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UNDERSTANDING THE DYNAMICS OF DISSOLVED ORGANIC CARBON AND MICROBIAL DIVERSITY IN SMALL HEADWATER STREAMS

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Abstract

Small headwater streams are an essential component of the hydrological cycle, connecting riverine and terrestrial ecosystems, and contributing to the flow of water, nutrients and organic matter to larger water bodies. While the connection between soils and streams have been extensively studied in terms of dissolved organic carbon and bacteria, little is known about the dynamics of these transfers. With this thesis we aim to explore and better characterize the dynamic connection that exists between terrestrial ecosystems and small headwater streams through a field study over a two-year period. First, we sought to quantitatively examine the dynamic transfer of dissolved organic carbon and bacteria from soils to streams, with particular interest in the effects of hydrologic events on these transfers. A second study evaluated the community composition resulting from these transfers and their fate in the aquatic continuum, notably in the streams biofilms. Finally, we investigated the importance of seasons and the biogeochemistry of streams on the dynamics of bacterial communities in this interconnected ecosystem. Overall, our results show that high flow events have a considerable impact on the transfers of carbon and bacteria from soils to headwater streams. We observed a concomitant mobilization of DOC and bacteria and in larger numbers during summer storms. These bacteria showed a largely terrestrial origin and were found further downstream in biofilms when the water level had returned to normal. Overall, our results support the importance of high flow events on the ecology of river systems year-round. Considering the potential impact of the ongoing global climate disruption on the frequency and intensity of precipitation, we should expect a change in the dynamics of DOC and bacterial communities in headwater streams and along the river continuum. These changes can have significant ecological consequences, including the disruption of food webs and changes in the composition and structure of riparian vegetation.

Zusammenfassung

Kleine Quellbäche sind ein wesentlicher Bestandteil des Wasserkreislaufs, sie verbinden Fluss- und Landökosysteme und tragen zum Fluss von Wasser, Nährstoffen und organischem Material in größere Gewässer bei. Während die Verbindung zwischen Böden und Bächen im Hinblick auf gelösten organischen Kohlenstoff und Bakterien eingehend untersucht wurde, ist über die Dynamik dieser Übertragungen wenig bekannt. Mit dieser Arbeit wollen wir die dynamische Verbindung zwischen terrestrischen Ökosystemen und kleinen Oberläufen durch eine Feldstudie über einen Zeitraum von zwei Jahren untersuchen und besser charakterisieren. Zunächst versuchten wir, den dynamischen Transfer von gelöstem organischem Kohlenstoff und Bakterien aus dem Boden in die Bäche quantitativ zu untersuchen, mit besonderem Interesse an den Auswirkungen hydrologischer Ereignisse auf diese Transfers. In einer zweiten Studie wurde die Zusammensetzung der aus diesen Übertragungen resultierenden Lebensgemeinschaften und ihr Verbleib im aquatischen Kontinuum, insbesondere in den Biofilmen der Bäche, untersucht. Schließlich untersuchten wir die Bedeutung der Jahreszeiten und der Biogeochemie der Bäche für die Dynamik der Bakteriengemeinschaften in diesem vernetzten Ökosystem. Insgesamt zeigen unsere Ergebnisse, dass Hochwasserereignisse einen erheblichen Einfluss auf den Transfer von Kohlenstoff und Bakterien aus dem Boden in die Oberläufe der Bäche haben. Wir beobachteten eine gleichzeitige Mobilisierung von DOC und Bakterien, und zwar in größerer Zahl während Sommergewittern. Diese Bakterien waren größtenteils terrestrischen Ursprungs und wurden weiter flussabwärts in Biofilmen gefunden, als sich der Wasserstand wieder normalisiert hatte. Insgesamt belegen unsere Ergebnisse die Bedeutung von Hochwasserereignissen für die Ökologie von Flusssystemen das ganze Jahr über. In Anbetracht der potenziellen Auswirkungen der anhaltenden globalen Klimastörung auf die Häufigkeit und Intensität von Niederschlägen sollten wir mit einer Veränderung der Dynamik von DOC und Bakteriengemeinschaften in Oberläufen und entlang des Flusskontinuums rechnen. Diese Veränderungen können erhebliche ökologische Folgen haben, einschließlich der Störung von Nahrungsnetzen und Veränderungen in der Zusammensetzung und Struktur der Ufervegetation.

General Introduction

River networks are the backbone of terrestrial ecosystems. They cover approximately 3% of the land surface but the amount of carbon they transport and emit to the atmosphere make them key actors in land carbon balance (Le Quéré et al., 2017; Raymond et al., 2013). Most of the organic and inorganic carbon found in streams and rivers were exported via subsurface flow paths originating in surrounding terrestrial ecosystems (Battin et al., 2008). In fact, stream water can originate from precipitation, snowmelt, subsurface flow, and groundwater discharge, but the latter is usually the most stable source of water for streams. Dissolved organic carbon (DOC) that comes in from surrounding soils can have drastic impacts on the ecology of streams. For instance, it affects food-webs structure by enhancing photosynthesis and respiration (Demars et al., 2020), and can alter water acidity (Buffam et al., 2007). Additionally, CO_2 that was terrestrially sequestrated by plants and transferred to streams through the subsurface flow path was shown to be rapidly transferred back to the atmosphere via physical gas exchanges (Campeau et al., 2019). This supports the important role that surface waters play in global carbon emissions. The terrestrial-aquatic transfers likely occur throughout the watershed but are reinforced in the most upland tributaries, where the high ratio between soil-stream interfaces and water volume promotes transfers from soils to streams: headwaters. As such, small headwater streams interlink terrestrial and aquatic biogeochemical cycles (Richardson, 2019). Despite their small size, they can account for up to 80% of the fluvial system and are essential for downstream ecosystems (Wipfli et al., 2007). Headwater streams are a source of water, organic matter, nutrients, and invertebrates for downstream ecosystems. Studies showed that first-order streams contribute 70% of the water volume and 65% of the nitrogen fluxes of second-order streams (Alexander et al., 2007). These contributions are reduced to 55% and 40% in higher-order streams. It is therefore crucial to understand the mechanisms that drive the ecology and functioning of such environments to better comprehend the processes occurring further downstream and the impact of carbon emissions from river networks on global climate.

Organic matter production, retention, and transport are primary drivers of biotic processes in streams. Since most streams are heterotrophic, which means they consume more organic matter than they produce, a portion of the carbon they use must come from allochtonous sources. Autochtonous and allochtonous DOM commonly exhibit different fluorescence properties due to their diverging molecular structure and can therefore be discriminated using fluorescence spectroscopy. High order streams with larger channels benefit from higher sunlight exposure

and therefore higher instream autotrophic production of organic matter. In contrast, headwater streams generally get lower light exposure due to canopy coverage resulting in a higher proportion of allochtonous organic matter (Richardson et al., 2005). Connections with the surrounding environment, and more precisely with terrestrial ecosystems, is therefore crucial to sustain life in small headwater streams. Allochtonous organic matter present in headwater streams are principally a result of leaching from particulate organic matter - such as wood debris and leaves which presence is favored by the canopy coverage of headwater streams - or transport from surrounding soils (Cole et al., 2007). The molecular structure of DOM determines how it reacts with the environment and its bioavailability (Minor et al., 2014). High uptake rates have been observed for simple DOM compounds such as acetate and glucose and low-molecular-weight DOM sources such as leaf leachates (Berggren et al., 2010; Mineau et al., 2016). On the other hand, soil leachate usually presents lower biodegradability. Regardless, the processes of transformation and mineralization of DOM rely substantially on the heterotrophic communities present in headwater streams.

Microbial communities – a group that comprises bacteria, archaea, and fungi - represent a vast majority of Earth's biodiversity and are essential components of all ecosystems. On a global scale, microbial species richness was estimated to reach 10^{12} species and represent a biomass of 89 Gt C (Bar-On et al., 2018; Locey & Lennon, 2016). With ongoing advances in genetic technologies, we have now a better understanding of the composition and distribution of microbial communities. Despite their ubiquitous distribution, bacteria and archaea communities present geographical patterns based on linear distance, latitude, depth, oxygen availability, and climatic conditions (Martiny et al., 2006). In river networks, microbial diversity varies depending on the position in the aquatic continuum (Cruaud et al., 2020; Ruiz-González, Niño-Garcia, et al., 2017). In headwater streams, microbial communities were shown to be strongly influenced by the inoculation of bacteria from surrounding soil ecosystems (Crump et al., 2012; Ruiz-González, Niño-García, et al., 2017). With increasing stream order, the proportion of typical freshwater taxa increases and bacterial diversity decreases (Besemer et al., 2013; Hermans et al., 2019). With their high diversity and strong influence on downstream ecosystems, headwaters have been described as reservoirs of microbial life for river networks (Besemer et al., 2013). Most microorganisms in aquatic environments reside in surface-attached biofilms (Battin et al., 2003). These biofilms consist of complex meta-communities of algae, bacteria, archaea, and other microorganisms that are attached through an extra-cellular matrix to any surface in a stream (Bengtsson et al., 2018). The interaction between microbial

autotrophs and heterotrophs within the matrix of biofilms have profound impacts on the energy fluxes in streams and rivers (Battin et al., 2016). Biofilm microbial communities are the result of inoculation from free-flowing communities and channel morphology (Benda et al., 2005; Besemer et al., 2007, 2012). The transfer of bacteria from soils to small streams therefore represents the primary source that significantly influences the taxa available for biofilm settlements and, in turn, potentially and very likely affect ecosystem processes.

Headwater streams are dynamic systems, defined by variations in physical, chemical, and biological conditions. Dynamics occur in response to seasonal fluctuations in temperature, precipitation, and other environmental factors. These changes can have a significant impact on the functioning of headwater streams, including their water quality, flow, and distribution and abundance of aquatic species. For example, the availability of light and the distribution of vegetation along the stream banks can change with season, affecting the distribution and abundance of photosynthetic organisms, while changes in precipitation can influence the flow and water levels in the stream. High flow events in headwater streams refer to periods of increased water discharge in the stream, which can occur as a result of heavy rainfall or snowmelt. Headwater streams were shown to exhibit drastic changes in discharge over short periods of time (Johnson et al., 2022). These events can have a significant impact on the physical, chemical, and biological conditions in headwater streams, and can play a key role in shaping the structure and function of these systems over time. During baseflow conditions, most of the water in headwater streams originate from groundwater with long residence times. On the other hand, during hydrological events, the water table raises and activates water flow through shallower, organic-rich soil layers that affects stream chemistry (Ledesma et al., 2018). Understanding the impacts of high flow events, in particular on DOC quality and concentration as well as on microbial communities, is important for managing and conserving these systems, as well as for predicting how they will respond to changes in their environment, such as land use change, climate change, or the introduction of pollutants. Considering the strong link bacterial community diversity and composition share with the DOM cycle in streams, one can assume that both will be impacted by hydrological events. However, the intricate interactions between DOM and bacterial communities in headwater streams remain poorly understood to date.

With this thesis, I aim to explore the variations of DOM and bacterial communities in the soil-river-biofilm continuum in regard to season and flow conditions. The project aimed to characterize the dynamic transfer of DOM and microbial life from catchment soils to small

streams and the fate of bacteria in biofilms of a larger stream, the Oberer Seebach. This monitoring was conducted throughout the year, after hydrological events (summer floods and snowmelt), and at varying flow conditions. I was thereby able to better appreciate the impact of these influxes on stream microbial community composition and diversity.

Chapter 1. Dynamic transfer of soil bacteria and dissolved organic carbon into small streams during hydrological events

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Abstract

Small headwater streams interlink catchment soils with the river network. As water makes its way from the hillslopes to the stream, it mobilizes dissolved organic carbon (DOC) and potentially soil microbes into stream water. In this study, we aimed at quantifying the dynamic transfer of DOC and microbial life, namely bacteria from catchment soils into streams. We hypothesized that increased soil saturation enhances the lateral inflow of bacteria and DOC into streams. To address this hypothesis, we sampled six first order streams and three soil transects at two different depths located within the pre-alpine Oberer Seebach (OSB) catchment in Austria over a duration of 2 years. We found a strong variation in DOC concentrations (range: 0.4 to 5.6 mg L^{-1}) and bacterial abundances (range: <500,000 to 3,863,000 cells mL^{-1}) measured by flow-cytometry. The highest values of DOC and bacterial cells occurred during high flow events. DOC concentration and bacterial abundance were correlated across all streams and seasons. In soils, DOC ranges were higher and were also correlated with bacterial abundance, while DOC concentrations were ~10 times higher per bacterial cell than in streams. Overall, we show that soils provide a dynamic inflow of bacteria and DOC to first order streams. Most probably, this results in a dynamic and reoccurring inoculation of small streams from catchment soils during runoff events. We propose that this dynamic microbial inoculation of small streams is potentially relevant for microbial community dynamics of downstream receiving waters.

Introduction

Small headwater streams are dynamic systems that interlink the terrestrial landscape with the fluvial network (Battin et al., 2008; Richardson & Danehy, 2007). As they receive water from hillslopes, they have been proposed as 'mirrors of the landscape', integrating the catchment heterogeneity of soils, geology and land-use into a combined biogeochemical signature (Bishop et al., 2008; Wipfli et al., 2007). Typically, headwater streams encompass 60% to 80% of stream networks flow length (Benda et al., 2005; Schumm, 1956). As they provide nutrients, woody debris, and dissolved and particulate organic carbon (DOC and POC, respectively) to downstream water bodies (Gomi et al., 2002; Wipfli et al., 2007), they play a crucial role for the ecology of downstream river networks. Also, through matter transport, headwater streams link the terrestrial and aquatic carbon cycle (e.g. Fasching et al. 2015; Raymond et al. 2013; Schelker et al. 2016). It is therefore critical to assess the ecology of headwater streams to understand the functioning of the rest of the river network.

Despite the important role headwaters play in aquatic networks, they remain understudied. Most first order streams are not fully represented on current maps (Benda et al., 2005). Also, headwater streams are influenced by environmental change (Wipfli et al., 2007). The strong link they share with terrestrial environment affect in particular stream DOC concentrations that show fluctuations throughout the year – being lower during winter and higher during summer (Dawson et al., 2008; Laudon et al., 2011). These seasonal variations are superimposed by discharge and temperature as secondary drivers that are also sensitive to climate change (Köhler et al., 2009; Sidle et al., 2000). Similar to DOC, bacterial population dynamics, such as for example the bacterial abundances in streams show recurring seasonal patterns (Hullar et al., 2006).

DOC provides carbon and energy for bacterial heterotrophs (Williams & Del Giorgio, 2005). Its variation influences bacterial biogeography (Findlay et al., 2008) and stream metabolism (Berggren & del Giorgio, 2015). In some aquatic systems, the prevalence of major bacterial clades has been shown to be significantly modified by the presence and composition of DOC (Amaral et al., 2016). Thus, variations in DOC and bacterial abundances throughout the year support the dynamic nature of headwater streams. However, the origin of these variations is hitherto not well understood.

Soils constitute one of the most important microbial habitats, containing a great diversity of bacteria. The microbes have crucial roles in nutrient cycling, maintaining soil fertility and soil carbon sequestration (Fierer, 2017; Philippot et al., 2013; Wagg et al., 2014). Soil microbes have a significant effect on biological processes as they produce CO_2 while respiring soil organic matter, influence soil acidity and regulate soil dynamics (Fierer, 2017). Within soils, microbial densities vary greatly. Microbial biomass is commonly one to two orders of magnitude lower in deeper soil horizons than at the soil surface (Federle et al., 1986; Fierer et al., 2003; Taylor et al., 2002).

Soils are connected to river networks by water flows that enter small streams through the riparian zone (Richardson & Danehy, 2007; Sidle et al., 2000). The relationship between soils and streams has been extensively studied regarding the seasonal variation in DOC concentrations. In forested catchments, snowmelt and storms mobilize DOC from the surrounding soils that reaches the streams through subsurface flow-paths and induce an increase in stream DOC concentration (Fasching et al., 2015; Laudon et al., 2004). At the same time, soils have been proposed to serve as critical reservoirs and thus source areas of microbes for surface waters (Crump et al., 2012; Hullar et al., 2006). The transport of microbes from soils to small streams was previously shown using DNA sequencing techniques and this transfer has been hypothesized to alter downstream microbial communities (Crump et al., 2012; Ruiz-González et al., 2015; Savio et al., 2015). Bacterial transfer to streams was also shown to vary throughout the year and in response to changes in temperature and precipitation (Hassell et al., 2018; Hermans et al., 2019; Teachey et al., 2019). However, DNA sequencing data does not provide quantitative information and, to this day, little is known about the dynamics and seasonality of bacterial abundances in small headwater streams (Richardson, 2019).

Here we propose that the dynamic connectivity between soils and streams acts as a principle origin of variation of bacterial abundances in headwater streams. To our knowledge, no previous study has analyzed bacterial abundances in soil water from catchment soils in order to compare it to nearby headwater streams and shed light on their transient connectivity. Thus, we aim to explore the dynamic link between soils and headwater streams in terms of DOC and bacterial abundances through time and at different flow conditions. We pose the following questions for this study: What is the soil contribution in terms of providing DOC and microbial life to small headwater streams? What are the temporal dynamics of these provisions? We hypothesize that DOC concentrations co-vary with bacterial abundances, as both are mobilized

from the soil simultaneously. In addition, we propose that varying flow conditions influence the degree of connectivity between soils and streams and thus control the DOC concentrations and bacterial abundances in small streams.

Methods

Study site

Sampling was carried out on six small headwater streams draining into Lake Lunz located in the eastern Alps near Lunz am See, Austria (Fig. 1). Lake Lunz receives most of its water from the Oberer Seebach Catchment. This catchment is approximately 25 km^2 and is largely pristine. Vegetation is dominated by *Fraxinus excelsior*, *Acer pseudoplatanus*, *Fagus sylvatica*, *Salix caprea* and *Picea abies* (Battin, 1999; Fasching et al., 2015). During the year 2018, air temperature averaged 8.96°C and precipitation averaged 1,500 mm. Snow cover can extend from November to May, but in some winters little snow is present. Between May 2010 and

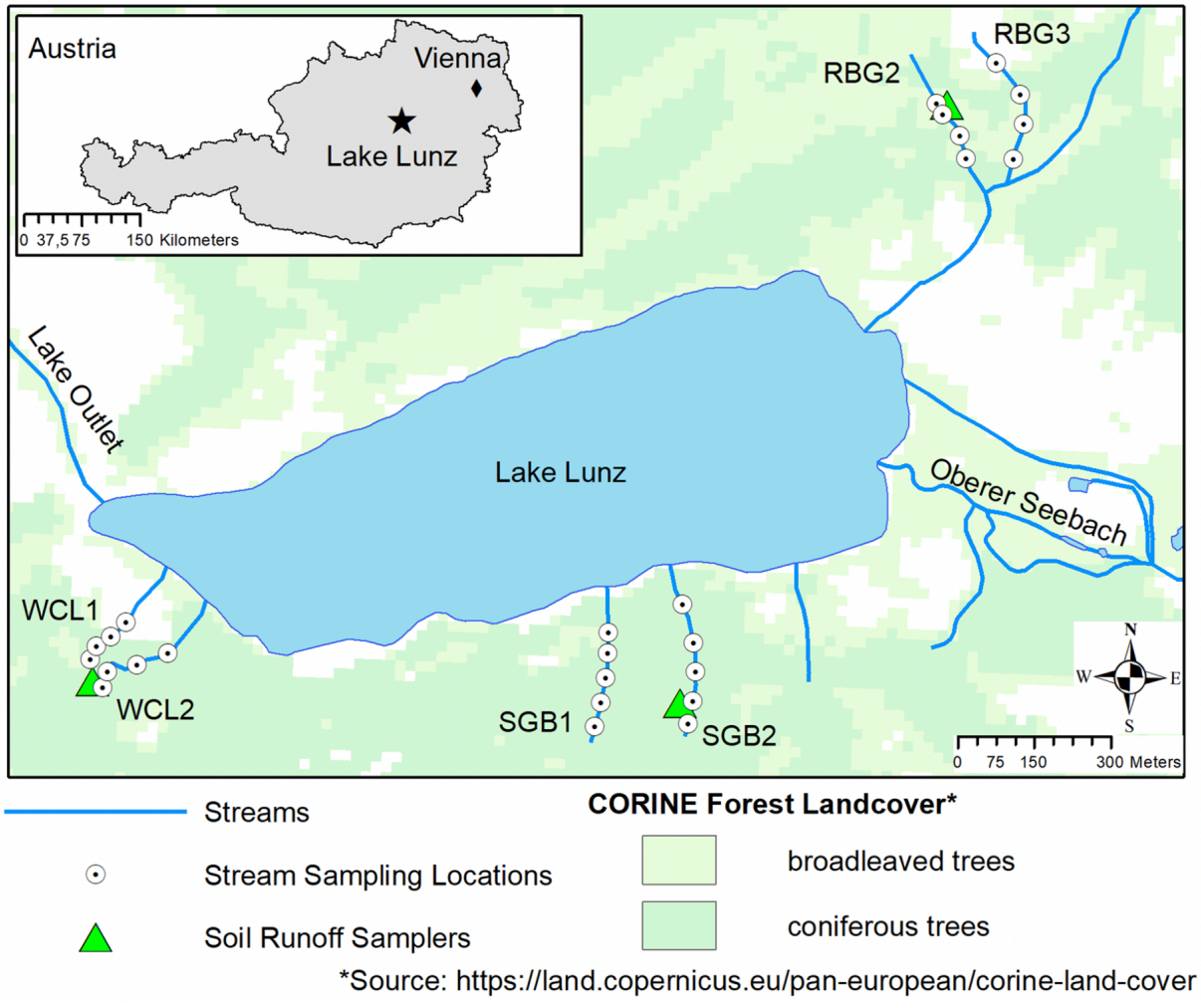

Fig. 1 Location of sampled first order streams near Lake Lunz in Austria. Dots represent stream sampling stations and green squares soil runoff samplers.

August 2013, the Oberer Seebach had an average DOC concentration of 1.56 ± 0.3 mg L^{-1} during autumn and of 1.79 ± 0.4 mg L^{-1} during summer (Fasching et al., 2015). The catchment is characterized by glacial alluvial deposits, which are underlain by a low-permeability layer of ancient lake sediment and calcareous rock (Battin, 1999).

Three hillslopes around Lake Lunz were selected for this study (Rehberg, RBG; Schlögelberg, SBG; and the WasserCluster Lunz slope, WCL; Fig. 1). On each hillslope, two headwater streams were chosen, referred to as RBG2 and 3; SBG1 and 2; and WCL1 and 2. The streams are of first stream order, small in size (width between 0.1 and 0.3 m) and enter Lake Lunz within 800 m of their flow length. All hillslopes are steep; the typical slope of the hillslopes is 29%.

Stream sample collection

All six streams were sampled for DOC every two weeks in spring, summer and fall, respectively and approx. every four weeks in wintertime from July 2017 to end of June 2019. Analyses of bacterial abundances started in May 2018. Additional samplings were performed during and following high flow events in order to characterize those events and compare the dynamics of the soil/stream connectivity at different flow conditions. At each sampling, the same four to five locations along each stream, with an approximate distance of 35 m between sites were sampled (Fig. 1).

The three hillslopes differed in orientation, aspect and land use (Fig. 1). WCL and SBG are located on a north-facing forested mountainside, whereas the RGB streams face towards the southwest. Land use of the SBG streams is mixed deciduous forest, with little forestry activity during the last two decades. The WCL hillslope is similarly forested, but WCL1 enters an open meadow towards the lake. Also, both WCL streams were temporarily affected by forestry machinery crossing the stream in summer 2017. Land cover of the RGB streams is also forest, except an open meadow uphill of RGB3, which is used for low intensity cattle grazing during summer.

Soil runoff collection

Soil water was collected at two different soil depths (approximately 30 and 50 cm, referred to A and B, respectively in the following) from each hillslope (RBG, SBG, and WCL). These samples were drawn from soil runoff samplers, which were installed in wet locations in close proximity (< 15 m) to at least one of the two streams on each hillslope (Fig. 1). Each soil runoff sampler consisted of two stainless steel soil water collectors of 2 m width. The collectors were

pressed laterally (angle approx. 10° to allow water flow) into the hillslope (30-40 cm) at the respective depth. The samplers intercept soil water from the unsaturated zone of the soil profile as it drains downwards along the hillslope. The sampling of the soil samplers was then performed by placing acid washed and pre-combusted (4 h, at ~450 °C) 2 L Schott bottles below the outflow of each sampler the day before sampling. Sampling was then done by homogenizing the content of the Schott bottles and filtering samples of soil water, similar to stream samples. During many occasions, Schott bottles were overflowing at the time of sampling, while at the driest summer conditions, some soil samplers also remained dry.

DOC Sampling

Stream water samples were filtered in the field on 0.7 µm pre-combusted glass-fiber filters (Whatmann GF/F) into 40 mL glass vials using a syringe and syringe-type filter holders. Vials were prepared 'organic carbon free' by acid-washing and subsequent combustion (450 °C, 4 h). The filtrate was analyzed for DOC concentration within 24 h following sampling using a Sievers 900 TOC Analyser (GE Analytical Instruments, Boulder, CO, USA) operated with an inorganic removal unit.

Bacterial cells counting

Unfiltered water samples were fixed for 2 h with paraformaldehyde (1% final sample concentration) within 6 h after sampling. Fixed samples were then diluted 1:2 for stream water and 1:10 for soil runoff water using deionized and sterile ultrapure water. In order to separate bacteria from larger inorganic particles, samples were sonicated in a bath for 5 min and immediately filtered using sterile bolting cloth of 20 µm mesh-size. This method yields the total number of bacteria in the water column, independent of their potential bond to a particle. The filtrate was kept at 4 °C until counting, which was done within 48 h.

SYBR Green I was prepared by diluting a 10,000X stock solution (prod. nr. 7563; Invitrogen, Life Technologies, Paisley, UK) with sterile Tris EDTA buffer (AMRESCO Inc., Solon, OH, USA) to a concentration of 100X and was used to stain the samples at a final concentration of 1X. Dye was stored at -20 °C. The staining was performed in the dark for at least 20 min prior to analysis. All measurements were performed with a Beckman Coulter CytoFLEX flow cytometer (Beckman Coulter GmbH, Krefeld, Germany) equipped with a laser providing 50 mW at 488 nm and five different fluorescent channels. The threshold to trigger events was set in green fluorescence (525 nm) as stained bacteria would emit green light when excited with

blue (488 nm). Bacterial cells were discriminated from the background using previously measured unstained samples and green fluorescence versus side scatter dot plots.

Hydrology

We obtained precipitation data from the records of the meteorological station at the Biological Station Lunz operated by the Zentralanstalt für Meteorologie und Geodynamik (ZAMG), Austria. Daily values represent the accumulated precipitation (in mm) for the past 24 h, measured at 7 am CET.

Variability in stream runoff was analyzed using data from the Oberer Seebach station at the inlet of Lake Lunz (Fasching et al., 2015). As such, this station is located upstream of the study streams which drain directly into Lake Lunz (Fig. 1). However, the Oberer Seebach station was assumed more representative of headwater streams as compared to the station at the lake outlet. Stream discharge (Q in m^3 s^{-1}) was derived from 10 min water level measurements by using well established rating curves (Fasching et al., 2015). From this data, daily average discharge was calculated and used for further analysis. As some data was missing due to malfunctioning of logger systems, missing daily discharge values were estimated from the gage at the lake outlet ($R^2 = 0.91$ for daily Q's from both stations).

We classified flow conditions by the level of stream discharge. Based on daily average discharge on the whole study period, we defined discharge higher than 90% of the time ($Q > 1.86$ m^3 s^{-1}) as high flow. This classification based on the flow percentile is similar to the definition used in earlier work at the OSB (Fasching et al., 2015). However, we note that our threshold value is different, due to other hydrological conditions during our study period as compared to the earlier study. Similarly, low flow was defined as all discharge lower than the high flow threshold ($Q \leq 1.86$ m^3 s^{-1}), thus effectively representing intermediate and low flows.

Data analysis

DOC concentrations and bacterial abundances were tested for differences between high and low flows. In order to avoid statistical inconsistencies due to the nested sampling design, only a mean value of DOC concentrations and bacterial abundance per stream and sampling event was used for this comparison. Tests for normal distribution were conducted using the Shapiro-Wilk test. As the results indicated non-normal data, we performed non-parametric Mann-Whitney U test to compare the average difference in DOC concentrations and bacterial abundances between high flow and low flow. We considered $p < 0.05$ as significant and $p <$

0.001 as highly significant. To compare the correlation between DOC and bacterial abundances from stream water with the one from soil water, we used an analysis of covariance with the type of sample as the nominal variable. Because of the large range of values measured for both DOC concentrations and bacterial abundances, data was log-transformed (log_{10}) before the analysis. When seasonal comparisons (also Mann-Whitney U test) were carried out, seasons were defined similar to earlier work (Fasching et al., 2015) by the month as follows: Spring from 01.03 to 31.05; summer from 01.06 to 31.08; autumn from 01.09 to 30.11; and winter from 01.12 to 28-29.02.

Results

DOC concentrations

DOC concentrations ranged from 0.39 to 5.56 mg L^{-1} with an overall mean and standard deviation of 1.16 ± 0.56 mg L^{-1} in stream water throughout the study period (Fig. 2). All streams presented an increase in DOC concentration of on average 17.9% between the most upstream and the most downstream sampling locations (Fig. 3). This increase along the streams' flow path was significant during low flow conditions (+18.1%, Mann-Whitney U, $p < 0.001$) but not during high flow conditions (+4.4%, $p = 1$). Further, stream DOC was found to be lower during the winter – with a mean concentration of 0.87 ± 0.2 mg L^{-1} – and higher during autumn and summer – with respective means of 1.16 ± 0.35 and 1.31 ± 0.53 mg L^{-1}. The higher values were measured during high flow events, especially summer storms (Fig. 4a). During storms, the mean DOC concentration increased significantly by 44% (Mann-Whitney U, $p < 0.001$), from 1.1 ± 0.36 to 1.57 ± 0.7 mg L^{-1} as compared to low flow conditions.

In soil runoff, measured DOC concentrations were significantly higher than in streams, ranging from 0.98 to 36.9 mg L^{-1} with an overall mean of 9.37 ± 8.21 mg L^{-1}, being eight times higher than the overall stream average. However, in soil water, DOC concentrations were not found to differ significantly between high flow and low flow conditions (Mann-Whitney U, $p = 0.9$) and seasons (pairwise Mann-Whitney U, $p = 0.38$) (Fig. 4c).

Bacterial abundances

Bacterial abundances ranged from 19,300 to 3,862,800 cells mL^{-1} with an overall mean of 394,000 ± 509,000 cells mL^{-1} in stream water throughout the year (Fig. 2). Concentrations were found to be lower during winter – with a mean abundance of 162,900 ± 69,500 cells mL^{-1} – and higher during summer – with a mean abundance of 488,700 ± 514,400 cells mL^{-1}. In contrast

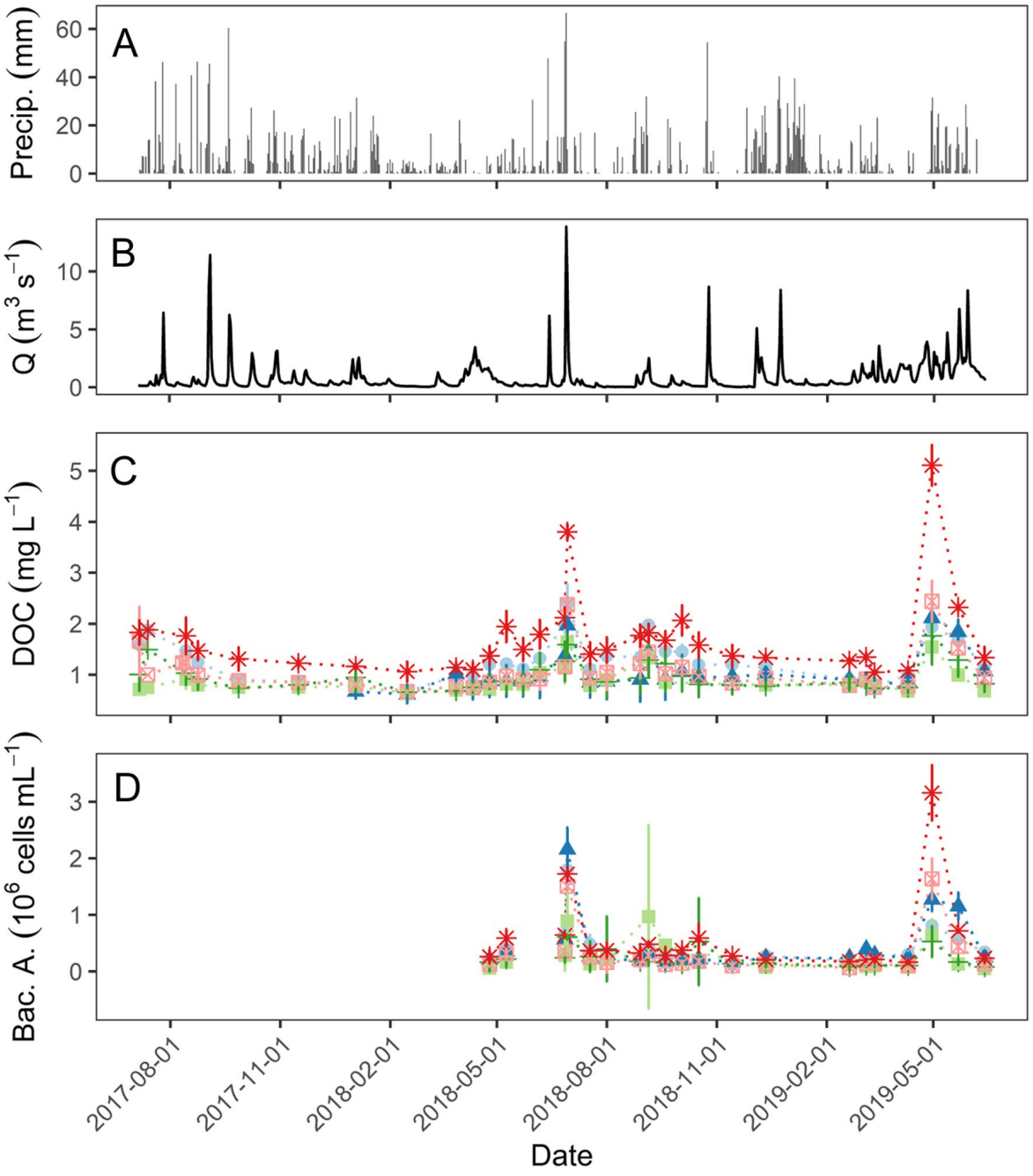

Fig. 2 (**a**) Daily precipitation and (**b**) daily discharge in the OSB, (**c**) DOC concentrations and (**d**) bacterial abundances throughout the study years. Symbols denote the streams as follows: blue dots RBG2, blue triangles RBG3, green filled squares SBG1, green pluses SBG2, red crossed squares WCL1, and red stars WCL2. Each symbol represents a mean, bars the standard deviation of all samples taken along each stream during one sampling occasion, respectively. The dataset includes a total number of 811 DOC samples and 547 bacterial samples.

to DOC, only some streams showed an increase in bacterial abundances between the most upstream and the most downstream sampling locations, while in others no consistent pattern was visible (Fig. 3b). Similar to DOC, high values were measured during high flow events (Fig. 4b), during which the mean bacterial abundance in streams increased significantly by 164.5%

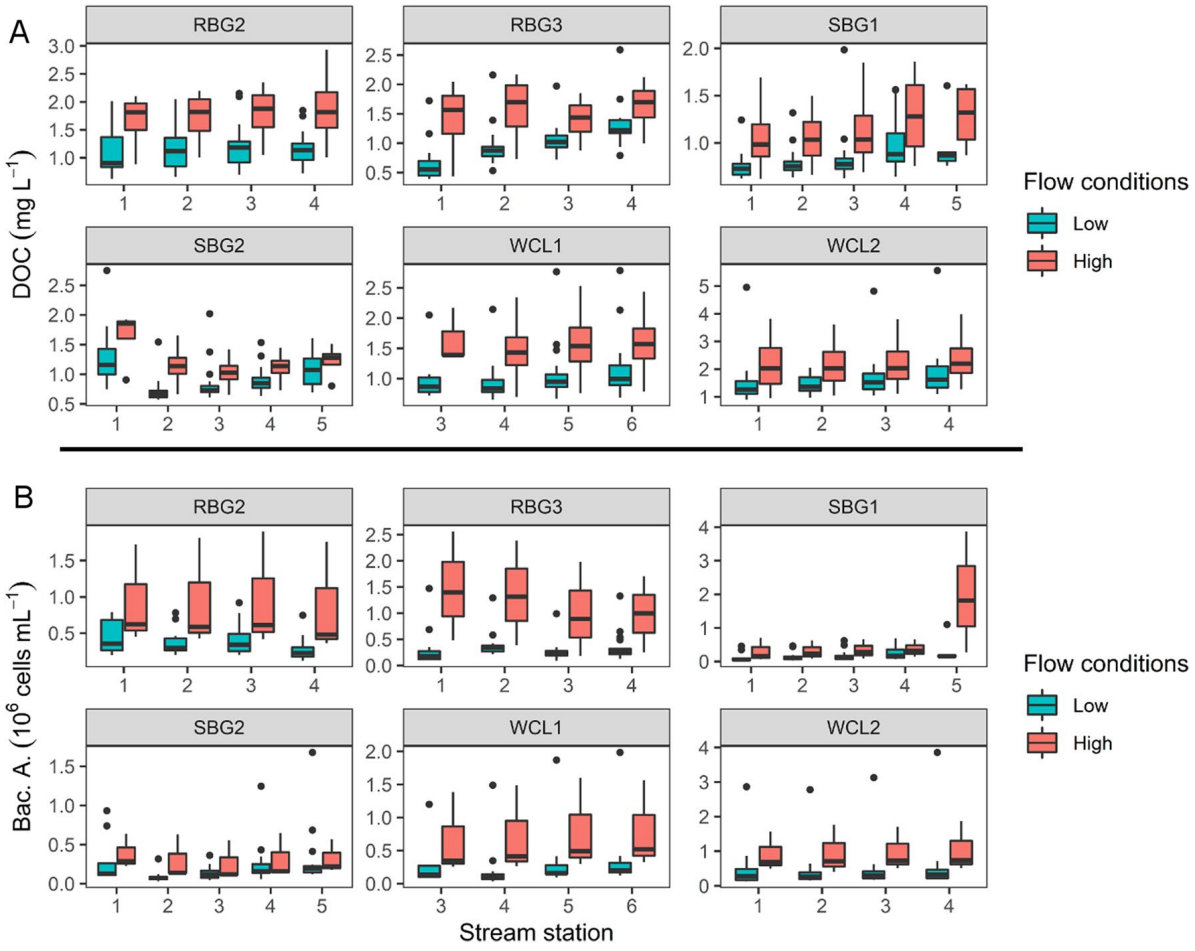

Fig. 3 (a) Changes of DOC concentration and (**b**) of bacterial abundances along the flow path of the six headwater streams at low and high flow conditions. Station numbers at X denote the location along the flow length, with one being closest to the streams source, while the highest number is closest to the outlet to Lake Lunz. Horizontal lines show the median, boxes the 25th to 75th percentiles, whiskers the 5th to 95th percentile range. Black dots are values outside the interquartile range.

(Mann-Whitney U, $p < 0.001$), from 304,700 ± 372,400 to 806,100 ± 620,700 cells µL^{-1} as compared to low flow conditions. In soil runoff, measured bacterial abundances were significantly higher than in streams ($p < 0.001$), ranging from 327,300 to 35,412,300 cells mL^{-1} with a mean of 4,450,400 ± 5,985,200 cells mL^{-1}, being ~10 times higher than the overall stream average. Similar to DOC, bacterial abundances were not found to be significantly different between high flow and low flow conditions in soil water ($p = 0.3$) (Fig. 4d).

Correlation between DOC and bacterial abundance

We investigated the relationship between DOC concentration and bacterial abundance in stream and soil water (Fig. 5). Log_{10} DOC was found to be positively correlated with log_{10} bacterial abundance in stream water ($r^2 = 0.61$, $p < 0.001$) and also in soil water ($r^2 = 0.34$, $p < 0.05$).

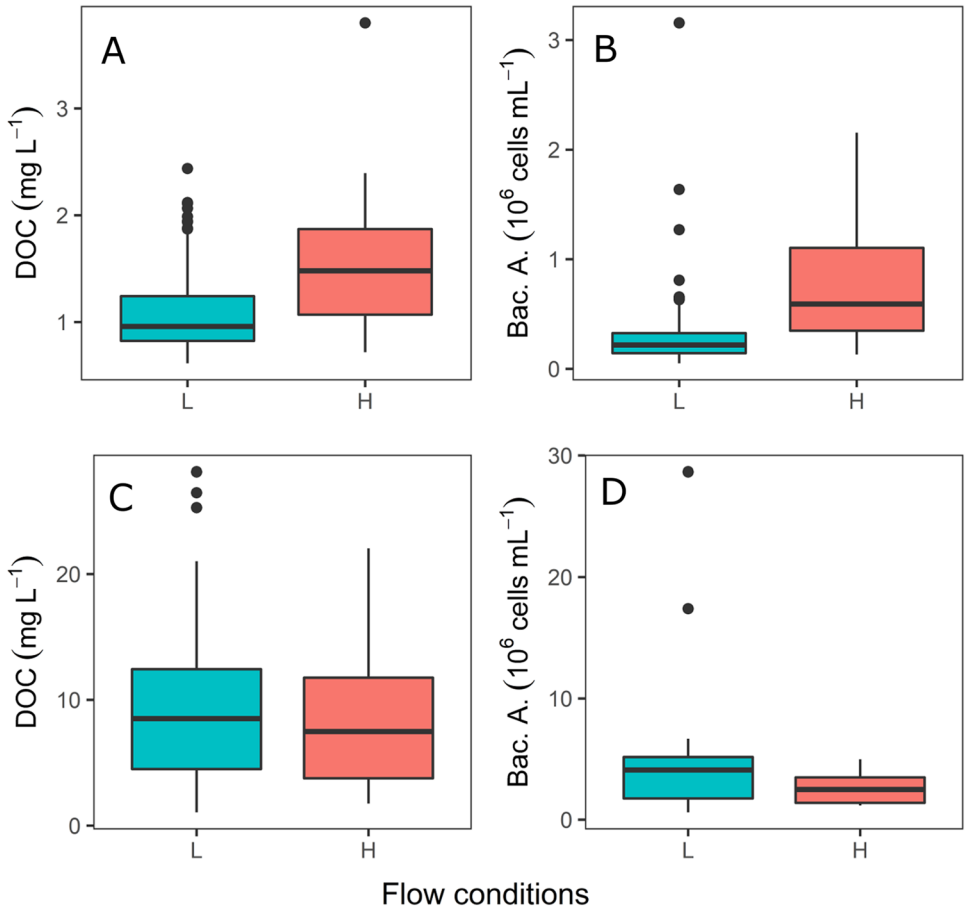

Fig. 4 Boxplots of (**a**) DOC concentration and (**b**) bacterial abundances in streams, and (**c**) DOC concentration and (**d**) bacterial abundances in soils at low flow (L) and high flow (H), respectively. Boxplot are the same as in Fig. 3.

The slopes of the regression lines were significantly different (0.35 for stream and 0.47 for soil water, respectively, $p < 0.01$) as well as their y-intercept ($p < 0.001$).

Discussion

The major finding of this study is that the mobilization of bacteria from catchment soils to headwater streams is controlled by hydrological variability. Specifically, we provide evidence that high-flow events, such as rainstorms, cause an enhancement of bacterial mobilization (Fig. 4). Further we show that the timing of increase and the magnitude are similar to the mobilization of DOC from catchment soils. But whereas the responses of DOC to storm events in first order streams have been well described in the past (Fasching et al., 2015; Hinton et al., 1998), we now demonstrate that dynamics similar to those of DOC are also controlling microbial abundances in small streams.

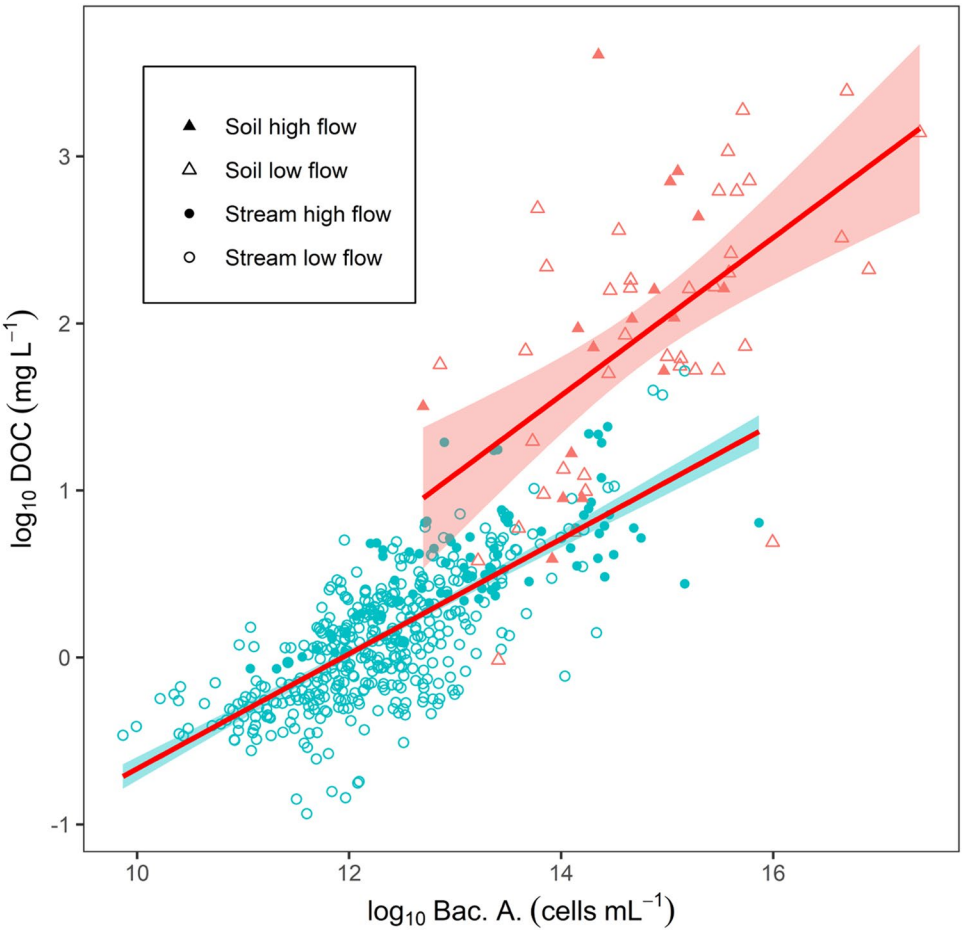

Fig. 5 Log_{10} bacterial abundance versus log_{10} DOC concentration in stream water (green circles/dots) and soil water (open/filled triangles). Open symbols depict low flow and filled symbols high flow conditions, respectively. Shaded areas show 95% confidence level for predictions of the linear model.

We observed an increase in DOC concentration at all streams along their flow length from their spring to the end of our study reach (Fig. 3a). We propose that this increase is the result of intensified soil contributions along the streams flow length. Our study was located in a karst region. Most permanently draining streams in this region are groundwater fed, as they would otherwise cease during dry spells. The increase of DOC along the streams indicates that our study streams are likely fed with spring water with low DOC that is then enriched along the flow at the steep hillslope by high DOC soil water. This conceptual model contrasts somewhat with the traditional view of first order streams being a direct link between catchment soils and streams in other regions (Schelker et al., 2012).

As an alternative explanation, enrichment of DOC along the flow length could also be generated by enhanced primary production that enhances DOC concentrations, especially during spring and summer low flow (Harjung et al., 2019). However, many previous studies have shown that

first order streams presented very low autochthonous primary production that accounted for less than 5% of the annual organic carbon inputs in these ecosystems (Fisher & Likens, 1973; Mulholland, 1997; Richardson, 2019). In addition, the fact that the increase was independent of season, i.e., that it was also present during the fall and winter, makes this explanation less probable. Further, if DOC deliveries from primary production were relevant, additional variation based on light availability for stream primary producers would be expected (Dodds et al., 1996; Hall et al., 2015). Our study streams differed in their light regimes (shading by closed forest canopy at SBG1, SBG2 and RGB2, while WCL1, WCL2 and RBG3 are at least partly open, Fig. 1), as well as their aspect. However, we were unable to identify a light-induced variation in our downstream DOC pattern. Thus, we conclude that increasing soil contributions along the flow length is the most probable explanation for the increasing DOC concentrations along our study streams. Interestingly, an increase in bacterial abundances along the stream flow path was also indicated for some streams (Fig. 3b) but this increase was not significant and generally weaker than the one observed for DOC concentrations. At present, we cannot explain these differences, but consider soil community dynamics or minor variations of how bacteria are mobilized from soils as compared to DOC as possible explanations. However, these minor differences appear to be overwhelmed during events resulting in synchrony in increase.

Our findings on the variation of DOC concentration and bacterial abundance with varying discharge expand our present knowledge on the dynamics of headwater streams. High flow events led to a significant increase in DOC concentration by 70.8% and in bacterial abundance by 32.4%. Previous work has described the increase of DOC concentration in streams during storms following enhanced soil contributions (e.g. Ågren et al. 2008; Fasching et al. 2015; Hinton et al. 1998).

The link between soils and streams in terms of microbial communities has been investigated in the past using sequencing techniques (Crump et al., 2012; Ruiz-González et al., 2015; Savio et al., 2015). However, few studies have investigated the temporal dynamics of bacterial abundances in streams (Hullar et al., 2006; Richardson, 2019). Here we found that high flow conditions, as they are commonly present in our study streams during rainstorms or snowmelt, caused a general increase in bacterial abundances. This pattern may be explained by increased deliveries of microbes from the catchment soils, similar to the mobilization of DOC. Alternatively high bacterial abundances could also be caused by the remobilization of in-stream bacteria in upstream reaches. The latter would be caused by high flow velocities transiently

enhancing the shear stress on benthic biofilms. The biofilm would then release bacteria during high flow (Paul et al., 2012; Stewart, 1993). However, some of our observational points were located at the very source area of the first order streams (upstream length < 20 m). These sites showed the same increase in bacterial abundance during events, as those further downstream. Thus, we suggest that provision of microbes from upstream reaches is small, as compared to soil contributions.

The water residence time is small in the study streams, ranging from minutes to hours at the maximum. For example, we quantified a mean flow velocity of 0.1 m s^{-1} across a distance of ~ 100 m in WCL1 at intermediate flow conditions. Assuming similar velocities for all streams suggest that the residence time in the study reaches rarely exceeds 2 h. We argue that this is too short for relevant bacterial growth in the free-flowing water column, especially during high flow events. Therefore, we attribute the dynamic increases in bacteria during runoff events in our streams to enlarged soil contributions, rather than instream growth.

Following these notions, we suggest that soil inoculation of headwater streams is a dynamic process that may be largely controlled by the intensity of hydrometeorological events. Consequently, we propose that most headwater streams are subject to a dynamic and reoccurring microbial inoculation from their catchment soils during storm events. This dynamic microbial inoculation of streams by soils may then have some impact on the development of the microbial communities in the downstream river network (Besemer et al., 2013; Ruiz-González et al., 2015; Savio et al., 2015; Widder et al., 2014). However, at present this potential impact remains largely unknown, as most previous studies on the role of soil inoculation on stream microbial communities did not consider the temporal dynamics of inoculation (Besemer et al., 2013; Monard et al., 2016; Ruiz-González et al., 2015; Widder et al., 2014).

The correlation we found between $\log_{10}$ DOC and $\log_{10}$ bacterial abundance in streams further supports our view that both constituents are mobilized concurrently from the catchment soils and that this mobilization is enhanced during high flow. The general mechanisms proposed for DOC mobilization from soils to streams include the flushing of DOC from the organic top soil (A-horizon) (Boyer et al., 1996), as well as from carbon rich riparian soils (Laudon et al., 2011; Schelker et al., 2013). Similarly, the abundance of soil bacteria has been suggested to vary with soil depth, with the highest abundance and microbial diversity present in the organic rich top soils (Fierer et al., 2003). This suggests that conceptual models developed for the mobilization of DOC from soils to streams (Boyer et al., 1996; Laudon et al., 2011; Weiler & McDonnell,

2006; Zarnetske et al., 2018) could potentially also be applied to explain the variation in bacterial abundances in streams in the future. Nevertheless, the high ratio between soil-stream interfaces and water volume in headwater streams promotes transfer from soil to streams but the relationship might be weaker in larger fluvial systems. Also, pre-alpine streams have low DOC concentrations (Fasching et al., 2015) in comparison to other fluvial ecosystems, such as boreal streams (Dawson et al., 2008; Laudon et al., 2011). Further investigations must be conducted to determine if the relationship between DOC concentrations and bacterial abundances also holds true in more DOC-rich environments.

Interestingly, the strong correlation of $\log_{10}$ DOC concentration per $\log_{10}$ bacterial abundance observed in streams was also present in soil water. Although the slopes and the Y-intercepts of the linear models for soils and streams were significantly different, the general pattern of increasing $\log_{10}$ abundance with increasing $\log_{10}$ DOC remained. However, the significant differences between the relationships likely indicate fundamental differences in ecosystem structure regarding the availability of carbon versus the size of the maintained bacterial population in stream versus soil ecosystems.

Among the main distinctions between lotic and terrestrial ecosystems is the difference in the ratio between dissolved and particulate organic carbon, with a larger ratio in streams versus soils (Grimm et al., 2003). Also, primary producers show stoichiometric and lifespan differences (lower C:N and P:N ratios, more short-lived in streams) (Grimm et al., 2003; Nowlin et al., 2008). Thus, one possible explanation for the observed difference in the relationships in our oligotrophic system would be that streams can maintain higher populations of primary producers during nutrient limitation. Further, the generally higher ratio of consumers versus producers in streams (Grimm et al., 2003) could then result in the difference of higher $\log_{10}$ bacterial abundance versus $\log_{10}$ DOC.

Alternatively, differences in the size of the maintained microbial population between soils and streams could also stem from differences in DOM composition between the two systems. In soils, degraded humic-like DOM components are typically dominant, while in streams labile, protein-like DOM is present along with humic substances (Fasching et al., 2015; Hutchins et al., 2017). This could also cause a general advantage in maintaining an extended microbial population in streams.

Future research should address two main aspects. First, the precise nature of the microbial communities that are delivered from soils to streams during hydrological events. At present, the community composition of these pulses of microbial life are not well understood (Monard et al., 2016; Ruiz-González et al., 2015). Second, there is currently only limited knowledge about the relevance of the dynamic and reoccurring microbial soil inoculation for stream microbial community development in the receiving waters (Besemer et al., 2013; Widder et al., 2014).

Overall, we show that soils provide a dynamic inflow of bacteria and DOC to first order streams resulting in a dynamic and reoccurring inoculation of stream microbial communities from catchment soils during runoff events. We propose that this process is potentially important for the development of microbial communities of downstream river networks. We suggest future research on the community composition and the potential downstream effects of this dynamic soil inoculation process.

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Chapter 2. Soil microbial inoculation during flood events shapes headwater stream microbial communities and diversity

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Abstract

Flood events are now recognized as potentially important occasions for the transfer of soil microbes to stream ecosystems. Yet, little is known about these '*dynamic pulses of microbial life'* for stream bacterial community composition (BCC) and diversity. In this study, we explored the potential alteration of stream BCC by soil inoculation during high flow events in six pre-alpine first order streams and the larger Oberer Seebach. During one year, we compared variations of BCC in soil water, stream water and in benthic biofilms at different flow conditions (low to intermediate flows versus high flow). Bacterial diversity was lowest in biofilms, followed by soils and highest in headwater streams and the Oberer Seebach. In headwater streams, bacterial diversity was significantly higher during high flow, as compared to low flow (Shannon diversity: 7.6 versus 7.9 at low versus high flow, respectively, $p < 0.001$). Approximately 70% of the bacterial Operational Taxonomic Units (OTUs) from streams and stream biofilms were the same as in soil water, while in the latter one third of the OTUs were specific to high flow conditions. These soil high-flow OTUs were also found in streams and biofilms at other times of the year. These results demonstrate the relevance of floods in generating short and reoccurring inoculation events for flowing waters. Moreover, they show that soil microbial inoculation during high flow enhances microbial diversity and shapes fluvial BCC even during low flow. Hence, soil microbial inoculation during floods could act as a previously overlooked driver of microbial diversity in headwater streams.

Introduction

Streams and rivers maintain large microbial communities that are essential for the functioning of fluvial ecosystems. Microbial communities in streams contribute significantly to biogeochemical cycles of essential nutrients, such as carbon and nitrogen that they respire, convert and metabolize (Tolkkinen et al., 2020), drive organic matter decomposition (Fasching et al., 2020) and carbon dioxide evasion (Kreutzweiser & Capell, 2003), and are critical for the transfer of carbon to higher trophic levels. Moreover, the capacity of aquatic microbes to reduce nutrient contamination often defines the suitability of water resources for the human use (Ward et al., 2018).

Stream microbial diversity is enormous (Besemer et al., 2013) and varies depending on the position in the aquatic continuum (Cruaud et al., 2020; Ruiz-González, Niño-Garcia, et al., 2017). In headwater streams, the furthest upstream tributaries in river networks, microbial community diversity is high and has been partly found to resemble soil microbial communities (Crump et al., 2012; Ruiz-González et al., 2015). This is likely caused by significant transport of soil bacteria from catchment soils into headwater streams (Caillon & Schelker, 2020; Mansour et al., 2018; Staley et al., 2016). Further downstream, bacterial richness has been observed to decrease while the proportion of typical freshwater taxa increases (Hermans et al., 2019; Savio et al., 2015). As directional dispersal occurs within the dendritic structure of river networks (Altermatt, 2013; Wisnoski & Lennon, 2020), headwater streams have been named as reservoirs of microbial diversity of river network metacommunities (Besemer et al., 2013).

A large part of the fluvial freshwater microbiome lives attached to surface biofilms (Battin et al., 2016; Geesey et al., 1978). Biofilms are stable physical structures in which bacteria live together with algae and other microbes (Battin et al., 2016). Although stream biofilms are assumed to assemble from the suspended bacterial community present in the water column, biofilm microbial assemblages are noticeably different from free-flowing communities (Besemer et al., 2012). As processes, such as species sorting, reduce stream biofilm diversity through competition, the biofilm diversity is believed to be maintained by a continuous inflow of microorganisms from upstream catchments (Besemer et al., 2012; Gautam et al., 2020). Vice versa, stream biofilms may also promote the dispersal of aquatic bacterial species within aquatic networks, for example when biofilms release microbes and thereby re-inoculate the free-flowing stream community with these taxa (Battin et al., 2016).

Bacterial communities in lotic systems show variations in their taxonomic composition over time in response to habitat conditions (Zeglin, 2015). Environmental variables such as nutrient availability (Olapade & Leff, 2005), water temperature (Adams et al., 2010), organic matter availability (Kobayashi et al., 2009), and, notably, water flow (Richardson & Danehy, 2007) can all influence community composition. However, the temporal dynamics of water flows affect not only the biophysicochemical conditions within fluvial systems, but also the direct transfer of microbes from the catchment soils into streams (Caillon & Schelker, 2020).

Soils are connected to river networks by water flows that enter streams through the riparian zone (Richardson & Danehy, 2007; Sidle et al., 2000). This connection is highly dynamic and is controlled by soil moisture (Penna et al., 2011) and precipitation (Sidle et al., 2000). Through this connection, soils provide nutrients, organic carbon and microbes to the stream ecosystem (Fasching et al., 2020; Risse-Buhl et al., 2020; Savio et al., 2015; Staley et al., 2016). Soil bacterial abundance and diversity is large, with some estimates suggesting up to 10^6 bacterial species in one gram of soil (Fierer, Bradford, et al., 2007). Thus, soils provide a potentially large source of microbial diversity for stream ecosystems, if soils become transiently connected to streams during hydrological events.

Previous work questioned if soil bacteria would be capable to cross the terrestrial-freshwater interface and successfully establish populations within freshwater ecosystems (Monard et al., 2016). However, other findings suggest that at least some microbes originating from soils can thrive in freshwater environments (Ruiz-González, Niño-García, et al., 2017). For example, Fenchel et al. (1994) found that soil microbes could develop in interstitial waters between soils particles. Further, more recent studies recognized a high overlap between the microbial communities of soils and freshwater environments (Crump et al., 2012; Hassell et al., 2018; Ruiz-González et al., 2015). Yet despite these notions, there is to date little knowledge on the precise spatiotemporal organization of soil microbial inoculation of stream ecosystems.

In this study, we aimed to explore the effects of the changes in the connectivity of catchment soils and streams caused by hydrological dynamics for the assembly of stream bacterial communities. Given the previously observed elevated bacterial abundances in streams during high flow events (Caillon & Schelker, 2020), we propose that soil microbial inoculation of streams takes place primarily during high flow events, when soil water contributions to streams are highest. We hypothesized that bacterial diversity in streams will be higher during high flow conditions, as compared to low flow. Furthermore, we proposed that bacterial communities in

soils and streams homogenize at high flow due to an increase in soil contributions to small streams during floods. We evaluated these hypotheses by comparing bacterial community composition and diversity in streams and soil water under different flow conditions.

Methods

Study site and sampling

We sampled soil water, stream water and benthic biofilms once a month and during high flow events over a period of five months, resulting in six sampling occasions, including two high flow events. Sampling was carried out in the Oberer Seebach Catchment, around Lake Lunz in the eastern Alps near Lunz am See, Austria (Fig. 1). This catchment is largely pristine and the Oberer Seebach (OSB) is a well-studied stream with meteorological and discharge data available (Ejarque et al., 2018; Fasching et al., 2015). The catchment is dominated by mixed deciduous forest with some meadows used for low intensity cattle grazing during summer. For this study, we selected three different hillslopes of the catchment (Rehberg, RBG; Schlögelberg, SBG; and the WasserCluster Lunz slope, WCL), each located within a radius of 1.5 km around

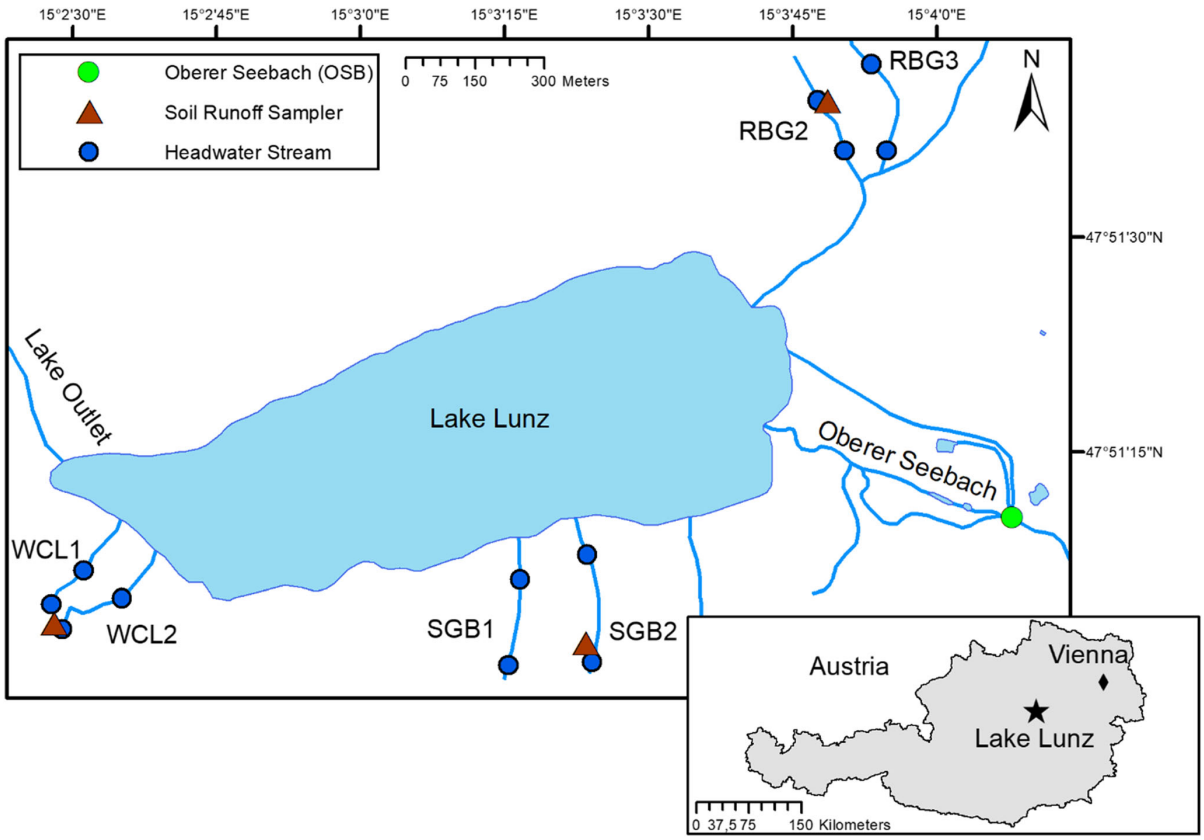

Fig. 1 Locations of sampled soil water, headwater streams, and Oberer Seebach near Lake Lunz in Austria. Dots represent stream sampling stations and brown triangles soil runoff samplers.

Lake Lunz to ensure equal weather conditions (i.e. air temperature, precipitation). On each hillslope, we sampled two headwater streams (Strahler order 1) and soil water from two different depths (around 20 cm and around 50 cm deep). All headwater streams have similar width (< 1 m), depth (< 10 cm) and slopes (mean 29%) and are reasonably fast flowing (flow velocity 0.1 m sec^{-1} at intermediate flow conditions; Caillon and Schelker 2020).

We drew soil water samples from soil runoff samplers, which were installed in wet locations in close vicinity (< 15 m) to the source of one headwater stream on each hillslope. Each soil sampler consisted of two stainless steel soil water collectors of 2 m width that were pressed laterally into the hillslope (30-40 cm) at the respective depth (Caillon & Schelker, 2020). Soil depth was not considered in this study and both samples were used as replicates. The samplers intercept soil water from the unsaturated zone of the soil profile as it drains downwards along the hillslope, essentially representing mobile soil water on its way towards the stream. We performed the sampling by placing acid washed and pre-combusted (4 h, at ~450 °C) 500 mL Schott bottles below the outflow of each sampler the day before sampling. During the driest summer conditions, some soil runoff samplers remained dry, reducing the number of soil water samples to 18.

In all six headwater streams, we collected stream samples at two different locations, one near the source of the stream (and the soil runoff samplers) and one ~400-500 m further downstream. Water samples were collected using pre-combusted (4 h, at ~450 °C) 500 mL Schott Bottles and sterile 15 mL syringes when necessary.

Additionally, we collected water from a larger stream, the OSB (Strahler order 3) at three different locations across the width of the stream. This stream was sampled in order to compare its bacterial community to the communities upstream in the headwaters and because of the extensive data available for it.

Finally, we also sampled biofilms from the OSB due to their implication for bacteria dispersal and the potential impact of soil inoculation on their communities. Sterile ceramic tiles (5 by 5 cm), glued on bricks to prevent them from erosion, served as substratum for biofilm growth in the stream. Tiles were installed on the streambed four weeks prior to sampling to allow for biofilm growth. During flood events, the water level of the OSB was too high to allow for the collection of the tiles and, therefore, we could only sample one biofilm sample at high flow

conditions. After sampling, tiles were kept at -20 °C in sterile plastic bags until further processing.

In total, we collected 96 samples for the characterization of bacterial communities at different flow conditions (soil water n = 18, headwater streams n = 58, OSB water n = 14, and OSB biofilms n = 6).

DNA extraction, PCR and Illumina sequencing

Water samples were kept at 4 °C and, within 10 h, were filtered in the lab on 0.2 µm mixed cellulose filters (Whatman ME24) and stored at -20 °C in 15 mL sterile tubes until further processing. We extracted DNA from the filters and the biofilms using the DNeasy PowerSoil extraction kit (Qiagen) following the manufacturer's protocol. This kit has been used successfully in earlier studies on stream water and biofilms (e.g. Kobayashi et al. 2009). Prior to our extraction, we cut the filters into pieces using ethanol-flamed tweezers and scissors, biofilms were removed from the tiles using ethanol-flamed razor blades and spatula. The V3-V4 region of the 16S rRNA gene was amplified using the bacteria specific primers 341F (5'-CCT ACG GGN GGC WGC AG-3') and 785R (5'-GAC TAC HVG GGT ATC TAA KCC-3') (Thijs et al., 2017). PCR amplification, Illumina MiSeq library preparation and sequencing using V3 chemistry and 2 x 300 bp paired-end reads was carried out by LGC Genomics GmbH (Berlin, Germany). Sequences are accessible at the National Center for Biotechnology Information Sequence Read Archive e under the accession number PRJNA685744.

Bioinformatics

We processed amplicon sequences as in Cholet et al. (2019). The first steps followed the recommendations of Schirmer et al. (2015). We used sickle v1.2 to perform quality trimming of paired-end reads using a 20 bp sliding window. We trimmed reads where the average quality score dropped below 20 and discarded reads below 10 bp length. Then, we applied BayesHammer (Nikolenko et al., 2013) from the Spades v2.5.0 assembler (Bankevich et al., 2012) to error correct the paired-end reads. Next, we used PANDAseq v2.4 (Masella et al., 2012) to assemble overlapping forward and reverse reads using a minimum overlap of 10 bp.

We followed the VSEARCH v2.3.4 pipeline for construction of Operational Taxonomic Unit (OTU) (Rognes et al., 2016). The pooled sequences were de-replicated, sorted and singletons discarded. We clustered sequences were clustered on a 97% identity level. We also performed *de novo* chimera detection by searching for potential parent reads, followed by reference-based

chimera detection using the Silva aligned version of the gold database (https://www.mothur.org/w/images/f/f1/Silva.gold.bacteria.zip). Then we matched the original barcoded sequences against the clean OTUs with a 97% similarity threshold to generate OTU tables. We derived the taxonomic assignment by classification against the SILVA SSU Ref NR v123 database at a 90% confidence threshold. OTUs that occurred less than 10 times in the dataset, were classified as chloroplasts, or were not classified as bacteria, were excluded from further analysis. Also, one biofilm sample had a low number of reads (85) and was removed from the dataset. The cleaned dataset consisted of 7 418 358 sequences clustered into 25 985 OTUs. All OTUs could be classified to a phylum or lineage.

Hydrology

Variability in stream runoff was analyzed using data from the OSB station at the inlet of Lake Lunz (Ejarque et al., 2018; Fasching et al., 2015). Stream discharge (Q in $m^3\ s^{-1}$) was derived from 10 min water level measurements by using well established rating curves (Fasching et al., 2015). From this data, daily average discharge was calculated and used for further analysis. As some data was missing due to malfunctioning of logger systems, missing daily discharge values were estimated from the gage at the lake outlet ($R^2 = 0.91$ for daily Q's from both stations).

Flow conditions were classified by the level of stream discharge similar to earlier work. In short, daily average discharge higher than 90% of the time in our study period ($Q > 1.86\ m^3\ s^{-1}$) was defined as high flow (Caillon & Schelker, 2020; Fasching et al., 2015). Low flow was defined as all discharges lower than high flow ($Q \leq 1.86\ m^3\ s^{-1}$) and thus effectively also includes intermediate flow conditions (Caillon & Schelker, 2020).

Statistical analysis

Statistical analyses were performed in R with the *phyloseq*, *stats*, and *vegan* packages (McMurdie & Holmes, 2013; Oksanen et al., 2019; R Core Team, 2020). Non-metric multidimensional scaling with Bray-Curtis distances was used to ordinate the samples based on their dissimilarity in community composition. ANOSIM analysis was carried out with 9,999 permutations to test the differences between habitat type and flow conditions.

Richness and Shannon diversity (the number equivalent of the Shannon entropy; Jost 2007) were calculated after rarefying to the lowest number of reads obtained from a sample (9,905 reads) to account for differences in sequencing effort. Shapiro-Wilk test for normal distribution on the data indicated that richness was normally distributed while Shannon diversity was not.

ANOVA and Tukey tests were used to compare the difference in richness between sample types and flow conditions. Mann-Whitney U tests and Kruskal-Wallis tests were used for Shannon diversity.

To identify potential habitat specialists and the impact of flow conditions on their dispersal, we calculated indicator values for each OTU using the *labdsv* R package (Roberts, 2019). We considered indicator species (in our case OTUs) as significant for indicator values > 0.7 and *p-values* < 0.05. Additionally, we carried out a fast expectation-maximization for microbial source tracking (FEAST) analysis on bacteria using the *FEAST* R package. This analysis allows to estimate the contribution of one habitat as a source, to another as a sink (Shenhav et al., 2019).

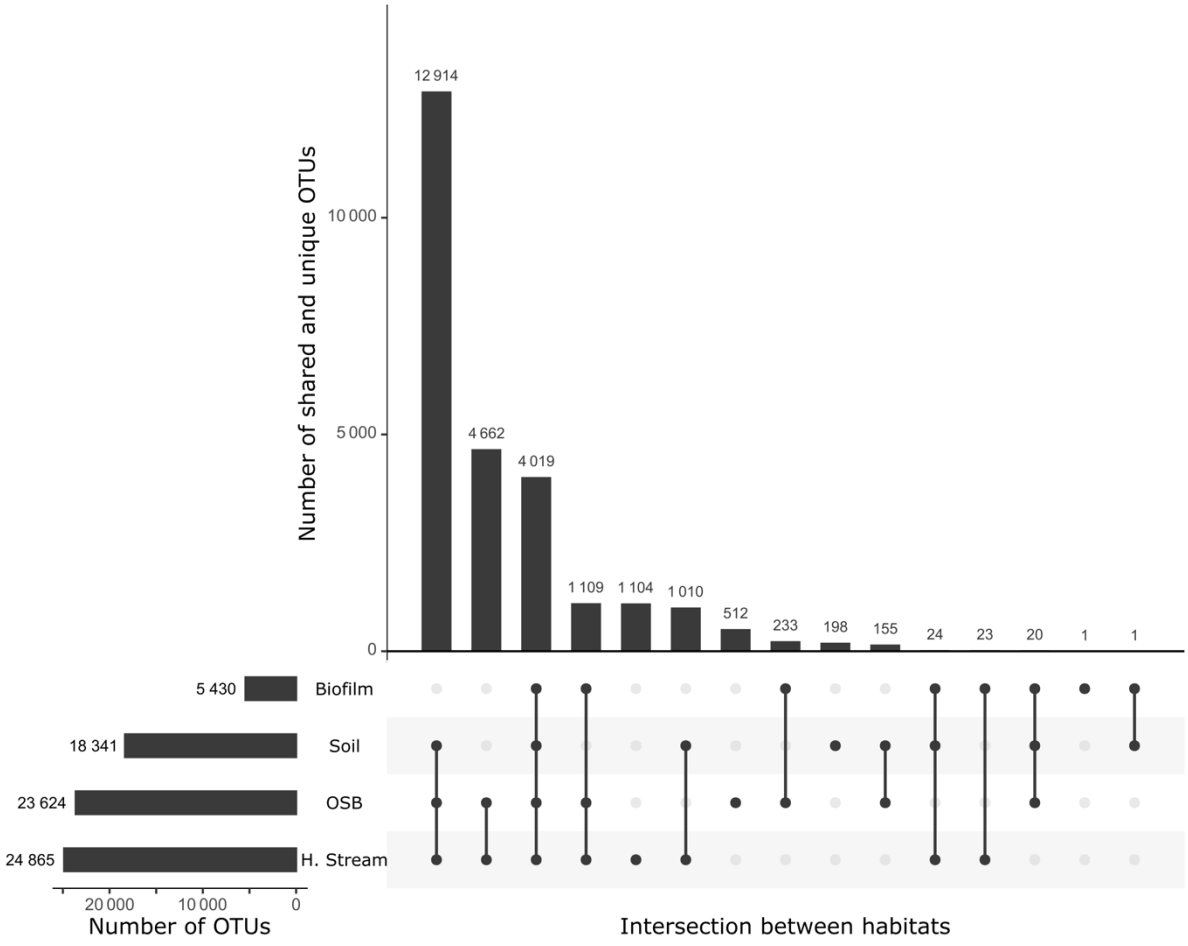

Fig. 2 Number of shared and unique OTUs between soil water, headwater streams (h. streams), OSB water, and biofilms bacterial communities. The total number of OTUs detected in each habitat is represented in the horizontal histogram. The vertical histogram represents the size of the intersection (the number of shared OTUs) between the habitats that are connected in the lower panel.

Results

Similarities in microbial communities across habitats

In total, we recovered 25,985 OTUs of which 18,341 were present in soil water, 24,865 in stream water, 23,624 in the OSB water and 5,430 in the biofilms. When we compared the different habitats, a large overlap was observed in the bacterial OTUs present in streams and the catchment soils (Fig. 2). Headwater streams, the OSB and soil water shared 12,914 common OTUs. Further, we found that 72% of OTUs found in headwater streams, 72% of OTUs found in the OSB and 75% of OTUs found in biofilms were also present in soil water. A strong overlap

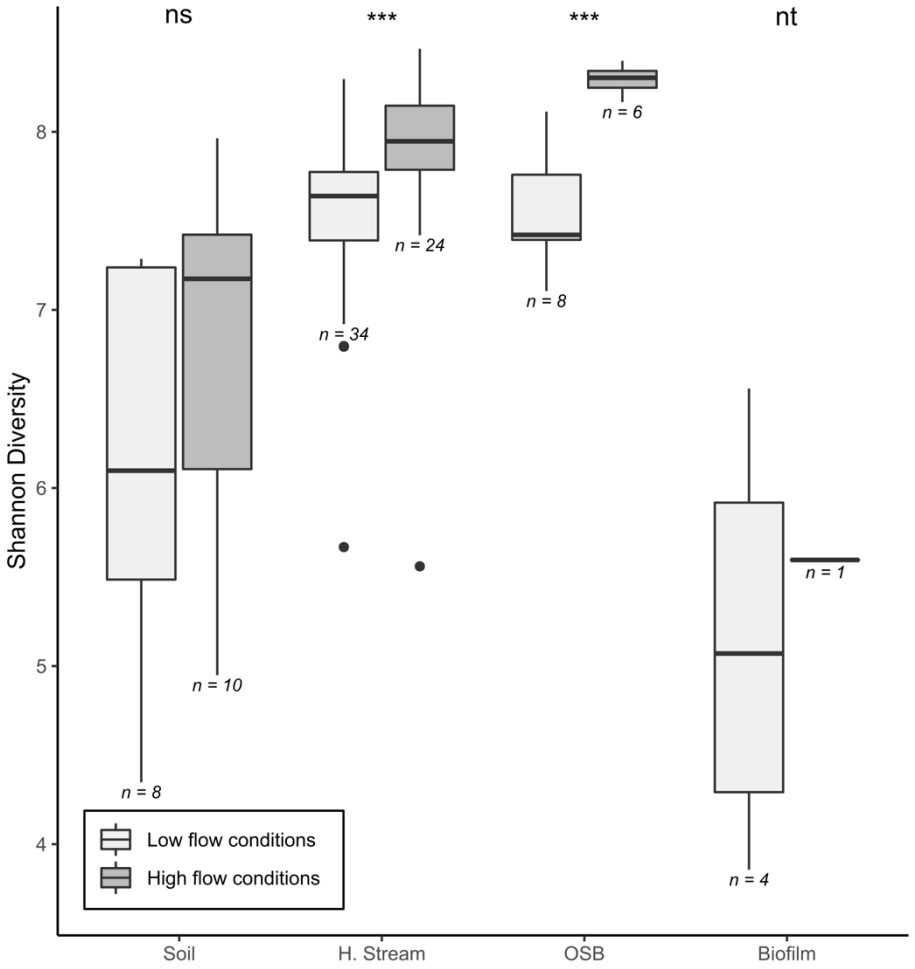

Fig. 3 Shannon diversity index for each habitat of the soil-stream-river interface. Shannon diversity was calculated for high and low flow conditions. Horizontal lines show the median, boxes the 25th to 75th percentiles, whiskers the 5th and 95th percentile range. Black points are values outside the interquartile range. Values presented below each boxplot refer to sample size. Test results presented at the top refer to the high versus low flow comparisons of each compartment; 'ns' denotes not significant, 'nt' not tested, and '***' a highly significant difference (Mann-Whitney U, $p < 0.001$). No statistical test was conducted on biofilm samples since there was only one biofilm at high flow. H. stream stands for headwater streams.

between bacterial communities was also observed between headwater streams and the OSB. These two habitats had 22,704 OTUs in common, which represents 91.3% of the headwater stream OTUs and 96.1% of the OSB OTUs. Biofilm communities represented a subset of the OSB OTUs, with only 49 OTUs occurring in biofilms but not in the OSB water.

Bacterial diversity

Bacterial richness varied strongly across the landscape (ANOVA, $p < 0.001$). In soil water samples, after rarefying the data, between 1,396 and 9,685 OTUs were identified (mean (±SD) 5,112 ± 2,369). In headwater streams, 2,589 to 14,497 OTUs were found (mean 8,032 ± 2,553), while in the OSB 2,591 to 16,606 OTUs (mean 9,428 ± 4,173) were present. In stream biofilms between 728 and 3,133 (mean of 2,098 ± 959) OTUs were identified and this habitat presented a substantially lower richness than all other habitats.

Flow conditions had a significant effect on the richness. During high flow events, the overall richness increased significantly by 37% (ANOVA, $p < 0.001$), from 6,352 ± 2,764 to 8,716 ± 3,455 OTUs as compared to low flow conditions. The strongest increase was measured in the OSB where the richness increased by 92% (ANOVA, $p < 0.001$) during high flow as compared to low flow. In contrast, we observed no difference in richness in soils and in biofilm samples during high flow versus low flow conditions, respectively.

We found Shannon diversity to be overall significantly higher in headwater streams and the OSB compared to soils and biofilm samples (Kruskal-Wallis, $p < 0.001$) (Fig. 3). During

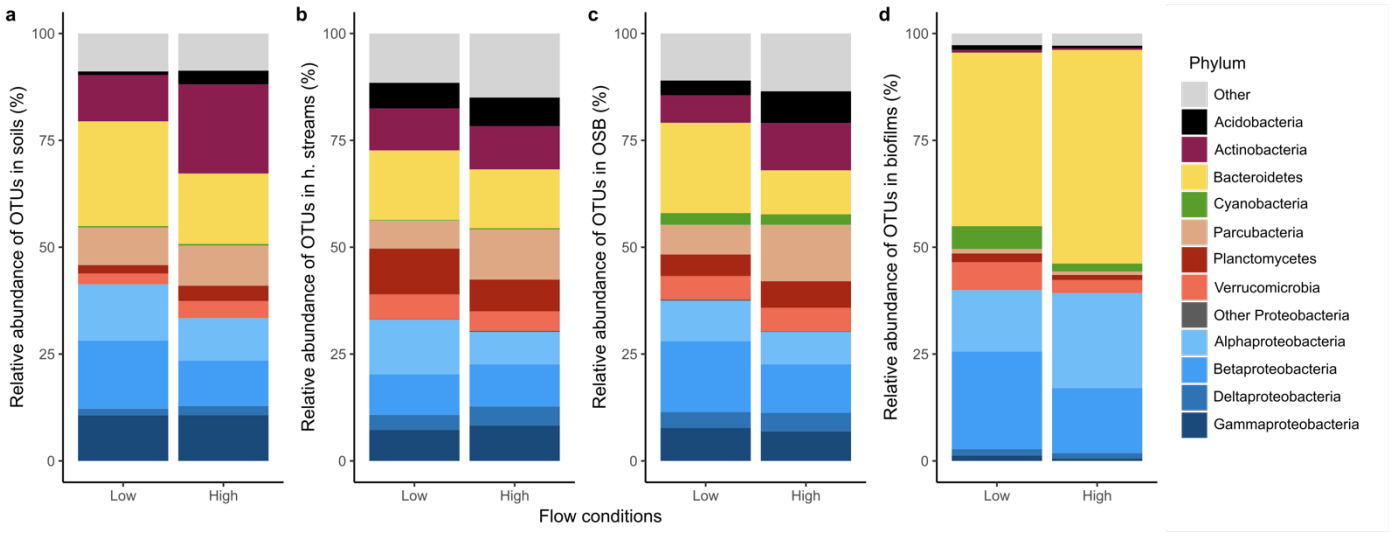

Fig. 4 Bacterial community composition expressed as fraction of total bacterial sequences in (**a**) soil water, (**b**) headwater streams, (**c**) the OSB, and (**d**) biofilm samples, during high and low flow, respectively. Displayed are the 12 most abundant phyla, the remaining phyla were classified as 'Other'.

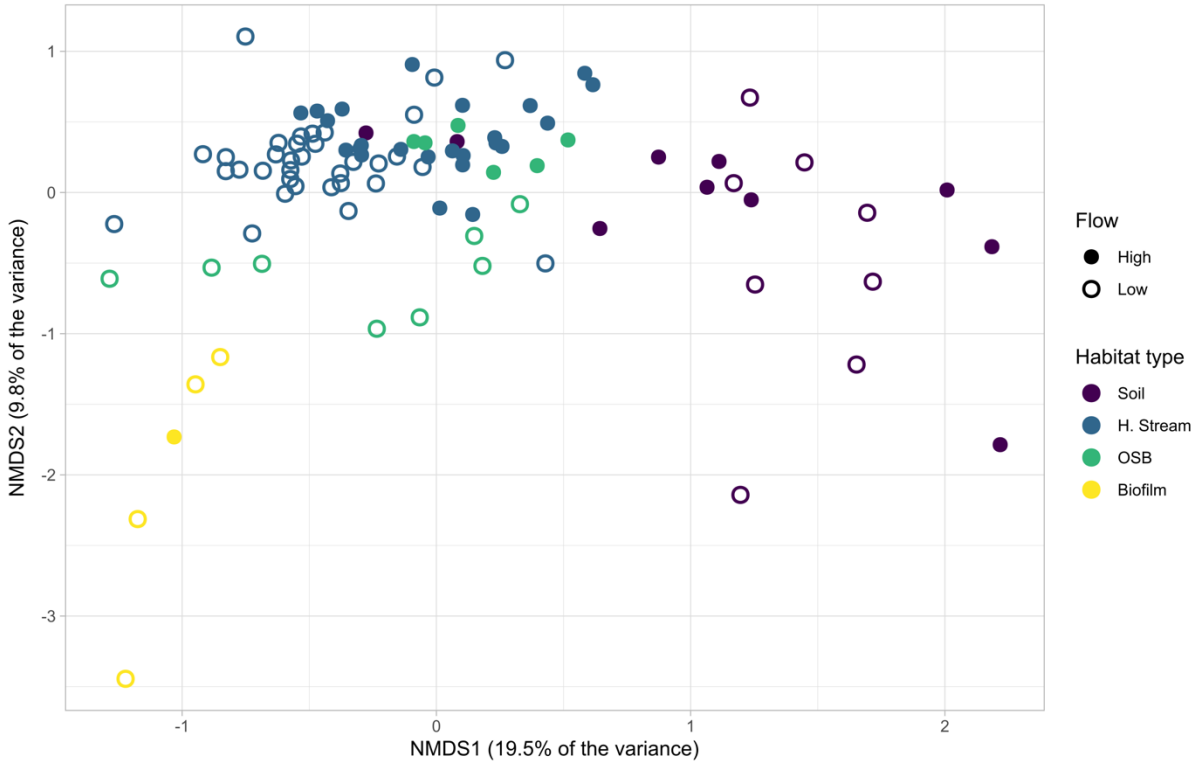

Fig. 5 Nonmetric multidimensional scaling of bacterial communities of different habitat types and high and low flow conditions. H. stream stands for headwater streams.

storms, the overall Shannon diversity increased significantly by 5% (Kruskal-Wallis, $p < 0.001$), from 7.2 ± 1.0 to 7.6 ± 1.0 as compared to low flow conditions. Shannon diversity did not appear to change with flow conditions in soils (Mann-Whitney U, $p = 0.32$) and biofilms individually. However, we quantified a significant increase in diversity in headwater streams and the OSB at high flow (+4% and +10% respectively, Mann-Whitney U, $p < 0.001$).

Bacterial communities varied in composition across soil water, streams and biofilm samples. While biofilm was dominated primarily by Bacteroidetes (42%, Fig. 4), soil and stream water were dominated by Bacteroidetes, Alpha- and Betaproteobacteria, Actinobacteria and a diverse set of less abundant phyla. Bacterial communities of headwater streams and OSB showed a higher proportion of Acidobacteria compared to soil water (Fig. 4). Bacteroidetes were the most abundant taxa in all habitats (20% in soil water, 15% in headwater streams, 16% in OSB, and 42% of biofilm sequences). Betaproteobacteria were more abundant in soil water than in headwater stream water and were more important under low than under high flow conditions. Alpha- and Gammaproteobacteria were also more important in soil water than in the stream water but showed no pattern with flow conditions. Parcubacteria presented similar proportions in soils, headwater streams and OSB (around 9% of bacterial sequences) and were almost absent

from biofilm communities (1% of sequences). Their proportion increased in all habitats except biofilms during high flow conditions.

The indicator species analysis identified 196, 110, 124 and 189 significant indicator OTUs ($p < 0.05$) for soils, headwater streams, the OSB, and biofilms, respectively. Soil indicator OTUs were predominantly Bacteroidetes, Actinobacteria and Betaproteobacteria (32%, 25% and 21%, respectively). Headwater stream indicator OTUs were mostly identified as Planctomycetes and Bacteroidetes (28% and 25%, respectively). Indicator OTUs of the OSB were Cyanobacteria and Betaproteobacteria (29% and 22%, respectively). Finally, biofilm indicator OTUs were predominantly Bacteroidetes and Alphaproteobacteria (56% and 17%, respectively).

During high flow events, community composition changed in all habitats (Fig. 4). Non-metric multidimensional scaling analysis showed that there was a statistical difference between bacterial communities based on habitat type (Fig. 5, ANOSIM, R = 0.71, $p < 0.001$). Overall, we found no difference in bacterial communities between high flow and low flow (ANOSIM, R = 0.09, $p < 0.001$) but within groups, small stream samples and the OSB showed a significant difference in their bacterial communities between high flow and low flow conditions (ANOSIM, R = 0.29 and 0.41, $p < 0.001$ and $p = 0.005$, respectively). At low flow, we observed a higher dissimilarity between habitat types in terms of bacterial communities than at high flow

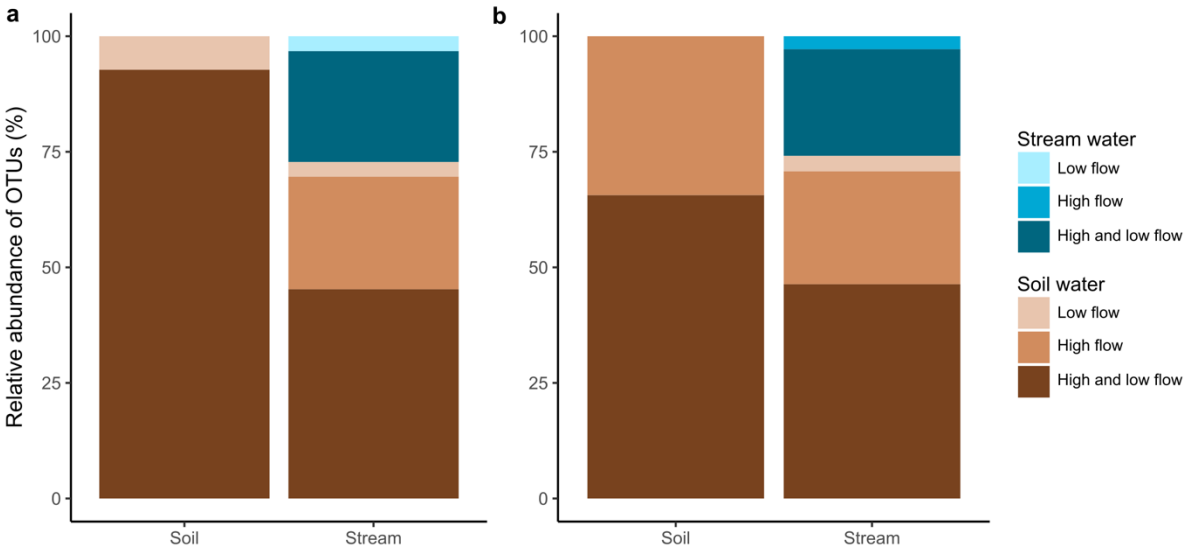

Fig. 6 Relative abundance of OTUs in soil water and headwater streams at (**a**) low flow and (**b**) high flow categorized by the farthest upslope habitat and flow conditions they were first detected in. Values are expressed as a fraction of the total OTUs for each habitat.

(ANOSIM, R = 0.87 and 0.50, respectively, $p < 0.001$), during which communities were more homogeneous between habitats.

Contribution of soil taxa to downstream aquatic habitats

At low flow, 189 out of 196 soil indicative OTUs were also found in headwater streams and 178 in the OSB. At high flow, this number increased to 196 soil indicative OTUs in headwater streams and 189 in the OSB.

Similarly, the FEAST analysis confirmed the contribution of soil water to the downstream habitats. Soils as sources contributed 77% to headwater streams and OSB communities, and 83% to biofilm communities. When the other habitats were considered as additional sources besides soil water, the contribution of headwater streams to OSB communities and of OSB to biofilm communities were substantially lower (28% and 21%, respectively).

To further investigate the relevance of soil inoculation for streams and biofilm, each OTU was assigned to the most upstream environment and the flow conditions it was detected in. With this analysis (Fig. 6), we found that 72% of the bacterial OTUs present in small streams and the OSB and 75% of OTUs present in stream biofilms were first detected in soil water. The approximate proportion of these soil OTUs in downstream environments did not change with flow conditions. However, when the origin of OTUs was analyzed for specific flow conditions, we detected a strong shift in the soil bacterial community during high flow as compared to low flow. During high flow, 34.4% of bacterial sequences consisted of OTUs that did not occur in soil water during low flow (Fig. 6). These high flow specific soil OTUs were also detected further downstream during high and low flow conditions, respectively. In streams, the fraction of specific high flow soil OTUs accounted for 33% of the sequences originating from soil and 24% of all OTUs detected in streams, independent of flow conditions (Fig. 6). On a phylum level, this fraction appeared to be dominated by Bacteroidetes, Planctomycetes and a combination of 'Other' phyla in soils and streams (13%, 16% and 20% respectively in soils and 11%, 19% and 18% respectively in streams).

Discussion

In this study we explored the effects of hydrological conditions on the soil microbial inoculation to streams, thereby complementing previous studies, which showed the importance of soil microbial inoculation on stream community composition (Besemer et al., 2013; Crump et al., 2012; Ruiz-González et al., 2015) and the impact of flow on the abundance of bacteria that are

mobilized from catchment soils (Caillon & Schelker, 2020). Our results demonstrate that flood events drive bacterial community composition in stream ecosystems. High flow events trigger a different soil microbial inoculation than during low flow conditions that influences aquatic microbial diversity (Fig. 6).

We found that bacterial communities in all habitats were dominated by apparently terrestrially derived OTUs, which on average accounted for > 70% of the sequences identified in aquatic communities and biofilms, this percentage was even higher according to the FEAST analysis. Only a few OTUs were unique to a given habitat type and strong overlaps were identified in the bacterial communities across habitats (Fig. 2 and 6). Additionally, soil indicator OTUs were detected in headwater streams and the OSB water column. This agrees with previous studies, which described a continuity between terrestrial and aquatic communities, suggesting that soils are a part of the stream network metacommunity (Crump et al., 2012; Hermans et al., 2019; Ruiz-González et al., 2015; Zeglin, 2015). In contrast to studies which indicated that the strong physical habitat differences between soils and aquatic habitats could prevent microbes from crossing the terrestrial-aquatic interface (Monard et al., 2016), our study supports the notion that many soil bacteria are present in the interstitial water between soil particles and could therefore be adapted to a planktonic life in aquatic environments (Fenchel et al., 1994). Furthermore, we observed an increase of bacterial diversity and richness between soil water and streams, which goes in line with the concept of headwater streams being the initial mixing and dispersing zones for communities originating from various upslope terrestrial environments (Besemer et al., 2013; Crump et al., 2012; Ruiz-González et al., 2015).

Community composition in all habitats agreed with earlier findings in freshwater systems typically dominated by Proteobacteria, Bacteroidetes, Actinobacteria and Cyanobacteria (Battin et al., 2016; Newton et al., 2011). Alpha-, Beta- and Gammaproteobacteria appeared to be slightly more abundant in soil water than in downstream habitats in the present study. While this is consistent with earlier studies for Gammaproteobacteria (Crump et al., 2012), it contrasts with previous findings for Betaproteobacteria, which were found to increase from soil water to downstream habitats (Crump et al., 2012; Ruiz-González et al., 2015). The decreasing abundance of Alphaproteobacteria contradicts findings from a tundra freshwater system (Crump et al., 2012), but agrees with findings from a boreal system (Ruiz-González et al., 2015). These diverging findings might indicate a significant influence of local environmental

conditions, such as the presence of permafrost, on the bacterial communities. Yet, overall, they agree on the strong observed overlap between soil water and nearby surface waters.

Bacteroidetes showed a decrease from soil water to downstream habitats, thereby contradicting earlier findings (Crump et al., 2012; Ruiz-González et al., 2015). Members of the Bacteroidetes are capable of degrading terrestrial organic matter by breaking down polymeric organic substances, which can be assumed to be advantageous in soil water and headwaters receiving large amounts of terrestrial organic matter (Besemer et al., 2013; Caillon & Schelker, 2020). Planctomycetes, which were common in the indicator OTUs for headwater streams, are now recognized to be common in both soil and freshwater habitats (Wiegand et al., 2018). Similar to Bacteroidetes, members of this phylum can efficiently degrade polymeric organic substances (Wiegand et al., 2018). Parcubacteria, which proportions increased during high flow events, were shown to have reduced genomes indicating a specialized, free-living or parasitic/symbiotic lifestyle (Wagner et al., 2015). Cyanobacteria were more important in the OSB than in soil water and headwater streams due to higher light availability in this broader stream.

Biofilms held an overall lower diversity than other habitats. Even though the biofilm OTUs were basically a subset of the suspended OSB community and almost all biofilm OTUs were first identified in upslope environments, the community composition was noticeably different from the other habitats and was dominated by Bacteroidetes. This indicates strong shifts in the relative abundance of phyla and is in line with the assumption that the biofilm environment selects for specific OTUs from the stream water (Besemer et al., 2012). Cyanobacteria are often an important component of stream biofilms exposed to light (Wagner et al., 2015) and have been suggested to shape bacterial diversity and community composition patterns through allelopathy (Besemer et al., 2013). Members of the Bacteroidetes are known to exhibit gliding motility, which might facilitate the colonization of surfaces and enable them to thrive in biofilms (Battin et al., 2016).

An earlier study of high flow events in small headwater streams revealed an increase in soil contributions to small streams in terms of bacterial abundances (Caillon & Schelker, 2020). Here we show that high flow events trigger an overall increase of richness and diversity in headwater streams and the OSB, and affect the bacterial community composition in all the studied habitats (Fig. 3-5). This is likely a mass effect of bacteria coming in large amounts from the soil and homogenizing communities between aquatic habitats (Mayr et al., 2020). This

notion is further supported by the observation of an increased number of soil indicator OTUs in stream water during flood events.

Alternatively, changes in diversity could also be caused by variations in physical and chemical parameters within each habitat (Fierer, Morse, et al., 2007; Fierer & Jackson, 2006). However, water residence times were shown to rarely exceed 2 h in our headwater streams (Caillon & Schelker, 2020) and we argue that this would be too short for relevant bacterial growth and changes in bacterial communities (Crump et al., 2007). The increasing proportion of Actinobacteria and Acidobacteria at high flow in streams, especially prominent in the OSB, rather support a larger soil contribution during high flow events. Indeed, these phyla are known to be more soil-associated (Lauber et al., 2009), even though some studies reported their presence in lakes and streams (e.g. Staley et al., 2016). Accordingly, typical freshwater phyla, such as Verrucomicrobia (Zwart et al., 2002), decreased in proportion during high flow events in streams.

Although bacterial diversity in soils was not found to be significantly different between high flow and low flow conditions (Fig. 3), we noted a high proportion of OTUs that were only detected in soils during high flow events (Fig. 6). We named these OTUs as 'high flow specific soil taxa' that accounted for 34% of soil sequences during flood events. Since high flow would likely mobilize particles and increase their abundance in soil water (Lenzi & Marchi, 2000), these high flow specific soil OTUs could represent taxa which live preferentially attached to particles. Streambed erosion by water flow might provide a continuous source of particles and attached bacteria stemming from the immediate vicinity of the stream. Particle-attached bacterial communities have repeatedly been found to harbor distinct communities, which differ from the free-living communities in the surrounding water (Besemer et al., 2005; Jackson et al., 2014; Savio et al., 2015).

An alternative explanation for some of our observations regarding enhanced microbial diversity and richness during high flows, could also be caused by groundwater fluctuations as a response to rainfall. During high flow events, hydrologic connectivity between surface soil water and shallow groundwater might be established (Detty & McGuire, 2010; Sidle et al., 2000), potentially augmenting the soil water community with additional taxa from shallow groundwater. Upwelling and mixing of this groundwater in the streambed could then lead to the transfer of groundwater bacteria to the stream (Boulton et al., 1998). Indeed, Parcubacteria, which have been observed to be one of the most important bacterial groups in a limestone karst

aquifer (Savio et al., 2019), were important during high flow in the present study potentially indicating groundwater inflow. However, given the steep gradients in the studied systems, such effects are likely less relevant as compared to direct microbial transfer by soil water into streams.

Overall, this study showed that flood events have a fundamental effect on the bacterial diversity and community composition of stream ecosystems. By enhanced soil contributions during such events, bacterial communities across habitats homogenized and diversity increased. These results demonstrate the need to integrate the hydrological events in fluvial microbial community studies.

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Chapter 3. Hydrological and biogeochemical controls on stream microbial composition and diversity in pre-alpine headwater streams

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Abstract

Microbial communities play a vital role in the functioning of stream ecosystems. However, the factors that control their composition and diversity in headwater streams remain poorly understood. In this study, we investigated the hydrological and biogeochemical controls on microbial composition and diversity at different seasons in the Oberer Seebach catchment in Austria. Here we show that bacterial community composition and diversity is strongly influenced by variations in precipitation and discharge. We found that the free-flowing bacterial communities found in streams during flood events differed depending on whether the event was the result of a summer storm or snowmelt. During summer high flow events, the bacterial diversity in the Oberer Seebach increased by 8% compared to communities during snowmelt. In soils and biofilms, bacterial diversity and species richness were more affected by dissolved organic carbon quality or pH than a change in discharge. Our results demonstrate that soil water, streams, and biofilm communities respond differently to an increase in discharge and a change in season despite them being intricately connected.

Introduction

Bacteria form one of Earth's most abundant taxonomic groups (Bar-On et al., 2018; Whitman et al., 1998). In the world's aquatic environments, the biomass of prokaryotes – a group composed of Bacteria and Archaea – was estimated to be 1.2×10^{29} cells (Whitman et al., 1998). Despite their ubiquitous distribution, bacterial communities still exhibit geographical patterns based on distance, climatic conditions, seasons, and environmental variables such as nutrient concentration, oxygen, and pH (Liu et al., 2019; Livermore & Jones, 2015; Zeglin, 2015).

In freshwaters, bacteria play a key role in global biogeochemical cycles (Battin et al., 2016; Tolkkinen et al., 2020), for example through organic matter decomposition and carbon dioxide evasion (Kreutzweiser & Capell, 2003), and are critical for the transfer of carbon to higher trophic levels (Hall & Meyer, 1998). The general dispersal pathway of riverine bacterioplankton is through downstream transport from headwater streams, the furthest upstream tributaries of river networks (Jones et al., 2020; Savio et al., 2015). As such, headwater streams, influence the biogeochemical characteristics of downstream fluvial networks by transferring nutrients and microbial communities from soils (Caillon & Schelker, 2020; Fasching et al., 2014; Jones et al., 2020; Schelker et al., 2016).

Headwater streams are highly dynamic (Blöschl & Sivapalan, 1995; Johnson et al., 2022) and present strong seasonality in terms of bacterial abundances (Caillon & Schelker, 2020), their microbial diversity (Caillon et al., 2021; Hullar et al., 2006), and dissolved organic carbon (DOC) concentrations in stream water (Dawson et al., 2008; Schelker et al., 2012). It is therefore crucial to study the effects of hydrologic variability and seasonality on headwater streams to better understand their role in shaping the ecology of the river network.

In streams and rivers, bacteria can either develop in the water column as free-flowing communities or attached to the substrate as biofilms (Besemer et al., 2012; Geesey et al., 1978). The key difference between these two types of microbial communities is the location and stability of the habitat that they occupy. Free-flowing microbial communities are more mobile and are subject to changes in the flow regime and other environmental conditions, while biofilm-attached microbial communities provide a stable habitat that can support the growth and persistence of microorganisms over time. In headwaters, bacterial communities display high diversity and have been found to resemble soil communities due to important transfers between terrestrial and aquatic environments (Caillon et al., 2021; Crump et al., 2012; Ruiz-

González et al., 2015). Further downstream, bacterial diversity decreases and typical freshwater taxa progressively become more dominant (Savio et al., 2015). Additionally, stream bacterial communities display temporal variations with season and flow conditions (Caillon et al., 2021; Hullar et al., 2006). For instance, it was shown that bacterial diversity increased in headwater streams during summer floods compared to low flow conditions, and that soil microbial inoculation to streams changed during these events (Caillon et al., 2021). Other studies revealed that bacterial communities were also shaped by environmental selection and season (Fierer et al., 2007; Hullar et al., 2006; Zeglin, 2015). The most important environmental factors that drive stream bacterial communities include metal concentration, seasonal temperature changes, hydrology, and DOC concentration and quality (Zeglin, 2015).

Dissolved organic matter concentration and composition is an important predictor of bacterial abundance and community composition in streams (Caillon & Schelker, 2020; Findlay et al., 2003; Olapade & Leff, 2005). It provides energy for heterotrophic bacteria and its fluctuations can influence bacterial community distribution and stream metabolism (Berggren & del Giorgio, 2015; R. H. Findlay et al., 2008; Williams & Del Giorgio, 2005). In forested catchments, snowmelt and storms induce a rising water table that creates increased flows towards headwater streams through the upper soil horizon, flushing DOC and bacteria from catchment soils to stream waters (Boyer et al., 1995; Caillon & Schelker, 2020; Fasching et al., 2015). During summer floods, it was shown that the concentration of DOC in headwater streams increase by 44% (Caillon & Schelker, 2020).

In this study, we aim to explore the seasonal differences in bacterial diversity when discharge increases across different seasons (namely spring snowmelt versus summer storms). We hypothesize that bacterial diversity increases in headwater streams during snowmelt with increasing transfers of carbon and bacteria from soils to small streams. As soil bacterial communities vary with changing seasons (Lipson & Schmidt, 2004), we expect to find different communities in streams during summer floods versus snowmelt. To explore these hypotheses, we compared bacterial communities in soil water, streams, and biofilms under different seasons and varying flow conditions.

Methods

Most of the methods used for this study are an extension of previous research conducted on the same sampling design (Caillon & Schelker, 2020; Caillon et al. 2021). Samples were added to an already extensive data set to study new aspects of stream dynamics, particularly during snowmelt.

Study site and sampling

Over the course of 32 months, spanning from June 2017 to January 2020, we collected samples of soil water, stream water, and benthic biofilms around lake Lunz in the Oberer Seebach catchment near Lunz am See, Austria. This catchment is predominantly pristine and well-researched, having accessible meteorological and discharge data (Ejarque et al., 2018; Fasching et al., 2015). It is characterized by a dominance of mixed decideous forest, complemented by occasional meadows for low intensity cattle grazing during the summer. To ensure consistent weather conditions (i.e. air temperature and precipitation), we selected three different hillslopes of the catchment within a 1.5 km radius around Lake Lunz (Rehberg, Schlögelberg, and the WasserCluster Lunz slope). On each of these hillslopes, we collected samples from two headwater streams of Strahler order 1, as well as soil water from two different depths (at 20 and 50 cm depth).

To obtain soil water samples, we used soil runoff samplers which were positioned within a short distance (<15 m) from the source of a single headwater stream on each hillslope. These samplers were designed with two stainless steel soil water collectors, each 2 m wide, inserted 30 to 40 cm laterally into the hillslope (Caillon & Schelker, 2020). Conceived to capture soil water as it flows downslope and into streams, the samplers were placed to collect samples in the unsaturated zone of the soil profile. To facilitate sampling, 500 mL Schott bottles, pre-treated by acid-washing and pre-combusting (4h at ~450°C), were positioned below the outflow of each sampler one day prior to collection. During the driest summer periods, some collectors remained dry, reducing the number of samples to 35.

Stream water samples were collected at two different locations in all six headwater streams – one near the stream source and the soil runoff samplers and another approximately 400-500 m downstream. For the collection, pre-combusted 500 mL Schott bottles and sterile 15 mL syringes were used. Each of the headwater streams shares comparable characteristics, with

similar width (<1 m), depth (<10 cm), and slopes (mean 29%). At intermediate flow conditions, these streams exhibit an approximate flow velocity of 0.1 m/sec (Caillon & Schelker, 2020).

Further, water samples were obtained from a larger stream, the Oberer Seebach (Strahler order 3), at three distinct locations spanning its width. The inclusion of this stream in the sampling design was essential to compare its bacterial community with those in the upstream headwaters and to leverage the availability of extensive data related to the Oberer Seebach. In headwaters and the Oberer Seebach, stream water pH and electrical conductivity were measured directly on-site using WTW probes (pH3310, Cond3310).

Finally, biofilms from the Oberer Seebach were also sampled. Sterile ceramic tiles (5 by 5 cm), glued on bricks to prevent them from erosion, served as substratum for biofilm growth in the stream. Tiles were installed on the streambed four weeks prior to sampling to allow for biofilm growth. During flood events, sampled biofilm aged between 1 to 4 weeks, depending on the date of the precedent sampling. However, the water level of the Oberer Seebach was too high at times to allow for the collection of the tiles and, therefore, only four biofilm samples could be sampled at high flow conditions. After sampling, tiles were kept at -20°C in sterile plastic bags until further processing.

In total, 235 samples were collected for the characterization of bacterial communities at different seasons and flow conditions (soil water n = 35, headwater streams n = 134, Oberer Seebach water n = 29, and Oberer Seebach biofilms n = 37). Since the sampling was carried out over several years, part of this dataset was already used to understand the dynamics of DOC and bacterial communities in headwater streams (Caillon et al., 2021; Caillon & Schelker, 2020).

DOC concentration and composition

Soil, headwater stream, and Oberer Seebach water for DOC analysis was filtered in the field through 0.7 µm pre-combusted glass-fiber filters (Whatmann GF/F) into acid-washed and pre-combusted 40 mL glass vials using syringes and syringe-type filter holders. The filtrates were kept at 4°C pending further analysis.

Filtrates were analysed for DOC concentration within 24 hours after sampling. From July 2017 to February 2019, analyses were performed using a Sievers 900 TOC Analyser (GE Analytical Instruments, Boulder, CO, USA) operated with an inorganic removal unit. Past this date, samples were analysed using a Shimadzu TOC-VCSH FA E200. We assessed dissolved organic

matter absorbance using a UV-VIS spectrophotometer (ShimadzuUV 17000) in 5 cm cuvettes. Ultraclean water (MilliQ) served as blank. Excitation emission matrices were generated using a fluorescence spectrophotometer (Hitachi F-7000) and 1 cm quartz cuvettes. Fluorescence intensities were measured at excitation wavelengths ranging from 250 to 450 nm and emission wavelengths from 250 to 550 nm. Excitation emission matrices were subtracted by blanks and corrected for the inner filter effect using corresponding absorbance measurements (Lakowicz, 2006). Manufacturer-provided built-in functions were used to correct all fluorescence measurements for wavelength-dependent lamp inefficiencies. Fluorescence index was calculated as the ratio of excitation at 370 nm of intensities emitted at 470–520 nm (Cory & McKnight, 2005); lower values are considered to be of terrestrial origin and higher values correspond to an aquatic origin (McKnight et al., 2001). Biological index, the ratio of emission 380 nm and 430 nm at excitation 310 nm, was used to indicate more recent production of DOM with lower values (Huguet et al., 2009). The humification index, as index increasing with humification, was calculated using the area under the emission spectra 435-480 nm divided by the peak area 300-345 nm from the spectra at excitation 254 nm (Ohno, 2002).

DNA extraction, PCR and Illumina sequencing

Bacterial community water samples were kept at 4°C and, within 10 hours, filtered on 0.2 µm mixed cellulose filters (Whatman ME24). Samples were stored at -20°C in 15 mL sterile tubes until further processing. DNA was extracted from the filters and the biofilms using the DNeasy PowerSoil extraction kit (Qiagen) following the manufacturer's protocol. Before the extraction process, filters were fragmented using ethanol-flamed tweezers and scissors, while biofilms were carefully removed from the tiles using ethanol-flamed razor blades and spatula. The V3-V4 region of the 16S rRNA gene was amplified using the bacteria specific primers 341F (CCT ACG GGN GGC WGC AG) and 785R (GAC TAC HVG GGT ATC TAA KCC) (Thijs et al., 2017). PCR amplification, Illumina MiSeq library preparation and sequencing using V3 chemistry and 2 x 300 bp paired-end reads was carried out by LGC Genomics GmbH (Berlin, Germany). Sequences are accessible at the NCBI XXX archive under the accession number XXX.

Bioinformatics

Following the same method than in Caillon et al. (2021), amplicon sequences were processed as in Cholet et al. (Cholet et al., 2019). The first steps follow the recommendations of Schirmer

et al. (Schirmer et al., 2015). Sickle v1.2 was used to perform quality trimming of paired-end reads using a 20 bp sliding window. Reads were trimmed where the average quality score dropped below 20 and reads below 10 bp length were discarded. Then, BayesHammer (Nikolenko et al., 2013) from the Spades v2.5.0 assembler (Bankevich et al., 2012) was applied to error correct the paired-end reads. PANDAseq v2.4 (Masella et al., 2012) was used to assemble overlapping forward and reverse reads using a minimum overlap of 10 bp.

For Operational Taxonomic Unit (OTU) construction, the VSEARCH v2.3.4 pipeline was followed (Rognes et al., 2016). The pooled sequences were de-replicated, sorted, and singletons discarded. Sequences were clustered on a 97% identity level. *De novo* chimera detection by searching for potential parent reads was performed, followed by reference-based chimera detection using the Silva aligned version of the gold database (https://www.mothur.org/w/images/f/f1/Silva.gold.bacteria.zip). The original barcoded sequences were then matched against the clean OTUs with a 97% similarity threshold to generate OTU tables. OTUs were taxonomically classified against the TaxAss database to the lowest taxonomic level possible (Rohwer et al., 2018). OTUs that presented less than three occurrences over all samples were removed from the dataset as well as OTUs classified as Archaea, Eukaryota, mitochondria, and chloroplasts. Also, one biofilm sample and all the samples from one of the headwater streams on the Rehberg hillslope (23 samples) presented very low number of reads compared to all the other samples and were removed from further analysis. The cleaned dataset consisted of 12 344 682 sequences clustered into 27 528 OTUs. In order to avoid bias from sequencing effort and enable samples comparison, relative abundances of each OTU per sample were used for all analyses.

Hydrology and seasonality

Data from the Oberer Seebach station, located at the inlet of Lake Lunz and previously studied by Ejarque et al. (2018) and Fasching et al. (2015), was used to analyse the variability in stream runoff. Stream discharge (Q in $m^3\ s^{-1}$) was determined through well-established curves, using water level measurements taken at 10-minutes intervals (Fasching et al., 2015). Using the available data, daily average discharge was computed and subsequently used for further analysis. In instances where data was missing due to logger system malfunctions, the absent daily discharge values were estimated from the gage at the lake outlet, which exhibited a strong correlation ($R^2 = 0.91$) with the daily discharge values at the inlet station.

Flow conditions were categorized based on the stream discharge level, following a similar approach as in previous studies (Caillon & Schelker, 2020; Fasching et al., 2015). Briefly, daily average discharge higher than 90% of the time in our study period ($Q > 1.68$ m^3 s^{-1}) was defined as high flow. Low flow was defined as all discharges lower than 25% of the time ($Q < 0.13$ m^3 s^{-1}) and all other discharges were defined as intermediate flow conditions (Caillon & Schelker, 2020). Each sampling day was described by time since a high flow event (that is, days since discharge in the Oberer Seebach > 1.68 m^3 s^{-1}; equals 0 during high flow events) and by the number of consecutive days of low flow conditions preceding sampling (days since discharge < 0.13 m^3 s^{-1}; equals 0 when discharge in the Oberer Seebach exceeds the low flow threshold).

Samples separated by flow conditions were subsequently divided into three seasons, namely winter, snowmelt, and summer. Samples from December to May were categorised as winter, all other months were categorized as summer. The snowmelt period was defined from the beginning of the discharge increase in late February 2019 to the return of base flow in early June 2019. Snowmelt samples were not further separated into high, intermediate, and low flow conditions.

Statistical analysis

Statistical analyses were conducted in R (R Core Team, 2020) with the *phyloseq*, *stats*, and *vegan* packages (McMurdie & Holmes, 2013; Oksanen et al., 2019). Non-metric multidimensional scaling with Bray-Curtis distances was used to ordinate the samples based on their dissimilarity in community composition. ANOSIM analysis was conducted with 9999 permutations to test the differences between habitat type and flow conditions.

A canonical correspondence analysis was performed on cube root transformed community data to evaluate the role of typical environmental variables (namely DOC concentration and composition, average daily precipitation and discharge at the Oberer Seebach, pH, electrical conductivity, number of days since the last high flow event, and length of drought on sampling day) (Amaral et al., 2016; Langenheder & Lindström, 2019; Zeglin, 2015) on community composition in each habitat and season. An ANOVA like permutation test for canonical correspondence analysis was performed to assess the significance of constraints.

Species richness and Shannon diversity (the number equivalent of the Shannon entropy; Jost 2007) were calculated after rarefying to the lowest number of reads obtained from a sample (6 761 reads) to account for differences in sequencing effort. Shapiro-Wilk test for normal

distribution on the data indicated that richness data was normally distributed while Shannon diversity data was not. ANOVA and Tukey test were used to compare the difference in species richness between seasons in each habitat. Shannon diversity was assessed using Mann-Whitney U tests and Kruskal-Wallis tests.

To compare community composition between seasons, we generated taxonomic heat trees using the R package *metacoder* (Foster et al., 2017). For the sake of readability of the trees, we selected only the 8 most abundant phyla, the 20 most abundant classes, and the 30 most abundant orders in the whole dataset. Even though these thresholds were chosen subjectively, they represented well the overall community and diversity. Any phyla, class, or order that were not selected were classified as "Other". Comparisons between communities of different seasons were performed with Wilcoxon Rank Sum tests.

Results

Bacterial community composition varied strongly in each habitat across the study period (Figure 1). Some of the largest variations, specifically in streams free flowing communities, were associated with an increase in discharge. In all habitats, communities were dominated throughout the year by bacteria of the phyla Gammaproteobacteria, Bacteroidota, and Alphaproteobacteria.

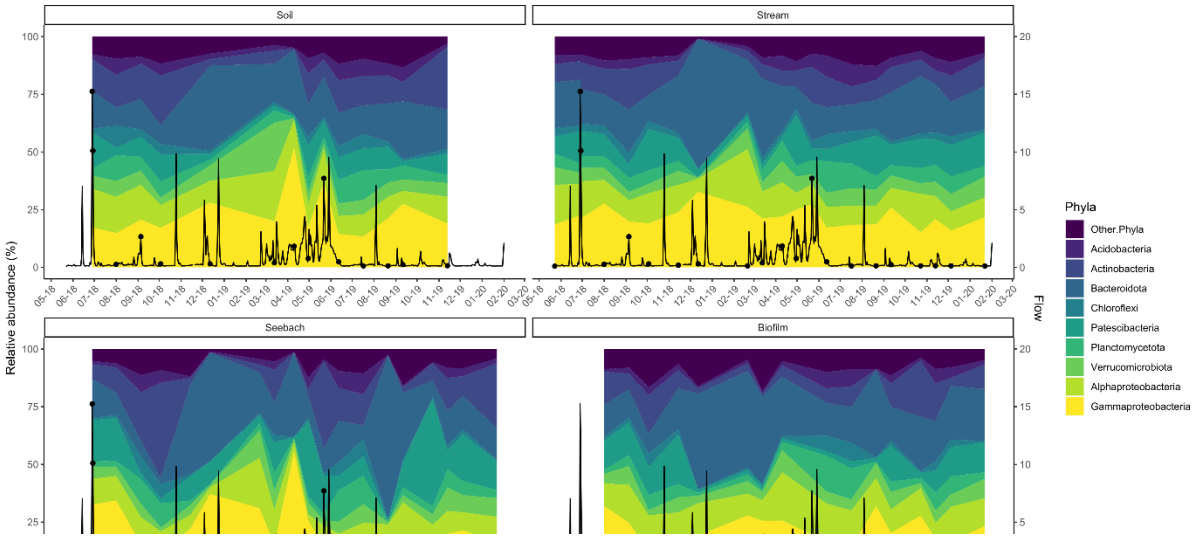

Fig. 1 Changes in bacterial community assemblages in soil water, headwater streams, the Oberer Seebach, and biofilm samples over the sampling period. Daily discharge in the Oberer Seebach is represented in black, with dots indicating sampling dates. Only the 9 most dominant phyla, accounting for more than 3% each of the total community, are labelled. The other are groups in Other Phyla.

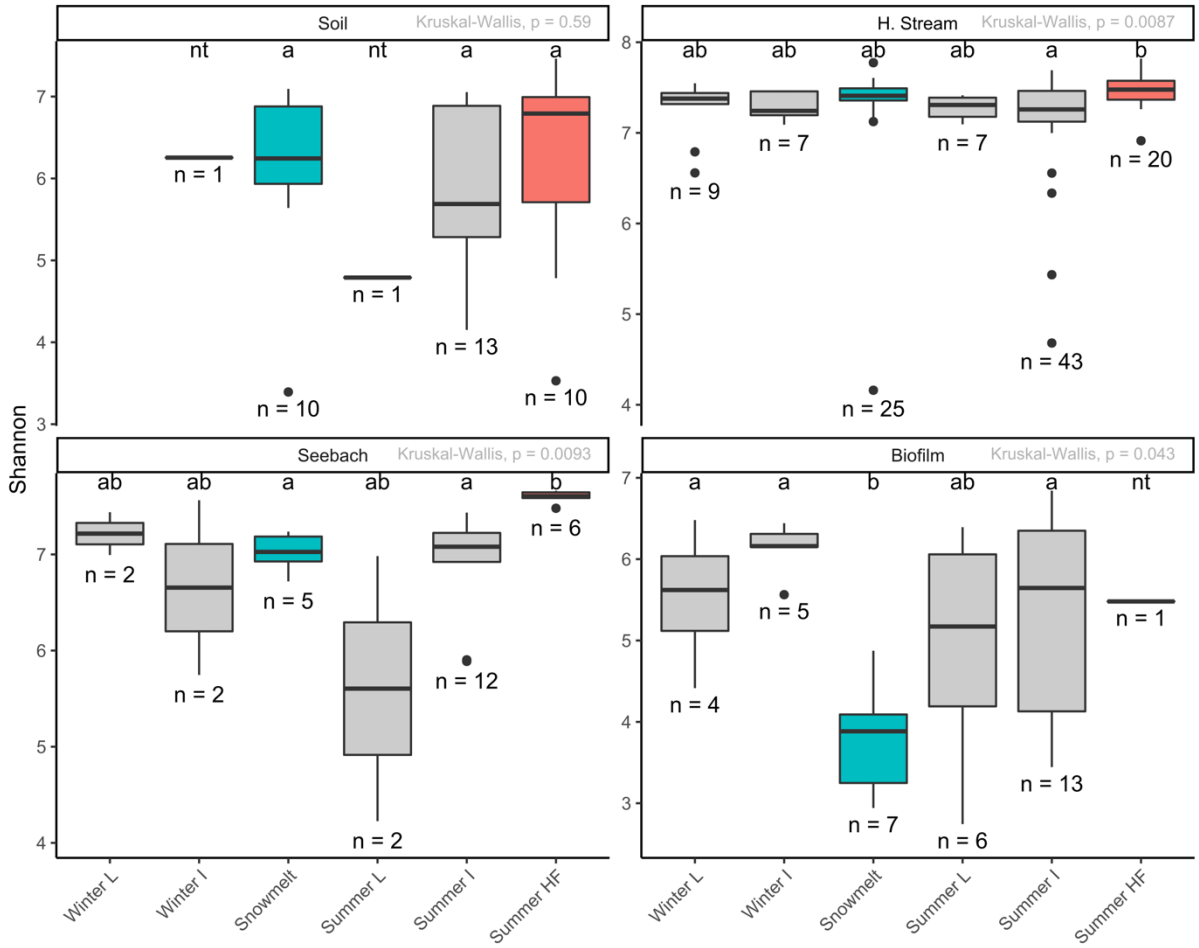

Fig. 2 Variation in Shannon diversity index between seasons and flow conditions in soil water, headwater streams, the Oberer Seebach, and biofilm samples. Horizontal lines show the median, boxes the 25th to 75th percentiles, whiskers the 5th and 95th percentile range. Black points are values outside the interquartile range. Values presented below each boxplot refer to sample size. Within each habitat, boxplot that do not share a letter are significantly different (pairwise Mann-Whitney, $p<0.05$). No statistical test was conducted on categories with only one sample and are therefore labelled "nt" for not tested.

We observed a significant difference in Shannon diversity between habitat types (Kruskal-Wallis, $p<0.001$). Overall, Shannon diversity ranged from 2.74 to 7.82 across habitats, with the lowest values in stream biofilms with a mean Shannon diversity of 5.06 ± 1.23 and the highest in headwater stream samples (mean 7.27 ± sd 0.49). Shannon diversity in each habitat responded differently to a change in season and flow conditions (Figure 2). For soil water samples, no statistical difference was observed in Shannon diversity with season and flow conditions. In biofilms, snowmelt presented lower Shannon diversity than other seasons and flow conditions, but the difference was only significant between snowmelt and winter intermediate flow conditions. Summer high flow conditions presented significantly higher Shannon diversity than snowmelt samples in the Oberer Seebach (+8%; pairwise Mann-Whitney test, $p<0.05$). We also observed an increase in microbial diversity in headwater

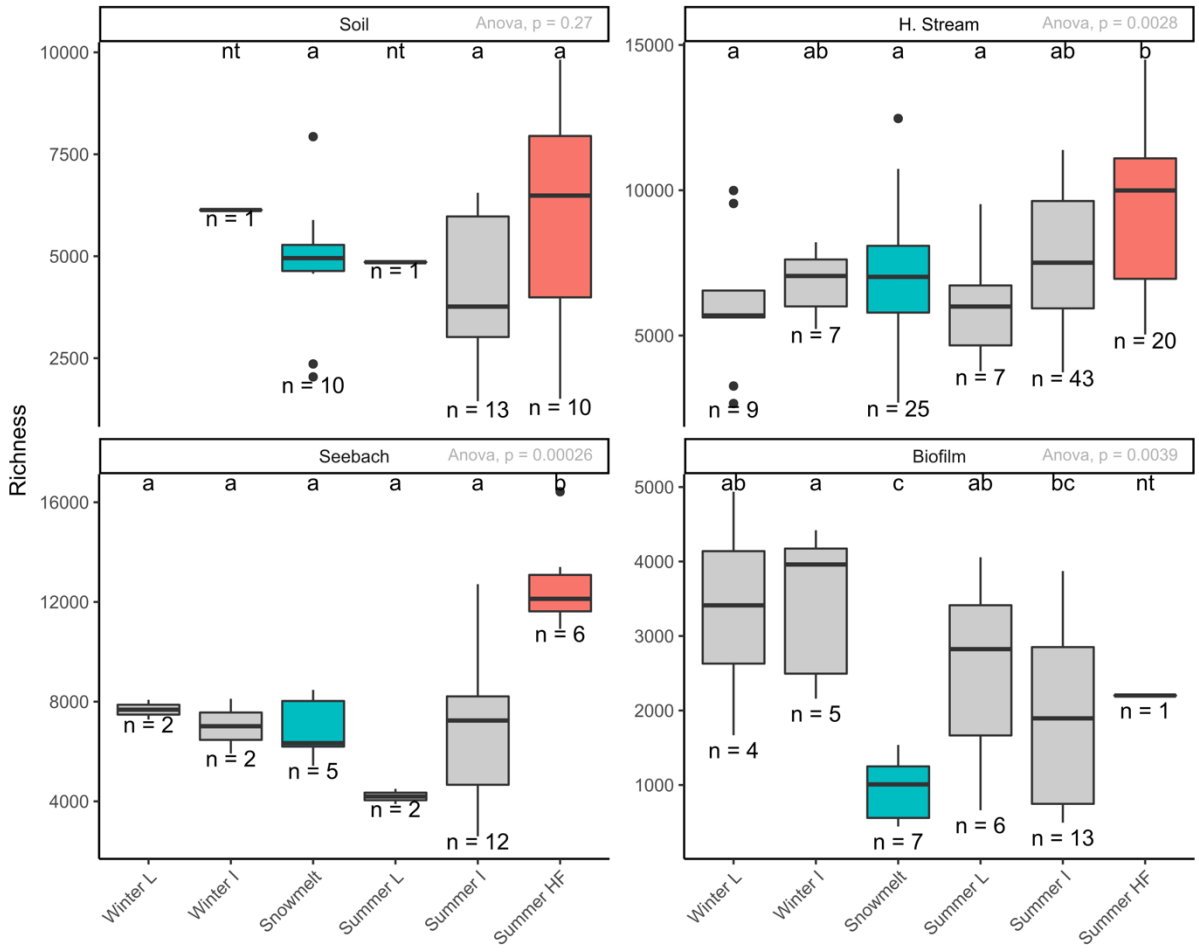

Fig. 3 Variation in bacterial species richness between seasons and flow conditions in soil water, headwater streams, the Oberer Seebach, and biofilm samples. Horizontal lines show the median, boxes the 25th to 75th percentiles, whiskers the 5th and 95th percentile range. Black points are values outside the interquartile range. Values presented below each boxplot refer to sample size. Within each habitat, boxplot that do not share a letter are significantly different (pairwise t-test, $p<0.05$). No statistical test was conducted on categories with only one sample and are therefore labelled "nt" for not tested.

streams, but this change was not significant (+2%; pairwise Mann-Whitney test, $p = 0.454$). Samples at low flow were not significantly different from samples at intermediate flow conditions in any habitat.

Similar to the Shannon diversity, bacterial species richness differed significantly between habitats (Figure 3, ANOVA, $p<0.001$). Biofilm samples presented the lowest richness (2211 ± 1321) followed by soil samples (4945 ± 2040), with the highest species richness in the Oberer Seebach and the headwater streams (7974 ± 3284 and 7554 ± 2377, respectively). In soil water, no significant difference in richness was observed between the seasons and flow conditions (ANOVA, $p = 0.27$). In headwater streams and in the Oberer Seebach samples, summer high flow conditions presented the highest species richness (9192 ± 2718 and 12743 ± 1986, respectively). In both habitats, species richness was significantly higher during summer high

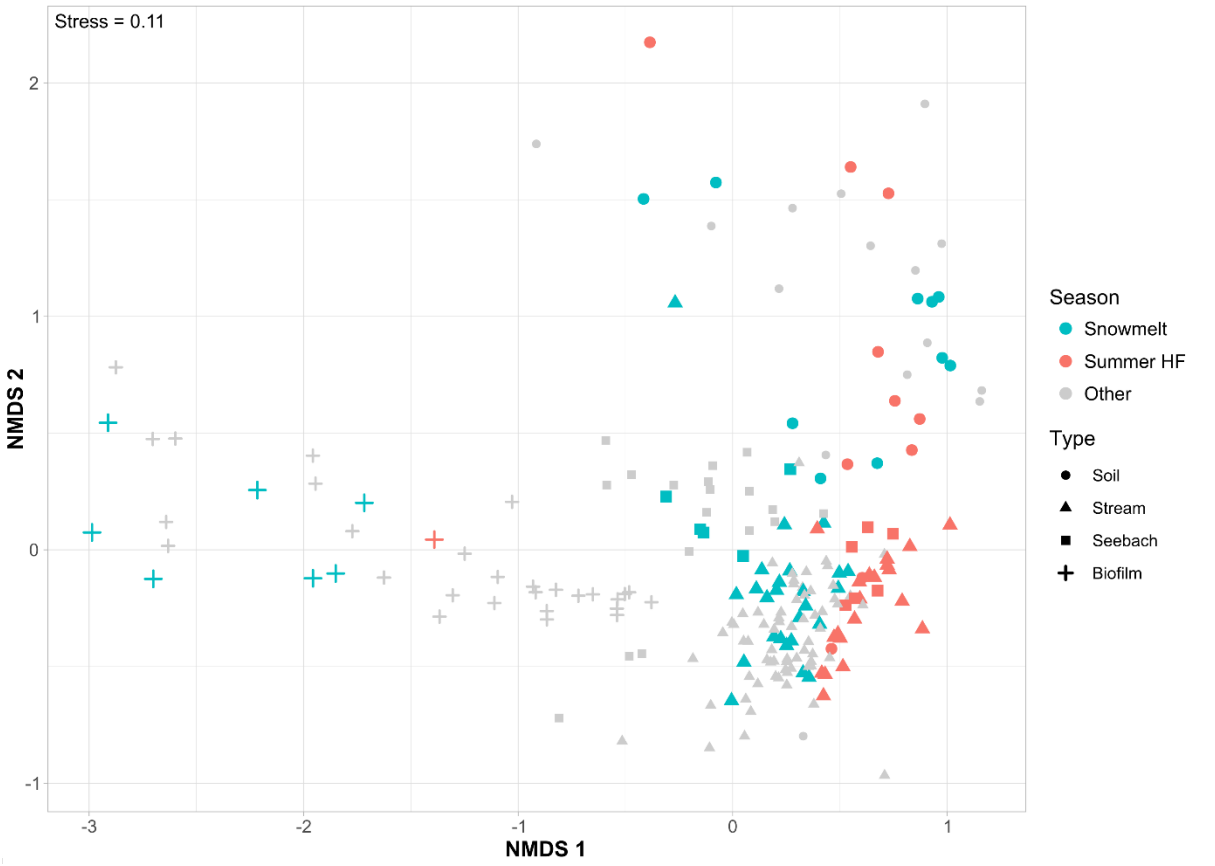

Fig. 4 Nonmetric multidimensional scaling of bacterial communities of different habitats categorised by season and flow conditions.

flow conditions than during the snowmelt (pairwise t-test, $p<0.05$). In biofilms, the lowest bacterial species richness was measured during snowmelt (944 ± 428). Biofilm species richness in snowmelt samples differed significantly from winter low and intermediate flow conditions as well as from summer low flow conditions (pairwise t-test, $p<0.05$).

Nonmetric multidimensional scaling showed different bacterial communities between habitats (Figure 4, ANOSIM, $R = 0.71$, $p<0.001$). When pooling all habitats, there was no significant difference between season categories (ANOSIM, $R = 0.03$, $p = 0.17$). When analysed for different habitats separately, there were significant differences between snowmelt and summer high flow communities (ANOSIM, soil water, $R = 0.23$, $p = 0.004$; headwater streams, $R = 0.38$, $p<0.001$; Oberer Seebach, $R = 0.73$, $p = 0.002$; biofilms, $R = 0.29$, $p = 0.25$).

Canonical correspondence analysis was used to further explore the observed compositional differences between seasons and their relationship to prominent environmental variables (Figure 5b-c). Constraints were significant in all habitats (Figure 5, ANOVA like permutation test, $p<0.001$). In soil water samples, DOC concentration, days of low flow conditions prior to sampling and fluorescence index were significant in explaining community composition (ANOVA, $p<0.05$). In headwater streams, DOC concentration, pH, electrical conductivity,

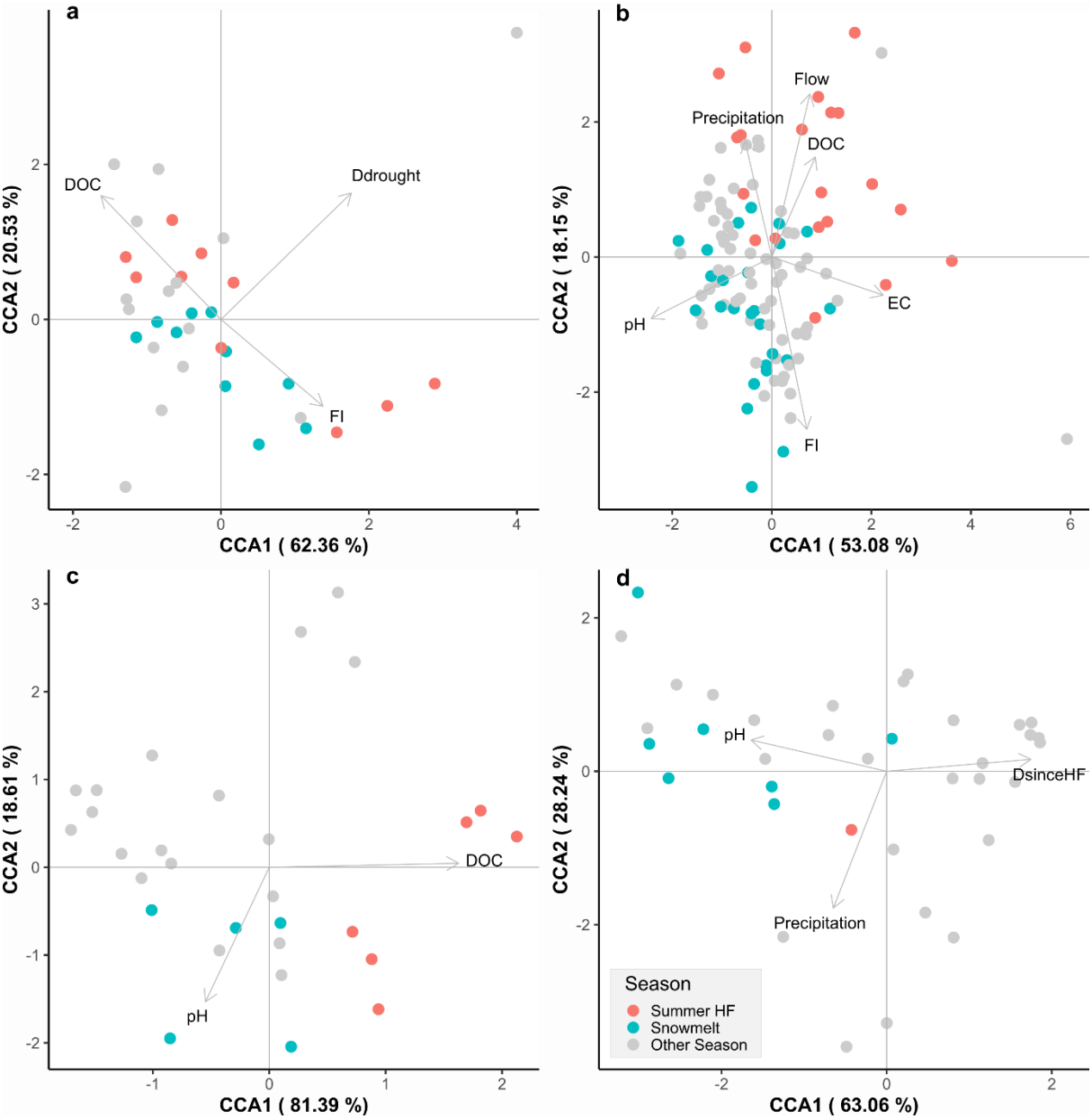

Fig. 5 Canonical Correspondence Analysis of bacterial communities in (**a**) soil water, (**b**) headwater streams, (**c**) Oberer Seebach, and (**d**) biofilm samples with environmental variables as arrows. The direction of the arrows represents the correlation between each variable and the canonical axes. The length of the arrows relates to the contribution of the variable to the axes. Environmental variables are the following: "Ddrought" for number of days with low flow conditions prior to sampling; "DOC" for dissolved organic carbon concentration; "DsinceHF" for number of days since the last high flow event; "EC" for electrical conductivity; "FI" for fluorescence index; "Flow" for discharge; pH; and precipitation.

fluorescence index, discharge, and precipitation were significant in explaining community composition (ANOVA, $p<0.05$). Typical summer high flow communities were favoured by high discharge, precipitation, low pH, low fluorescence index, and high DOC concentrations, whereas snowmelt samples corresponded to high pH and low DOC concentrations. In the Oberer Seebach, only DOC concentration and pH significantly explained community composition (ANOVA, $p<0.05$). Higher DOC concentration was associated with summer high

flow samples and higher water pH with snowmelt samples. In biofilms samples, only pH of the surrounding water of the Oberer Seebach was significant in explaining community composition (ANOVA, $p<0.05$). Precipitation and the number of days since the last high flow event were the next two environmental variables in order of contribution but were not significant ($p = 0.137$ and 0.144, respectively). Snowmelt samples appeared to be mostly correlated with pH in the surrounding Oberer Seebach water as well as with repetitive high flow events (low number of days since last high flow). Dissolved organic matter biological index and humification index, as well as the number of days since the last high flow event never appeared to be significantly explaining community composition in any habitat.

The taxonomic groups responsible for the observed differentiation of samples in the canonical correspondence analysis were explored using heat trees (Foster et al., 2017) (Figure 6). In soil water, only minor differences were observed in bacterial communities between seasons and flow conditions. Summer high flow conditions were associated with a significant increase in bacteria of the order Verrucomicrobiales compared to snowmelt and other seasons (Wilcoxon Rank Sum test, $p<0.05$). Additionally, summer high flow soil samples presented more Bacteroidia of the order Chitinophagales than other seasons and more Bacteroidia of the order Flavobacteriales than snowmelt samples (Wilcoxon Rank Sum test, $p<0.05$). During snowmelt, soil samples presented more bacteria of the order Bacteroidales than during summer high flow conditions and more bacteria of the order Microtrichales (class Acidimicrobiia) and class Polyangia (phylum Myxococcota) than during other seasons. Inversely, both summer high flow conditions and other seasons presented significantly more bacteria of the order Xanthomonadales (class Gammaproteobacteria) than during snowmelt.

In headwater streams, summer high flow conditions presented significantly higher proportions of bacteria of the classes Parcubacteria and Chlamydiae than snowmelt and other seasons (Wilcoxon Rank Sum test, $p<0.05$). The snowmelt period in headwater streams was associated with an increase in bacteria of the classes Acidimicrobiia and Alphaproteobacteria - notably the order Rhodobacterales - compared to summer high flow conditions (Wilcoxon Rank Sum test, $p<0.05$). Compared to other seasons, snowmelt showed an increase in the relative abundance of Saccharimonadales (order of Patescibacteria), Acidimicrobiia (class of Actinobacteria), and Polyangiales (order of Myxococcota) in headwater streams.

In the Oberer Seebach, summer high flow communities showed a strong difference with the communities of snowmelt and other seasons. Overall, they comprised more bacteria of the phyla

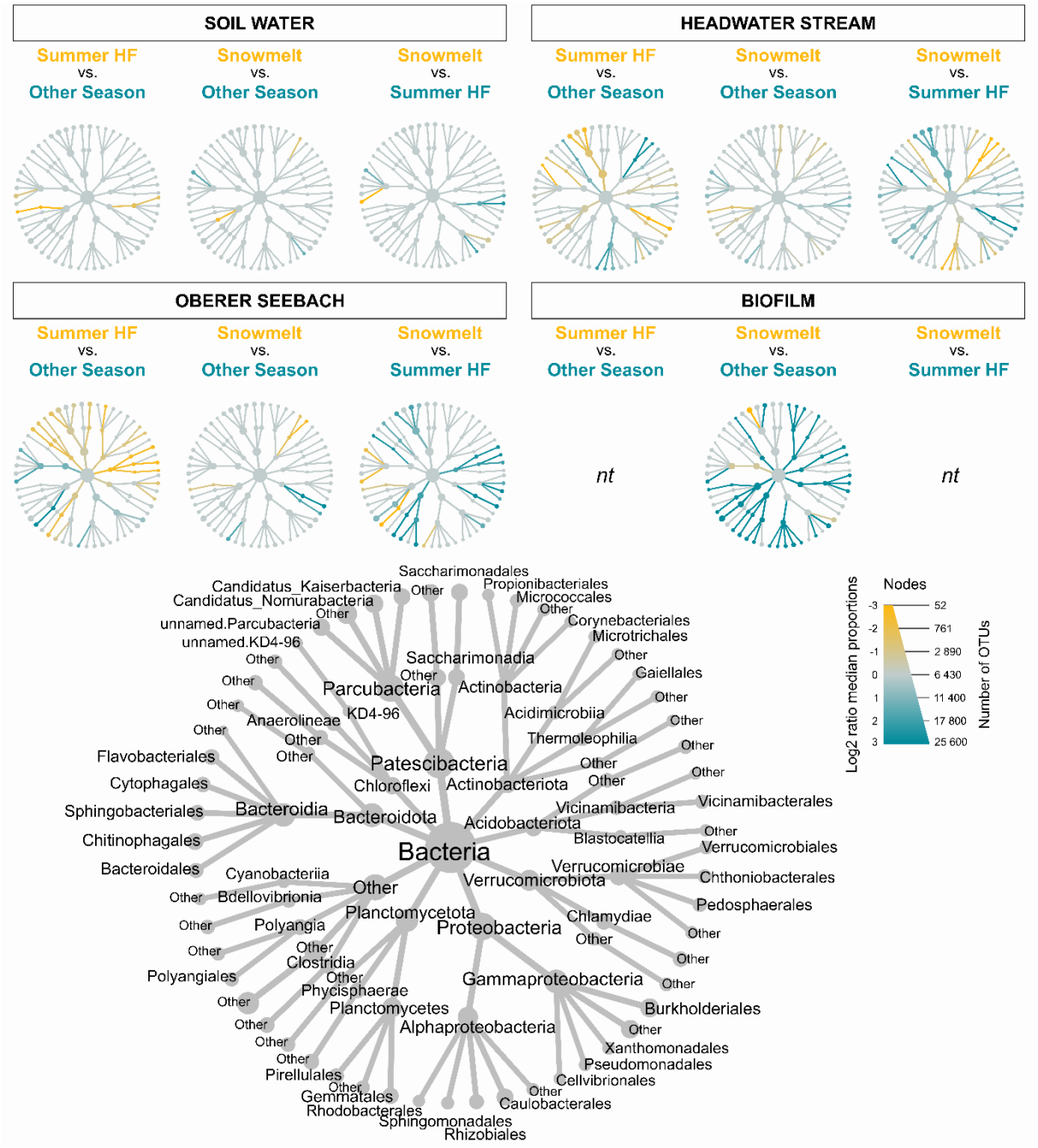

Fig. 6 Differential heat trees showing the differences in bacterial community composition between seasons in each habitat. Colours indicate which taxa are more important in each treatment written above each tree (Wilcoxon Rank Sum test, $p<0.05$). The size of each node is relative to the number of OTUs observed in each taxon on the whole dataset (with all habitats combined).

Acidobacteriota, Chloroflexi, and Planctomycetota, and of the class Parcubacteria than those seasons (Wilcoxon Rank Sum test, $p<0.05$). In the case of snowmelt, we observed an increase in the proportion of bacteria of the class Clostridia (Firmicutes) and orders Rhodobacterales, Flavobacteriales, and Bacteroidales compared to summer high flow conditions (Wilcoxon Rank Sum test, $p<0.05$).

In biofilms, only snowmelt and other season communities could be compared statistically because of limited data on biofilm samples during summer high flows. Overall, snowmelt biofilm samples presented less of most of the taxa compared to other seasons (Wilcoxon Rank Sum test, $p<0.05$). Indeed, during snowmelt, we observed a decrease in bacteria of the phyla Planctomycetota, Verrucomicrobiota, Acidobacteria, Actinobacteriota, Chloroflexi, and of the class Alphaproteobacteria compared to other seasons. On the contrary we observed more Bacteroidia and other Parcubacteria in snowmelt biofilm samples than during other seasons (Wilcoxon Rank Sum test, $p<0.05$).

Discussion

In this study, we explored the changes in bacterial diversity in the soil-stream interface with changing season and flow conditions. The major finding of this study is that, despite the increasing discharge during snowmelt, this event induces a different stream bacterial community than during summer high flow events (Figure 2-4, 6). These results complement previous studies that showed the impact of hydrological variability on stream bacterial transfer and diversity (Caillon et al., 2021; Caillon & Schelker, 2020 ; Hassell et al., 2018 ; Mishra et al., 2021).

Our results demonstrate that soil water, streams, and biofilm communities respond differently to an increase in discharge and a change in season despite them being intricately connected. Here we show that, as opposed to the other habitats, the diversity and relative abundance of bacterial phyla in the total soil water bacterial community remained stable with hydrological variations (Figure 2-6). This corroborates results from previous studies that showed no difference in the relative abundance of present bacterial phyla and classes in soil with increased precipitation (Barnard et al., 2015). In our dataset, soil water communities were more affected by the concentration and origin (fluorescence index) of dissolved organic matter (Figure 5), as shown in previous studies (Caillon & Schelker, 2020; Judd et al., 2006). Furthermore, one of the few differences we observed in community composition in soil water was a decrease in the relative abundance of the order Xanthomonadales during snowmelt compared to other seasons and flow conditions (Figure 6). These bacteria have been proposed to participate in the degradation of terrestrial leaves in aquatic environments which would well go in line with the observed decrease in early spring, when the relevance of fall leaves as a carbon source is diminishing (Kim & Liesack, 2015). Additionally, bacteria of the order Flavobacterales (phyla

Bacteroidetes), that have been associated with plant roots and were suggested to be able to degrade complex organic compounds (Kolton et al., 2013), also increased in relative abundance during summer high flow and other seasons compared to snowmelt. We propose that dissolved organic matter concentration and composition may modify soil water bacterial community composition through the seasonal strengthening and decline of these groups.

In headwater streams and the Oberer Seebach, flow variations had a greater impact on the bacterial communities than they did in soil water. In particular, flow affected Shannon diversity and bacterial species richness (Figure 2-3). Summer high flow enhanced bacterial diversity, a pattern that was not observed during the snowmelt. According to the CCA, DOC concentrations were a main driver of diversity in both habitats during summer high flows (Figure 5). With this, our results support our previous work that demonstrated a strong correlation between DOC concentrations and bacterial abundances in these headwater streams (Caillon & Schelker, 2020). Along with the NMDS, the CCA clearly separated high flow events during summer and other seasons from the snowmelt in both headwater streams and the Oberer Seebach. During snowmelt, the bacterial communities of the two habitats did not differ significantly from the other seasons and appeared to be mainly driven by pH.

In the headwater streams we observed an increase in the relative abundance of three soil-associated groups during snowmelt compared to other seasons - namely Saccharimonadales, Acidimicrobiia, and Polyangiales (Kessler et al., 2019; Lin et al., 2020; Rüthi et al., 2020). This observation confirms the enhanced connectivity between catchment soils and streams when discharge is increased (Figure 6). Furthermore, Saccharimonadales have been reported to be associated with high phosphorus concentrations in soils (Mason et al., 2021) and it is well admitted that phosphorus, and notably phosphate, reach their peak concentrations in stream water during snowmelt events due to flushing from surrounding soil environments while biological demand is still low (L. Guo et al., 2004; O'Donnell et al., 2021). Despite a higher connectivity between soils and streams when discharge increases, snowmelt induced a different bacterial community than summer high flow events in both headwater streams and the Oberer Seebach (Figure 5-6). The relative abundance of Parcubacteria increased in both habitats during summer high flow compared to snowmelt and other seasons even though their populations have been shown to be relatively stable over time and seasons (Vigneron et al., 2020). This group, along with bacteria of the class Chlamydiae, have been proposed to be involved in carbon and nitrogen cycling and the degradation of complex organic matter (F. Guo et al., 2020; Vigneron

et al., 2020). This would support our assumption that higher DOC concentrations drive bacterial diversity in stream water (Figure 5). A stronger soil signal was observed in the Oberer Seebach during summer high flow events compared to other habitats and seasons, including snowmelt, with the significant increase of Acidobacteriota, Chloroflexi, and Planctomycetota in their relative abundance (Figure 6). These groups are typical for the soil environment and have a very limited ability to colonize aquatic habitats, such as sediments (Tolotti et al., 2020). It is surprising that the same groups were not observed in higher amounts during snowmelt.

We propose that the strong shift we observed in biofilm bacterial community composition between snowmelt and other seasons is primarily due to a depletion of bacteria from biofilms when discharge increase as opposed to an increase in bacterial diversity during other seasons. Indeed, the snowmelt samples were negatively correlated with the number of days since the last high flow (Figure 5). It was previously shown that biofilm biodiversity can be reduced by enhanced flow velocity due to stream hydrodynamics (Besemer et al., 2009). Although implications for the stream biogeochemistry in the weeks following snowmelt can well be anticipated, we found no direct evidence in our current dataset to support this claim. Further, our findings support the notion that biofilm communities constitute an important contribution to energy flows and nutrient cycling in streams (Battin et al., 2003). With the reduction in biofilm bacterial diversity during snowmelt, we propose that ecosystem functioning may also be temporarily reduced following such events.

Considering the projected changes in climate for Europe, we can anticipate significant shifts in the timing, intensity, and frequency of snowmelt and summer storm events, leading to alterations in stream runoff regimes from snow-dominated to rain-dominated (Beniston, 2012; Blöschl et al., 2017). In the light of our results, these changes may lead to a shift in soil contributions and streams bacterial community composition as well as in the importance of snowmelt-derived nutrients and organic matter. The microbial response to these changes in hydrological conditions can have cascading effects on carbon and nutrient cycling in stream ecosystems, influencing their overall functioning and ecological dynamics. During spring snowmelt, headwater streams typically experience a peak in gross primary production; however, with reduced snow cover, it was shown that ecosystem respiration increased causing a shift from autotrophy (carbon sink) to heterotrophy (carbon source), potentially leading to cascading effects on climate change (Ulseth et al., 2018).

It is important to note that using environmental DNA to study bacterial communities in an ecosystem only describes the presence or absence of bacterial taxa. While DNA-based analyses can identify the presence of bacterial species, RNA-based methods are able to describe which portion of the community is actively contributing to the ecosystem functioning. With short term environmental variation such as variation in precipitation and discharge, RNA-based methods are more likely to identify changes in a community compared to total-DNA (Blazewicz et al., 2013; Girvan et al., 2003). Yet, it remains that DNA-based methods have been successfully used in the past to describe the total bacterial community composition and allow for an extensive comprehension of dispersal of bacteria between habitats, independent of ecosystem functioning (Caillon et al., 2021; Crump et al., 2012).

Our study agrees with others that reported seasonal changes in bacterial communities in soil water and streams ecosystems (Hullar et al., 2006; Portillo et al., 2012). We observed strong differences between seasons but more importantly between flow conditions. The variations in community composition we observed between snowmelt and summer high flow events were mainly explained by DOC concentration and origin in stream water. Climate change is projected to cause significant fluctuations in precipitation and extreme events (IPCC, 2023). Our results demonstrate that these events are key occasions to shape stream microbial communities, potentially leading to far-reaching ecological consequences for stream ecosystems in the near future.

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General Conclusion

The dynamics of DOC and bacterial communities in streams are highly dependent on connection to the surrounding terrestrial ecosystem as well as seasonal fluctuations in hydrology. With this thesis, I aimed to better characterize this connection by studying the fate of bacterial communities, both quantitatively and qualitatively, in the soil-stream-biofilm continuum throughout the year and at different flow conditions. I found that DOC and bacterial communities were concomitantly mobilized from surrounding soils to headwater streams, and in larger numbers during high flow events (Chapter 1). This mobilization had significant impacts on the bacterial community composition in the whole stream continuum and throughout the year (Chapter 2). I also determined that the impact of flooding on stream bacterial communities depended on the origin of the hydrological variation, whether from summer storms of snowmelt (Chapter 3). Considering the key role that headwater streams play in the whole river network via downstream transfer of energy, nutrients, organic matter, sediments, and organisms (Ferreira et al., 2022; Richardson, 2019; Wipfli et al., 2007), such dynamic connection with surrounding soils should not be overlooked.

Previous studies had shown that bacterial communities in headwater streams could be traced back to surrounding terrestrial ecosystems, and especially soils (Crump et al., 2012; Ruiz-González et al., 2015). As such, soils are a part of the stream network metacommunity and headwater streams have been described as ‘mirrors of the landscape’ (Bishop et al., 2008). Here I found that more than 70% of bacteria found in streams and stream biofilms were first identified in soils. Even though most of the bacteria in the soil-stream-biofilm continuum originated in soils, each habitat had its own individual bacterial community composition and diversity. I observed an increase in bacterial species richness and diversity from soils to headwater streams. Thus, although soil water samples had on average 10 times the bacterial abundance of streams, their diversity remained low in comparison, probably due to the predominance of some taxa relative to others in this habitat. These results highlight the role of headwater streams for the rest of the river network as initial mixing and dispersing zones for bacterial communities originating in surrounding soils. Additionally, the high proportion of soil-derived bacteria in biofilm communities support the importance of these transfers in the ecology of whole river systems. Indeed, bacterial communities in biofilms are formed from free-living communities in the surrounding water and are essential for in-stream processes, energy fluxes, and

biogeochemical cycles (Battin et al., 2008; Besemer, 2015). The lower diversity observed in biofilms compared to the other habitats is due to the more selective nature of these environments where certain bacteria thrive and others are lost to competition (Besemer et al., 2007; Flemming et al., 2016). Overall, the incremental significance of soil inputs along the course of headwater streams was evidenced by the observed increase in DOC concentration from source to outlet of our headwater streams without possible internal production through photosynthesis as was shown by the water residence time in our sampling system.

Although previous studies suggested the relevance of soil microbial communities for the stream network microbial diversity, they largely neglected the dynamic nature of these transfer processes and did not characterize the transfer of microorganisms in a quantitative manner. By studying the soil-stream-biofilm continuum over a period of two years, I was able to better appreciate the variations in DOC and bacterial community composition and abundance with changing seasons and flow conditions. I found that both DOC concentration and bacterial abundance varied throughout the year in headwater streams - being higher during summer and lower during winter (Köhler et al., 2009) – and measured the highest numbers during summer high flow events. Thus, the mobilization of bacterial communities and DOC from soils to headwater streams is fundamentally controlled by hydrological variability. Soils bacterial communities, on the contrary, appeared to be much more stable through time, both in numbers and in composition. I also found that soil DOC concentration was reasonably stable as flow rose, suggesting that the observed DOC increase in streams was due to increased mobilization rather than increased production during rain events. This strong mobilization of carbon and bacteria during summer high flow events induced a strong shift in bacterial richness and diversity in free-flowing communities. Thus, and as expected, I detected a greater share of bacterial groups typical of terrestrial ecosystems in streams during these episodes such as Acidobacteriota, Chloroflexi, and Planctomycetota. In fact, communities homogenized among habitats when flow increased in response to the magnitude of soil inputs. Additionally, 34.4% of the community found in soils during summer high flow events was specific to these episodes and found further downstream, in headwaters and biofilms, all year long. This underscores the long-term importance of summer high flow events on the microbial community composition of streams and biofilms, and by extension on the ecology of the entire river system.

Surprisingly, snowmelt-induced high flow episodes did not lead to an increase in the diversity and richness of bacterial taxa in streams as observed during summer flood events. It remains

that the increased connection between soil and streams during snowmelt is still observable with the detection of typical soil species in headwaters, albeit different from those found during summer floods - namely Saccharimonadales, Acidimicrobiia, and Polyangiales. The fact that diversity and species richness remain stable during snowmelt may be due to lower bacterial activity in soils at lower temperatures and with less carbon allocated from photosynthesis to the soil (Kaiser et al., 2010). Thus, the strong link between soil and streams is maintained year-round, but the resulting bacterial communities differ with the seasons. Indeed, I observed changes in bacterial community composition in each of the studied habitats throughout the year. In soil water and headwater streams, the composition of the bacterial communities could in part be explained by DOC concentration. It is well known that DOC has a strong impact on bacterial communities (Berggren & del Giorgio, 2015; Findlay et al., 2008) and I observed higher proportions of carbon-degrading bacteria (e.g. Parcubacteria and Chlamydiae) in streams when DOC concentrations increased. In addition, stream bacterial communities showed sensitivity to the proportion of DOC in the water originating from surrounding soils (represented by the fluorescence index) that also partially explained bacterial community composition. As expected, given the short residence times in the studied streams, the proportion of DOC from autotrophic production (i.e. biological index) did not explain any of the bacterial communities identified in our study.

While this thesis confirms and quantifies the dynamic link that exists between soils and headwater streams, emphasizing the significance of these transfers to the microbial ecology of river systems as a whole, further studies should be conducted to better understand the fate of the incoming bacterial communities and dissolved organic carbon in the hours, days, and weeks following high flow events. I am aware that studies of bacterial communities based solely on environmental DNA do not allow us to qualify the active part of these communities, which may indeed have an impact on the ecology of the environments studied. These studies must rather be completed with RNA and enzymatic activity assessments. DNA-based analyses have the advantage of giving a comprehensive idea of bacterial species dispersal between habitats, independent of ecosystem functioning. Finally, it would be interesting to replicate this experiment in contrasting environments to better understand the effect of climatic conditions and human activities on the transfers of bacteria and carbon to small headwater streams.

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