency, hyporheic zone, heterotrophic microorganisms, extracellular enzymes, microbial respiration

Zusammenfassung

Klimawandel sowie Wasserentnahmen haben das Abflussregime verändert und zu einer Verringerung der Sommerabflüsse in Fließgewässern geführt. Dies fördert Fließunterbrechungen und führt dazu, dass viele ehemals perennierende Fließgewässer intermittierend werden. Verkürzte Fließperioden und Wasserdefizite nehmen Einfluss auf heterotrophe Mikroorganismen. Derzeit gibt es nur wenig Kenntnis über die Auswirkungen von Fließunterbrechungen, insbesondere im gemäßigten Klima. Diese Studie untersucht die unmittelbaren (während der abflusslosen Periode) und langfristigen (während der Fließperiode) Auswirkungen von verkürzten Fließperioden auf die Aktivität von mikrobiellen Gemeinschaften im Burgenland und in der Steiermark. Hyporheische Sedimente von perennierenden und intermittierenden Bächen wurden während unterschiedlicher Fließbedingungen untersucht. Extrazelluläre enzymatische Aktivität und mikrobielle Atmung wurden verwendet um Stressreaktionen von heterotrophen Organismen auf die Austrocknung festzustellen. Die Ergebnisse zeigen keine signifikante sofortige oder langfristige Abnahme der heterotrophen mikrobiellen Aktivitäten in intermittierenden Bächen. Während der abflusslosen Periode zeigten intermittierende Bäche eine signifikant höhere mikrobielle Atmung (0,58 µgC/g/h) und Phosphatase-Aktivität (214,49 nmol/g/h) als perennierende Bäche (0,43 µgC/g/h bzw. 138,35 nmol/g/h). Während der Fließperiode gab es keine signifikanten Unterschiede in der Aktivität der intermittierenden und perennierenden Bäche. Die Aufrechterhaltung der heterotrophen Aktivität in der hyporheischen Zone wird durch die Erhaltung eines relativ hohen Feuchtigkeitsgehalts von etwa 12% während der abflusslosen Zeit begründet. Ausreichend feuchte Bedingungen im Sediment werden durch einen hohen Gehalt an organischer Substanz und Feinsediment begünstigt. Diese Studie festigt die Bedeutung der hyporheischen Zone als feuchtes Rückzugshabitat für die Mikroben während der abflusslosen Periode.

Schlagwörter: intermittierende Fließgewässer, hyporheische Zone, heterotrophe Mikroorganismen, extrazelluläre Enzymaktivität, mikrobielle Atmung

1 Introduction

1.1 Intermittent streams

More than 50% of the global river network have no permanent surface flow and dry out during times of low water supply (Datry et al., 2014). Such rivers are called intermittent streams (Bayly and Williams, 1973; Datry et al., 2017). While parts of these rivers cease to flow due to natural reasons such as bed porosity/transmission loss, evapotranspiration and freezing of surface and shallow subsurface zone (Datry et al., 2017; Larned et al., 2010), many formerly perennial streams became intermittent due to anthropogenic influences. A changing climate as well as water abstractions and land use changes have led to an altered flow regime and a reduction of the summer flows, promoting intermittency (Datry et al., 2014; Wilby et al., 2006). In near future, the number and length of intermittent streams in regions affected by climate change and human alterations will increase even further (Döll and Schmied, 2012; Larned et al., 2010).

1.2 Intermittent streams- Hydrological phases and their effects

Intermittent streams are characterized by their typical dry and wet phases (Fig. 1), responding to the hydrological seasonality by expanding during wet periods and contracting during dry periods (Bernal et al., 2013). In the first phase, flow gradually decreases until the flow gets disrupted as shallow surface habitats (riffles) start to dry, resulting in the creation of isolated pools (Acuña et al., 2005; Costigan et al., 2017; Sabater et al., 2016). These pools can either persist during the period of non-flow (Fig. 2b) or start to dry as well (Fig. 2c), depending on the duration of the drying, the morphology of the pool and the presence of vertical interactions due to remaining subsurface flow (Gómez et al., 2017; Sabater et al., 2016). Over longer periods of flow recession, the surface drying can be followed by subsurface drying (Costigan et al., 2017; Sabater et al., 2016). After the dry phase, flow resumes again due to the occurrence of rainfall, the input of meltwater or the decrease of evapotranspiration (Costigan et al., 2017; von Schiller et al., 2017). This cycle creates a high diversity of shifting mosaics including lotic (flowing), lentic (non-flowing) and terrestrial (dry) habitats along time and space (DATRY ET AL., 2017).

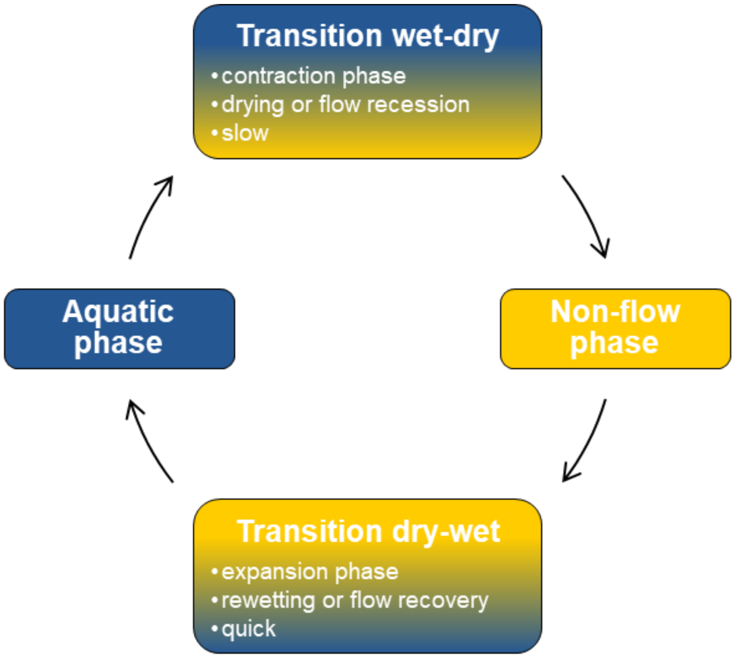

Figure 1: The hydrological phases of intermittent streams based on SABATER ET AL. (2016).

During the contraction phase, the hydrological connectivity is disrupted (BERNAL ET AL., 2013) along three dimensions, longitudinally, laterally and vertically (BOULTON ET AL., 2017), affecting the biochemistry as well as aquatic organisms (LARNED ET AL., 2010; SABATER ET AL., 2016). Longitudinally, the downstream transport of sediments, organic matter, and biota gets blocked (BOULTON ET AL., 2017). Consequently, the nutrient and organic matter supply is reduced due to the reduction of the nutrient transport from upstream (VON SCHILLER ET AL., 2017). On the other hand, the spatial variation of nutrient concentrations increases due to flow fragmentation (ACUÑA ET AL., 2005; BERNAL ET AL., 2013; VON SCHILLER ET AL., 2011). With decreasing discharge and velocity, the water residence time gets prolonged (VON SCHILLER ET AL., 2017). Moreover, the temperature of the remaining water is increased, thus enhancing the microbial metabolism (BERNAL ET AL., 2013). Both biotic and abiotic factors increase the potential for nutrient and organic matter retention (BERNAL ET AL., 2013; VON SCHILLER ET AL., 2017). Laterally, the interaction between floodplain and main channel gets interrupted as the water level decreases, leading to an isolation of aquatic habitats along the riparian zone and in the floodplain area (BOULTON ET AL., 2017). The vertical connectivity due to upwelling and downwelling water exchanges, and with that the transport of oxygen and nutrients, between the surface and subsurface zone gets disrupted as surface flow stops (Boulton et al., 2017; von Schiller et al., 2017). As the stream dries up, the water content in the sediments decreases and the oxygen content rises, as the sediments get exposed to the atmosphere. Consequently, the partial drying of the sediments creates a zone for nitrification coupled with denitrification (Baldwin and Mitchel, 2000).

Figure 2: Three hydrological phases in an intermittent stream (a) flowing, (b) contraction (non-flowing) and (c) dry (non-flowing).

During prolonged reduction of water supply, the stream enters the dry phase. This creates an oxygenated environment, which favors aerobic nitrogen and phosphorous processes (Cavanaugh et al., 2006; Dieterich and Anderson, 1998; Gómez et al., 2012; von Schiller et al., 2017), while anaerobic processes, such as denitrification, are ceased (Baldwin and Mitchel, 2000; Gómez et al., 2012). As the sediment moisture is essential for the microbes living on and in the sediments, the low water availability could lead to a decreasing microbial activity (e.g. Gionchetta et al., 2019; Marxsen et al., 2010; Zoppini and Marxsen, 2010), through direct physiological effects, lowered microbial mobility and reduced diffusion of soluble substrates (Amalfitano et al., 2008; Bernal et al., 2013; Humphries and Baldwin, 2003).

1.3 Microbes- Introduction and survival strategies

Microbes play a major role in the aquatic nutrient cycle, in particular of carbon, nitrogen and phosphorous (Romaní et al., 2017). They can be divided into autotrophic and heterotrophic organisms. While autotrophic microorganisms can synthesize their own biomass using inorganic substances, either using light energy (photoautotrophic) or chemical energy (chemoautotrophic), the heterotrophic organisms depend on organic food sources (FUCHS, 2014; MADIGAN AND MARTINKO, 2009).

Microorganisms produce and release extracellular enzymes which decompose the organic matter into smaller soluble molecules (BELL ET AL., 2013; CUNHA ET AL., 2010). The products of the enzymatic degradation are, among others, glucose, phosphate and amino acids, which are used by the microbes for growth and metabolism (GERMAN ET AL., 2011). Among all enzymatic activities, ß-glucosidase, leucin-aminopeptidase and phosphatase are widely distributed and studied in the aquatic environment. ß-glucosidase is known to hydrolyse ß-linked disaccharides of glucose, celluhexose and carboxymethylcellulose. Leucin-aminopeptidase is an enzyme which hydrolyses a large number of peptides and amino acid amides. Both of their activity is mainly associated with heterotrophic microorganisms. In contrast, phosphatase activity can originate from bacteria, but also from phytoplankton and zooplankton. This enzyme can mediate the hydrolysis of esters and anhydrides of phosphoric acid (CHROST, 1991; CUNHA ET AL., 2010; ROMANÍ AND SABATER, 2000). The extracellular enzymes are not only important to the microbes themselves, but also to the entire ecosystem, as they are involved in many biochemical processes, such as mineralization, processing, stabilization and destabilization of organic matter and nutrient cycling (BALDWIN AND MITCHELL, 2000; BELL ET AL., 2013; BRUNKE AND GONSER, 1997; MARX ET AL., 2001).

During stream desiccation, microbes are exposed to high amounts of stress. The main reason for that is the semipermeable membrane, which creates an equilibrium between the water content outside and inside of the cell. Losing sediment moisture means dehydration of the cell and will lead to death. Microbes can be either resistant to stress conditions or adapt the stress to survive and remain active (SCHIMEL ET AL., 2007). An example of a resistance strategy would be more robust, thicker cell walls (SABATER ET AL., 2017; TIMONER ET AL., 2014) like those of gram-positive bacteria. Acclimation requires redirecting nutrients and energy from growth into survival (SCHIMEL ET AL., 2007).

The production of osmolytes, organic compounds like amino compounds, can help microbes to survive desiccation by stabilizing membranes and reducing the water potential (CSONKA, 1989; SCHIMEL ET AL., 2007). Another possibility is to produce an extracellular polymeric substance (EPS) (GIONCHETTA ET AL., 2019; ROBERSON AND FIRESTONE, 1992). The EPS consists of polysaccharides, proteins, lipids, nuclear acids and humic substances and can be secreted by microbes or released due to cell lysis. The EPS fulfills multiple functions. It is a structural element of biofilms, and it is used to form those biofilms and attach them to surfaces. Further to that, it acts as a protective barrier against oxidation, ultraviolet radiation and desiccation by producing a highly hydrated microenvironment around the organism leading to a higher tolerance in environments with water deficit (FLEMMING AND WINGENDER, 2010; MORE ET AL., 2014).

All those strategies have high costs of nutrients and energy and can alter the microbial community composition and the physiology and create a shift of carbon, nutrient and energy fluxes in the ecosystem. Over extended stress periods, microbes can learn to adapt to those conditions to easier acclimate in case of desiccation (SCHIMEL ET AL., 2007). If the stress level gets too severe and they are not resistant or cannot acclimate, microbes try to take refuge in remaining wet habitats such as pools, leaf-litter packs and subsurface sediments (BOGAN ET AL., 2017; SABATER ET AL., 2016) or enter dormant stages. Once beneficial ecosystem conditions are restored, dormant organisms regain their activity again (ROMANÍ ET AL., 2017; SCHIMEL ET AL., 2007).

1.4 Aim and relevance

The changing climate as well as human pressures enhance intermittency and increase the number and length of intermittent streams. The desiccation of the sediments, resulting from the intermittency, affects the organisms including microbes, which live there. Those microbes perform important ecosystem functions, like the decomposition of organic matter and phosphorous, mineralization of nitrogen, production of oxygen or carbon dioxide, and they are a food source to other organisms. A water deficit in the sediments could lead to a reduction of the activity of those microbes and therefore, affect the whole system. But, there is still limited knowledge about the effects of stream desiccation on the sediment microbes.

Most of the existing studies are done in the Mediterranean climate region, for example ACUÑA ET AL. (2015), AMALFITANO ET AL. (2008), GIONCHETTA ET AL. (2019) and TIMONER ET AL. (2012). These studies showed that heterotrophic microbes were able to maintain their activity to some extend during the non-flowing period (ACUÑA ET AL., 2015; AMALFITANO ET AL., 2008; GIONCHETTA ET AL., 2019) and were able to recover rapidly after flow resumption (MARXSEN ET AL., 2010; TIMONER ET AL., 2012). Nonetheless, decreasing bacterial abundance, biomass and metabolic functions were reported due to the desiccation stress (AMALFITANO ET AL., 2008; TIMONER ET AL., 2012). The intensity of the effect on the microbes is linked to the duration of the non-flowing period (ACUÑA ET AL., 2015; TIMONER ET AL., 2012).

Studies in the temperate region are rare. MARXSEN ET AL. (2010) examined microbial communities in streambed sediments experimenting with the sediments of a Mediterranean and a temperate stream, but this study focuses more on the recovery after desiccation. However, intermittency not only affects the Mediterranean region, but also temperate regions, where decreased run-offs and extended desiccation periods have been observed, especially in headwater streams (SUTHERLAND ET AL., 2008; WILBY ET AL., 2006). Unlike the Mediterranean region, the temperate region is typically characterized by less severe contraction phases. Therefore, temperate streams do not dry out completely, but tend to preserve some water content in the sediments during the non-flowing period (BERNAL ET AL., 2013; MARXSEN ET AL., 2010). However, communities of Mediterranean streams, with their typical occurring wet-dry cycles, had longer time to adapt to those conditions than communities in temperate streams, which are only recently affected (MARXSEN ET AL., 2010).

The hyporheic zone, an ecotone between the surface stream and the groundwater (BOULTON ET AL., 1998), can act as an important refuge habitat for the microbes during the non-flowing period. The hyporheic refuge theory was first imposed by WILLIAMS AND HYNES (1974) for macroinvertebrates. They predicted that macroinvertebrates migrate into deeper sediment layers as the conditions on the surface become unfavorable. Later, FEBRIA ET AL. (2012) discovered that microorganisms as well use the hyporheic zone as a sort of refuge from desiccation. These subsurface sediments remain longer hydrated during contraction periods than surface sediments, which will allow the microbes to survive and maintain their activity longer even during times of prolonged drought (FEBRIA ET AL., 2012; GIONCHETTA ET AL., 2019).

This study aimed to investigate the effects of drying on the activity of the microbial community in the hyporheic zone of temperate streams. We took hyporheic sediment samples from both intermittent and perennial reaches before and at the end of the dry period to analyse short- and long-term effects of drying on hyporheic biofilms.

1.5 Research questions and hypotheses

Q1: What are the immediate effects (days to weeks) of stream desiccation on the heterotrophic microbial activity (respiration, enzymatic activity) in hyporheic sediments?

H1: During the non-flowing phase, the respiration and enzymatic activity is decreased in the dry sediments of intermittent streams compared to the wet sediments of perennial streams.

Q2: Are there any long-term effects (months to years) on the heterotrophic microbial community living in hyporheic sediments due to the desiccation stress in intermittent streams?

H2: During the flowing phase before flow cessation, the respiration and enzymatic activity in the wet sediments of intermittent streams is lower than in the wet sediments of perennial streams.

Q3: Which factors buffer the effects of intermittency in the hyporheic sediments?

H3: Higher amounts of organic matter and fine sediments reduce the effects of desiccation by maintaining a high sediment moisture content.

2 Material and Method

2.1 Study reaches

The sampling sites are distributed over Burgenland and Styria. The sites in Burgenland belong to the bioregion Östliche Flach- und Hügelländer, whereas the streams in Styria are part of the bioregion Grazer Feld und Grabenland. The streams of these regions are characterized by a pluvial discharge regime and a substrate of mainly upper tertiary sediments and sedimentary rocks (Wimmer et al., 2012). Both bioregions in the south-east of Austria are influenced by the Illyrian climate. An average annual temperature of around 10°C and a summer average temperature of around 20°C are typical in this area. Compared to the northern regions, the annual precipitation is noticeable higher reaching values from 700 to 1000 mm (Fally and Fischer, 2015; Killian et al., 1994; Sauberer et al., 2017).

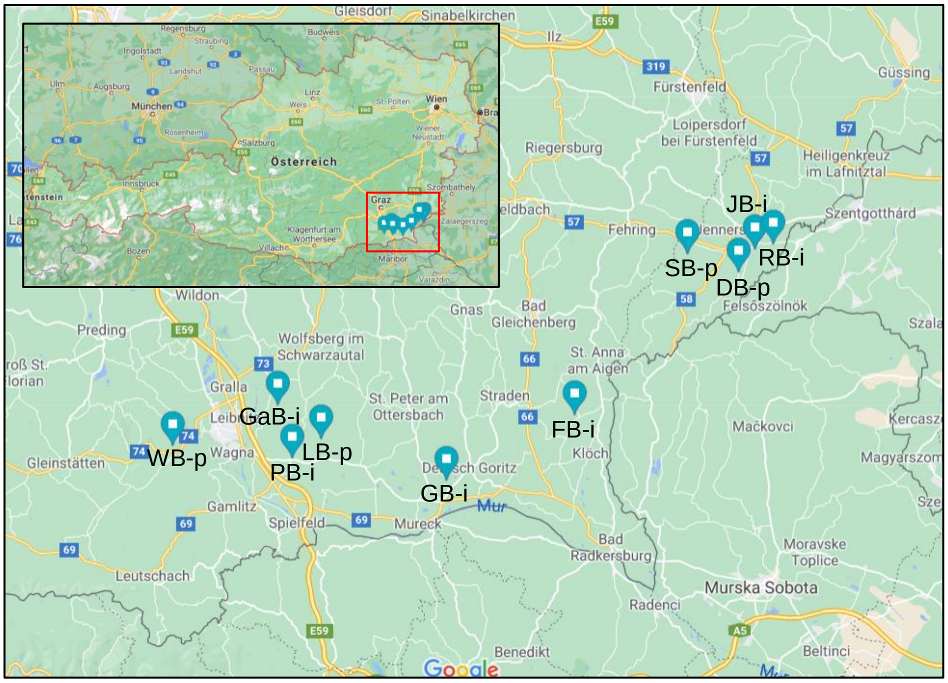

Figure 3: Map of all sampling sites.

A total of ten streams were observed including five intermittent streams and five comparable perennial streams (Fig. 3, Table 1). The streams resemble each other regarding their size, discharge and the nearby agricultural land use. Four of the streams are located in Burgenland, with Reithschuhlbach and Joachimsbach as intermittent and

Drosenbach and Schwabenbach as perennial streams (Fig. 4). Reithschuhlbach is located in the eastern part of Neumarkt an der Raab in Southern Burgenland. The river is intermittent with pools between dry sections. The river stretch is mainly covered by closed canopy and has an approximately width of 2.5 m. The second intermittent river in Burgenland is the Joachimsbach, which has small pools and a step-pool character. The river stretch is located within a forest, in the western part of Neumarkt an der Raab, and is therefore mainly covered by closed canopy. Similar to Reithschuhlbach, the river width is around 2.5 m. Drosenbach is situated south of St. Martin an der Raab. The stretch is shaded by a closed canopy and has a width of around 3 m. Schwabenbach is located west of St. Martin an der Raab, right next to the street. The stretch has an approximately width of 3 m and is only partly shaded by canopy.

Figure 4: Sampled perennial and intermittent streams of Burgenland (a) Reithschuhlbach, (b) Joachimsbach, (c) Drosenbach and (d) Schwabenbach.

The remaining six streams are located in Styria, with Gatterbach, Pfaffenbach and Glaunigbach as intermittent and Wellingbach, Lieberbach and Fruttnerbach as perennial river section (Fig. 5). Gatterbach is situated east of St. Nikolai ob Draßling in the South-East of Styria. This intermittent river is characterized by pools between the dry sections and larger grain sizes with a high amount of gravel and fine pebbles. The stretch is located in a forest, but is only partly shaded with no closed canopy. In contrast, the Pfaffenbach is characterized by smaller grain sizes with a high amount of clay and silt.

The river stretch is situated in a forest south of Gatterbach and north of St. Veit am Wongau. The last intermittent stream in Styria is the Glaunigbach in the western part of Ratschendorf. The river stretch is around 4 m wide and is shaded by a closed canopy. Welingbach is located in the northern part of Heimschuh. It has a river width of around 4 m, is partly shaded by a canopy and is characterized by a high amount of course sandy sediments. Lieberbach is situated in the southern part of St. Nikolai ob Draßling and north east of Pfaffenbach. The stretch is shaded by a canopy and has a stony surface substrate. Fruttnerbach was classified as perennial stream, but the river section dried out during the summer months and was then sampled in September as intermittent stream. The stream is located in the northern part of Tieschen. It is covered by a closed canopy and has typical pools in between the dry sections.

Figure 5: Sampled perennial and intermittent streams of Styria (a) Wellingbach, (b) Gatterbach, (c) Pfaffenbach, (d) Lieberbach, (e) Glaunigbach and (f) Fruttnerbach.

Table 1: Description of the sampling sites.

Name	ID	Type	Location	Coordinates
Reithschuhlbach	RB-i	intermittent	Burgenland	46°55'35.2"N 16°10'27.6"E
Joachimsbach	JB-i	intermittent	Burgenland	46°55'23.7"N 16°09'11.7"E
Drosenbach	DB-p	perennial	Burgenland	46°54'17.4"N 16°08'03.7"E
Schwabenbach	SB-p	perennial	Burgenland	46°55'08.2"N 16°04'35.7"E
Wellingbach	WB-p	perennial	Styria	46°46'10.0"N 15°29'24.3"E
Gatterbach	GaB-i	intermittent	Styria	46°48'03.3"N 15°36'36.3"E
Lieberbach	LB-p	perennial	Styria	46°46'30.6"N 15°39'34.8"E
Pfaffenbach	PB-i	intermittent	Styria	46°45'34.3"N 15°37'35.4"E
Glaunigbach	GB-i	intermittent	Styria	46°44'33.9"N 15°48'05.1"E
Fruttnerbach	FB-i	perennial /intermittent	Styria	46°47'37.0"N 15°56'51.9"E

2.2 Sampling design

The sampling took place at three times (Fig. 6), once during the flow period in May (6.-7. May) 2019 and twice during the non-flowing period in July (15.-16. July) and September (2.-3. September) 2019. The hydrological phases were defined by the climatic conditions. Water as well as sediment samples were collected at each stream during all sampling dates. The water samples were used to determine chemical parameters like DOC, nitrite, nitrate, ammonium and soluble reactive phosphorus (SRP) as phosphate. At each site, five subsamples of sediments were taken with a shovel at a depth of approximately 10 to 20 cm. In September 2019, an additional sampling of benthic sediments took place. Therefore, the upper 5 cm were sampled at five places at each site. Only areas with fine sediment accumulations were sampled and the sediments were sieved in the field with a 4 mm sieve (ALLISON ET AL., 2007; ČERNOHLÁVKOVÁ ET AL., 2009). To avoid contamination, the sampling equipment (shovel, sieve and bucket) was cleaned in the respective stream before sampling. The sampling process lasted two days. The already sampled sediments were stored at cool temperatures with the help of ice and ice packs over the whole period. Upon arrival at the lab, the samples were stored at 4°C (ČERNOHLÁVKOVÁ ET AL., 2009; DEFOREST, 2009).

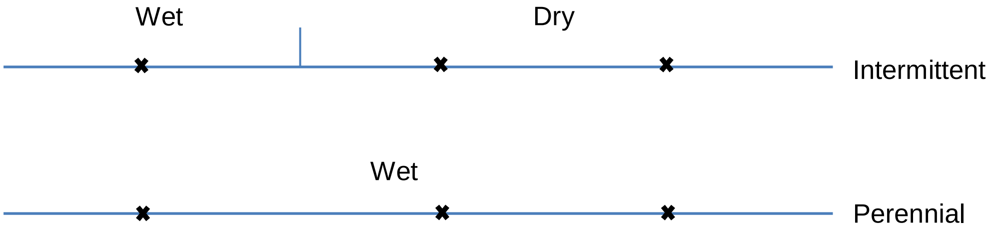

Figure 6: Scheme of the sampling design.

2.3 Water content, organic matter and sediment characteristics

The water content was determined as the difference between fresh and dry weight. The sediments were dried at 75°C until constant weight. To determine the organic matter, the sediments were muffled at 450°C for four hours to receive the ash free dry weight (COULSON ET AL., 2021; GIONCHETTA ET AL., 2019). To accurately display the grain size distribution, the sediments were sieved through sieves with the mesh sizes 2 mm, 1 mm, 0.5 mm and 0.125 mm. The proportion of the fine sediments was calculated using the sediment fraction below 0.125 mm. Water content, organic matter and proportion of the fine sediments were expressed as percent of the dry weight.

2.4 C/N-POP Analysis

To analyse the nutrients, dry sediments and only the fine fraction below 0.5 mm were used. The organic phosphorous in the sediment was determined adapting the method described by LABRY ET AL. (2013). Briefly, 0.1 – 0.2 g of sediment was weighted into microwave tubes. 2.5 ml of potassium peroxodisulfate ($K_2S_2O_8$) and 20 ml of Milli-Q water were added. After a reaction time of 10 minutes, the tubes with the samples and blanks were placed in the microwave at 1600 W (10 min heating up to 175°C, 10 min at 175°C and 15 min cooling). The extract was filled into 50 ml tubes with Milli-Q until an end volume of 32.5 ml. Before measuring the organic phosphorous via continuous-flow analysis (CFA, Alliance instruments), the samples were diluted 10-fold to an end volume of 10 ml. The results were expressed as µg/g DW after multiplying by the extract volume and division by sediment dry weight. The C:N ratio was determined adapting the methods described by CIFUENTES ET AL. (1996) and MICHENER AND LAJTHA (2008). 1 mg of sediment and standard was weighted into tin capsules. Hydrochloric acid (HCl, 10 M) was added, to remove all inorganic carbon, under the fume hood until foam started to form. The samples were mixed gently and more HCl was added until the foam started to disappear. The samples were dried at 60°C for 24 hours on a heating plate. After drying, the tin capsules were folded to balls and analysed in the elemental analyzer (EA 1110 CE Instruments).

2.5 Microbial respiration

According to the colorimetric CO_2 detection method from MicroResp described by ROWELL (1995) and CAMPELL ET AL. (2003), 0.7 g of fresh sediment was weighted into the deep-well microplates. The detection plates, containing an indicator gel consisting of agar, potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$) and cresol red, were read in the 96-well Microplate Reader (VARIOSCAN FLASH, Thermo Fisher Scientific) at 570 nm and assembled onto the deep-well plates with a seal containing air holes. The plates were incubated six hours at 25°C. After the incubation period, the plates were read again in the microplate reader at 570 nm. The principle of this method is that the microbes in the sediment release CO_2 due to respiration. This CO_2 reacts with the indicator gel and the color changes from pink to yellow due to a changing pH as CO_2 reacts with bicarbonate. This color change can be measured in the microplate reader comparing the readings after the incubation time with a starting measure. The microbial respiration was determined using three subsamples and three analytical reps. The results were expressed as µg CO_2-C/g/h and calculated using following formulas:

$$\%CO_2 = A + B \div (1 + D \times Ai)$$

Where A= -0.2265, B=-1.606, D=-6.771

$$CO_2\ production\ rate = \frac{\left(\frac{(\%CO_2 \div 100) \times vol \times (44 \div 22.4) \times (12 \div 44) \times \left(273 \div (273 + T)\right)}{fw \times (\%dw \div 100)}\right)}{incubation\ time}$$

with

Ai…normalized absorbance data ((At6-At0) x mean (At0)),

vol…headspace volume in the well in µl,

T…incubation temperature in °C,

fw…fresh weight of sediment per well in g,

%dw…sediment sample % dry weight for intermittent and perennial streams.

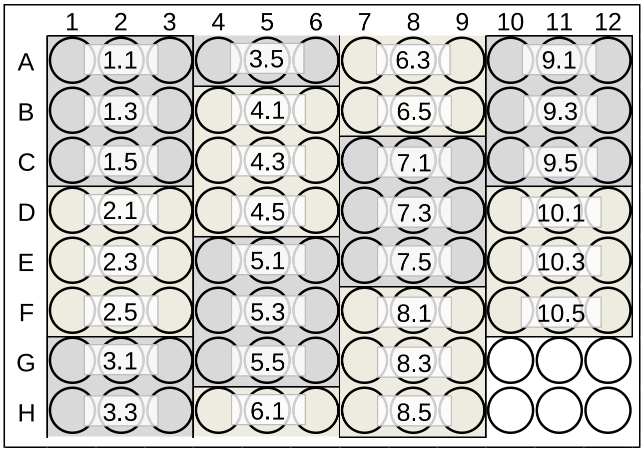

Figure 7: Detection plate layout on 96-well microplate for the determination of the respiration.

2.6 Extracellular enzymatic activity (EEA)

The extracellular enzyme activity was assessed using modifications of the fluorescence enzyme assay methods described by Bell et al. (2013), German et al. (2011), Gionchetta et al. (2019) and Stemmer et al. (2004). Four different enzyme activities, related to the degradation capacity of C (β-D-glucosidase, β-D-xylosidase), N (leucin-aminopeptidase) and P (phosphatase) compounds, were analysed in the sediments. In general, carbon, nitrogen and phosphorous rich substrates bound with a fluorescence dye are added to sediments. As the bond is intact there is no fluorescence. Then the enzymes of the microbes start to break these bonds, the dye gets released and starts to fluoresce. Two fluorescent dyes were used, methylumbelliferyl (MUF) for the carbon and phosphorous compounds and aminomethylcoumarin (AMC) for the nitrogen compounds.

In short, 1 g of fresh sediment was weighted into 15 ml tubes and filled with 5 ml of autoclaved stream water. To provide a more homogenous suspension and to release previous immobilized enzymes on humic colloids, an ultrasonication was done for 3 minutes. 200 µl of each homogenized sample was pipetted into 96-well black microplates. This was repeated four times for every sample to analyse all four enzymatic activities. For each fluorescent dye (MUF and AMC) and each location (Burgenland and Styria) a standard row with a quench control was done. Due to high amounts of particular and organic matter in the sediments the turbidity can be increased, which causes a so called "quenching" effect and interferes with the fluorescence readings. The quench control consists of homogenized sediment samples, which are added to the standard row to minimize this effect (GERMAN ET AL., 2011). Therefore, 200 µl of homogenized samples (once for the Burgenland streams and once for the Styria streams) were pipetted into the wells and mixed with 50 µl of the standard concentration. 250 µl of a blank, consisting of the pure autoclaved water used for the standard row, were pipetted into the wells as well. Right before the first reading in the 96-well Microplate Reader (VARIOSCAN FLASH, Thermo Fisher Scientific) 50 µl of the respective substrates (2 mmol) were added to the samples. The fluorescence was measured at 365/450 nm excitation/emission for MUF and 380/440 nm excitation/emission for AMC. The plates were incubated for one hour in the dark before measuring again in the microplate reader. The enzymatic activity was determined using five subsamples and two analytical reps. The results were expressed as nmol/g/h and calculated using following formula:

$$EEA = \frac{(ct1 - ct0) \times vol \times df \times 1000}{incubation\ time \times dw}$$

with

ct1…concentration of second measurement converted from the fluorescence data in µmol/l,

ct0… concentration of first measurement converted from the fluorescence data in µmol/l, vol…volume of autoclaved stream water in l,

df…dilution factor by adding the substrate (df=1.25),

dw…dry weight of sediment in g.

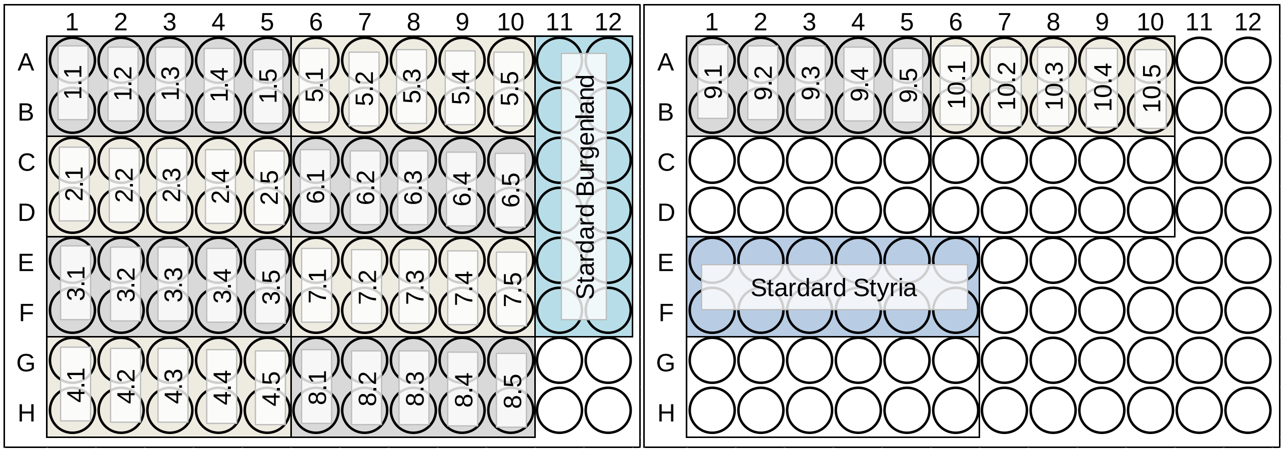

Figure 8: Plate layout on 96-well black microplates for the determination of EEA. This layout is repeated four times (once for each enzyme) with the MUF standard on the Phos plate and the AMC standard on the Leu plate.

The described method was used to determine the extracellular enzymatic activity of the samples in May and July 2019. The enzymatic activity of the last sampling process was determined using an adaption of the method, which provides more precise results of the enzymatic activity especially for dry sediments (SEE 4.1 IMMEDIATE EFFECTS). 1 g of fresh sediment was weighted into 15 ml tubes and filled with 4 ml of autoclaved stream water. This process is repeated four times to determine the four enzymatic activities. A standard row was prepared for each fluorescent dye (MUF and AMC). In each sample, 120 µl of substrate (10 mmol) was added and the samples were incubated for one hour in the dark. After the incubation time, 2 ml of glycine buffer (0.2 M, pH 10.4) was added to all samples, standards and blanks to stop the reaction. Samples were centrifuges for 2 min at 2000 rpm to reduce the turbidity. 250 µl of the standards, blanks and supernatant of each sample were pipetted into 96-well black microplates and read in the microplate reader at 365/450 nm excitation/emission for MUF and 380/440 nm excitation/emission for AMC. The enzymatic activity was determined using five subsamples and two analytical reps. The results were expressed as nmol/g/h and calculated using following formula:

$$EEA = \frac{c \times vol \times df \times 1000}{incubation\ time \times dw}$$

with

c…concentration converted from the fluorescence data in µmol/l,

vol…volume of autoclaved stream water in l,

df…dilution factor by adding the substrate (df=1.53),

dw…dry weight of sediment in g.

2.7 Statistical analysis

Differences among stream types and regions were analysed using the non-parametric Mann-Whitney U test at a significance level of 0.05 for the sediment parameters and the non-parametric Kruskal-Wallis test at a significance level of 0.05 for the water chemistry parameters. Correlations between the water content, organic matter content, fine sediment content, organic phosphorous content, C:N ratio, microbial respiration and extracellular enzymatic activity were determined by Kendall's tau-b correlation and tested at a two tailed significant level at the significance level of 0.01 and 0.05. Correlations with the enzymatic activities were only tested for the non-flowing period in September, excluding the sampling dates May and July, due to the use of different methods to determine the extracellular enzymatic activity. The strength of the relationship was defined as followed:

τ= below ± 0.2…no correlation

τ= ±0.2 to ± 0.5…low correlation

τ= ±0.5 to ± 0.7…moderate correlation

τ= ±0.7 to ± 0.9…strong correlation

τ= above ± 0.9… very strong correlation

All statistical analyses were performed using SPSS Statistics 26.0 for Windows.

3 Results

3.1 Water chemistry

During the flow period in May, the nitrogen concentrations, ammonium, nitrite and nitrate, were similar across the stream types (Kruskal-Wallis test, p>0.05, n=20, Table 2), but the soluble reactive phosphorus and DOC differed significantly. The intermittent streams showed a significantly lower phosphate concentration (Kruskal-Wallis test, p=0.021, n=20), while the DOC was observed to be significantly higher (Kruskal-Wallis test, p=0.009, n=10). Comparing the regions, the streams in Styria had a significantly higher nitrate concentration (Kruskal-Wallis test, p=0.002, n=20) and a significantly lower phosphate concentration (Kruskal-Wallis test, p=0.031, n=20). The ammonium and nitrite concentration as well as the DOC were similar across the regions (Kruskal-Wallis test, p>0.05, n=20; p>0.05, n=10).

Table 2: Mean nutrient concentrations of the water during flow period in intermittent and perennial streams (n=2). DOC, dissolved organic carbon.

Sites	**Stream type**	**Region**	**NH_4^+** [µg/l]	**NO_2^-** [µg/l]	**NO_3^-** [µg/l]	**PO_4^{3-}** [µg/l]	**DOC** [mg/l]
RB	Intermittent	Burgenland	14.7	10.6	1016.5	14.2	6.29
JB	Intermittent	Burgenland	177.5	36.4	1879.3	22.0	8.06
DB	Perennial	Burgenland	53.8	17.9	1012.5	21.0	4.50
SB	Perennial	Burgenland	28.1	11.0	1287.4	20.6	4.02
WB	Perennial	Styria	9.0	5.8	1510.1	10.7	1.55
GaB	Intermittent	Styria	7.8	5.3	2150.8	8.9	6.30
LB	Perennial	Styria	88.5	99.0	1740.2	23.1	5.04
PB	Intermittent	Styria	7.4	9.2	1518.9	8.0	8.68
GB	Intermittent	Styria	28.3	24.1	3992.0	8.7	6.11
FB	Perennial	Styria	67.0	26.0	4997.0	16.4	2.63

3.2 Water content, organic matter and sediment characteristics

During the flow period in May and in the early non-flowing period in July, no significant differences of the water content were observed between the hyporheic sediments of intermittent and perennial streams (Mann-Whitney U test, p>0.05, n=50; Fig. 9). During the non-flowing period in September, the intermittent streams showed a significant lower water content (Mann-Whitney U test, p=0.000, n=45), indicating a desiccation event of the hyporheic sediments. The median of the intermittent steams was around 12%, while the perennial streams had a median water content of around 20%. The lowest water content was measured in Fruttnerbach (FB-i) in September with an average of 8.24%.

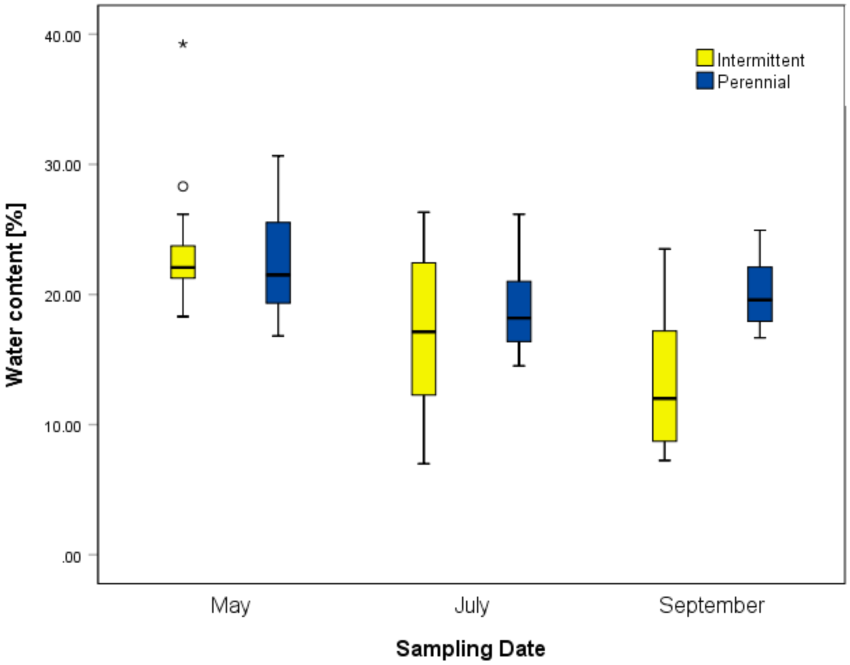

Figure 9: Water content in % during flow (May) and non-flow periods (July and September) comparing intermittent and perennial streams (n= 25 per stream type, exception: perennial streams in September n=20). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

Comparing the streams of the region Burgenland and Styria during the non-flowing periods, significant differences were detected (Fig. 10). In July, intermittent streams had significant lower water content than perennial streams in Burgenland (Mann-Whitney U test, p=0.002, n=20), while there was no difference among the stream types in Styria (Mann-Whitney U test, p>0.05, n=30). In September, the water content of intermittent streams was significantly lower in Burgenland (Mann-Whitney U test, p=0.009, n=20) and Styria (Mann-Whitney U test, p=0.008, n=25) than in perennial streams.

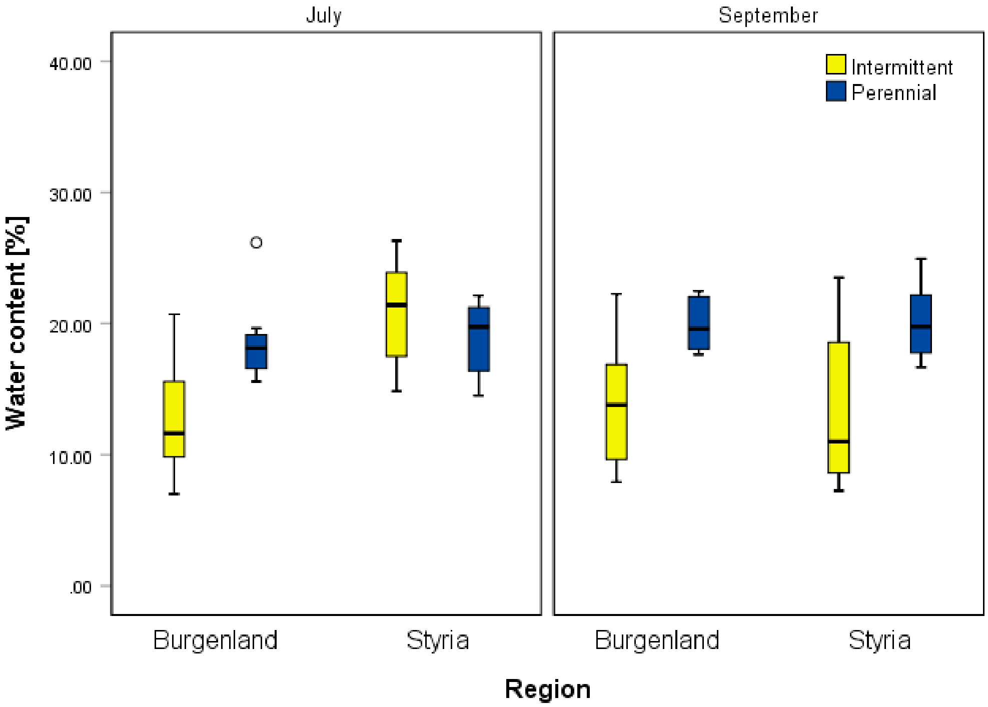

Figure 10: Water content in % during the non-flow periods (July and September) comparing intermittent and perennial streams across the regions Burgenland and Styria (n= 10 per stream type in Burgenland and n=15 per stream type in Styria, exception: perennial streams in Styria in September with n=10). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

Regarding the organic matter content, no significant differences were observed during the flow period (Mann-Whitney U test, p>0.05, n=50, Fig. 11), but it differed significantly during the non-flowing period in July (Mann-Whitney U test, p=0.005, n=50) and September (Mann-Whitney U test, p= 0.00, n=45). In July, the intermittent streams had a median organic matter content of 1.3% and the perennial streams of 0.66%. In September, the median values were 1.87% for the intermittent streams and 0.57% for the perennial streams. The highest organic matter content was measured in Pfaffenbach (PB-i) with 3.53% and an average of 3.1% in July. In contrast the lowest average organic matter content in July was observed in Lieberbach (LB-p) with a value of 0.48%. Comparing the steams per region, significant differences were detected between the regions in intermittent streams during all sampling dates (Mann-Whitney U test, p=0.31, n=25; p=0.10, n=25; p=0.00, n=25, Fig. 12). The intermittent streams in Styria tended to have higher contents of organic matter in the hyporheic sediments, with medians around 2.2%, than the streams in Burgenland, with medians around 1.0%.

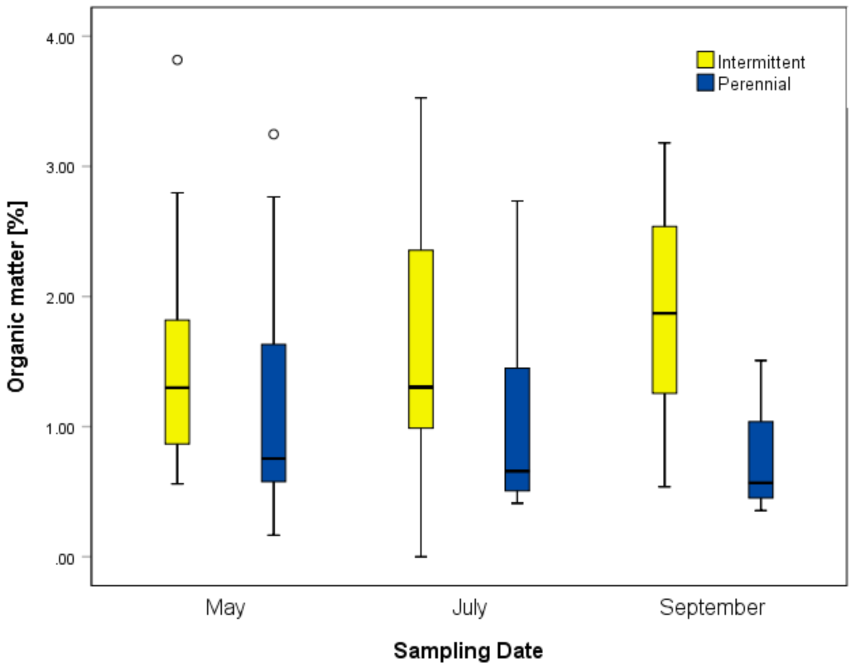

Figure 11: Organic matter content in % during flow (May) and non-flowing periods (July and September) comparing intermittent and perennial streams (n=25 per stream type, exception: perennial streams in September with n=20). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

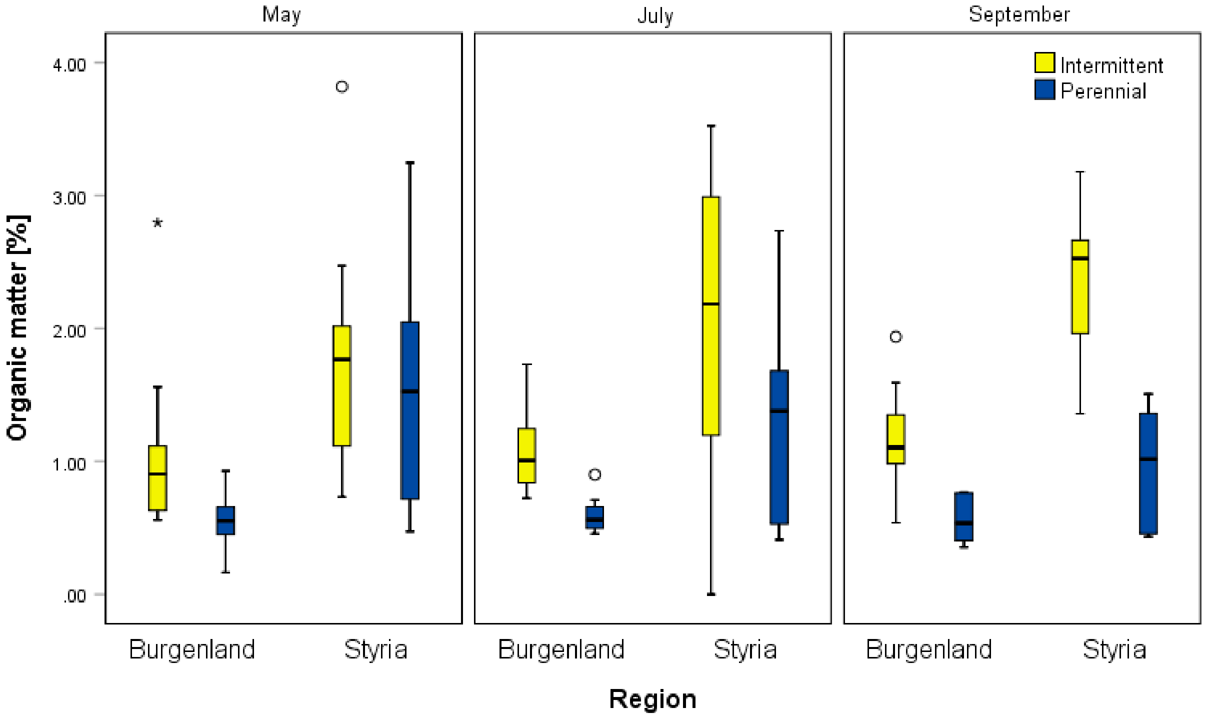

Figure 12: Organic matter content in % during flow (May) and non-flowing periods (July and September) comparing intermittent and perennial streams across the regions Burgenland and Styria (n=10 per stream type in Burgenland, n=15 per steam type in Styria, exception: perennial streams in Styria in September with n=12). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

The proportion of the fraction of fine sediments below 0.125 mm did not differ significantly among the regions Burgenland and Styria in intermittent streams (Mann-Whitney U test, p>0.05, n=15, Fig. 13). The intermittent streams in Styria tended to have a slightly higher content of fine sediments, with a median of 2.95%, than in Burgenland, with a median of 2.4%. The highest content of fine sediments was measured in Pfaffenbach (PB-i) with a value of 4.4% and an extreme value with a content of 17.89%. In contrast, the highest value observed in Burgenland was in Joachimsbach (JB-i) with 3.06%. There was no significant difference between the stream types in Burgenland (Mann-Whitney U test, p>0.05, n=12) and Styria (Mann-Whitney U test, p>0.05, n=18).

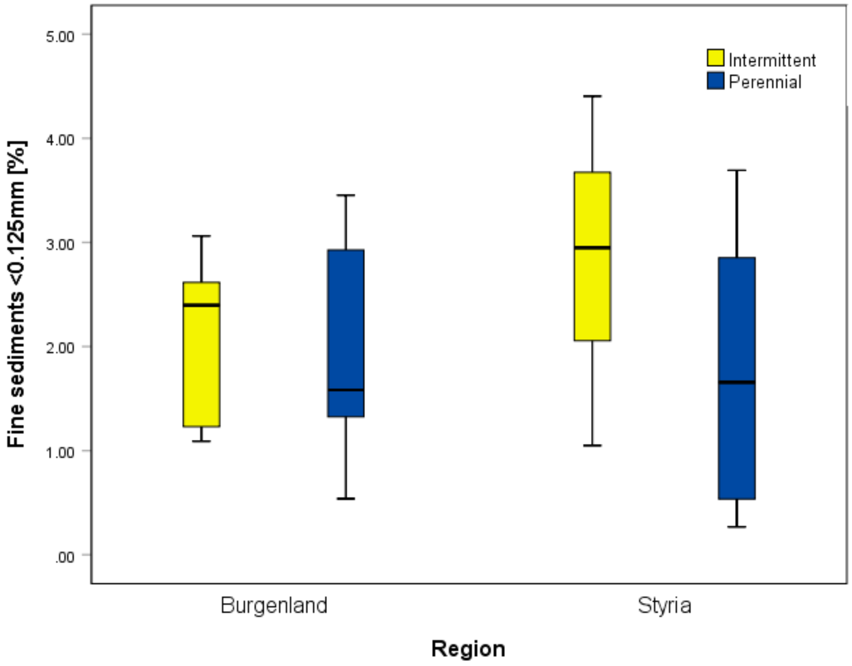

Figure 13: Content of fine sediments below 0.125 mm in % comparing intermittent and perennial streams in Burgenland and Styria (n=6 per stream type in Burgenland and n=9 per stream type in Styria). Shown are the 10, 25, 50, 75, and 90% percentiles.

3.3 C/N-POP Analysis

The C:N ratios were similar across all sites (Mann-Whitney U test, p>0.05, n= 30, Fig. 14). The highest average C:N ratios were observed in Reithschuhlbach (RB-i) with 7.38, followed by Glaunigbach (GB-i) with 6.98. The lowest average C:N ratios were detected in Wellingbach (WB-p) with 2.74 and Drosenbach (DB-p) with 2.85. Both of these streams contained samples which showed values below 1.

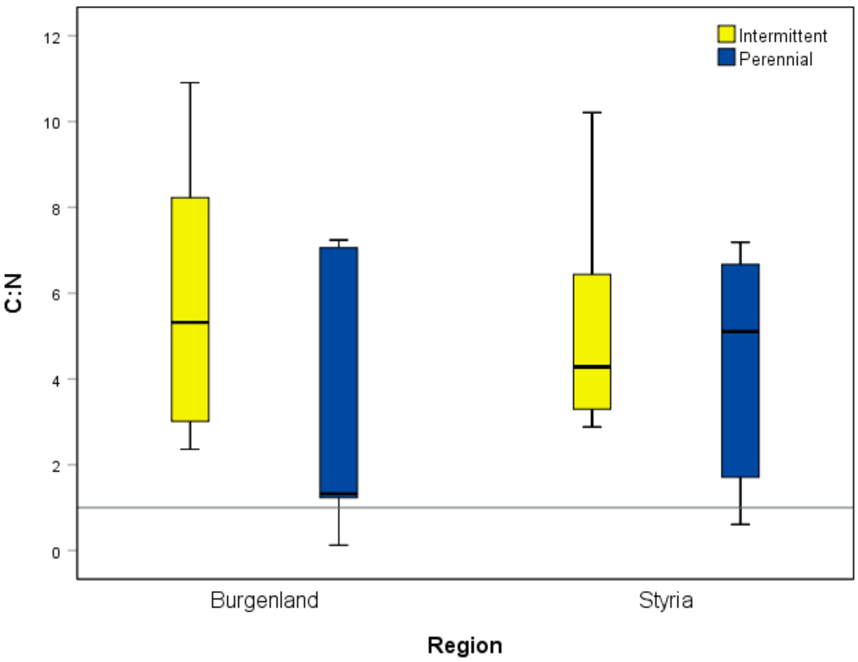

Figure 14: C:N ratios of the organic fraction in the sediments comparing intermittent and perennial streams in Burgenland and Styria (n=6 per stream type in Burgenland and n=9 per stream type in Styria). Shown are the 10, 25, 50, 75, and 90% percentiles. The grey line is indicating a C:N ratio of 1.

Regarding the distribution of organic phosphorous in the sediments, a significant difference was observed among the stream types (Mann-Whitney U test, $p=0.002$, $n=30$). The intermittent streams showed a higher median value of 458.96 µg/g compared to the perennial streams with a median of 258.15 µg/g. Comparing the streams per region, no significant difference was detected among the streams in Burgenland (Mann-Whitney U test, $p>0.05$, $n=12$, Fig. 15), but the intermittent streams in Styria had a significant higher organic phosphorous content (Mann-Whitney U test, $p=0.005$, $n=18$). The intermittent streams showed no difference across the region (Mann-Whitney U test, $p>0.05$, $n=15$). The highest average organic phosphorous content was observed in Pfaffenbach (PB-i) with a value of 780.32 µg/g. In contrast, the lowest average was found with 231.21 µg/g in Drosenbach (DB-p). The overall highest content of organic phosphorous was detected in Glaunigbach (GB-i) with 997.02 µg/g, followed already by Pfaffenbach (PB-i) with 985.30 µg/g.

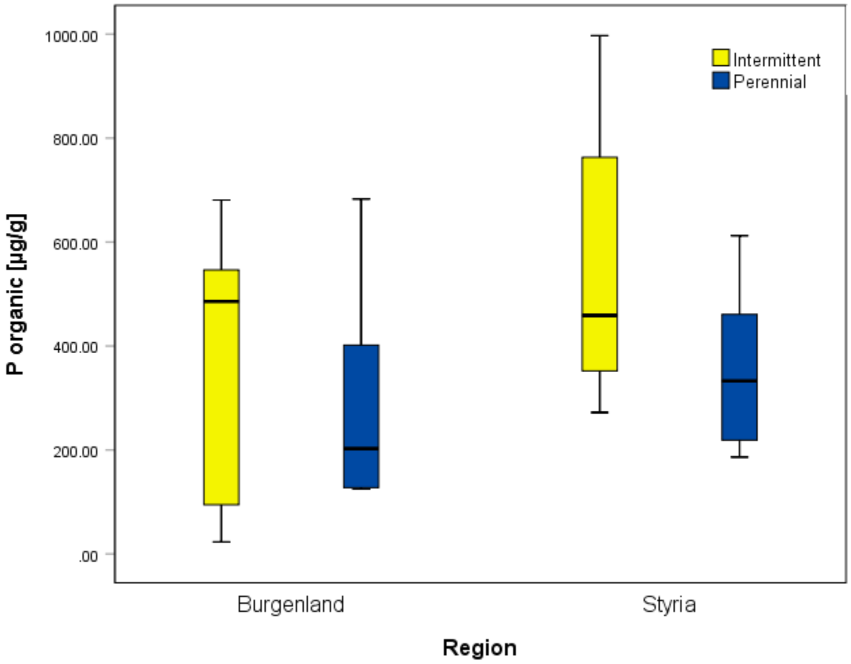

Figure 15: Amount of organic phosphorous in the sediments in µg/g comparing intermittent and perennial streams in Burgenland and Styria (n=6 per stream type in Burgenland and n=9 per stream type in Styria). Shown are the 10, 25, 50, 75, and 90% percentiles.

3.4 Microbial respiration

Across all sites, intermittent and perennial streams showed similar respiration rates in May and July (Mann-Whitney U test, p>0.05, n=30, Fig. 16), but differed significantly in September (Mann-Whitney U test, p=0.002, n=27). There, the median CO_2 production of intermittent streams was 0.58 µgC/g/h and of perennial streams 0.43 µgC/g/h. The highest microbial respiration rate was measured in Fruttnerbach (FB-i) in Styria during the non-flow period in September with an average of 0.86 µgC/g/h.

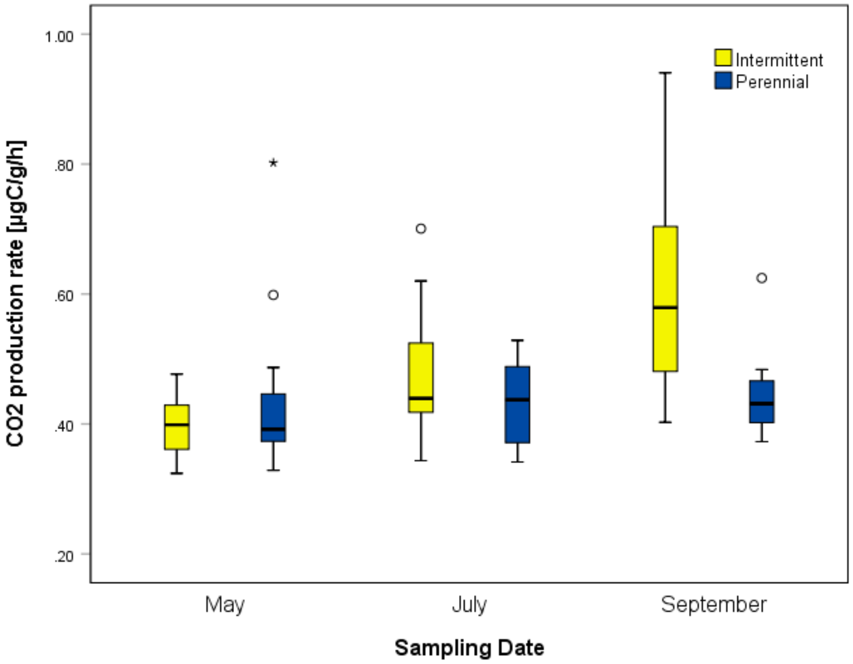

Figure 16: CO_2 production rate in µg C/g/h during flow (May) and non-flow periods (July and September) comparing intermittent and perennial streams (n= 15 per stream type, exception: perennial streams in September n=12). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

In July, in the early non-flowing period, a significant higher CO_2 production was observed in the intermittent streams compared to the perennial streams in Burgenland (Mann-Whitney U test, p=0.026, n=12, Fig. 17). There, the median respiration rate was 0.56 µgC/g/h in intermittent streams and 0.37 µgC/g/h in perennial streams. The streams in Styria showed similar respiration rates across all stream types in July (Mann-Whitney U test, p>0.05, n=18), whereas a significant higher respiration rate was detected in the intermittent streams in September (Mann-Whitney U test, p=0.05, n=15), with a median of 0.58 µgC/g/h compared to 0.43 µgC/g/h of perennial streams.

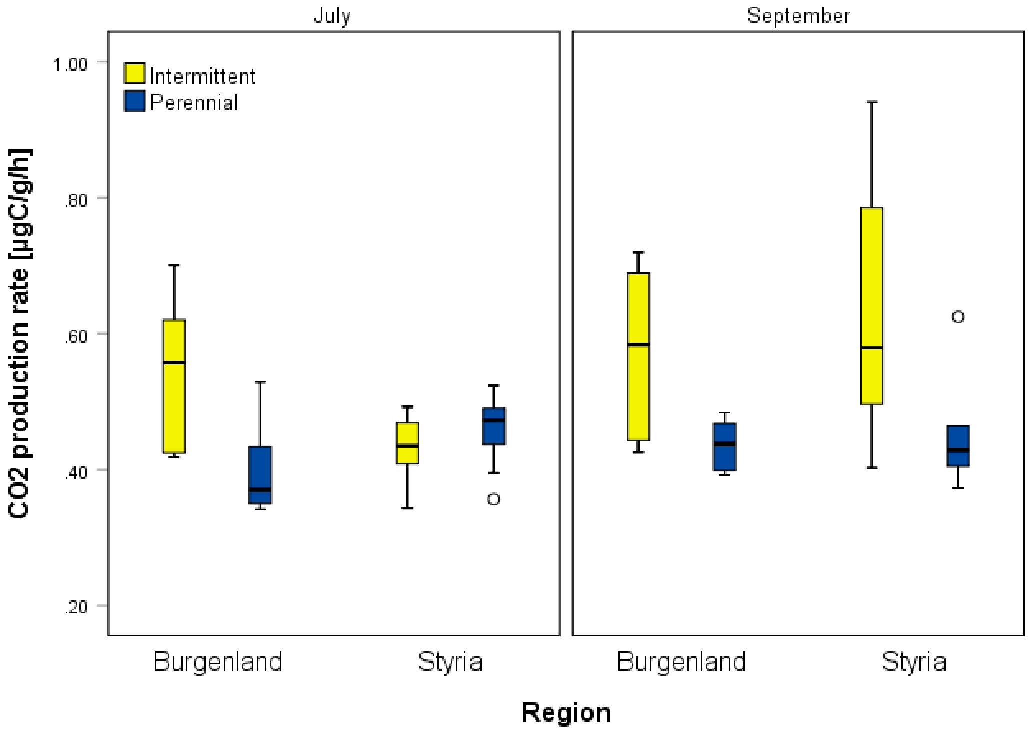

Figure 17: CO_2 production rate in µg C/g/h during the non-flow periods (July and September) comparing intermittent and perennial streams across the regions Burgenland and Styria (n=6 per stream type in Burgenland and n=9 per stream type in Styria, exception: perennial streams in Styria in September with n=6). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

3.5 Extracellular enzymatic activity (EEA)

All potential extracellular enzymatic activities of intermittent and perennial streams were observed to be similar in May and July (Mann-Whitney U test, p>0.05, n=50, Fig. 18). The highest activities were observed for leucin-aminopeptidase and phosphatase. β-D-xylosidase and β-D-glucosidase showed much lower activities.

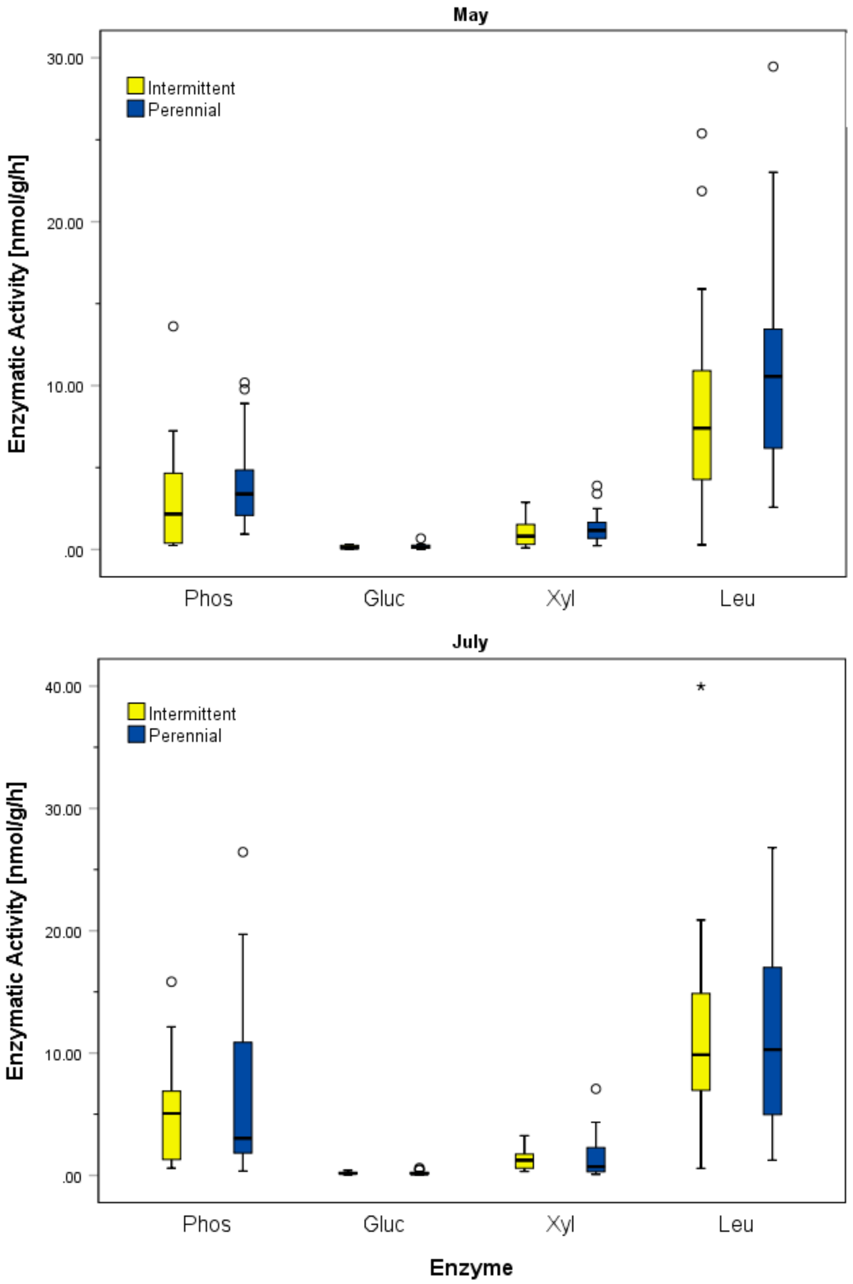

Figure 18: Potential extracellular enzymatic activities of phosphatase (Phos), β-D-glucosidase (Gluc), β-D-xylosidase (Xyl) and leucin-aminopeptidase (Leu) in nmol/g/h during the flow (May) and early non-flow period (July) comparing intermittent and perennial streams (n=25 per stream type). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

In September, a significant higher phosphatase activity was detected in intermittent streams (Mann-Whitney U test, p=0.038, n=45, Fig. 19). There, the median phosphatase activity was 214.49 nmol/g/h for intermittent streams and 138.35 nmol/g/h for perennial streams. The other enzymatic activities, leucin-aminopeptidase, β-D-glucosidase and β-

D-xylosidase, did not differ significantly (Mann-Whitney U test, p>0.05, n=45). As described in 2.6 EXTRACELLULAR ENZYMATIC ACTIVITY, differences in methods have been used. In September, a different trend in the activity of the enzymes was observed. The highest potential activity was measured for phosphatase, followed by β-D-glucosidase, leucin-aminopeptidase and β-D-xylosidase.

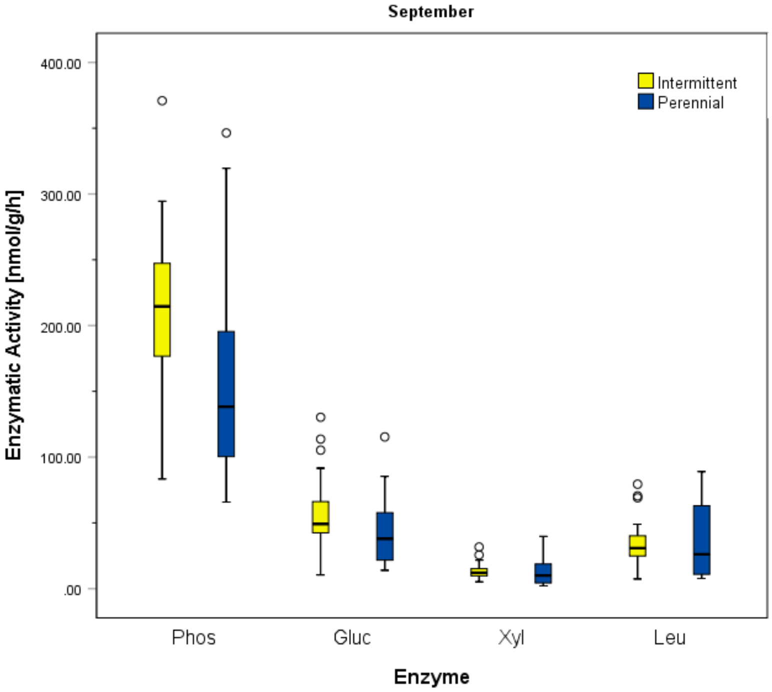

Figure 19: Potential extracellular enzymatic activities of phosphatase (Phos), β-D-glucosidase (Gluc), β-D-xylosidase (Xyl) and leucin-aminopeptidase (Leu) in nmol/g/h during the non-flow period in September comparing intermittent and perennial streams (n=25 for intermittent streams and n=20 for perennial streams). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

The regions Burgenland and Styria reacted differently in the non-flowing periods. If a different significance across the streams types was detected, the intermittent streams in the Burgenland region had significant higher enzymatic activities, while the intermittent streams in Styria were observed with lower enzymatic activities compared to the perennial streams in the respective region (Fig. 20). In Burgenland, significant differences were detected for the enzymes phosphatase, β-D-xylosidase and leucin-aminopeptidase in July and September (Mann-Whitney U test, p=0.000, n=20). The β-D-glucosidase showed similar activities across all streams in July (Mann-Whitney U test, p>0.05, n=20), but differed significantly in September with higher activities in the intermittent streams (Mann-Whitney U test, p=0.000, n=20). In Styria, the enzymatic activities of phosphatase and leucin-aminopeptidase differed significantly among stream types in July (Mann-Whitney U test, p=0.009, n=30; p=0.007, n=30). In September, β-D-xylosidase and leucin-aminopeptidase had significant lower activities in intermittent streams (Mann-Whitney U test, p=0.007, n=25; p=0.008, n=25). β-D-glucosidase showed similar activities across the streams in July and September (Mann-Whitney U test, p>0.05, n=25).

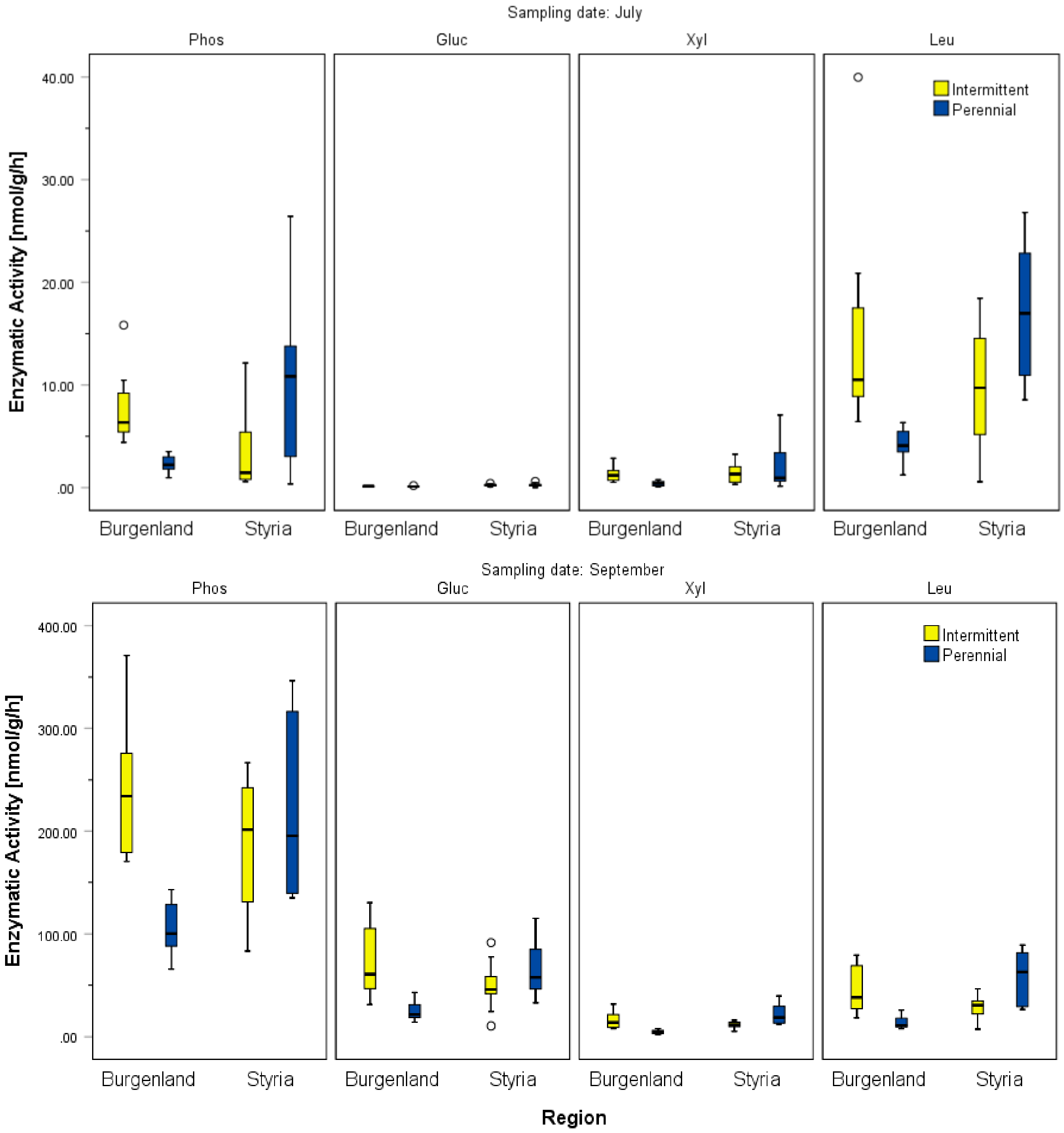

Figure 20: Potential extracellular enzymatic activities of phosphatase (Phos), β-D-glucosidase (Gluc), β-D-xylosidase (Xyl) and leucin-aminopeptidase (Leu) in nmol/g/h during the non-flow periods (July and September) comparing intermittent and perennial streams across the regions Burgenland and Styria (n= 10 per stream type in Burgenland and n=15 per stream type in Styria, exception: perennial streams in Styria in September with n=10). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

3.6 Correlations

For intermittent streams, negative correlations were detected between water content and respiration (n=45) and between water content and the enzymatic activity of phosphatase and leucin-aminopeptidase (n=25, Table 3). The organic matter was found to be negative correlated with the enzymes phosphatase, β-D-glucosidase and leucin-aminopeptidase (n=25). The activity of β-D-glucosidase and β-D-xylosidase were moderately negatively correlated with the amount of fine sediments present (n=15). The water content of the sediments was positively influenced by the amount of organic matter (n=75) and fine sediments (n=45) present. A positive relationship between the amount of fine sediments and the organic matter was detected (n=45). Both the organic matter as well as the fine sediments showed a positive correlation with the organic phosphorous in the sediments (n=45, n=15). A moderate to strong positive correlation was found between all the enzymes, except between β-D-xylosidase and leucin-aminopeptidase activity (n=25).

Table 3: Correlation table for the parameters of the intermittent streams showing the correlation coefficient (τ) and the significance level (p). The colours indicate the significance level with dark grey for 0.01 and light grey for 0.05. WC, water content; OM, organic matter; Fines, fine sediments <0.125 mm; POP, particulate organic phosphorous; Resp, microbial respiration; Phos, phosphatase; Gluc, β-D-glucosidase; Xyl, β-D-xylosidase; Leu, leucin-aminopeptidase.

	WC		**OM**		**Fines**		**POP**		**C:N**	
	τ	**p**	**τ**	**p**	**τ**	**p**	**τ**	**p**	**τ**	**p**
WC	/	/	0.204	0.010	0.226	0.032	0.147	0.161	-0.129	0.220
OM	0.204	0.010	/	/	0.382	0.000	0.288	0.006	-0.100	0.342
Fines	0.226	0.032	0.382	0.000	/	/	0.298	0.005	-0.131	0.219
POP	0.147	0.161	0.288	0.006	0.298	0.005	/	/	0.298	0.005
C:N	-0.129	0.220	-0.100	0.342	-0.131	0.219	0.298	0.005	/	/
Resp	-0.503	0.000	0.079	0.445	-0.180	0.086	-0.131	0.213	-0.020	0.852
Phos	-0.407	0.004	-0.373	0.009	-0.429	0.026	-0.276	0.151	0.181	0.347
Gluc	-0.133	0.350	-0.380	0.008	-0.581	0.003	-0.276	0.151	0.219	0.255
Xyl	-0.082	0.592	-0.203	0.185	-0.590	0.005	-0.205	0.329	0.077	0.714
Leu	-0.420	0.003	-0.387	0.007	-0.295	0.125	-0.333	0.083	0.314	0.102

	Resp		Phos		Gluc		Xyl		Leu	
	τ	p	τ	p	τ	p	τ	p	τ	p
WC	-0.503	0.000	-0.407	0.004	-0.133	0.350	-0.082	0.592	-0.420	0.003
OM	0.079	0.445	-0.373	0.009	-0.380	0.008	-0.203	0.185	-0.387	0.007
Fines	-0.180	0.086	-0.429	0.026	-0.581	0.003	-0.590	0.005	-0.295	0.125
POP	-0.131	0.213	-0.276	0.151	-0.276	0.151	-0.205	0.329	-0.333	0.083
C:N	-0.020	0.852	0.181	0.347	0.219	0.255	0.077	0.714	0.314	0.102
Resp	/	/	0.314	0.102	0.162	0.400	0.128	0.542	0.257	0.181
Phos	0.314	0.102	/	/	0.553	0.000	0.403	0.009	0.720	0.000
Gluc	0.162	0.400	0.553	0.000	/	/	0.610	0.000	0.380	0.008
Xyl	0.128	0.542	0.403	0.009	0.610	0.000	/	/	0.299	0.052
Leu	0.257	0.181	0.720	0.000	0.380	0.008	0.299	0.052	/	/

While intermittent streams tended to show a higher respiration activity with decreasing water content in the sediments (r^2= 0.570, n=45, Fig. 21), perennial streams showed higher activities with rising water content (r^2= 0.243, n=41). The CO_2 production rate of intermittent streams clustered with the perennial streams at water contents of around 20%, until the hyporheic sediments started to dry to water contents around 10%.

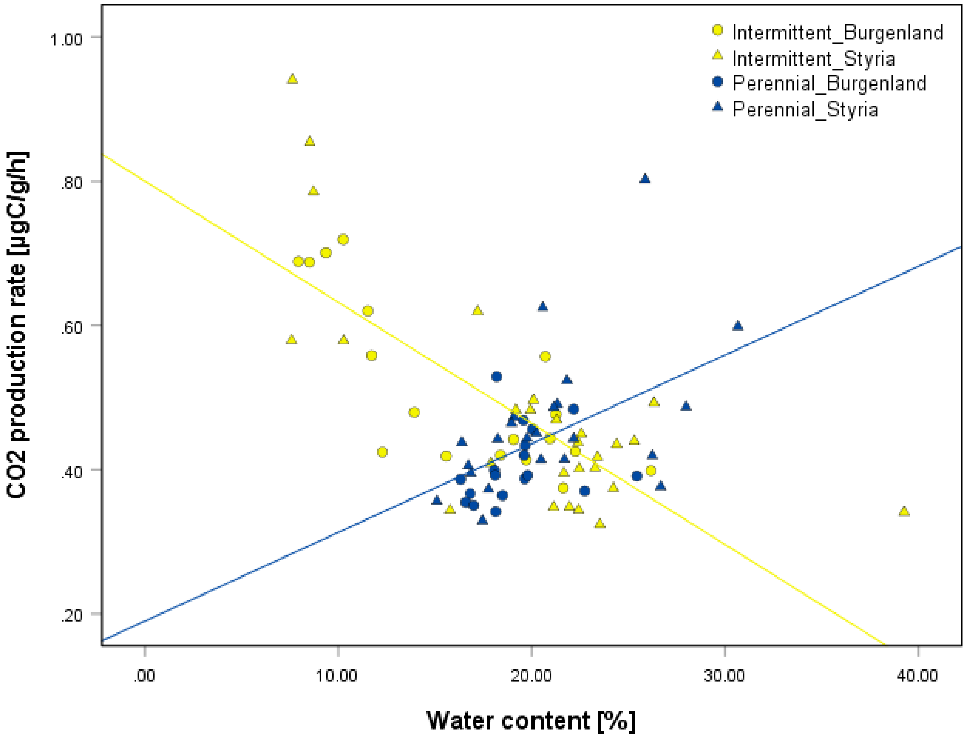

Figure 21: Relationship of the water content in % and the CO2 production rate in µg C/g/h comparing intermittent and perennial streams in Burgenland and Styria (n=86). Shown are the flowing and non-flowing periods and the fit lines for intermittent (yellow) and perennial (blue) streams.

A similar trend was observed for the phosphatase activity during the non-flow period in September (Fig. 22). The activity tended to increase with decreasing water content in intermittent streams (r^2=0.346, n=25) and increase with rising water content in perennial streams (r^2=0.281, n=20).

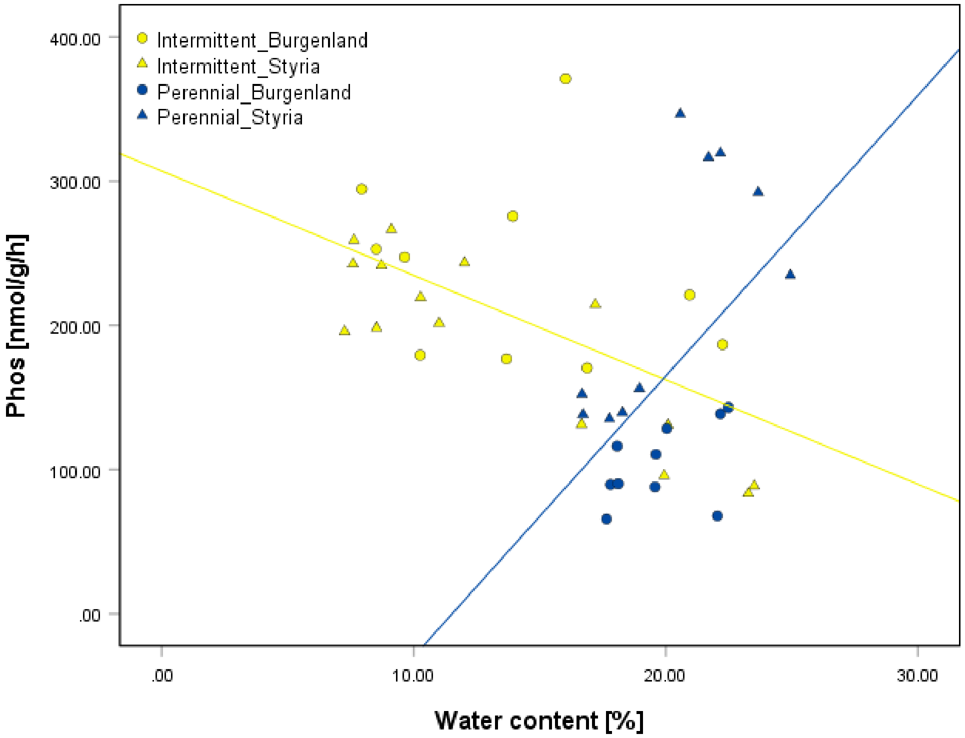

Figure 22: Relationship of the water content in % and the phosphatase activity in nmol/g/h comparing intermittent and perennial streams in Burgenland and Styria (n=45). Shown are the non-flowing period in September and the fit lines for intermittent (yellow) and perennial (blue) streams.

3.7 Benthic sediments in September

During the non-flow period in September, benthic sediments of intermittent streams showed a significant lower water content compared to perennial streams (Mann-Whitney U test, p=0.000, n=45, Fig. 23). The median of intermittent streams was around 11%, while perennial streams had a median water content of around 19%. The lowest water content was measured in Fruttnerbach (FB-i) with 2.70% and an average of 6.26%. Regarding the organic matter content, intermittent streams were characterized by a significant higher content than perennial streams (Mann-Whitney U test, p=0.000, n=45, Fig. 23). Intermittent streams showed a median of 2.03% compared to 0.69% in perennial streams. The highest organic matter content was observed in Fruttnerbach

(FB-i) with 4.68% and an average of 3.25%. The lowest average content was detected in Schwabenbach (SB-p) with 0.54%.

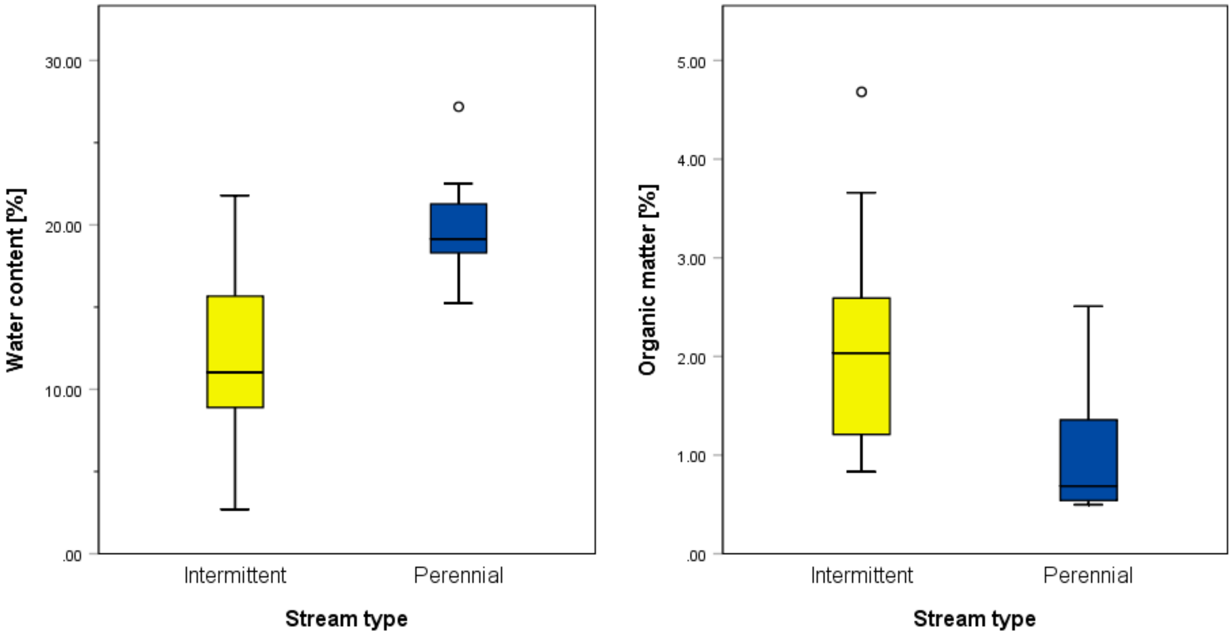

Figure 23: Water content and organic matter content in % during non-flow period in September comparing the benthic sediments of intermittent and perennial streams (n=25 for intermittent streams and n=20 for perennial streams). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

Across all sites, intermittent and perennial streams showed similar respiration rates during the non-flow period in September (Mann-Whitney U test, $p>0.05$, $n=27$, Fig. 24). The median CO_2 production of intermittent streams was 0.43 µgC/g/h and of perennial streams 0.70 µgC/g/h. The highest average activity was measured in Schwabenbach (SB-p) with 0.82 µgC/g/h, followed by Fruttnerbach (FB-i) with 0.76 µgC/g/h.

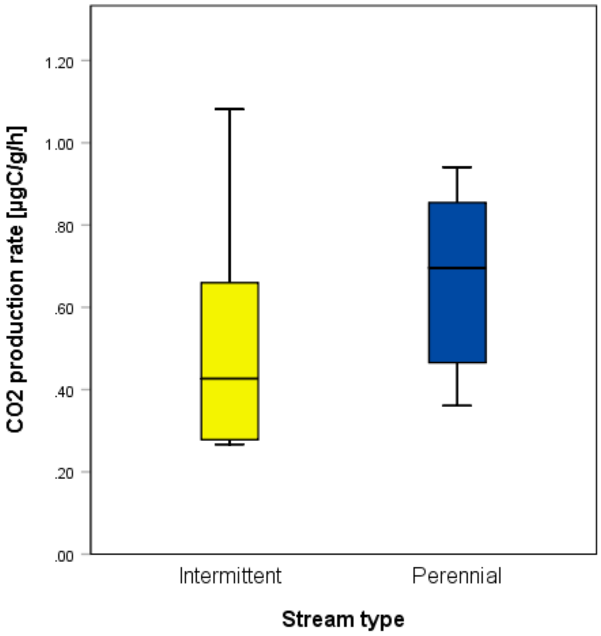

Figure 24: CO_2 production rate in µg C/g/h during the non-flow period in September comparing benthic sediments of intermittent and perennial streams (n=15 for intermittent streams and n=12 for perennial streams). Shown are the 10, 25, 50, 75, and 90% percentiles.

In September, a significant lower phosphatase activity was detected in the benthic sediments of intermittent streams compared to perennial streams (Mann-Whitney U test, p=0.00, n=45, Fig. 25). There, a median phosphatase activity of 552.21 nmol/g/h in intermittent and 636.16 nmol/g/h in perennial streams was observed. The other enzymatic activities, leucin-aminopeptidase, β-D-glucosidase and β-D-xylosidase, did not differ significantly (Mann-Whitney U test, p>0.05, n=45). The highest potential extracellular enzymatic activity was measured for phosphatase, followed by β-D-glucosidase and leucin-aminopeptidase. β-D-xylosidase showed the lowest activities across all sites.

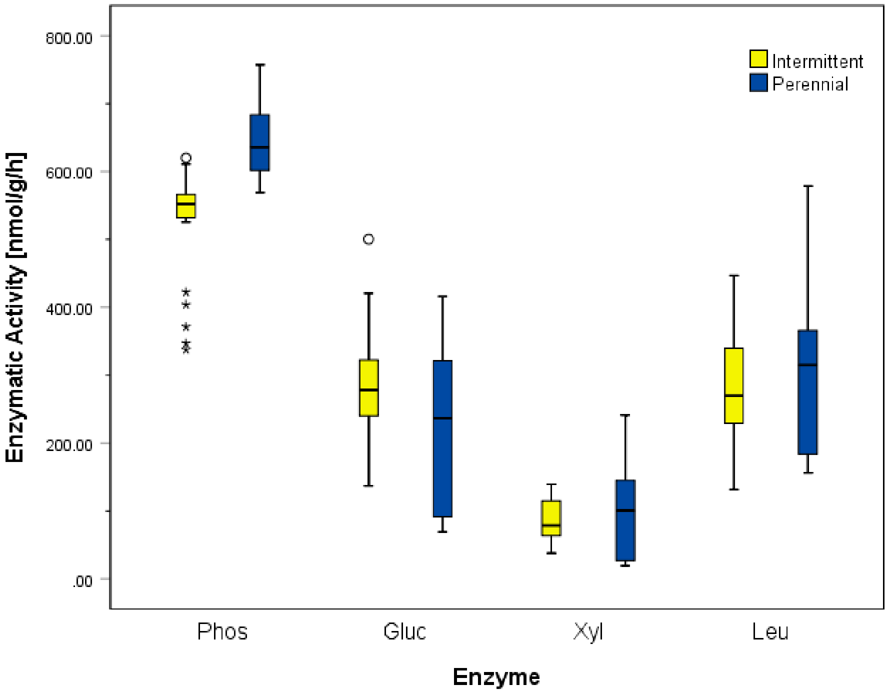

Figure 25: Potential extracellular enzymatic activities of phosphatase (Phos), β-D-glucosidase (Gluc), β-D-xylosidase (Xyl) and leucin-aminopeptidase (Leu) in nmol/g/h during the non-flow period in September comparing benthic sediments of intermittent and perennial streams (n=25 for intermittent streams and n=20 for perennial streams). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

For benthic sediments of intermittent streams, negative correlations were detected between water content and enzymatic activities, except leucin-aminopeptidase (n=25, Table 4). The leucin-aminopeptidase activity was observed to be negative correlated to the amount of organic matter present (n=25). The microbial respiration was positively influenced by the organic matter amount (n=15). A low to strong positive relationship was found between all the extracellular enzymatic activities (n=25).

Table 4: Correlation table for the parameters of benthic intermittent stream sediments showing the correlation coefficient (τ) and the significance level (p). The colours indicate the significance level with dark grey for 0.01 and light grey for 0.05. WC, water content; OM, organic matter; Resp, microbial respiration; Phos, phosphatase; Gluc, β-D-glucosidase; Xyl, β-D-xylosidase; Leu, leucin-aminopeptidase.

	WC		**OM**		**Resp**	
	τ	**p**	**τ**	**p**	**τ**	**p**
WC	/	/	0.127	0.375	-0.162	0.400
OM	0.127	0.375	/	/	0.486	0.012
Resp	-0.162	0.400	0.486	0.012	/	/
Phos	-0.407	0.004	-0.173	0.225	-0.086	0.656
Gluc	-0.413	0.004	-0.020	0.889	0.010	0.961
Xyl	-0.427	0.003	-0.047	0.744	-0.086	0.656
Leu	-0.267	0.062	-0.327	0.022	-0.295	0.125

	Phos		Gluc		Xyl		Leu	
	τ	p	τ	p	τ	p	τ	p
WC	-0.407	0.004	-0.413	0.004	-0.427	0.003	-0.267	0.062
OM	-0.173	0.225	-0.020	0.889	-0.047	0.744	-0.327	0.022
Resp	-0.086	0.656	0.010	0.961	-0.086	0.656	-0.295	0.125
Phos	/	/	0.593	0.000	0.553	0.000	0.607	0.000
Gluc	0.593	0.000	/	/	0.840	0.000	0.440	0.000
Xyl	0.553	0.000	0.840	0.000	/	/	0.440	0.000
Leu	0.607	0.000	0.440	0.000	0.440	0.000	/	/

3.8 Comparison of benthic and hyporheic sediments

During the non-flowing period in September, a similar water content and amount of organic matter were observed across all sediment depths (Mann-Whitney U test, p>0.050, n=50). Differences in the water content were only detected looking at the minimal water content. While hyporheic sediments never dropped to moisture contents below 8%, the lowest observed water content of benthic sediments was 2.7%. Across both sediment depths, the water content was significantly decreased and the organic matter was significantly increased in intermittent streams compared to perennial streams.

In the hyporheic zone, all potential extracellular enzymatic activities were significantly lower than in benthic sediments (Mann-Whitney U test, p=0.000, n=50). Differences between the sediment depths varied from around 3 times, observed for phosphatase concentration in intermittent streams with a median of 214.49 nmol/g/h in the hyporheic zone and 592.21 nmol/g/h in surface sediments, up to 12 times, detected for leucin-aminopeptidase in perennial streams with a median of 26.18 nmol/g/h in the hyporheic zone and 314.95 nmol/g/h in surface sediments, respectively. Microbial respiration showed similar activities in intermittent streams across sediment depth (Mann-Whitney U test, p>0.050, n=30), but differed significantly in perennial streams (Mann-Whitney U test, p=0.010, n=24). There, the median CO_2 production of hyporheic sediments was 0.43 µgC/g/h and of benthic sediments 0.70 µgC/g/h. While most of the microbial activity could be maintained during the desiccation process of benthic sediments in September, the phosphatase activity was significantly decreased during that period in intermittent streams (Fig. 26). In comparison, phosphatase activity and microbial respiration were significantly increased in hyporheic sediments during the non-flow period in intermittent streams.

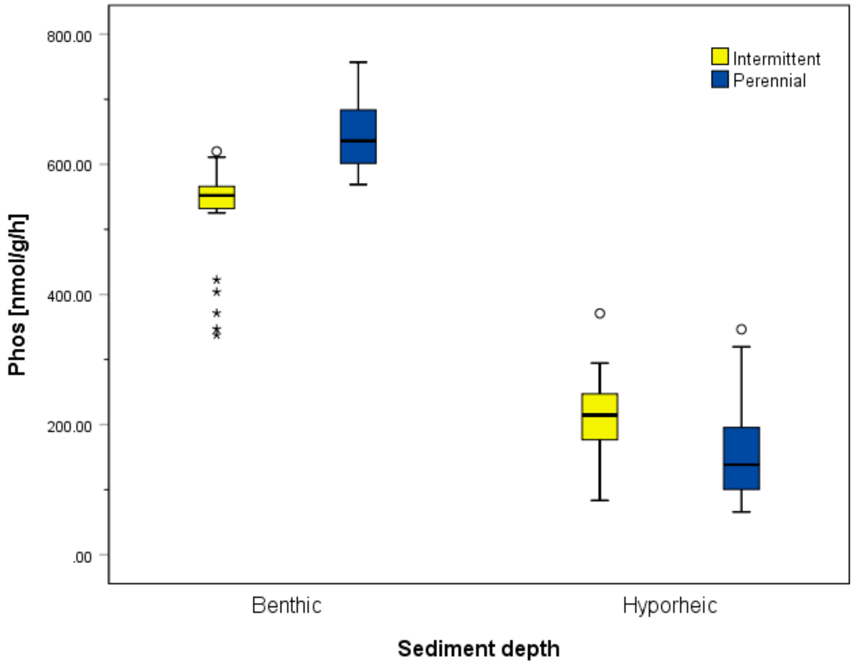

Figure 26: Potential extracellular enzymatic activities of phosphatase (Phos) in nmol/g/h during the non-flow period in September comparing benthic and hyporheic sediments of intermittent and perennial streams (n=25 per sediment depth in intermittent streams and n=20 per sediment depth in perennial streams). Shown are the 10, 25, 50, 75, and 90% percentiles and outliers.

4 Discussion

4.1 Immediate effects

Contrary to the hypothesis, the heterotrophic activity was maintained during the non-flowing period. The activity of intermittent streams was mostly similar or increased compared to perennial streams. These discoveries correspond with findings of ACUÑA ET AL. (2015) and TIMONER ET AL. (2012). ACUÑA ET AL. (2015) found out that non-flow periods had an immediate effect on autotrophic processes, but the effect on heterotrophic processes were delayed and completed only after crossing the ecological threshold of complete desiccation. Heterotrophic microbes tend to have a higher capacity to withstand desiccation, especially in the hyporheic zone (TIMONER ET AL., 2012).

The water content of the hyporheic stream sediments was maintained at a level high enough to support the microbial activity during the non-flow period. The hyporheic sediments of intermittent streams still showed a similar water content compared to perennial streams in July and only decreased in September. However, even then some moisture was preserved in the sediments, never reaching water contents below 8%. GIONCHETTA ET AL. (2019) observed essential moisture contents even after a long-term drought in the hyporheic zone. While the sediments moisture in the surface zone was reduced to 0.5%, the hyporheic sediments could maintain 2.5% of water content. Consistent with the sediment moisture, extracellular enzymatic activities were significantly repressed in the benthic sediments, but not in the hyporheic zone. COULSON ET AL. (2021) studied the effects of desiccation ranging from 4 to 105 days on the hyporheic communities and microbial processes in outdoor hyporheic flumes. Regardless of the drought duration, high moisture contents of 5.2 ± 2.4% could be maintained and microbial activities were not significantly reduced. AMALFITANO ET AL. (2008) concluded that the hyporheic waters could sustain the sediment moisture during non-flow periods and support the microbial activity during that time. Furthermore, MARXSEN ET AL. (2010) and OPREI ET AL. (2019) found the temperate climate region as a factor to maintain high moisture contents as well. MARXSEN ET AL. (2010) compared sediments of a temperate and a Mediterranean stream and observed a less complete desiccation and higher moisture content in the temperate climate region. OPREI ET AL. (2019) concluded that the temperate climate can delay harmful complete desiccation by preserving an essential level of water during non-flow periods via rainfall events, which often interrupt summer dry periods in the temperate region.

Consistent with the water content, respiration and extracellular enzymatic activity of intermittent streams were similar in July, while the respiration and phosphatase activity were increased in September compared to perennial streams. In intermittent streams, the respiration, phosphatase and leucin-aminopeptidase activity were negatively related to the percentage of the water content. In contrast, the activity of β-D-glucosidase and β-D-xylosidase were not affected by the water content.

The negative relationship and unexpected increase of respiration and phosphatase activity could be explained by findings of GIONCHETTA ET AL. (2019). They concluded that in the drying of the hyporheic zone favourable conditions for microorganisms co-occur, such as a high remaining water content combined with an increased oxygen availability compared to the water saturated conditions. Consistent with the results, ACUÑA ET AL. (2015) observed an increase in the phosphatase activity, while TIMONER ET AL. (2012) detected a negative relationship of phosphatase and leucin-aminopeptidase activity with the percentage of the water content. During the non-flowing period, TIMONER ET AL. (2012) and GIONCHETTA ET AL. (2019) observed an average water content of 0.6 ± 0.4% and 2.51% in the hyporheic sediments, respectively. These finding were comparatively lower than the measured water content during this study, which ranged from 8.24% to 20.69%.

Throughout the non-flow period, the vertical water exchange, which is important for the exchange of organic matter and nutrients (BOULTON ET AL., 1998), is ceased. Due to the missing water exchange, microbes could rely more on internal cycling, driving them to produce more enzymes. During the analysis of the enzymatic activity, water was added to the dry sediments, which initiated a short rewetting. This process could lead to a release of the produced enzymes and to the detected increased enzymatic activity in intermittent streams compared to perennial streams. An enhanced enzymatic activity immediately after a rewetting event to values higher than during the flowing period has already been observed by GIONCHETTA ET AL. (2019) and SABATER ET AL. (2016). This effect was most prominent for dry sediments sampled in July. The used method had an estimated time gap of around one hour between the water addition and the substrate addition, which could enhance the rewetting effect. In September, the enzymatic activity was analysed with a slightly different method (SEE 2.6 EXTRACELLULAR ENZYMATIC ACTIVITY). During this analysis, the substrate was added directly to the suspension, diminishing the time gap and a potential rewetting effect. Alongside the time aspect other differences between the methods were detected. In the method used in May and July, the enzymatic activity was measured directly after substrate addition and then again after one hour. For the method applied in September only one measurement was done. One hour after substrate addition, a buffer was added to stop the ongoing reaction. After the buffer addition, the samples can either be stored or measured directly. Samples analysed with the method in May and July could not be stored during the procedure. Differences between the two methods were not only observed in the execution, but in the results as well. The method applied in May and July led to overall lower enzymatic activities compared to the method of September. Further to that, a different trend in the activities was detected. In September, phosphatase showed much higher activities than leucin-aminopeptidase, while in May and July, leucin-aminopeptidase tended to be higher. All these differences between the methods made a comparison of the enzymatic activities between the sampling dates impossible. Nevertheless, the decision to apply the new method in September was made because of the reduction of the time gap and rewetting issue.

Comparing the activity of hyporheic sediments with the surface sediments, a slight difference can be observed. While the microbial activity could be maintained in the hyporheic sediments during the non-flow period, phosphatase activities were suppressed in the benthic sediments. Consistent with the results, GIONCHETTA ET AL. (2019) observed a reduction of enzymatic activities, like β-D-glucosidase and β-D-xylosidase, and bacterial viability in the benthic sediments. These responses were stronger and more significant in the surface than in the hyporheic zone, where the sediments were almost not affected. GIONCHETTA ET AL. (2019) suggested greater resistance of the hyporheic sediments (SEE 4.3 BUFFERING EFFECTS OF THE HYPORHEIC ZONE AND THE CLIMATE).

4.2 Long-term effects

In the hyporheic sediments of intermittent streams, no long-term effects were observed, in contrast to the hypothesis. The respiration and extracellular enzymatic activity were similar during the flow period in intermittent and perennial streams.

Microbial activities tend to recover very quickly after flow resumption (BERNAL ET AL., 2013; ROMANÍ ET AL., 2013), due to their ability of being metabolically active even in sediment with reduced water content (AMALFITANO ET AL., 2008). MARXSEN ET AL. (2010) observed a rapid recovery of all investigated microbial activities, like β-D-glucosidase and leucin-aminopeptidase, in sediments of a temperate intermittent stream. At the beginning of their study, microbial activities were suppressed due to desiccation of the sediments. Microbial activities were reestablished to unaffected conditions only after four days of rewetting. After merely four hours, the activity of β-D-glucosidase showed already values comparable to sediments that were not exposed to dryness. Slower recovery rates were observed in the sediments of a Mediterranean stream, where a much more intense drying occurred. GIONCHETTA ET AL. (2019) compared previously dry sediments after 165 days of rewetting with water saturated sediments. While the microbial activity in surface sediments could not fully recover, no difference between the treatments was observed in the hyporheic sediments. The β-D-xylosidase activity not only achieved the same values as the control but also surpassed them.

The ability of microbes to withstand the desiccation effects of non-flow periods and to recover quickly from it, is likely linked to the duration of the non-flow period and the time they microbes had to adapt to it (ACUÑA ET AL., 2015; TIMONER ET AL., 2020). ACUÑA ET AL. (2015) observed a relationship of the duration of the non-flow period with the resistance and resilience of the community respiration. Resistance was considered as an indicator of the capacity of minimal change and resilience was used as an indicator of the ability to recover rapidly after disturbance. Concerning the resistance of the community respiration, treatments of 1, 3 and 6 days of non-flow conditions showed resistance values around 80%, whereas resistance decreased to 25% at 12 and 20 days. TIMONER ET AL. (2020) compared the resistance and resilience of biofilms of intermittent and perennial streams. After a drying period of 31 days, all stream sediments were rewetted. Community respirations in intermittent streams were suppressed after drying, but after a few days of rewetting, similar activities as the non-affected situation were achieved. Their study concluded that the resistance to the non-flow period, and the ability to recover from it, were related to the biofilms' previous adaptation to desiccation.

4.3 Buffering effects of the hyporheic zone and the climate

As hypothesized, higher amount of organic matter and finer grain sizes had a positive effect on the remaining water content of the sediments of intermittent streams, therefore providing better conditions for the microbes during the non-flowing period. These results are consistent with SENITZA (2019), inspecting the microbial activity in surface sediments, where a similar relationship between water content, organic matter and fine sediments was found.

ZLATANOVIĆ ET AL. (2018), studying the effects of sediments structures on stream metabolism resistance and resilience, observed that different sediment structures influenced the sediment moisture due to variable evaporation and as a result shaped community composition changes. Hydrological disconnection due to the non-flowing period in intermittent streams could increase spatial heterogeneity of dissolved organic matter (DOM) processing dynamics, especially in the hyporheic zone (HARJUNG ET AL., 2018). HARJUNG ET AL. (2018) discovered two time periods with disproportionately high rates for dissolved organic matter processing during the non-flow period in the hyporheic zone, a short pulse at the beginning of disconnection and a longer time period during fragmentation and dry phase. HARJUNG ET AL. (2018) and SHUMILOVA ET AL. (2019) detected that DOM leached from sediments was mainly of microbial origin, suggesting high potential bioavailability. HARJUNG ET AL. (2018) concluded that under non-flow conditions, the hyporheic zone becomes a sink for DOM. Correspondingly, the results of this study showed that intermittent streams had significantly higher amounts of organic matter than perennial streams during the non-flowing period.

The hyporheic zone can act as a humid refuge during the non-flow periods, as a still high water content can be maintained in the sediments. The conservation of some water content in the sediments is mainly linked to streambed characteristics such as organic matter content and sediment grain size (GIONCHETTA ET AL., 2019; ROMANÍ ET AL., 2013). Corresponding with the results of this thesis, GIONCHETTA ET AL. (2019) found out that hyporheic sediments are more resistant to long-term droughts primarily due to the conservation of a slightly higher water content than in surface sediments. Throughout their study, different sediment textures were measured and a higher amount of clay was detected in the hyporheic zone, which not only could retain water, but also form a reserve of organic matter, by the formation of clay-organic complexes. ROMANÍ ET AL. (2013) concluded that bacteria surviving droughts are typically associated with these humid refuges like the hyporheic zone.

Next to the amount of organic matter and fine sediments preserving some moisture, especially in the hyporheic zone, climate related variables can also drive buffering effects during the non-flowing period. The climate change is known to have different impacts on intermittent streams in different geographical regions (SHUMILOVA ET AL., 2019). Streams in the temperate climate region are characterized by a less severe desiccation leading to maintenance of higher water content in the sediments compared to Mediterranean streams (MARXSEN ET AL., 2010). Typical rainfall events during summer dry period in the temperate region have a positive effect on the sediment moisture and help preserving just an essential level of moisture for microbes (SEE 4.1 IMMEDIATE EFFECTS) to remain active (OPREI ET AL., 2019). PAILLEX ET AL. (2020) modeled the change of intermittency in alpine stream networks. The best model predicted flow intermittency based on average temperature and water origin. A strong correlation between flow intermittency and temperature, with streams having higher average temperature more likely to dry, was observed. The study concluded that headwater intermittency is likely driven by complex interactions between climate, precipitation, water source and geology.

The hyporheic zone as a humid refuge during the non-flowing period is important for the microbes by increasing their resistance to drought events. The positive impact of the climate-related variables in the temperate region could be diminished by the ongoing climate change by increasing air temperatures and the duration of non-flowing periods and reducing summer precipitation.

5 Conclusion

A changing climate interacting with other human pressures, like water abstractions and land-use changes, enhances intermittency and increases the number and length of intermittent streams in the near future. The desiccation of the sediments, resulting from the intermittency, could lead to a reduction of microbial activity in these zones. Those microbes mediate essential ecosystem processes such as the decomposition of organic matter and the cycling of carbon, nitrogen and phosphorous. Thus, the reduction of their activity could affect the whole system. This study highlights the importance of the hyporheic zone as a refuge habitat for microbes during the non-flowing period. The heterotrophic activity of the microbes could be maintained during the non-flowing period. Their resistance is mainly linked to the preservation of sufficient moisture in the hyporheic sediments. Streambed characteristics such as high organic matter content and fine sediments promote the maintenance of minimum water content in the sediments. Overall, the understanding of the effect of the dry-wet cycle on microbial dynamics is still limited. Future research in the production of extracellular polymeric substances (EPS) and the abundance of bacteria will provide a better understanding of the microbial processes during the non-flowing period. Furthermore, a prolonged study period could give more insight into the ecological effects of the whole dry-wet cycle. Artificial drying experiments or sampling of a larger regional and climate gradient of streams could help examine the relevance of the role of the hyporheic zone in more detail.

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Appendix

Descriptive statistics: Mean ± Standard Deviation

Water chemistry July				
Sites	**NH4+** [µg/l]	**NO2-** [µg/l]	**NO3-** [µg/l]	**PO43-** [µg/l]
RB	120.3	2.2	<100.0	57.3
JB	21.2	5.6	125.1	6.6
DB	90.1	27.9	337.1	36.9
SB	13.7	6.2	331.1	24.7
WB	2.7	2.9	1300.1	22.4
GaB	28.7	12.9	933.4	30.9
LB	14.2	24.8	2708.2	88.0
PB	17.8	10.0	486.1	21.8
GB	29.8	30.0	1549.9	17.5
FB	24.9	36.2	1465.7	62.0

Water chemistry September				
Sites	**NH4+** [µg/l]	**NO2-** [µg/l]	**NO3-** [µg/l]	**PO43-** [µg/l]
RB	45.3	8.4	167.1	17.1
JB	121.3	10.6	183.9	2.5
DB	892.0	22.4	<100.0	69.0
SB	2.3	5.1	202.3	4.5
WB	3.6	5.3	1433.4	24.3
GaB	30.0	24.1	1097.7	18.0
LB	26.0	10.1	745.2	35.8
PB	101.3	7.3	<100.0	27.1
GB	183.0	45.8	1187.9	33.4
FB	NDA	NDA	NDA	NDA

Hyporheic sediments May 1/2

Sites	WC [%]	OM [%]	Fines [%]	Porg [µg/g]	C:N
RB	21.84 ± 2.67	1.38 ± 0.87	1.59 ± 0.74	367.98 ± 298.80	7.38 ± 4.01
JB	19.98 ± 1.75	0.78 ± 0.21	2.68 ± 0.36	404.03 ± 294.63	4.33 ± 2.24
DB	21.20 ± 2.59	0.69 ± 0.17	2.74 ± 0.83	231.21 ± 149.31	2.85 ± 3.70
SB	20.13 ± 2.79	0.45 ± 0.18	1.07 ± 0.46	349.59 ± 293.82	3.25 ± 3.45
WB	26.33 ± 1.07	1.54 ± 0.14	0.53 ± 0.38	332.73 ± 242.10	2.74 ± 3.41
GaB	22.59 ± 1.27	0.94 ± 0.23	2.71 ± 0.73	366.91 ± 93.38	4.48 ± 1.94
LB	19.16 ± 1.36	0.63 ± 0.10	2.16 ± 0.77	334.00 ± 132.84	4.89 ± 2.84
PB	27.13 ± 6.90	2.50 ± 0.77	8.47 ± 8.19	780.32 ± 196.94	3.88 ± 1.38
GB	23.27 ± 2.85	1.63 ± 0.23	1.86 ± 0.98	560.26 ± 378.39	6.98 ± 3.00
FB	25.23 ± 4.35	2.55 ± 0.52	3.11 ± 0.97	413.70 ± 24.02	4.76 ± 2.50

Hyporheic sediments May 2/2

Sites	Resp [µgC/g/h]	Phos [nmol/g/h]	Gluc [nmol/g/h]	Xyl [nmol/g/h]	Leu [nmol/g/h]
RB	0.40 ± 0.03	7.11 ± 3.81	0.26 ± 0.01	1.89 ± 0.83	15.98 ± 7.22
JB	0.44 ± 0.03	2.94 ± 0.73	0.25 ± 0.06	1.13 ± 0.35	10.72 ± 5.12
DB	0.39 ± 0.00	2.85 ± 1.29	0.35 ± 0.18	0.82 ± 0.30	5.65 ± 3.52
SB	0.37 ± 0.00	1.56 ± 0.67	0.26 ± 0.02	0.76 ± 0.20	7.53 ± 2.93
WB	0.43 ± 0.06	4.67 ± 3.16	0.07 ± 0.03	2.19 ± 1.46	20.04 ± 7.34
GaB	0.34 ± 0.01	0.32 ± 0.13	0.05 ± 0.03	0.30 ± 0.10	3.35 ± 1.73
LB	0.39 ± 0.06	2.85 ± 1.17	0.04 ± 0.02	1.15 ± 0.54	12.06 ± 4.48
PB	0.37 ± 0.03	0.40 ± 0.09	0.07 ± 0.03	0.24 ± 0.08	4.42 ± 2.70
GB	0.43 ± 0.03	3.99 ± 1.97	0.06 ± 0.02	1.32 ± 0.50	9.28 ± 2.64
FB	0.62 ± 0.18	8.69 ± 0.98	0.13 ± 0.03	1.79 ± 0.51	8.72 ± 2.28

Hyporheic sediments July

Sites	WC [%]	OM [%]	Resp [µgC/g/h]	Phos [nmol/g/h]	Gluc [nmol/g/h]	Xyl [nmol/g/h]	Leu [nmol/g/h]
RB	10.81 ± 3.26	0.86 ± 0.12	0.51 ± 0.16	7.74 ± 2.36	0.10 ± 0.04	1.73 ± 0.68	20.22 ± 11.77
JB	14.27 ± 4.12	1.33 ± 0.27	0.58 ± 0.04	7.65 ± 4.66	0.16 ± 0.01	0.80 ± 0.29	8.43 ± 1.40
DB	20.25 ± 3.37	0.64 ± 0.18	0.43 ± 0.09	2.46 ± 1.04	0.10 ± 0.02	0.39 ± 0.28	4.32 ± 1.63
SB	16.72 ± 0.93	0.55 ± 0.06	0.36 ± 0.02	2.03 ± 0.60	0.13 ± 0.04	0.41 ± 0.27	3.96 ± 1.89
WB	21.30 ± 0.92	1.42 ± 0.10	0.49 ± 0.04	1.76 ± 1.26	0.25 ± 0.03	0.52 ± 0.30	11.22 ± 2.65
GaB	20.12 ± 5.23	1.61 ± 0.89	0.43 ± 0.08	1.66 ± 0.74	0.15 ± 0.05	1.50 ± 1.12	8.72 ± 4.38
LB	15.86 ± 0.97	0.48 ± 0.05	0.40 ± 0.04	13.90 ± 7.35	0.10 ± 0.08	1.57 ± 1.34	19.78 ± 6.25
PB	23.77 ± 1.43	3.10 ± 0.53	0.40 ± 0.05	0.76 ± 0.30	0.28 ± 0.04	0.53 ± 0.13	5.30 ± 3.77
GB	18.92 ± 2.72	1.53 ± 1.00	0.45 ± 0.04	7.83 ± 2.92	0.28 ± 0.08	2.19 ± 0.73	15.19 ± 2.74
FB	19.19 ± 2.74	2.08 ± 0.50	0.80 ± 0.55	14.92 ± 4.15	0.40 ± 0.19	4.22 ± 1.75	19.91 ± 6.51

Hyporheic sediments September

Sites	WC [%]	OM [%]	Resp [µgC/g/h]	Phos [nmol/g/h]	Gluc [nmol/g/h]	Xyl [nmol/g/h]	Leu [nmol/g/h]
RB	11.20 ± 3.57	0.86 ± 0.21	0.62 ± 0.12	288.26 ± 49.89	93.61 ± 32.51	21.72 ± 8.59	61.61 ± 16.37
JB	16.80 ± 4.99	1.47 ± 0.30	0.53 ± 0.16	186.85 ± 19.99	50.11 ± 13.77	11.12 ± 2.70	25.96 ± 7.58
DB	21.26 ± 1.35	0.68 ± 0.12	0.47 ± 0.01	113.19 ± 33.40	26.85 ± 10.00	5.14 ± 2.28	18.06 ± 7.14
SB	18.24 ± 0.78	0.43 ± 0.08	0.40 ± 0.01	94.46 ± 19.98	24.01 ± 10.21	4.08 ± 1.38	9.58 ± 1.39
WB	22.60 ± 1.71	1.39 ± 0.09	0.49 ± 0.11	301.85 ± 42.12	82.04 ± 23.69	30.50 ± 7.30	77.77 ± 10.81
GaB	NDA	NDA	NDA	NDA	NDA	NDA	NDA
LB	17.67 ± 0.99	0.51 ± 0.12	0.41 ± 0.05	144.25 ± 9.29	46.50 ± 12.60	13.14 ± 0.74	38.80 ± 15.41
PB	20.69 ± 2.82	2.62 ± 0.66	0.46 ± 0.05	106.02 ± 23.29	31.23 ± 16.15	7.44 ± 3.21	17.56 ± 6.43
GB	11.61 ± 3.53	1.94 ± 0.47	0.59 ± 0.02	224.26 ± 18.40	67.89 ± 18.05	13.67 ± 1.93	33.00 ± 4.21
FB	8.24 ± 0.78	2.53 ± 0.17	0.86 ± 0.08	232.27 ± 33.43	47.68 ± 10.51	10.87 ± 2.36	36.54 ± 7.85

Benthic sediments September

Sites	WC [%]	OM [%]	Resp [µgC/g/h]	Phos [nmol/g/h]	Gluc [nmol/g/h]	Xyl [nmol/g/h]	Leu [nmol/g/h]
RB	9.53 ± 2.52	0.95 ± 0.13	0.27 ± 0.01	566.91 ± 9.82	305.93 ± 20.39	100.70 ± 20.91	346.20 ± 23.45
JB	13.59 ± 4.33	1.66 ± 0.50	0.54 ± 0.47	548.07 ± 18.29	288.36 ± 34.81	87.76 ± 18.54	313.14 ± 41.00
DB	19.74 ± 1.34	0.69 ± 0.21	0.71 ± 0.24	621.31 ± 36.54	158.35 ± 76.97	70.80 ± 52.28	412.74 ± 147.16
SB	17.12 ± 1.51	0.54 ± 0.02	0.82 ± 0.06	602.34 ± 20.84	85.50 ± 5.85	25.72 ± 1.26	175.53 ± 11.54
WB	21.58 ± 0.61	1.42 ± 0.09	0.65 ± 0.22	720.72 ± 32.87	295.02 ± 46.92	190.55 ± 35.06	388.43 ± 42.36
GaB	NDA	NDA	NDA	NDA	NDA	NDA	NDA
LB	20.30 ± 3.90	1.16 ± 0.80	0.47 ± 0.16	627.91 ± 43.66	330.52 ± 62.16	96.08 ± 30.27	269.13 ± 65.11
PB	17.39 ± 2.63	2.68 ± 0.50	0.58 ± 0.14	376.49 ± 36.29	180.01 ± 37.98	45.02 ± 7.35	179.23 ± 59.69
GB	12.66 ± 2.95	1.84 ± 0.27	0.47 ± 0.13	543.24 ± 11.34	242.82 ± 21.25	66.36 ± 14.74	244.19 ± 29.22
FB	6.26 ± 2.36	3.25 ± 0.93	0.76 ± 0.13	587.66 ± 35.83	410.55 ± 67.38	122.28 ± 9.82	310.18 ± 94.75

Non-parametric tests: Kruskal-Wallis and Mann-Whitney U

Water chemistry May								
Paramteter	across stream type				across region			
	Chi-sq	**df**	**p**	**N**	**Chi-sq**	**df**	**p**	**N**
NH4+	1.463	1	0.226	20	1.929	1	0.165	20
NO2-	1.042	1	0.307	20	0.381	1	0.537	20
NO3-	0.023	1	0.880	20	9.524	1	0.002*	20
PO43-	5.321	1	0.021*	20	4.670	1	0.031*	20
DOC	6.818	1	0.009*	10	0.045	1	0.831	10

Hyporheic sediments May												
Paramteter	across stream type				Burgenland: across stream type				Styria: across stream type			
	U	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**
WC	284	-0.553	0.580	50	49	-0.076	0.971	20	104	-0.353	0.744	30
OM	219	-1.814	0.070	50	17	-2.495	0.011*	20	98	-0.601	0.567	30
Fines	78	-1.178	0.252	30	17	-0.160	0.937	12	24	-1.155	0.277	18
Porg	72	-1.680	0.098	30	16	-0.320	0.818	12	24	-1.457	0.161	18
C:N	75	-1.555	0.126	30	8	-1.601	0.132	12	34	-0.547	0.605	18
Resp	129	0.684	0.512	30								
Phos	404	1.775	0.076	50								
Gluc	351	0.747	0.455	50								
Xyl	392	1.543	0.123	50								
Leu	375	1.213	0.225	50								

Hyporheic sediments July												
Paramteter	across stream type				Burgenland: across stream type				Styria: across stream type			
	U	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**
WC	345	0.631	0.528	50	90	3.024	0.002*	20	70	-1.763	0.081	30
OM	169	-2.784	0.005*	50	3	-3.553	0.000*	20	65	-1,970	0.050	30
Resp	97	-0.643	0.539	30	4	-2.242	0.026*	12	56	1.369	0.190	18
Phos	348	0.689	0.491	50	0	-3.780	0.000*	20	175	2.592	0.009*	30
Gluc	258	-0.609	0.543	50	37	-0.983	0.353	20	106	0.392	0.717	30
Xyl	249	-1.232	0.218	50	7	-3.250	0.000*	20	133	0.850	0.412	30
Leu	306	-0.126	0.900	50	0	-3.780	0.000*	20	177	2.675	0.007*	30

Hyporheic sediments September												
Paramteter	across stream type				Burgenland: across stream type				Styria: across stream type			
	U	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**
WC	407	3.586	0.000*	45	84	2.570	0.009*	20	122	2.607	0.008*	25
OM	49	-4.591	0.000*	45	7	-3.250	0.000*	20	3	-3.994	0.000*	25
Resp	28	-3.025	0.002*	27	7	-1.761	0.093	12	10	-2.003	0.050*	15
Phos	159	-2.079	0.038*	45	0	-3.780	0.000*	20	95	1.109	0.285	25
Gluc	170	-1.827	0.068	45	2	-3.628	0.000*	20	101	1.442	0.160	25
Xyl	172	-1.209	0.227	45	1	-3.704	0.000*	20	100	2.638	0.007*	25
Leu	210	-0.914	0.361	45	4	-3.477	0.000*	20	122	2.607	0.008*	25

September								
Paramteter	intermittent: across sediment depth				perennial: across sediment depth			
	U	**Z**	**p**	**N**	**U**	**Z**	**p**	**N**
Water content	262	-0.980	0.327	50	187	-0.352	0.738	40
OM	333	0.398	0.691	50	260	1.623	0.108	40
Resp	82	-1.265	0.217	30	116	2.540	0.010*	24
Phos	623	6.025	0.000*	50	400	5.410	0.000*	40
Gluc	625	6.063	0.000*	50	384	4.977	0.000*	40
Xyl	550	5.863	0.000*	50	378	4.815	0.000*	40
Leu	625	6.063	0.000*	50	400	5.41	0.000*	40