



DISSERTATION | DOCTORAL THESIS

Titel | Title

Influence of resource and consumer diversity on the quality and microbial processing of fine particulate organic matter during leaf breakdown in streams

verfasst von | submitted by

Pratiksha Acharya M.Sc.

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Doktorin der Naturwissenschaften (Dr.rer.nat.)

Wien | Vienna, 2026

Studienkennzahl lt. Studienblatt |
Degree programme code as it appears on the
student record sheet:

UA 796 605 299

Dissertationsgebiet lt. Studienblatt | Field of
study as it appears on the student record
sheet:

Environmental Sciences

Betreut von | Supervisor:

Univ.-Prof. Mag. Dr. Christian Griebler

Mitbetreut von | Co-Supervisor:

Dr. Katrin Attermeyer

“उद्देश्य के लिनु, उडी छुनु चन्द्र एक।”

महाकवि देवकोटा

“Look deep into nature and then you will understand everything better”

Albert Einstein

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor sincerely, Dr. Katrin Attermeyer, for giving me the opportunity to work in her lab at WasserCluster Lunz (WCL) and guiding me throughout this academic journey. Thank you for the space to grow, learn, and explore, to have broader conversations about science. My sincere thanks goes to my thesis committee members, Prof. Christian Griebler (official main supervisor), Prof. Gabriele Weigelhofer, and Prof. Michael Danger for serving on my committee and providing necessary feedback for my projects.

I am very grateful to Dr. Simon Vitecek (project partner and thesis committee member) for his continuous support and belief in me and my work, for his helpful advice during the experimental phases, and for his statistical guidance. Apart from science, thanks for the deep life talks and for making sure I was alive during sampling and the sample processing phase at the lab.

Many thanks to Dr. Luca Zoccarato and Dr. Christian Würzbacher, who guided me from the first glance into the bioinformatics aspects of the project; it was a great pleasure to collaborate with, who guided me from the first glance in the bioinformatics aspects of the project. It was a great pleasure to collaborate with them. I am also grateful to all my co-authors and anonymous reviewers for providing thoughtful feedback and critical comments on the manuscripts.

I truly appreciate the help from my lab members and friends who supported and assisted me throughout my research. I am grateful to my colleagues, Benjamin Misteli, Gertraud Steniczka, Theresa Reichenpfader, and Samuel-Karl Kaemmer, for helping me in the lab and with field work. I also want to express sincere gratitude towards Martin and Libor for all those insights into fatty acids. I am grateful to Veronika and Romana for all the administrative support during my stay at Lunz. Many, many thanks to WasserCluster Lunz for funding my research.

My special thanks go to Mourine Yegon (partner in crime in the lab), with whom it was a joy to collaborate and to walk each step of this journey together, and end up being friends. Cheers to all those inspirations and hurdles we shared, which made me stronger and more dedicated. I am deeply thankful to Anna and Robert for their immense support, concern, and care to me until you guys were there at Lunz. Angela, you are a great host as well as an amazing listener,

cheers to all those sleepovers at your place. Libbi, you are such a cheerful person - stay as you are, I must admit, those chit-chats at WCL corridors are locked in my memories. Leonie, your personal mails and texts gave me a lot of joy specially on those days when I needed them the most. Stefano, you are smart, kind, and goofy (in a positive way), and science needs more of that. Another special person I will never forget is Cihelio, who joined the group at the final stretch of this PhD journey. Thanks for the deep dives and rambles about ecology. I was fortunate to share an office with you all (Mourine, Stefano, and Cihelio). When I was feeling panicked and lost, you guys assured me that I would figure things out and all would be fine. Thank you very much, guys, for understanding, believing, and motivating me throughout the journey, and giving me beautiful memories.

Another thanks goes to all those incredible minds at conferences and courses including the SEFS (Bolu: Turkey and Newcastle: UK), PLPF (Landau: Germany), Survivor's Guide to Stable Isotope Ecology course (Sicily: Italy) and Circle U. Thematic Seminar (Berlin, Germany) and SIL Austria Meeting (Salzburg, Austria), I am grateful not only for these enriching opportunities but also the wonderful people I met along the way.

Finally, I am deeply grateful to my family and friends back in Nepal and Germany for their unconditional support and motivation despite the distance. My warmest thanks go to my mother, Sabita, and father, Mayanath, who kept telling me throughout the journey, "Everything happens for a reason, as good times and hard times will also pass away," which helped me to develop a 'keep going' attitude over time. My dearest brother, Pramod, who was there for me whenever needed, either emotionally or financially, I know you are the best gift from my parents to me ever. Moreover, last but not least, my lovely husband Michael, for witnessing all my rise and fall, and being there for me; your immense love and support were priceless throughout the entire journey. Thanks to all of you for being my pillar during all these years, which has formed the foundation of my academic endeavors. Without your unwavering faith and love for me, none of this would have been possible.

Last but not least, heartfelt condolences to all the insects we sacrificed in the name of research for this project.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	I
OVERVIEW OF THE DISSERTATION	IV
ABSTRACT	VI
ZUSAMMENFASSUNG	VII
INTRODUCTION	1
CHAPTER I:	16
Leaf Conditioning and Shredder Activity Shape Microbial Dynamics on Fine Particulate Organic Matter Produced During Decomposition of Different Leaf Litter in Streams	16
CHAPTER II:	30
Distinct bacterial communities affiliated with two types of shredder-produced particles in streams	30
CHAPTER III:	44
Shredder identity matters: Taxon-specific gut microbiota in aquatic shredders drives bacterial diversity during leaf breakdown in streams	44
DISCUSSION	73
APPENDIX	85
Supplementary material Chapter I-III	85

OVERVIEW OF THE DISSERTATION

The following thesis is presented as a cumulative dissertation. First, a general introduction establishes which processes drive leaf litter breakdown in streams, including the roles of biodiversity and the different environmental factors that influence this process. Based on this knowledge base, I present my contribution to the field in three chapters in which I investigate the microbial dynamics of fine particulate organic matter (FPOM) produced during leaf litter breakdown in streams. Chapters 1 and 2 are manuscripts published in international, peer-reviewed journals, whereas Chapter 3 has been submitted to the scientific peer-reviewed journal **ISME Communications**.

Chapter I

"Leaf Conditioning and Shredder Activity Shape Microbial Dynamics on Fine Particulate Organic Matter Produced During Decomposition of Different Leaf Litter in Streams" is a publication authored by Acharya, P., Yegon, M. J., Haferkemper, L., Misteli, B., Griebler, C., Vitecek, S., & Attermeyer, K. It is published in the Journal **Microbial Ecology** under DOI <https://link.springer.com/article/10.1007/s00248-025-02515-2>. This chapter discusses microbial dynamics of FPOM produced during the decomposition of different leaf litter in streams, and my contribution to the research in this field was conducting the experiment, sample collection, data visualization, formal analyses, writing the original draft, and review & editing.

Chapter II

"Distinct bacterial communities affiliated with two types of shredder-produced particles in streams" is a publication authored by Acharya, P., Yegon, M. J., Griebler, C., Vitecek, S., & Attermeyer, K. It is published in the journal **FEMS Microbiology Ecology** under DOI <https://academic.oup.com/femsec/article/101/10/fiaf091/8255872>. This paper examines the distinct bacterial communities associated with two types of shredder-produced FPOM. My contribution to the research in this field was conducting the experiment, data curation, formal analyses, investigation, methodology, resources, software, validation, data visualization, writing the original draft, and review & editing.

Chapter III

"Shredder identity matters: Taxon-specific gut microbiota in aquatic shredders drives bacterial diversity during leaf breakdown in streams" is the manuscript draft submitted to the **ISME Communications** journal on **23.12.2025**. This paper, authored by Acharya, P., Yegon, M. J., Zoccarato, L., Griebler, C., Vitecek, S., & Attermeyer, K., shows the shredder-taxon-specific bacterial communities in their gut and faecal pellets. My contribution to the research in this field was conducting the experiment, sample collection, formal analyses, data visualization, writing the original draft, and review & editing.

At the end of this thesis, I bring together and synthesize the findings from all three chapters and place them in the context of stream ecology and stream ecosystem functioning in a general discussion. There, I consider how my work within the broader scientific context connects to relevant ecological theories and critically examine the methodological strengths and limitations. Lastly, I identify remaining challenges and outline potential future research directions that could expand our understanding of the processes and mechanisms shaping freshwater detrital food webs.

ABSTRACT

Stream ecosystems depend heavily on the input and breakdown of terrestrial leaf litter, a process primarily driven by the interplay between heterotrophic microbial communities and detritivore-shredders. Microorganisms colonize and decompose leaves, thereby improving the nutritional value and, consequently, the palatability of leaf litter for other consumers, such as shredders. The shredders, in turn, consume the leaves, producing fine particulate organic matter (FPOM) through leaf fragmentation and defecation. Microorganisms further colonize this FPOM, and together, FPOM and its associated microbial communities serve as a food source for downstream organisms, such as collectors and filter-feeders. Recent evidence highlights the ubiquitous importance of microorganisms in the processing of coarse particulate organic matter (CPOM, particle size > 1mm). However, there is limited knowledge about the microbial dynamics specifically associated with the shredder-produced FPOM types, i.e., shredded leaves and faecal pellets. My thesis investigates the microbial dynamics underlying the transformation of CPOM into FPOM in response to three factors: oxygen availability during microbial colonization, leaf quality, and shredder identity. I conducted two laboratory feeding experiments where I explored how microbial communities differed among 1) FPOM produced by one shredder taxon (*Sericostoma* sp.) from leaves of alder, beech, or maple conditioned under oxic or anoxic environments and 2) oxic-conditioned alder leaves processed by three shredder taxa (*Allogamus auricollis*, *Potamophylax latipennis*, and *Sericostoma flavicorne*). Specifically, I assessed microbial activities (in the first experiment), microbial community composition of shredder-produced FPOM collected in both experiments, and also linked shredder gut microbiomes and FPOM-associated microbial communities (in the second experiment). Across the three chapters, I found that microbial conditioning reduced the C/N ratio of low-quality leaves (beech and maple), but C/N stayed constant during gut passage, and that shredded leaves and faecal pellets differ in their microbial activities (Chapter I). I also found that microbial colonization under varying oxygen levels and gut passage led to distinct bacterial communities colonizing two different FPOM types (Chapter II). The second experiment showed shredder-specific differences in the gut microbiota and consequently, in the faecal pellets produced by different shredder taxa (Chapter III). Overall, my thesis reveals a huge diversity in shredder-produced FPOM with respect to FPOM quality, activity of FPOM-associated microbiota, and bacterial community composition. This suggests that bacterial assemblages on different FPOM types and in the guts of different shredders may employ a wide range of metabolic pathways, which possibly contribute diversely to leaf litter decomposition (LLD) in streams, with potential implications for the stream microbiome, downstream nutrient cycling, and food-web dynamics.

ZUSAMMENFASSUNG

Fließgewässerökosysteme sind in hohem Maße auf den Eintrag und Abbau von terrestrischem Laub angewiesen, ein Prozess, der hauptsächlich durch das Zusammenspiel heterotropher mikrobieller Gemeinschaften und detritivorer Zerkleinerer ("Shredder") gesteuert wird. Mikroorganismen besiedeln und zersetzen das Laub und verbessern dadurch dessen Nährwert und damit auch dessen Attraktivität für andere Konsumenten, insbesondere für Shredder. Diese wiederum konsumieren das Laub und produzieren dabei durch Fragmentierung und Defäkation feines partikuläres organisches Material (FPOM). Das produzierte FPOM wird anschließend erneut mikrobiell besiedelt und stellt gemeinsam mit den assoziierten Mikroorganismen eine wichtige Nahrungsquelle für stromabwärts lebende Organismen, wie Sammler („Collector“) oder sogar kleine Fische, dar. Neuere Studien heben die zentrale Rolle von Mikroorganismen bei der Verarbeitung von grobem partikulärem organischem Material (CPOM, Partikelgröße > 1 mm) hervor, dennoch ist derzeit noch wenig über die spezifische mikrobielle Dynamik bekannt, die mit den von Shreddern produzierten FPOM-Typen, d. h. zerkleinerten Blättern und Kotpellets, verbunden ist. In meiner Dissertation untersuche ich die mikrobielle Dynamik, die an der Umwandlung von CPOM in FPOM beteiligt ist, und zwar in Abhängigkeit von drei Faktoren: Sauerstoffverfügbarkeit während der mikrobiellen Besiedlung, Blattqualität und Shredder-Identität. Ich führte zwei Fütterungsexperimente im Labor durch, in denen ich untersuchte, wie sich mikrobielle Gemeinschaften unterschieden zwischen 1) FPOM, produziert von einem Shredder-Taxon (*Sericostoma* sp.) aus Blättern von Erle, Buche oder Ahorn, die unter oxischen oder anoxischen Bedingungen konditioniert worden waren, und 2) oxisch konditionierten Erlenblättern, zerkleinert von drei verschiedenen Shredderarten (*Allogamus auricollis*, *Potamophylax latipennis* und *Sericostoma flavicorne*). Konkret untersuchte ich die mikrobiellen Aktivitäten (im ersten Experiment), die Zusammensetzung der mikrobiellen Gemeinschaften des von Shreddern produzierten FPOM, das in beiden Experimenten gesammelt wurde, und untersuchte den Zusammenhang zwischen den Darmmikrobiomen der Shredder und den FPOM-assoziierten mikrobiellen Gemeinschaften (im zweiten Experiment). Über alle drei Kapitel hinweg fand ich, dass die mikrobielle Konditionierung das C/N-Verhältnis von Blättern niedriger Qualität (Buche und Ahorn) verringerte, das C/N-Verhältnis jedoch während der Passage durch den Darm konstant blieb, und dass sich zerkleinerte Blätter und Kotpellets in ihren mikrobiellen Aktivitäten unterschieden (Kapitel I). Ich konnte zudem zeigen, dass mikrobielle Besiedlung unter unterschiedlichen Sauerstoffbedingungen sowie die Darmpassage zu verschiedenen bakteriellen Gemeinschaften auf zwei unterschiedlichen FPOM-Typen führte (Kapitel II). Im zweiten Experiment konnte ich shredder-spezifische Unterschiede in der Darmmikrobiota und folglich auch in den von ihnen produzierten Kotpellets nachweisen (Kapitel III). Zusammenfassend zeigt meine Dissertation eine große Diversität an

shredder-produziertem FPOM hinsichtlich Qualität, mikrobieller Aktivität und bakterieller Gemeinschaftszusammensetzung. Dies deutet darauf hin, dass bakterielle Gemeinschaften auf unterschiedlichen FPOM-Typen sowie in den Därmen verschiedener Shredder eine Vielzahl metabolischer Wege nutzen, die auf unterschiedliche Weise zum Abbau von Laubstreu (Leaf Litter Decomposition, LLD) in Fließgewässern beitragen und potenziell Auswirkungen auf den stromabwärtigen Nährstoffkreislauf sowie die Dynamik von Nahrungsnetzen haben.

INTRODUCTION

Freshwater ecosystems

Freshwaters such as lakes, rivers, and streams (Wilson & Carpenter, 1999) cover only 2.5% of the earth's surface (Carpenter et al., 2011), yet they provide essential ecosystem services (Maltby & Acreman, 2011) that are crucial to the river health and even history, survival, and well-being of humankind (Wantzen et al., 2016). Within these systems, streams are dynamic, interconnected networks that regulate hydrological and biogeochemical cycles by transporting water, sediments, nutrients, and organic matter from terrestrial to marine environments (Richardson, 2019). Despite containing merely 0.00015% of the Earth's water and having limited algal production, headwater streams show tremendous variability in water chemistry, temperature, velocity, and resource availability, and provide niches for a wide range of organisms and thereby contribute to landscape-level biodiversity (Leroy & Marks, 2006; Yue et al., 2022). Headwater streams are hotspots of biodiversity, supporting diverse species including riparian vegetation, bryophytes, fishes, aquatic insects, and invertebrates, as well as microorganisms (Lecerf & Chauvet, 2008; Phillips et al., 2017; Richardson & Danehy, 2007). They interlink terrestrial and aquatic biogeochemical cycles through the storage, transport, and decomposition of terrigenous detritus (Harvey et al., 2023; Loreau et al., 2003; Richardson, 2019; Richardson & Hanna, 2021). In addition to their ecological roles, streams and headwaters contribute significantly to human welfare through the provision of multiple ecosystem services, including freshwater supply, water purification and regulation, and recreational opportunities. Indeed, they are important to Earth's environmental and biogeochemical networks.

Leaf litter as a cross-ecosystem connector

Stream ecosystems are spatially connected to their terrestrial surroundings through the flux of materials and energy (Allan et al., 2021a; Dahlin et al., 2021; Loreau et al., 2003). These aquatic-terrestrial linkages are bidirectional: streams receive substantial inputs of organic matter from riparian vegetation while simultaneously exporting nutrients and emerging aquatic insects (post-metamorphosis) to surrounding terrestrial ecosystems (Fig. 1; Dahlin et al., 2021; Sullivan & Manning, 2019; Richardson, 2019; Richardson & Hanna, 2021). Such reciprocal exchanges link local ecosystem processes across larger spatial scales and are central to meta-ecosystem theory, which offers a framework for understanding spatial flows across ecosystems (Allan et al., 2021a; Harvey et al., 2023; Loreau et al., 2003; Richardson & Sato, 2015). Within this framework, ecosystems are viewed as interconnected systems rather than isolated units, with functioning that depends not only on local processes but also on spatial subsidies and feedbacks from adjacent systems (Loreau et al., 2003). Streams, in particular,

act as integrative corridors that collect, transform, and redistribute resources across terrestrial-aquatic boundaries, thereby playing a crucial role in biogeochemical cycles at the landscape level.

One well-recognized class of carbon subsidies is the transport of coarse particulate organic matter (CPOM), such as leaf litter, from terrestrial ecosystems to forested headwater streams (Richardson & Sato, 2015; Vannote et al., 1980). In shaded and nutrient-poor streams, detrital carbon (C) pathways are critical because primary production is limited (Pérez et al., 2021). Substantial amounts of organic carbon in the form of leaf litter enter the streams and are gradually broken down and processed in the stream detrital food web (Fig.1). This process is an indispensable component of the global C cycle (Boyer et al., 2016), and includes microbial decomposition of terrestrial detritus in streams, which results in a net global efflux of 1.8 Pg C/year into the atmosphere (Battin et al., 2009; Raymond et al., 2013). Detrital input supports a significant portion of total system secondary production (Wallace et al., 1997) and profoundly influences both food webs and carbon cycling (Gessner et al., 2010) as well as the nutrient budget (Fisher & Likens, 1973; Argerich et al., 2008) of streams. Consequently, leaf litter is the primary source of energy and nutrients for heterotrophic communities in stream detrital food webs (Vannote et al., 1980). It further supports higher trophic levels and diverse stream biota and, therefore, is important for the functioning and persistence of stream ecosystem communities (Fig. 1; Lidman, 2015; Richardson & Sato, 2015; Swan, 2021). Indeed, leaf litter decomposition is one of the most commonly measured ecosystem functions in lotic ecosystems and has been suggested as a measure of river ecosystem health (Gessner & Chauvet, 2002).

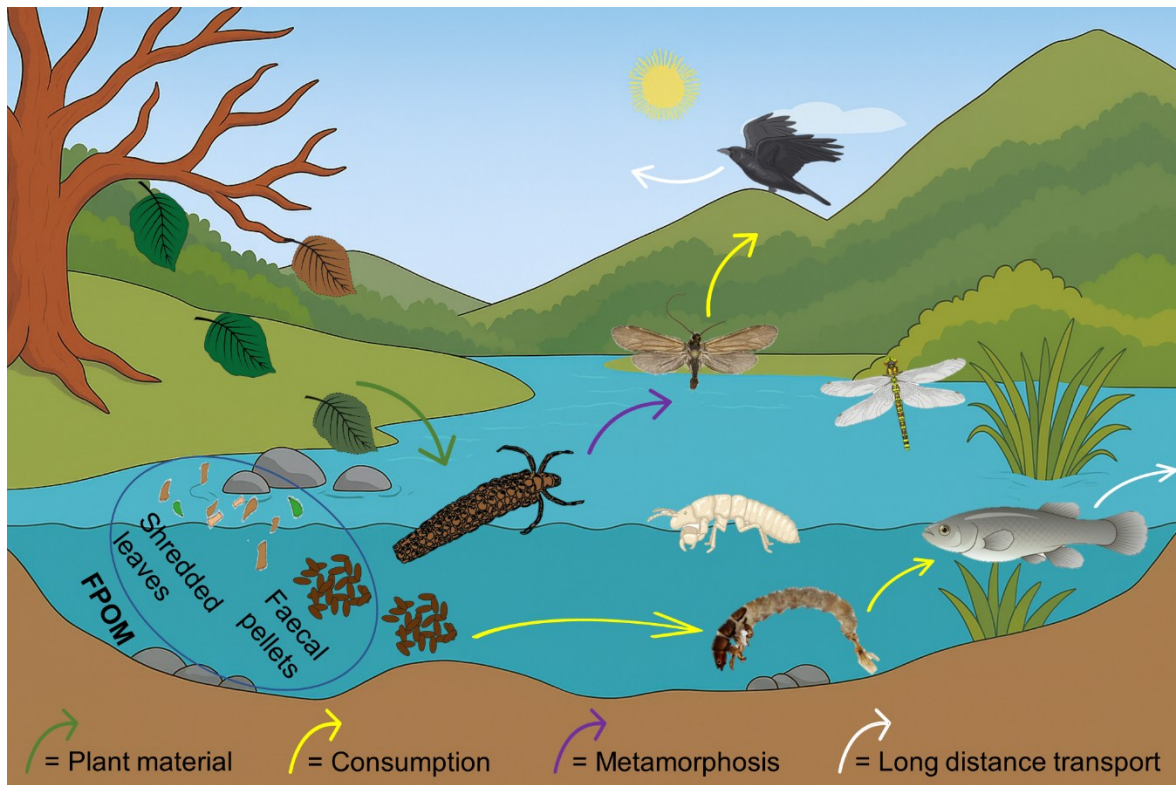


Figure 1. A detrital food web showing leaf litter linking the terrestrial and aquatic systems. Adapted after Dahlin et al. (2021) and created using <https://chat.openai.com> and Biorender (www.biorender.com).

Leaf litter decomposition in headwater streams

Headwater streams provide a wide range of microhabitats, such as submerged terrestrial leaves, macrophytes, suspended organic matter, and gravel, in which diverse microbial communities, including fungi, bacteria, archaea, protists, and viruses, thrive (Zeglin et al., 2015). These microorganisms together comprise the stream microbiome, which plays a crucial role in processing organic matter and regulating carbon storage and export from the system. The stream microbiome is particularly important in regulating the decomposition of terrigenous organic matter, which is a key ecosystem function within stream-detritus-based food webs. In general, leaf litter decomposition (LLD) in streams is a complex process that integrates physical, chemical, and biological factors, and collectively fuels the stream ecosystems and maintains their functioning (Monroy et al., 2022). It usually occurs in three stages: leaching, conditioning, and fragmentation (Fig. 2; Gessner et al., 1999). Leaves (i.e., terrestrial coarse particulate organic matter, CPOM > 1mm) undergo rapid loss of soluble compounds within 24 hours. In fact, within a few days of immersion, these CPOM are colonized by microbial communities and then subsequently macroinvertebrate-shredders (aquatic consumers) fragment CPOM into fine particulate organic matter (FPOM, < 1 mm; e.g., small detritus fragments, faecal pellets) and release additional nutrients (e.g., excreted ammonia) (Cummins, 1974; Graça, 2001; Prather et al., 2013). These combined activities of microbial

decomposers and shredder communities further regulate how quickly and efficiently leaf litter decomposes, thereby influencing nutrient cycling and carbon export from riverine networks (Battin et al., 2016; García-Palacios et al., 2021; Handa et al., 2014). Therefore, understanding the independent and interactive effects of leaf litter, microbes, and shredders on LLD and nutrient turnover is of great interest to predict the consequences of a changing environment on stream ecosystem functioning.

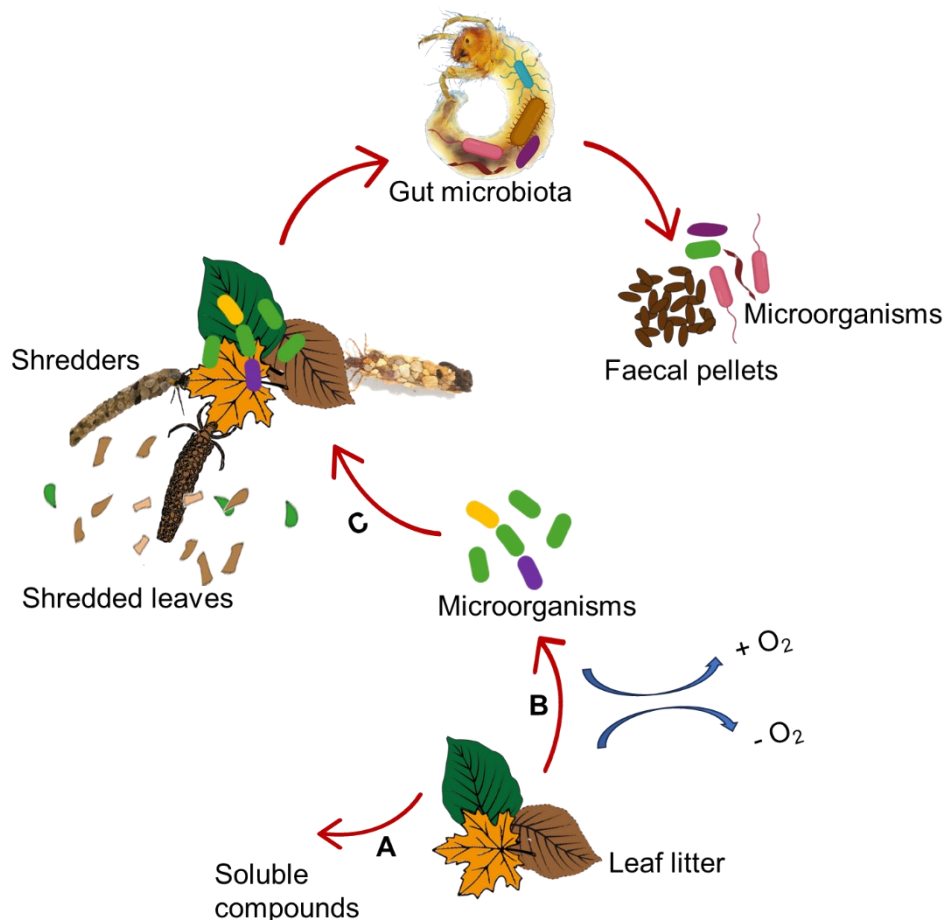


Figure 2. Conceptual understanding of leaf litter breakdown in streams followed by leaching (A), microbial colonization (B), and shredder fragmentation (C). Adapted after Bernabe' et al (2018) and created using Biorender (www.biorender.com).

Leaf litter decomposition with a spotlight on the microbial realm

Submerged leaves in streams are important microhabitats that support different microbial communities, often in response to their elemental composition and other traits. Such inhabiting microorganisms play a crucial role in the initial stage of LLD in streams by using immediately available leachate from these leaf resources and then colonizing and decomposing the submerged leaves (Bärlocher & Kendrick, 1975). Aquatic fungi, particularly hyphomycetes, and heterotrophic bacteria are important because they colonize leaf litter with a wide range of

nutritional qualities, including differences in carbon-to-nitrogen (C/N) ratios and fatty acid composition, and use the more recalcitrant leaf resources such as cellulose and lignin. Here, it is important to recognize that not all fallen leaves in streams are the same. For instance, leaf litter from tree species such as alder (*Alnus glutinosa*) or beech (*Fagus sylvatica*) differs substantially in its elemental composition (i.e., stoichiometry) and other properties (e.g., toughness) (Gessner, 2010). In fact, the composition and colonization speed of the microorganisms depend on leaf traits (e.g., C/N ratio, lignin content, fatty acids, toughness) and environmental conditions, including temperature and nutrient concentrations (Fenoy et al., 2024; García-Palacios et al., 2016; Gonçalves et al., 2024). Overall, the conditioning process softens the structural tissues and, through the biomass of fungi and bacteria, enhances the nutritional content of the submerged leaves with proteins, fatty acids, and essential elements such as nitrogen, phosphorus (Bärlocher & Kendrick, 1975; Funck et al., 2014; Manning et al., 2015; Newman et al., 2015; Torres-Ruiz & Wehr, 2010). This improves the quality of leaf resources (and probably their palatability) and makes them attractive to aquatic consumers (i.e., detritivore macroinvertebrates) that need to meet their nutritional requirements (Allan et al., 2021a; Gonçalves et al., 2024). Often, microbial leaf biofilms include taxa that are specialized in degrading complex biopolymers, such as lignin, cellulose, hemicellulose, and chitin: bacterial taxa active in leaf litter decomposition include *Sphingomonas*, *Flavobacterium*, *Pseudomonas*, and *Cellvibrio* that are primarily responsible for breaking down recalcitrant plant-derived compounds (Khomutovska et al., 2024; Rastogi et al., 2012; Silverman et al., 2017; Tláškal et al., 2016). Together, these efficient and diverse metabolic pathways in the stream microbiome speed up the breakdown of CPOM by shredders (Danger et al., 2012; Gessner, 2010). However, microbial metabolic responses may differ with leaf resource traits, thereby affecting the rate and intensity of shredder-mediated processing of CPOM into FPOM.

Additionally, environmental conditions (temperature, nutrient availability, hydrology, and dissolved oxygen) are important regulators of LLD in both terrestrial and aquatic ecosystems as they influence LLD by altering the CPOM quality and activity and community composition of decomposers (Fenoy et al., 2024; García-Palacios et al., 2016; Gonçalves et al., 2024; Handa et al., 2014; Wardle et al., 2006). These factors, therefore, can play a key role in shaping the structure and function of the whole ecosystem over time. Streams can show high variability in dissolved oxygen concentration, leading to oxic conditions in fast-moving, oxygen-rich riffles and anoxic conditions in slow-moving pools and in hyporheic sediments, where oxygen may be limited (Battin et al., 2016; Kristensen et al., 1995). Such small-scale habitat differences strongly influence which microbes grow and how they function during LLD (Clivot et al., 2013; Medeiros et al., 2009). Oxygenated areas are usually dominated by aerobic fungi and bacteria, which quickly break down organic matter with their higher biomass and activity that possibly

speed-up CPOM breakdown (Manning et al., 2015; Medeiros et al., 2009). In contrast, low-oxygen or anoxic zones are typically dominated by anaerobic microbes such as *Propionivibrio*, *Alkalibacter*, and *Paludibacter*. These organisms depend on fermentation and anaerobic respiration, and decompose organic matter more slowly (Karačić et al., 2024; Qiu et al., 2017; Silverman et al., 2017). Overall, studies have shown that the efficiency of LLD is interlinked with the complexity of the decomposer community and, thereby, that microbial succession is a major factor in nutrient cycling within stream ecosystems (Handa et al., 2014; Wardle et al., 2006).

Among the many biological factors involved in LLD, aquatic macroinvertebrates, particularly shredders, play an important role (Fig. 2; Cummins, 1974; Graça, 2001; Wallace et al., 1997). While shredders are traditionally recognized for mechanically fragmenting CPOM into FPOM (Cummins, 1974; Graça, 2001; Prather et al., 2013), their influence extends far beyond physical breakdown. Through feeding, shredders modify the microbial environment of leaf litter in several ways. First, shredding increases the surface area of leaf material, promoting microbial colonization and improving access to organic substrates (Hieber et al., 2002). Second, gut passage exposes ingested material to taxon-specific physicochemical conditions and digestive enzymes (Engel & Moran, 2013; Lange et al., 2023; Plante et al., 2023), thereby selectively altering both substrate quality and associated microbial communities before material is egested as faecal pellets (Graça, 2001; Prather et al., 2013). Together, these processes accelerate the CPOM breakdown, mobilize nutrients, and thereby shape the composition, activity, and functional potential of FPOM-associated microbial communities within stream food webs (Allan et al., 2021b; Prather et al., 2013). Despite the ecological significance of these processes, most research on LLD focuses on CPOM decomposition under different environmental conditions by shredders and microorganisms, whereas if and how shredder feeding activity influences FPOM chemical composition or the activity and composition of microbial communities associated with FPOM has not been investigated in depth. The fact that shredders themselves are taxonomically and functionally diverse in their feeding behavior, digestion efficiency, and habitat preferences has also not been given full attention so far (Dekanová et al., 2023; Grasser et al., 1992; Thomsen & Friberg, 2002; Urbanič et al., 2005; Waringer & Malicky, 2017). These traits likely affect microbial community composition in the gut and faecal pellets, as well as how quickly FPOM can be further decomposed/used; however, it is still not clear how shredder diversity influences the functioning of stream ecosystems.

Beyond fragmentation, shredders influence microbial communities more directly through gut-mediated processes. In the shredders' gut, ingested particles go through various physicochemical transformations that likely restructure the microbial community, possibly through selective digestion or seeding by resident gut microbes (Aira et al., 2022; Plante et al.,

2023). Such gut-mediated effects are well-documented across detritivores, including earthworms (Aira et al., 2008, 2022), polychaetes (Dale et al., 2019), shrimp (Lau et al., 2002), marine deposit feeders (Plante et al., 2010), and insect larvae (Ayayee et al., 2022; Chen et al., 2016). Furthermore, research on gut microbiomes in aquatic insects highlights the dynamic nature of microbial succession across developmental (= ontogenetic) stages and feeding modes (Chen et al., 2016; Kroetsch et al., 2020; Sontowski & van Dam, 2020; Yun et al., 2014). In fact, the insect gut is characterized by redox conditions, enzymatic activity of gut-microbiota, and an internal filtering process that selectively filters microbial taxa and reduces overall diversity while enriching resilient or metabolically flexible groups (Ayayee et al., 2022; Engel & Moran, 2013; Lange et al., 2023; Plante et al., 2023). When it comes to stream detritivores, gut passage can enhance the nutritional quality of egested material (Newman et al., 2015; Stelzer et al., 2003) and, in turn, alter biochemical pathways relevant to FPOM transport and decomposition within the stream continuum. Also, the gut environment may differ depending on the shredder's ontogenetic stages, with variation of gut microbiome over time -aligning with broader principles of microbial ecology: host-specific microbial succession (Ayayee et al., 2022; Engel & Moran, 2013; Lange et al., 2023; Plante et al., 2023). The composition of host-associated microbiomes is shaped by both host ontogenetic stage, physiology, and ingested substrates (Harris, 1993; Chen et al., 2016; Pechal & Benbow, 2016; King, 2018). Yet, there is a major knowledge gap regarding community composition of gut microbiota of Trichopteran shredder taxa, which have a prominent role in processing of terrestrial organic matter in headwater streams (Basaguren et al., 2002; Elliott, 1969; Grassler et al., 1992; Urbanič et al., 2005; Waringer & Malicky, 2017). In particular, knowledge on the development of the gut microbiome of freshwater shredders across ontogenetic stages is still lacking.

Independent of the finer mechanics associated with shredder-mediated fragmentation and gut passage, faecal pellets, one type of shredder-produced FPOM, become a key substrate for the microbiota, which drive their further decomposition. Studies on particulate matter in river networks have shown distinct microbial assemblies in free-living and particle-associated fractions (Crump et al., 1999; Riemann & Winding, 2001; Savio et al., 2015). It is usually assumed that shredded leaves, another type of shredder-produced FPOM, host microbial communities similar to those in coarse leaf particles. However, after gut-mediated transformations, faecal pellets can host diverse microbial communities capable of degrading polysaccharides, proteins, lignin derivatives, and microbial necromass (Gu et al., 2024; Wang et al., 2022; Wang et al., 2024). In all cases, as microbes colonize the FPOM, they alter the nutritional value of particles - typically reducing C/N ratio (Newman et al., 2015) - and stimulate heterotrophic metabolism (Manning et al., 2015; Pope et al., 2020). In fact, the microbial-enriched FPOM serves as an important food resource for downstream communities, including filter-feeders (Wallace et al., 1997). Apart from serving as a local resource, FPOM can act as

a transport vehicle that connects microbial communities across hydrological gradients and links headwaters to downstream ecosystems (Sadeghi et al., 2024; Stadler & Del Giorgio, 2022; Stegen et al., 2016). In the context of meta-ecosystem theory, FPOM transport can therefore be a strong example of ecosystem connectivity, where biological processes in one ecosystem compartment can influence ecological functioning far beyond their original location and function (Harvey et al., 2023; Loreau et al., 2003; Richardson & Sato, 2015). Therefore, FPOM can be seen as a link between microbial processes in the catchment-scale carbon cycling and enhancing organic matter turnover within the riverine continuum (Grossart et al., 2020; Catalán et al., 2018). Despite this, the importance of FPOM origin and FPOM-associated microbial ecology is still poorly understood.

Taken together, this thesis was motivated by several critical knowledge gaps. First, although FPOM is recognized as a major pathway for carbon and nutrients in streams, it remains conceptually underrepresented and under-investigated compared to CPOM in LLD research. Second, the influence of leaf quality and microbial conditioning (under different oxygen levels) on FPOM quality and microbial activity remains poorly understood. Third, there is limited knowledge about how these factors affect the composition of bacterial communities associated with FPOM. Finally, the influence of shredder species on shaping FPOM microbial communities during gut passage has been rarely examined, especially for dominant Trichoptera shredders in headwater streams.

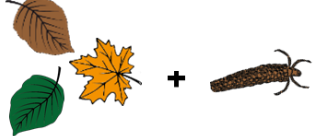
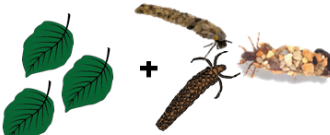
Objectives of the thesis

With this thesis, I primarily aim to investigate the combined influence of resource (leaf species and leaf conditioning under oxic versus anoxic conditions) and consumer diversity (shredder species) on the quality and microbial processing of FPOM during leaf breakdown in streams. Particularly, employing laboratory feeding experiments, the objective was to elucidate the chemical composition, activity, and community composition of bacteria in shredder-produced FPOM, including the gut microbiota during leaf breakdown (see Table 1).

In Chapter I (Leaf Conditioning and Shredder Activity Shape Microbial Dynamics on Fine Particulate Organic Matter Produced During Decomposition of Different Leaf Litter in Streams), I specifically addressed the question on how the CPOM composition, manipulated through leaves of different deciduous tree species, and oxygen availability, i.e., either oxic or anoxic conditioning of these leaves, affect shredder-produced FPOM properties and FPOM-associated microbial communities. As evident from past studies, shredder activity, encompassing both shredding behaviour along with feeding preference, and gut passage, produces FPOM with higher C/N ratios by stripping N from leaf particles. Hence, employing a laboratory feeding experiment approach, I examined protein production and respiration of

FPOM-associated microorganisms as a biotic response to FPOM composition and considered two types of shredder-produced FPOM: FPOM from mechanically shredded leaves, mimicking shredder feeding behaviour, as well as their faecal pellets.

Table 1. Scheme illustrating the research objectives, experimental framework (Experiments I and II), and their correspondence to Chapters (Papers I–III).

Experiments	Manipulated factors	Chapters	Samples	Parameters measured
I	Resource diversity  Includes microbial colonization of leaves under oxic/anoxic condition	I	 Shredded leaves  Faecal pellets	Composition (C/N, fatty acids) Microbial respiration and Bacterial protein production
		II	 Shredded leaves  Faecal pellets	Total Bacterial community composition (DNA)
II	Shredder diversity  Includes microbial colonization of leaves under oxic condition	III	 Shredded leaves  Larvae gut  Faecal pellets	Total and active Bacterial community composition (DNA and RNA)

In Chapter II (Distinct bacterial communities affiliated with two types of shredder-produced particles in streams), I extended prior knowledge on differences among biochemical composition and microbial activity of shredder-produced FPOM due to selective digestion and microbial filtering in the gut. Bearing that in mind, the two types of shredder-produced FPOM would be colonized by distinct bacterial communities. Specifically, I evaluated how leaf species, leaf conditioning under different oxygen levels, and gut passage affect the diversity and composition of bacterial communities associated with shredder-produced FPOM in a microcosm feeding experiment, and identified indicative bacterial taxa dominating shredder-produced FPOM during leaf litter breakdown.

In Chapter III (Shredder identity matters: Taxon-specific gut microbiota in aquatic shredders drives bacterial diversity during leaf breakdown in streams), I determined for the first time the information on the gut microbiome of shredders (*Allogamus* sp., *Potamophylax* sp., and *Sericostoma* sp.), all from the order Trichoptera, which is one of the most important functional feeding groups in headwater streams (Grasser et al., 1992; Urbanič et al., 2005; Waringer et al, 2017). I specifically investigated here how shredder-mediated leaf litter decomposition influences the composition, diversity, persistence, and potential ecological functions of particle-attached bacterial communities contributing to the stream microbiome.

References

- Aira, M., Pérez-Losada, M., Crandall, K. A., & Domínguez, J. (2022). Composition, Structure and Diversity of Soil Bacterial Communities before, during and after Transit through the Gut of the Earthworm *Aporrectodea caliginosa*. *Microorganisms*, 10(5), Article 5. <https://doi.org/10.3390/microorganisms10051025>
- Aira, M., Sampedro, L., Monroy, F., & Domínguez, J. (2008). Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. *Soil Biology and Biochemistry*, 40(10), 2511–2516. <https://doi.org/10.1016/j.soilbio.2008.06.010>
- Allan, J. D., Castillo, M. M., & Capps, K. A. (2021a). Carbon Dynamics and Stream Ecosystem Metabolism. In J. D. Allan, M. M. Castillo, & K. A. Capps (Eds.), *Stream Ecology: Structure and Function of Running Waters* (pp. 421–452). Springer International Publishing. https://doi.org/10.1007/978-3-030-61286-3_14
- Allan, J. D., Castillo, M. M., & Capps, K. A. (2021b). Detrital Energy and the Decomposition of Organic Matter. In J. D. Allan, M. M. Castillo, & K. A. Capps, *Stream Ecology* (pp. 177–224). Springer International Publishing. https://doi.org/10.1007/978-3-030-61286-3_7
- Ayayee, P. A., Wesner, J. S., & Ouellette, S. P. (2022). Geography, taxonomy, and ecological guild: Factors impacting freshwater macroinvertebrate gut microbiomes. *Ecology and Evolution*, 12(12), e9663. <https://doi.org/10.1002/ece3.9663>
- Baldy, V., Gessner, M. O., & Chauvet, E. (1995). Bacteria, Fungi and the Breakdown of Leaf Litter in a Large River. *Oikos*, 74(1), 93. <https://doi.org/10.2307/3545678>
- Bärlocher, F., & Kendrick, B. (1975). Leaf-conditioning by microorganisms. *Oecologia*, 20(4), 359–362. <https://doi.org/10.1007/BF00345526>
- Basaguren, A., Riaño, P., & Pozo, J. (2002). Life history patterns and dietary changes of several caddisfly (Trichoptera) species in a northern Spain stream. *Archiv Fur Hydrobiologie*, 155, 23–41. <https://doi.org/10.1127/archiv-hydrobiol/155/2002/23>
- Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M., & Packmann, A. I. (2016). The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, 14(4), 251–263.
- Bernabé, T. N., de Omena, P. M., Santos, V. P. D., de Siqueira, V. M., de Oliveira, V. M., & Romero, G. Q. (2018). Warming weakens facilitative interactions between decomposers and detritivores, and modifies freshwater ecosystem functioning. *Global Change Biology*, 24(7), 3170–3186.
- Carpenter, S. R., Stanley, E. H., & Vander Zanden, M. J. (2011). State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes. *Annual Review of Environment and Resources*, 36(1), 75–99. <https://doi.org/10.1146/annurev-environ-021810-094524>
- Chen, B., Teh, B.-S., Sun, C., Hu, S., Lu, X., Boland, W., & Shao, Y. (2016). Biodiversity and Activity of the Gut Microbiota across the Life History of the Insect Herbivore *Spodoptera littoralis*. *Scientific Reports*, 6(1), 29505. <https://doi.org/10.1038/srep29505>
- Clivot, H., Danger, M., Pagnout, C., Wagner, P., Rousselle, P., Poupin, P., & Guérol, F. (2013). Impaired Leaf Litter Processing in Acidified Streams. *Microbial Ecology*, 65(1), 1–11. <https://doi.org/10.1007/s00248-012-0107-x>
- Crump, B. C., Armbrust, E. V., & Baross, J. A. (1999). Phylogenetic Analysis of Particle-Attached and Free-Living Bacterial Communities in the Columbia River, Its Estuary, and the Adjacent Coastal Ocean. *Applied and Environmental Microbiology*, 65(7), 3192–3204. <https://doi.org/10.1128/AEM.65.7.3192-3204.1999>
- Cummins, K. W. (1974). Structure and Function of Stream Ecosystems. *BioScience*, 24(11), 631–641. <https://doi.org/10.2307/1296676>
- Dahlin, K. M., Zarnetske, P. L., Read, Q. D., Twardochleb, L. A., Kamoske, A. G., Cheruvilil, K. S., & Soranno, P. A. (2021). Linking terrestrial and aquatic biodiversity to ecosystem function across scales, trophic levels, and realms. *Frontiers in Environmental Science*, 9, 692401.
- Dale, H., Solan, M., Lam, P., & Cunliffe, M. (2019). Sediment microbial assemblage structure is modified by marine polychaete gut passage. *FEMS Microbiology Ecology*, 95(5), fiz047. <https://doi.org/10.1093/femsec/fiz047>
- Danger, M., Cornut, J., Elger, A., & Chauvet, E. (2012). Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. *Freshwater Biology*, 57(5), 1017–1030. <https://doi.org/10.1111/j.1365-2427.2012.02762.x>
- Dekanová, V., Novíkmec, M., Svitková, I., & Svitok, M. (2023). Functional diversity of shredders, not species richness, drives the decomposition rate of leaf litter in ponds. *Frontiers in Ecology and Evolution*, 11. <https://doi.org/10.3389/fevo.2023.1286672>
- Elliott, J. M. (1969). Life History and Biology of *Sericostoma personatum* Spence (Trichoptera). *Oikos*, 20(1), 110. <https://doi.org/10.2307/3543750>

- Engel, P., & Moran, N. A. (2013). The gut microbiota of insects – diversity in structure and function. *FEMS Microbiology Reviews*, 37(5), 699–735. <https://doi.org/10.1111/1574-6976.12025>
- Fenoy, E., Moya-Laraño, J., Rubio-Ríos, J., Moyano-López, F. J., & Casas, J. J. (2024). Litter quality modulates the effects of environmental drivers on microbial decomposition and home-field advantage in headwater streams. *Freshwater Biology*, 69(12), 1760–1772.
- Funck, J.-A., Bec, A., Perrière, F., Vincent, F., & Danger, M. (2014). Aquatic hyphomycetes: A potential source of polyunsaturated fatty acids in detritus-based stream food webs. *Fungal Ecology*, 13. <https://doi.org/10.1016/j.funeco.2014.09.004>
- García-Palacios, P., Handa, I. T., & Hättenschwiler, S. (2021). Plant Litter Decomposition in Terrestrial Ecosystems Compared to Streams. In C. M. Swan, L. Boyero, & C. Canhoto (Eds.), *The Ecology of Plant Litter Decomposition in Stream Ecosystems* (pp. 101–126). Springer International Publishing. https://doi.org/10.1007/978-3-030-72854-0_6
- García-Palacios, P., McKie, B. G., Handa, I. T., Frainer, A., & Hättenschwiler, S. (2016). The importance of litter traits and decomposers for litter decomposition: A comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology*, 30(5), 819–829. <https://doi.org/10.1111/1365-2435.12589>
- Gessner, M. O. (2010). Functional leaf traits and biodiversity effects on litter decomposition in a stream: Reply. *Ecology*, 91(6), 1869–1871.
- Gonçalves, S., Feckler, A., Pollitt, A., Baschien, C., Michael, J., Schreiner, V. C., ... & Bundschuh, M. (2024). Elevated fungicide and nutrient concentrations change structure but not function of aquatic microbial communities. *Environmental Toxicology and Chemistry*, 43(6), 1300–1311.
- Graça, M. A. S. (2001). The Role of Invertebrates on Leaf Litter Decomposition in Streams—A Review. *International Review of Hydrobiology*, 86(4–5), 383–393. [https://doi.org/10.1002/1522-2632\(200107\)86:4/5<383::AID-IROH383>3.0.CO;2-D](https://doi.org/10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D)
- Grasser, U., Wolfram, G., & Moog, O. (1992). *The role of Allogamus auricollis (Trichoptera: Limnephilidae) in benthic communities of a 4th-order crystalline mountain stream with some ecological notes.*
- Gu, J., Qiu, Q., Yu, Y., Sun, X., Tian, K., Chang, M., Wang, Y., Zhang, F., & Huo, H. (2024). Bacterial transformation of lignin: Key enzymes and high-value products. *Biotechnology for Biofuels and Bioproducts*, 17(1), 2. <https://doi.org/10.1186/s13068-023-02447-4>
- Handa, I. T., Aerts, R., Berendse, F., Berg, M. P., Bruder, A., Butenschoten, O., Chauvet, E., Gessner, M. O., Jabiol, J., Makkonen, M., McKie, B. G., Malmqvist, B., Peeters, E. T. H. M., Scheu, S., Schmid, B., van Ruijven, J., Vos, V. C. A., & Hättenschwiler, S. (2014). Consequences of biodiversity loss for litter decomposition across biomes. *Nature*, 509(7499), 218–221. <https://doi.org/10.1038/nature13247>
- Harvey, E., Marleau, J. N., Gounand, I., Leroux, S. J., Firkowski, C. R., Altermatt, F., ... & Fortin, M. J. (2023). A general meta-ecosystem model to predict ecosystem functions at landscape extents. *Ecography*, 2023(11), e06790.
- Hieber, M., & Gessner, M. O. (2002). Contribution of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83(4), 1026–1038. [https://doi.org/10.1890/0012-9658\(2002\)083\[1026:COSEFA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1026:COSEFA]2.0.CO;2)
- Karačić, S., Palmer, B., Gee, C. T., & Bierbaum, G. (2024). Oxygen-dependent biofilm dynamics in leaf decay: An in vitro analysis. *Scientific Reports*, 14(1), 6728.
- Khomutovska, N., Jasser, I., Sarapultseva, P., Spirina, V., Zaitsev, A., Masłowiecka, J., & Isidorov, V. A. (2024). Seasonal dynamics in leaf litter decomposing microbial communities in temperate forests: A whole-genome-sequencing-based study. *PeerJ*, 12, e17769. <https://doi.org/10.7717/peerj.17769>
- Kristensen, E., Ahmed, S. I., & Devol, A. H. (1995). Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? *Limnology and Oceanography*, 40(8), 1430–1437. <https://doi.org/10.4319/lm.1995.40.8.1430>
- Kroetsch, S. A., Kidd, K. A., Monk, W. A., Culp, J. M., Compson, Z. G., & Pavey, S. A. (2020). The effects of taxonomy, diet, and ecology on the microbiota of riverine macroinvertebrates. *Ecology and Evolution*, 10(24), 14000–14019. <https://doi.org/10.1002/ece3.6993>
- Lange, C., Boyer, S., Bezemer, T. M., Lefort, M.-C., Dhami, M. K., Biggs, E., Groenteman, R., Fowler, S. V., Paynter, Q., Verdecia Mogena, A. M., & Kaltenpoth, M. (2023). Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. *The ISME Journal*, 17(11), 1798–1807. <https://doi.org/10.1038/s41396-023-01500-2>
- Lau, W. W. Y., Jumars, P. A., & Armbrust, E. V. (2002). Genetic Diversity of Attached Bacteria in the Hindgut of the Deposit-Feeding Shrimp Neotrypaea (Formerly Callinassa) californiensis (Decapoda: Thalassinidae). *Microbial Ecology*, 43(4), 455–466.

- Lecerf, A., & Chauvet, E. (2008). Diversity and functions of leaf-decaying fungi in human-altered streams. *Freshwater Biology*, 53(8), 1658–1672. <https://doi.org/10.1111/j.1365-2427.2008.01986.x>
- Leroy, C. J., & Marks, J. C. (2006). Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biology*, 51(4), 605–617. <https://doi.org/10.1111/j.1365-2427.2006.01512.x>
- Lidman, J. (2015). *Decomposition of leaf litter in headwater streams.: Effects of changes in the environment and contribution of microbial and shredder activity on litter decomposition.* <https://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-105488>
- Loreau, M., Mouquet, N., & Holt, R. D. (2003). Meta-ecosystems: a theoretical framework for a spatial ecosystem ecology. *Ecology Letters*, 6(8), 673–679.
- Maltby, E., & Acreman, M. (2011). Ecosystem Services of Wetlands: Pathfinder for a new paradigm. *Hydrological Sciences Journal – Journal Des Sciences Hydrologiques*, 56, 1341–1359. <https://doi.org/10.1080/02626667.2011.631014>
- Manning, D. W. P., Rosemond, A. D., Kominoski, J. S., Gulis, V., Benstead, J. P., & Maerz, J. C. (2015). Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates. *Ecology*, 96(8), 2214–2224. <https://doi.org/10.1890/14-1582.1>
- Medeiros, A. O., Pascoal, C., & Graça, M. a. S. (2009). Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biology*, 54(1), 142–149. <https://doi.org/10.1111/j.1365-2427.2008.02101.x>
- Monroy, S., Larrañaga, A., Martínez, A., Pérez, J., Molinero, J., Basaguren, A., & Pozo, J. (2022). Temperature Sensitivity of Microbial Litter Decomposition in Freshwaters: Role of Leaf Litter Quality and Environmental Characteristics. *Microbial Ecology*. <https://doi.org/10.1007/s00248-022-02041-5>
- Newman, M. M., Liles, M. R., & Feminella, J. W. (2015). Litter Breakdown and Microbial Succession on Two Submerged Leaf Species in a Small Forested Stream. *PLOS ONE*, 10(6), e0130801. <https://doi.org/10.1371/journal.pone.0130801>
- Phillips, D. N., Contaminants, W. S. F. R. to, & Hamilton, is based at N. in. (2017, June 6). *Functional biodiversity in streams*. NIWA. <https://niwa.co.nz/publications/water-and-atmosphere/vol14-no1-march-2006/functional-biodiversity-in-streams>
- Plante, C. J., Feipel, S., & Wilkie, J. L. (2010). DISTURBANCE EFFECTS OF DEPOSIT FEEDING ON MICROALGAL COMMUNITY STRUCTURE AND MECHANISMS OF RECOLONIZATION1: DISTURBANCE AND RECOVERY OF BENTHIC MICROALGAE. *Journal of Phycology*, 46(5), 907–916. <https://doi.org/10.1111/j.1529-8817.2010.00883.x>
- Plante, C. J., Hill-Spanik, K. M., & Emerson, R. (2023). Inputs don't equal outputs: Bacterial microbiomes of the ingesta, gut, and feces of the keystone deposit feeder *Ilyanassa obsoleta*. *FEMS Microbiology Ecology*, 99(1), fiac152.
- Pope, C. A., Halvorson, H. M., Findlay, R. H., Francoeur, S. N., & Kuehn, K. A. (2020). Light and temperature mediate stimulation of heterotrophic activity on decomposing leaf litter. *Freshwater Biology*, 65(7), 1210–1222. <https://doi.org/10.1111/fwb.13465>
- Prather, C. M., Pelini, S. L., Laws, A., Rivest, E., Woltz, M., Bloch, C. P., Del Toro, I., Ho, C.-K., Kominoski, J., Newbold, T. A. S., Parsons, S., & Joern, A. (2013). Invertebrates, ecosystem services and climate change: Invertebrates, ecosystems and climate change. *Biological Reviews*, 88(2), 327–348. <https://doi.org/10.1111/brv.12002>
- Qiu, Y.-L., Tourlousse, D., Matsuura, N., Ohashi, A., & Sekiguchi, Y. (2017). Draft Genome Sequence of *Paludibacter jiangxiensis* NM7 T, a Propionate-Producing Fermentative Bacterium. *Genome Announcements*, 5, e00667-17. <https://doi.org/10.1128/genomeA.00667-17>
- Rastogi, G., Sbodio, A., Tech, J. J., Suslow, T. V., Coaker, G. L., & Leveau, J. H. J. (2012). Leaf microbiota in an agroecosystem: Spatiotemporal variation in bacterial community composition on field-grown lettuce. *The ISME Journal*, 6(10), 1812–1822. <https://doi.org/10.1038/ismej.2012.32>
- Richardson, J. S. (2019). Biological Diversity in Headwater Streams. *Water*, 11(2), 366. <https://doi.org/10.3390/w11020366>
- Richardson, J. S., & Danehy, R. J. (2007). A Synthesis of the Ecology of Headwater Streams and their Riparian Zones in Temperate Forests. *Forest Science*, 53(2), 131–147. <https://doi.org/10.1093/forestscience/53.2.131>
- Richardson, J. S., & Hanna, D. E. L. (2021). Leaf Litter Decomposition as a Contributor to Ecosystem Service Provision. In C. M. Swan, L. Boyero, & C. Canhoto (Eds.), *The Ecology of Plant Litter Decomposition in Stream Ecosystems* (pp. 511–523). Springer International Publishing. https://doi.org/10.1007/978-3-030-72854-0_22

- Richardson, J. S., & Sato, T. (2015). Resource subsidy flows across freshwater–terrestrial boundaries and influence on processes linking adjacent ecosystems. *Ecohydrology*, 8(3), 406–415. <https://doi.org/10.1002/eco.1488>
- Riemann, L., & Winding, A. (2001). Community Dynamics of Free-living and Particle-associated Bacterial Assemblages during a Freshwater Phytoplankton Bloom. *Microbial Ecology*, 42(3), 274–285. <https://doi.org/10.1007/s00248-001-0018-8>
- Sadeghi, J., Venney, C. J., Wright, S., Watkins, J., Manning, D., Bai, E., Frank, C., & Heath, D. D. (2024). Aquatic Bacterial Community Connectivity: The Effect of Hydrological Flow on Community Diversity and Composition. *Environments*, 11(5), Article 5. <https://doi.org/10.3390/environments11050090>
- Savio, D., Sinclair, L., Ijaz, U. Z., Parajka, J., Reischer, G. H., Stadler, P., Blaschke, A. P., Blöschl, G., Mach, R. L., Kirschner, A. K. T., Farnleitner, A. H., & Eiler, A. (2015). Bacterial diversity along a 2600 km river continuum. *Environmental Microbiology*, 17(12), 4994–5007. <https://doi.org/10.1111/1462-2920.12886>
- Silverman, J. D., Washburne, A. D., Mukherjee, S., & David, L. A. (2017). A phylogenetic transform enhances analysis of compositional microbiota data. *eLife*, 6, e21887. <https://doi.org/10.7554/eLife.21887>
- Sontowski, R., & van Dam, N. M. (2020). Functional variation in dipteran gut bacterial communities in relation to their diet, life cycle stage and habitat. *Insects*, 11(8), 543.
- Stadler, M., & Del Giorgio, P. A. (2022). Terrestrial connectivity, upstream aquatic history and seasonality shape bacterial community assembly within a large boreal aquatic network. *The ISME Journal*, 16(4), 937–947. <https://doi.org/10.1038/s41396-021-01146-y>
- Stegen, J. C., Fredrickson, J. K., Wilkins, M. J., Konopka, A. E., Nelson, W. C., Arntzen, E. V., Chrisler, W. B., Chu, R. K., Danczak, R. E., Fansler, S. J., Kennedy, D. W., Resch, C. T., & Tfaily, M. (2016). Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nature Communications*, 7(1), 11237. <https://doi.org/10.1038/ncomms11237>
- Stelzer, R. S., Heffernan, J., & Likens, G. E. (2003). The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology*, 48(11), 1925–1937. <https://doi.org/10.1046/j.1365-2427.2003.01141.x>
- Sullivan, S. M. P., & Manning, D. W. P. (2019). Aquatic–terrestrial linkages as complex systems: Insights and advances from network models. *Freshwater Science*, 38(4), 936–945. <https://doi.org/10.1086/706071>
- Swan, C. M. (2021). Biodiversity and Plant Litter Decomposition in Streams. In C. M. Swan, L. Boyero, & C. Canhoto (Eds.), *The Ecology of Plant Litter Decomposition in Stream Ecosystems* (pp. 129–142). Springer International Publishing. https://doi.org/10.1007/978-3-030-72854-0_7
- Thomsen, A. G., & Friberg, N. (2002). Growth and emergence of the stonefly *Leuctra nigra* in coniferous forest streams with contrasting pH. *Freshwater Biology*, 47(6), 1159–1172. <https://doi.org/10.1046/j.1365-2427.2002.00827.x>
- Tláškal, V., Voříšková, J., & Baldrian, P. (2016). Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiology Ecology*, 92(11), fiw177. <https://doi.org/10.1093/femsec/fiw177>
- Torres-Ruiz, M., & Wehr, J. D. (2010). Changes in the nutritional quality of decaying leaf litter in a stream based on fatty acid content. *Hydrobiologia*, 651(1), 265–278. <https://doi.org/10.1007/s10750-010-0305-9>
- Urbanič, G., Toman, M. J., & Krušnik, C. (2005). Microhabitat type selection of caddisfly larvae (Insecta: Trichoptera) in a shallow lowland stream. *Hydrobiologia*, 541(1), 1–12. <https://doi.org/10.1007/s10750-004-4314-4>
- Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(1), 130–137. <https://doi.org/10.1139/f80-017>
- Wallace, J. B., Eggert, S. L., Meyer, J. L., & Webster, J. R. (1997). Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science*, 277(5322), 102–104.
- Wang, L., Li, C., Wang, X., Wang, G., Shang, S., Dou, Z., & Luo, Y. (2022). Gut lignocellulose activity and microbiota in Asian longhorned beetle and their predicted contribution to larval nutrition. *Frontiers in Microbiology*, 13, 899865.
- Wang, X., Li, J., Zheng, J., Zhao, L., Ruan, C., Zhang, D., & Pan, X. (2024). Polysaccharide preferred minority-dominant community assembly and exoenzyme enrichment in transparent exopolymer particles: Implication for global carbon cycle in water. *Science of The Total Environment*, 914, 169976. <https://doi.org/10.1016/j.scitotenv.2024.169976>
- Wantzen, K. M., Ballouche, A., Longuet, I., Bao, I., Bocoum, H., Cissé, L., Chauhan, M., Girard, P., Gopal, B., Kane, A., Marchese, M. R., Nautiyal, P., Teixeira, P., & Zalewski, M. (2016). River

- Culture: An eco-social approach to mitigate the biological and cultural diversity crisis in riverscapes. *Ecohydrology & Hydrobiology*, 16(1), 7–18. <https://doi.org/10.1016/j.ecohyd.2015.12.003>
- Wardle, D. A., Yeates, G. W., Barker, G. M., & Bonner, K. I. (2006). The influence of plant litter diversity on decomposer abundance and diversity. *Soil Biology and Biochemistry*, 38(5), 1052–1062.
- Waringer, J., & Malicky, H. (2017). The larvae of *Sericostoma bergeri* Malicky, 1973 and *Sericostoma herakles* Malicky, 1999 (Trichoptera, Sericostomatidae). *ZooKeys*, 695, 123–133. <https://doi.org/10.3897/zookeys.695.14531>
- Wilson, M. A., & Carpenter, S. R. (1999). ECONOMIC VALUATION OF FRESHWATER ECOSYSTEM SERVICES IN THE UNITED STATES: 1971–1997. *Ecological Applications*, 9(3).
- Yue, K., De Frenne, P., Van Meerbeek, K., Ferreira, V., Fornara, D. A., Wu, Q., Ni, X., Peng, Y., Wang, D., Heděc, P., Yang, Y., Wu, F., & Peñuelas, J. (2022). Litter quality and stream physicochemical properties drive global invertebrate effects on instream litter decomposition. *Biological Reviews*, 97(6), 2023–2038. <https://doi.org/10.1111/brv.12880>
- Yun, J.-H., Roh, S. W., Whon, T. W., Jung, M.-J., Kim, M.-S., Park, D.-S., Yoon, C., Nam, Y.-D., Kim, Y.-J., Choi, J.-H., Kim, J.-Y., Shin, N.-R., Kim, S.-H., Lee, W.-J., & Bae, J.-W. (2014). Insect Gut Bacterial Diversity Determined by Environmental Habitat, Diet, Developmental Stage, and Phylogeny of Host. *Applied and Environmental Microbiology*, 80(17), 5254–5264. <https://doi.org/10.1128/AEM.01226-14>
- Zeglin L.H. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Front Microbiol* 2015;6:454. <https://doi.org/10.3389/fmicb.2015.00454>

CHAPTER I:

Leaf Conditioning and Shredder Activity Shape Microbial Dynamics on Fine Particulate Organic Matter Produced During Decomposition of Different Leaf Litter in Streams

RESEARCH



Leaf Conditioning and Shredder Activity Shape Microbial Dynamics on Fine Particulate Organic Matter Produced During Decomposition of Different Leaf Litter in Streams

Pratiksha Acharya^{1,2} · Mourine J. Yegon^{1,3} · Leonie Haferkemper^{1,4} · Benjamin Misteli¹ · Christian Griebler² · Simon Vitecek^{1,3,5} · Katrin Attermeyer^{1,2}

Received: 9 September 2024 / Accepted: 11 March 2025
 © The Author(s) 2025

Abstract

Leaf litter decomposition (LLD) is a key ecosystem function where invertebrate shredders produce large amounts of fine particulate organic matter (FPOM) that serves as a substrate for microbial assemblages. Here, we explore the shredder-produced FPOM composition and activity of FPOM-associated microbial communities in response to different leaf species and their conditioning. In a laboratory experiment, we fed leaves of different elemental compositions (alder, beech and maple), conditioned under oxic or anoxic conditions, to caddisfly larvae (*Sericostoma* sp.). We hypothesized differences in FPOM elemental and fatty acid composition and FPOM-associated microbial activity among the leaf species, conditioning, and two types of shredder-produced FPOM, i.e. shredded leaves and faecal pellets. Our results suggest that leaf conditioning and shredder activity play pivotal roles in shaping FPOM composition and FPOM-associated microbial activity. We observed lower C/N ratios with high-C/N litter (beech and maple leaves) after conditioning and no change in the elemental composition of the faecal pellets compared to the leaves. However, we observed differences in microbial fatty acid proportions and composition on leaves and faecal pellets with significantly higher fractions of bacterial fatty acids on faecal pellets than on leaves. We also noted a significant impact of leaf conditioning on the microbial activity of shredded leaves and faecal pellets, with a higher microbial growth efficiency observed on faecal pellets compared to ingested leaves. These findings highlight the crucial influence of leaf species and conditioning on the activity of shredder-produced FPOM, emphasizing the complex interplay between leaf properties and fate and microbial processes in streams.

Keywords Leaves · Faecal pellets · Microbial assemblage · FPOM composition · Microbial activity

Introduction

Substantial amounts of organic matter enter headwater streams from the surrounding riparian ecosystems—primarily in the form of leaf litter [1] that is fragmented into smaller particles [2]. Leaf litter enters the streams as coarse particulate organic matter (CPOM > 1 mm) with a wide range of nutritional quality, including differences in carbon-to-nitrogen (C/N) ratios and fatty acid composition. Some types of CPOM can provide readily available nutrients, labile organic molecules and low carbon-to-nitrogen ratios (hereafter, C/N ratios). However, other CPOM contains recalcitrant compounds and is hardly degradable with high C/N ratios. In all cases, fresh CPOM entering streams is first colonized by aquatic microbial communities. The rate of microbial colonization and community composition depends on the

✉ Katrin Attermeyer
 Katrin.Attermeyer@univie.ac.at

¹ Wassercluster Lunz – Biological Station, Dr. Carl Kupelwieser-Prom. 5, 3293 Lunz Am See, Austria

² Department of Functional and Evolutionary Ecology, Unit Limnology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

³ Institute for Hydrobiology and Water Management (IHG), University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33/DG, 1180 Vienna, Austria

⁴ Department of Aquatic Ecology, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland

⁵ Department of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

above-mentioned leaf properties and the environmental conditions. This process is known as “conditioning” [3, 4] which reduces the C/N ratio and thereby enhances the nutritional quality of the leaves [5, 6]. Furthermore, microorganisms colonizing the leaves can synthesize specific fatty acids (FAs), including linoleic acid (LIN, 18:2n-6), α -linolenic acid (ALA, 18:3n-3), palmitoleic acid (16:1n-7c), vaccenic acid (18:1n-7c) and oleic acid (18:1n-9) [7]. These FAs are typically biomarkers for aquatic hyphomycetes (e.g. LIN) and heterotrophic bacteria, which further help aquatic consumers meet their nutritional requirements. In general, microbial growth on leaves can facilitate the subsequent CPOM consumption by invertebrate shredders [3, 8]. As shredders consume CPOM, they produce fine particulate organic matter (FPOM < 1 mm) via two pathways: as small leaf litter fragments during the shredding of the leaves, also known as sloppy feeding [8, 9] and as faecal pellets during defecation [9, 10]. These two types of FPOM significantly contribute to stream ecosystem functioning, with faecal pellets accounting for up to two-thirds of FPOM in headwater streams [10], forming an essential component of the organic matter budget [11]. However, most of the existing studies on leaf litter decomposition (LLD) have focused on CPOM decomposition by shredders and microorganisms, whereas shredder-produced FPOM types and their associated microbial communities have been largely neglected.

Previous studies addressing both terrestrial and aquatic ecosystems revealed environmental conditions and CPOM quality as the main drivers of LLD [12, 13]. Environmental conditions can affect the LLD both directly by altering temperature and nutrients and indirectly by altering the CPOM quality and/or the decomposer community composition [14]. In streams, dissolved oxygen concentrations fluctuate spatially, leading to oxic conditions in flowing or turbulent sections and anoxic conditions in hyporheic sediments or slow-flowing sections or stagnant pools where leaves accumulate during autumnal leaf fall on stream beds [15, 16]. This in turn influences CPOM processing [17]. Oxic waters typically support higher microbial biomass and activities, including eukaryotes that potentially boost CPOM decomposition [18]. In contrast, anaerobic metabolic processes, i.e. fermentation and anaerobic respiration in anoxic zones, are mostly driven by bacteria-dominated microbial communities and characterized by low microbial activity [18, 19]. Thus, indirect effects of stretch-scale differences in oxygen availability, in combination with primary qualitative indicators such as CPOM C/N ratios, may influence LLD by shredders, shredder-produced FPOM types and their composition. Nevertheless, the effects of direct and indirect qualitative differences

of CPOM on the shredder-produced FPOM-associated microbial activity have not been well described yet. These effects are not included in the current conceptual understanding of LLD in streams.

Shredder-produced FPOM has been described and investigated as an important resource for filter feeders [20, 21]. However, the shredder-produced FPOM also serves as a food source as well as a physical habitat [22, 23] for microorganisms. FPOM-associated microbial communities play a key role as FPOM decomposers [24]. Additionally, their colonization ultimately alters the nutritional value of FPOM by decreasing the C/N ratio [5, 25] and thus contributes to energy transfer and organic matter turnover in the stream's food web [26]. Yet, if and how shredder activity influences FPOM composition and activity of FPOM-associated microbial communities remains to be investigated.

In this study, we aimed to test whether CPOM C/N ratios are mirrored in shredder-produced FPOM and if and how these compositional differences influence the activity of FPOM-associated microbial communities. Therefore, we conducted a lab experiment to investigate how the CPOM composition, manipulated through leaves of different deciduous tree species, and oxygen availability, i.e. either oxic or anoxic conditioning of these leaves, affect shredder-produced FPOM properties and FPOM-associated microbial communities. We specifically focused on the protein production and respiration of FPOM-associated microorganisms as a biotic response of FPOM composition and considered the two types of shredder-produced FPOM—FPOM from mechanically shredded leaves, mimicking the feeding behaviour, and faecal pellets. We hypothesized that (i) C/N ratios differ between oxic- and anoxic-conditioned leaves and the shredded leaves versus the faecal pellets; (ii) fatty acid composition differs between the conditioned leaves and the two types of shredder-produced FPOM, i.e. shredded leaves and faecal pellets. These compositional differences should be reflected in the microbial activities and we additionally hypothesized that (iii) microbial activities differ among the leaf species, leaf conditioning and the two types of shredder-produced FPOM.

Materials and Methods

We carried out a 25-day feeding experiment in indoor microcosms (12 °C, 11:13 light:dark period) with a single shredder species (Trichoptera: *Sericostoma* sp.) fed with anoxic- and oxic-conditioned leaves of different riparian tree species. We collected shredder-produced FPOM and analyzed its composition as well as the activity of FPOM-associated microbial communities.

Experiment Preparation

We collected naturally abscised leaves around Lunz am See, Austria (47°51' N, 15°04' E) during the period of maximal leaf fall in October 2021. We selected three tree species commonly found in Central Europe covering a range of CPOM quality: alder (*Alnus glutinosa* (L.) Gaertn.), beech (*Fagus sylvatica* L.) and maple (*Acer platanoides* L.), where alder serves as our high-quality CPOM and maple and beech as lower quality CPOM based on C/N ratios (supplementary; Table S1). We dried the collected leaves at room temperature and stored them in dry and dark conditions. For the experiment, we cut leaf discs of 18 mm in diameter from all three leaf species using a cork borer.

We packed 0.802 ± 0.003 (mean $\pm$ SD) g leaf discs of each leaf species in mesh bags (1 mm mesh size) for either anoxic or oxic conditioning. For anoxic conditioning, we immersed the mesh bags in clay slurry (unfired pottery clay; Glorex AG, Füllinsdorf, Switzerland) mixed 1:1 with 500 μ m hand-sieved stream water from Oberer Seebach (OSB), a relatively pristine second-order forested stream located in the pre-alpine region around Lunz am See, for 4 weeks. The mixture was gently shaken every 3 days. Before the start of the experiment, we gently washed the conditioned leaves with filtered stream water to remove the excess sediments. For oxic conditioning, we submerged the mesh bags in freshly collected 500 μ m-filtered stream water from OSB, which was continuously aerated. We kept both conditioning set-ups in the dark at 12 °C and monitored the dissolved oxygen concentration (WTW Oxi 315i; Xylem Analytics, Weilheim, Germany) in both conditioning treatments to confirm oxic (8.4 ± 1.0 mg L⁻¹) and anoxic (below 1.3 ± 0.5 mg L⁻¹) conditions, respectively. Despite measuring low concentrations of oxygen in the clay slurry, we refer to this treatment as “anoxic” as slight dissolved oxygen introduction likely occurred during the electrode movement, and additional analyses of the bacterial community on the anoxic-conditioned leaves show that the biofilm was dominated by obligate anaerobic bacteria (data not shown). We took subsamples of all leaf species immediately after termination of conditioning for analyses of CPOM quality as well as a baseline for microbial response assessments.

We collected *Sericostoma* sp. larvae of similar size (head capsule width 2.0 ± 0.1 mm) from Unterer Seebach (USB; 47°51'29" N 15°2'4.74" E) and acclimated them in separate food-grade plastic cups (polylactic acid (PLA), volume 4 cl) at 12 °C in filtered stream water (0.7 μ m, 450 °C, 4 h precombusted Whatmann GF/F filters). Larvae were starved for 24 h before the start of the feeding experiment.

Microcosm Setup

Each microcosm consisted of a food-grade white plastic bucket (1100 mL volume and 122 mm bottom diameter), a steel mesh (pore size 1 mm) installed at a height of 4.5–5 cm from the bottom and 750 mL stream water. We added ten shredders to each microcosm and fed them with different conditioned leaves, so that we obtained a total of 30 microcosms (3 leaf species $\times$ 2 conditionings $\times$ 5 replicates = 30). We collected the faecal pellets on the 5th, 15th and 25th day (5d, 15d and 25d) by separating each larva from each microcosm for 24 h in a plastic cup with filtered stream water before returning them to the microcosms. However, we pooled pellets from two larvae from each microcosm to have enough material for our measurements, providing us with five samples per microcosm and sampling. We analyzed bacterial protein production (BPP) and microbial respiration (MR) immediately, while samples for particulate organic carbon (POC), particulate nitrogen (PN) content and fatty acid (FA) composition were stored at –80 °C until further processing. We also collected the leftover leaf discs at the end of the experiment for POC, PN and FA analyses and stored them at –80 °C until further processing (see details in supplementary).

FPOM Composition

We measured the C and N concentrations in freeze-dried (Virtis™ Genesis Freeze Dryer, for minimum 24 h) leaf samples and faecal pellets on a Flash HT Plus CHNS/O elemental analyzer (Thermo Fisher Scientific, Bremen, Germany). Additionally, we analyzed the FA composition after lipid extraction and derivatization to fatty acid methyl esters (FAMES) that were quantified as FAMES using gas chromatography (GC) following the procedures described in Guo et.al [27]. We identified 47 single FAs using reference standards and calibration curves and more details on both procedures can be found in the supplementary material. For both analyses, faecal pellets per two larvae per microcosm were insufficient, so we pooled two larvae's faecal pellets from five microcosms for each treatment (faecal pellets of 2 larvae $\times$ 5 microcosms per treatment = faecal pellets from 10 larvae), resulting in a single sample per treatment for both analyses.

FPOM-Associated Microbial Activities

We incubated finely cut anoxic or oxic conditioned leaf discs from different leaf species, here referred to as “shredded leaves,” and pooled faecal pellets from two larvae per microcosm for MR and BPP measurements. We quantified respiration in our samples via oxygen consumption over time with a needle-type O₂ microsensor (Microx 4, Presens GmbH,

Regensburg, Germany). We converted the amount of consumed O_2 to $\mu g C L^{-1} d^{-1}$ by using a conversion factor of 1 [28]. Here, we express MR for shredded leaves and faecal pellets as $\mu g C h^{-1} gDW^{-1}$ and $ng C h^{-1} 2 animals^{-1}$, respectively. In addition, we determined BPP via the incorporation of L-Leucine [4,5- 3H] into the protein fraction using the protocol of [29] and [30]. We reported BPP as $\mu g h^{-1} gDW^{-1}$ for shredded leaves and $ng h^{-1} 2 animals^{-1}$ for faecal pellet samples (see details in supplementary).

Data Analysis

To test our first hypothesis on differences in C/N ratios between anoxic- versus oxic-conditioned leaves and between the two types of shredder-produced FPOM, we used non-parametric Wilcoxon signed-rank tests (function *wilcoxon.test*; *stats* package). In the first test, we compared C/N ratios from anoxic versus oxic conditioning paired by each leaf species (three pairs), while in the second test, we compared C/N ratios of leaves versus faecal pellets paired by the treatments and included data from the first and last faecal pellet sampling (12 pairs). Due to the small number of comparisons, we ran non-parametric tests here.

To test our second hypothesis and compare the FPOM FA composition between different groups, relative proportions (%FAME) of the most abundant single FAs (> 1% of total FAME) in leaves and faecal pellets as well as the FA categories omega-3 polyunsaturated fatty acids (n-3 PUFA) and sum of all bacterial fatty acids (BFA) were used. We first conducted a Principal Component Analysis (PCA) (function *prcomp*; *stats* package) to visualize compositional differences in FA profiles between the leaves and faecal pellets based on the proportional FAME data (*ggplot2* package) [31] in R [32] after the variables had been scaled. Ellipses represented 70% intervals around centroids of collected FPOM. We also applied non-parametric multivariate analysis of variance (PERMANOVA) on the most abundant single FAs to assess the effects of conditioning, FPOM types and their interaction using “Mahalanobis” dissimilarities as a distance measure (function *adonis2*; *vegan* package). The assumption of homogeneous within-group dispersion was tested (function *betadisper*; *vegan* package) and was fulfilled for all groups.

For our third hypothesis regarding the differences in microbial activities between FPOM type, leaf species and conditioning, we conducted several tests. For the MR and BPP of the shredded leaves, we ran a linear model using leaf species and conditioning as fixed factors. For the MR of the faecal pellets, we tested the differences between leaf species, conditioning, time and their interactions as fixed factors and microcosm ID as a random factor in a linear mixed model (function *lmer*; *lme4* package) [33], aiming to account for potential variation among microcosms. The dataset for the

BPP of faecal pellets is too small and does not permit the use of linear mixed models; therefore, we tested for effects of leaf species and conditioning in a simple linear model (see supplementary methods description for more information).

Finally, we assessed differences in microbial growth efficiency (MGE) by running a linear model (function *lm*; *stats* package) using FPOM type, leaf species and conditioning as fixed factors. We calculated MGE (in %) for both shredded leaves and faecal pellets as [34]:

$$MGE = \frac{BCP}{MR + BCP} \times 100 \quad (1)$$

where BCP=bacterial carbon production and MR=microbial respiration. Since the bacterial bulk C to bulk protein ratio is 0.86 on average, we calculated BCP from BPP by multiplying it by 0.86 [29]. We calculated the MGE to allow comparisons of MR and BPP among FPOM types, as measurements on faecal pellets could not be based on the dry weight of organic matter. Therefore, we now have the standardized units.

Afterwards, we ran an analysis of variance (ANOVA) to evaluate each model fit and report the significant factors. Further, we calculated the effect size (function *effectsize*; *effectsize* package) [35] after running each model fit to identify the strongest influencing fixed factor. We checked the goodness of fit of all the MR, BPP and MGE models (normality of residuals, homogeneity of variance and collinearity) using graphical procedures and Shapiro–Wilk tests. Data transformation (i.e. log, square root) was performed wherever necessary to meet the assumptions. We calculated the estimated marginal means and the upper and lower limits of the 95% confidence interval (function *emmeans*; *emmeans* package) [36] and reported adjusted *p*-values after using a multivariate *t*-distribution approach for pairwise comparisons among the groups (function *pairs*; *emmeans* package).

All parameters are reported as means $\pm$ standard deviation throughout the manuscript; otherwise, they would be mentioned individually. For all the data analyses and visualizations, we used R 4.2.3 [32].

Results

Elemental Compositional Change in Shredded Leaves and Faecal Pellets

We found no differences between the C/N ratios of oxic- or anoxic-conditioned leaves (Fig. 1a; Wilcoxon signed-rank test; $V = 1$; p -value = 0.5; $df = 2$), in contrast to our hypothesis. However, both oxic and anoxic leaf conditioning decreased the molar C/N ratio of the high-C/N CPOM (58.92 to 36.78 and 54.60 to 41.36 for beech and maple, respectively) while the alder C/N ratio remained at a similar level before and after conditioning (19.12 to 18.34) (Fig. 1).

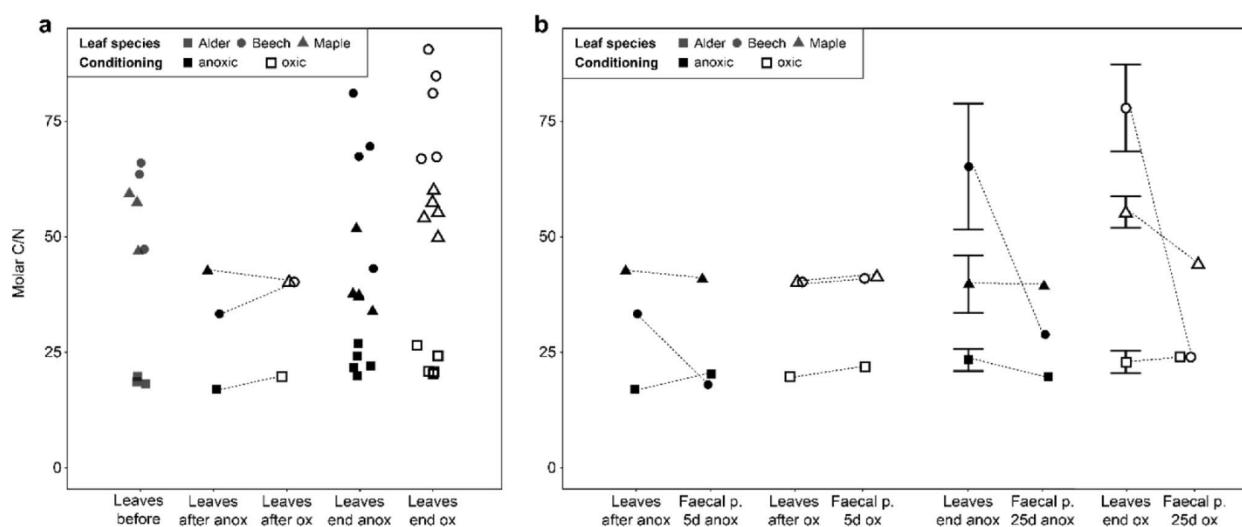


Fig. 1 Molar C/N ratios of leaves for all sampling times (**a**) and of leaves and faecal pellets from first and last sampling (**b**). In **a**, data are shown for the originally collected leaves (before), the leaves directly after anoxic or oxic conditioning (after anox or after ox) with paired comparisons used for testing changes between anoxic and oxic conditioning (indicated by dashed lines connecting datapoints) and the leaves at the end of the 25-day feeding experiment (end) with individual values for information. In **b**, data are shown for the start leaves and 5d faecal pellets for anoxic and oxic conditioning, as well

as the 25d leaves (=end) and faecal pellets for anoxic and oxic conditioning. The dashed lines connecting datapoints indicate paired comparisons between time points. The mean of the five replicates of leaves (displayed as mean with standard error) was used for paired comparisons with faecal pellets at the same time point. The oxic or anoxic conditioned samples are indicated by either black (anoxic) or white (oxic) and the three leaf species are shown by three different symbols

At the end of the feeding experiment, the C/N ratio of shredded oxic and anoxic-conditioned beech leaves increased approximately twofold, exceeding the C/N ratio from before conditioning. In contrast to our hypothesis, we also detected mean C/N ratios in the faecal pellets that were similar to those of the conditioned leaves for alder and maple treatments, and only anoxic-conditioned beech decreased by 7.9 (Fig. 1b; Wilcoxon signed-rank test; $V=23$, p -value = 0.23, $df=11$).

Fatty Acid Compositional Changes in Shredded Leaves and Faecal Pellets

The PCA of the relative proportions (% of total FAME) of the 20 most abundant individual FAs showed a clear separation between shredded leaves and faecal pellets (Fig. 2a). The PC1 accounted for 39.62% of the variation and was strongly correlated with the FAs, 17:0 (eigen values = 0.32), 20:3n-6 (0.32), 18:1n-6 (0.31), 18:3n-6 (−0.31) and 21:0 (−0.29), palmitic acid (16:0; 0.29), 24:0 (−0.24), linoleic acid (LIN; 18:2n-6; −0.24), 22:0 (−0.23) and 22:1n-6 (−0.21). The PC2 accounted for 14.96% of the variation and was strongly correlated with the FAs, alpha-linolenic acid (ALA; 18:3n-3; −0.49), stearic acid (18:0; 0.40), 15:1n-5 (−0.33), 18:3n-6 (−0.27), 16:0 (−0.25) and 21:0 (0.23). The PERMANOVA analysis showed that the FA composition of shredded leaves was significantly different from

those of faecal pellets (Table 1). We found no difference in the FA composition of shredder-produced FPOM between oxic and anoxic conditioning (Table 1). The proportion of n-3 PUFA was six times higher on shredded leaves than on faecal pellets (10.3 ± 4.7 versus $1.7 \pm 1.4\%$; type III ANOVA; $F_{(1,26)} = 48.08$; $p < 0.001$), while the proportion of BFA was five times higher on faecal pellets than on shredded leaves (11.8 ± 6.8 versus $2.2 \pm 0.6\%$; type III ANOVA; $F_{(1,26)} = 12.19$; $p < 0.05$) (Fig. 2b).

Microbial Activities of Shredded Leaves and Faecal Pellets

We observed a combined effect of leaf species and conditioning on both microbial respiration (MR) and bacterial protein production (BPP) on shredded leaves (Tables 2 and 3). Generally, MR was lower on anoxic-conditioned leaves whereas BPP was higher on anoxic-conditioned leaves, except for shredded beech leaves where we observed approximately six times higher BPP on the oxic-conditioned than anoxic-conditioned shredded beech leaves (Table S3). We measured the highest MR of $61.95 \pm 8.28 \mu\text{gC h}^{-1} \text{gDW}^{-1}$ on oxic-conditioned shredded maple leaves (Fig. 3a; Table S3) and the lowest MR of $24.66 \pm 2.78 \mu\text{gC h}^{-1} \text{gDW}^{-1}$ on anoxic-conditioned shredded beech leaves (Fig. 3a; Table S3). Furthermore, we found the highest BPP of $0.69 \pm 0.12 \mu\text{g h}^{-1} \text{gDW}^{-1}$ on oxic-conditioned shredded beech leaves and the lowest BPP

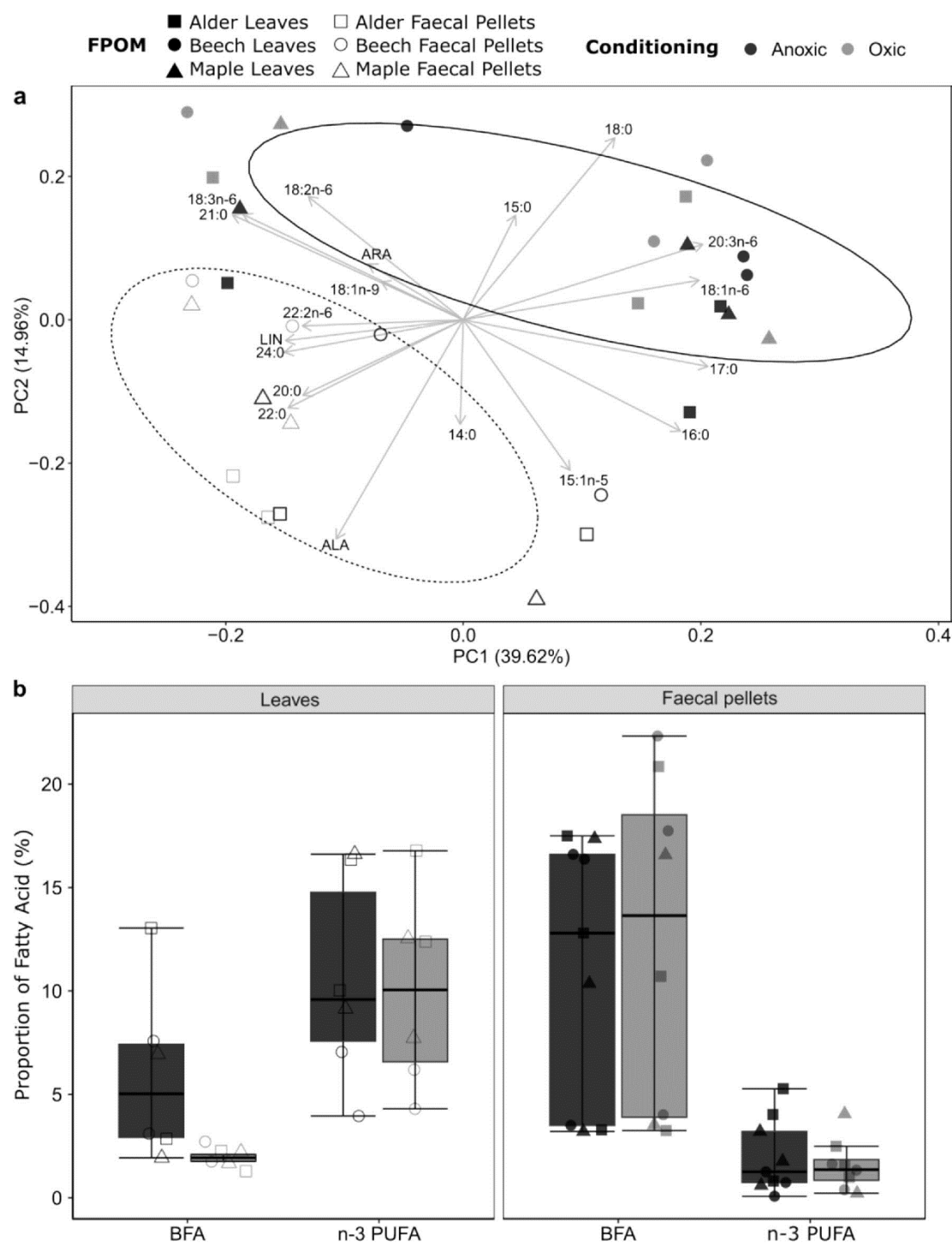


Fig. 2 Changes in fatty acid (FA) composition of shredded leaves and faecal pellets during leaf breakdown with a bivariate plot based on a principal component analysis of all collected FPOM and their fatty acid compositions **(a)** and boxplots of relative proportions of sums of bacterial fatty acids (BFA) and omega-3 polyunsaturated fatty acids (n-3 PUFA) **(b)**. All plots contain the faecal pellets data from all three timepoints and the leaves data from start and end of the feed-

ing experiment. The two colours distinguish the samples from oxic or anoxic conditioning. The three different empty symbols indicate three leaf species, and the three different filled symbols indicate faecal pellets obtained from shredders after feeding those three leaf species. In **a**, dotted and solid line of ellipses (70% confidence interval) encircle the shredded leaves and faecal pellets collected during the experiment, respectively

Table 1 Effects of FPOM type, conditioning and their interaction on FA composition as assessed by PERMANOVA. Abbreviations: SS, sum of squares; R^2 , coefficient of determination; df, degrees of freedom

Factors	df	SS	R^2	F-value	p-value
FPOM types	1	27.272	0.057	1.645	0.001
Conditioning	1	15.651	0.033	0.967	0.160
FPOM type: Conditioning	1	19.701	0.041	1.191	0.198
Residual	25	413.375	0.868		

Statistically significant p -values (<0.05) are shown in bold

Table 2 Effects of leaf species, conditioning and their interactions on MR of shredded leaves during feeding experiment based on linear model analysis. Abbreviations: St, standardized; SS, sum of squares; df, degrees of freedom

Factors	St. coefficient	SS	df	F-value	p-value
Conditioning	0.03	0.010	1	0.686	0.416
Leaf species	0.62	0.548	2	18.377	<0.001
Conditioning: Leaf species	0.39	0.219	2	12.268	<0.001
Residuals		0.343	23	7.349	

Statistically significant p -values (<0.05) are shown in bold

of $0.03 \pm 0.02 \mu\text{g h}^{-1} \text{gDW}^{-1}$ on oxic-conditioned shredded alder leaves (Fig. 3b, Table S3).

There was also a combined effect of leaf species, conditioning and time on MR on faecal pellets (Tables 4 and S5). Further, we found different MR and BPP on faecal pellets between the oxic and anoxic conditioning and leaf

species on 5d (Tables 3, 4, S5, S6 and S7). BPP in 5d faecal pellets from shredders feeding on oxic-conditioned leaves was lower than from anoxic-conditioned leaves (Table S3). However, these differences were no longer evident in 15d and 25d faecal pellets with similar MR and BPP for most leaf species and conditioning, (Tables S3, S5, S6 and S7) and with microbial activity decreasing over time (Table S3).

We additionally calculated the microbial growth efficiency (MGE) from MR and BPP to be able to compare microbial activities on shredded leaves versus faecal pellets and test their differences. The MGE was significantly higher on faecal pellets ($13.4 \pm 12.1\%$) compared to shredded leaves at the start ($0.81 \pm 0.79\%$) (type III ANOVA; $F_{(1,35)} = 4.97$; $p < 0.05$; see Tables S8, S9 for full model output, Fig. 4). Additionally, we found an effect of leaf species on MGE of both leaves and faecal pellets (type III ANOVA; $F_{(2,35)} = 3.41$; $p < 0.05$; Fig. 4).

Discussion

In the present study, we explored the nutritional composition and microbial activities of shredder-produced FPOM in response to leaf species and conditioning. Overall, our results indicate that CPOM properties, such as the C/N ratio, are partially reflected in shredder-produced FPOM. Conditioning enhanced the C/N ratios for low-quality CPOM. Yet, we neither found differences between oxic- or anoxic-conditioned leaves nor observed changes after digestion in shredder's guts, in contrast to our hypotheses. Nevertheless, we detected clear differences between the two types of shredder-produced FPOM, i.e. shredded leaves and faecal

Table 3 Effect of leaf species, conditioning and their interactions on BPP of shredded leaves and faecal pellets collected on different sampling times during the feeding experiment based on linear model analysis. Abbreviations: St, standardized; SS, sum of squares; df, degrees of freedom

FPOM type	Time	Factors	St. coefficient	SS	df	F-value	p-value
Shredded leaves	Start	Conditioning	0.77	0.315	1	79.936	<0.001
		Leaf species	0.83	0.474	2	60.112	<0.001
		Conditioning: Leaf species	0.94	1.368	2	173.378	<0.001
		Residuals		0.094	24		
Faecal pellets	5d	Conditioning	0.79	5.148	1	61.633	<0.001
		Leaf species	0.52	1.441	2	8.627	0.003
		Conditioning: Leaf species	0.30	0.574	2	3.437	0.058
		Residuals		1.337	16		
	15d	Conditioning	0.31	0.391	1	6.587	0.021
		Leaf species	0.48	0.819	2	6.906	0.007
		Conditioning: Leaf species	0.85	5.052	2	42.609	<0.001
		Residuals		0.889	15		
	25d	Conditioning	0.21	13.368	1	4.463	0.050
		Leaf species	0.54	60.486	2	10.098	0.001
		Conditioning: Leaf species	0.30	21.787	2	3.637	0.048
		Residuals		50.915	17		

Statistically significant p -values (<0.05) are shown in bold

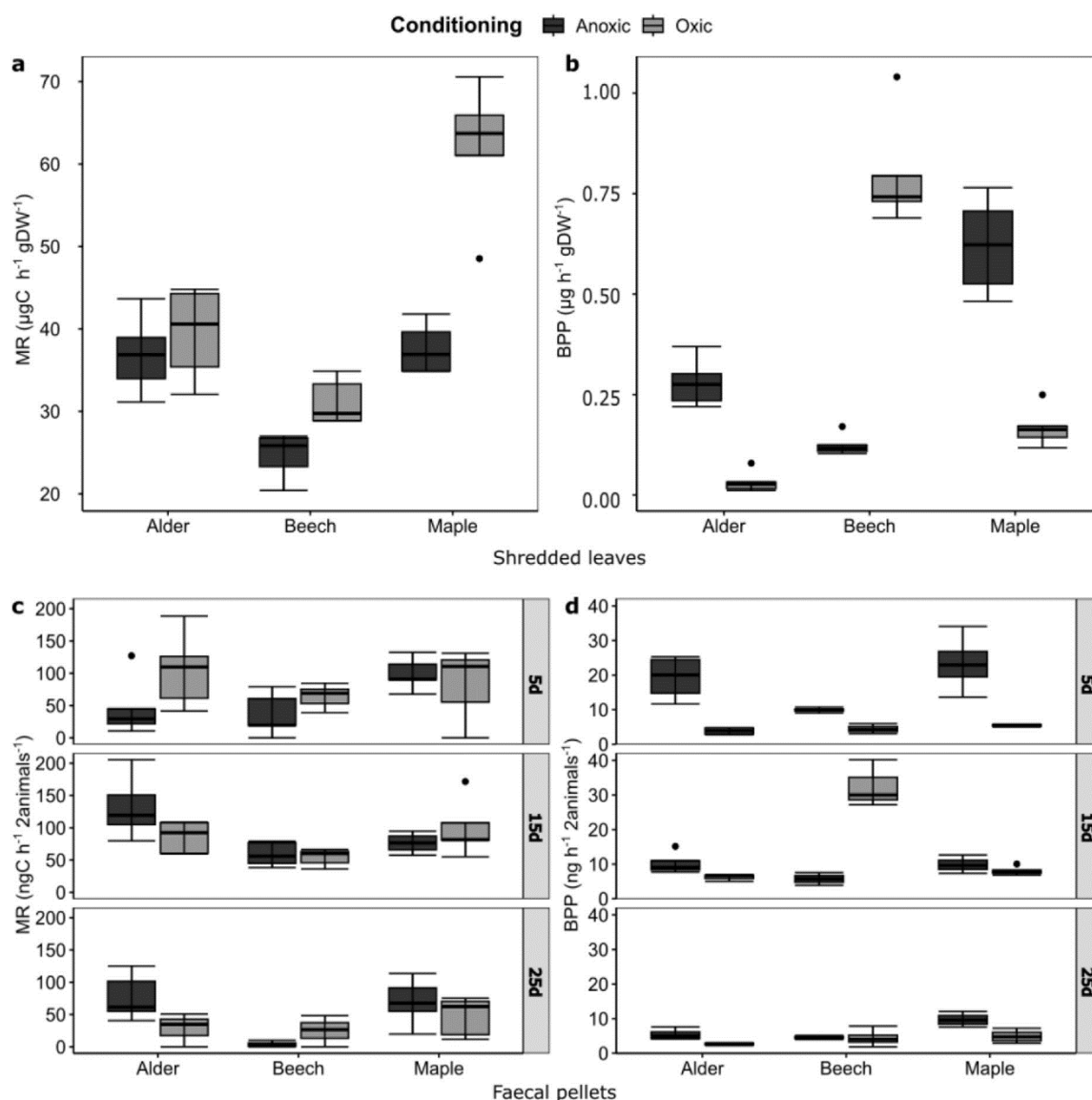


Fig. 3 Microbial respiration (MR) (a) and bacterial protein production (BPP) (b) on shredded leaves at the start of the experiment. The microbial respiration (c) and bacterial protein production (d) on faecal pellets collected on the 5th day (5d), 15th day (15d) and 25th day (25d) of the feeding experiment. The two colours distinguish the

samples from oxic or anoxic conditioning. The boxplots visualize the median of each treatment (line), the first and third quartiles (hinges) and the $1.5 \times$ inter-quartile ranges (whiskers), and the dots indicate outside values greater than $1.5 \times$ and less than $3 \times$ the interquartile range

Table 4 Model fit of a linear mixed model describing effects of leaf species, conditioning, time and their interactions on MR of faecal pellets. Abbreviations: St, standardized; SS, sum of squares; MS, mean of squares; df (Num; Den), degrees of freedom (numerator; denominator)

Factors	St. coefficient	SS	MS	df (Num;Den)	F-value	p-value
Conditioning	0.01	127.9	127.9	1;28.0	0.152	0.700
Leaf species	0.33	11,427.3	5713.6	2;27.9	6.779	0.004
Time	0.31	15,864.1	7932.0	2;41.3	9.411	<0.001
Leaf species: Conditioning	0.04	965.2	482.6	2;27.9	0.573	0.571
Leaf species: Time	0.15	6056.9	1514.2	4;40.8	1.797	0.148
Conditioning: Time	0.17	7250.3	3625.1	2;41.2	4.301	0.020
Leaf species: Conditioning: Time	0.21	9036.5	2259.1	4;40.8	2.680	0.045

Statistically significant *p*-values (<0.05) are shown in bold

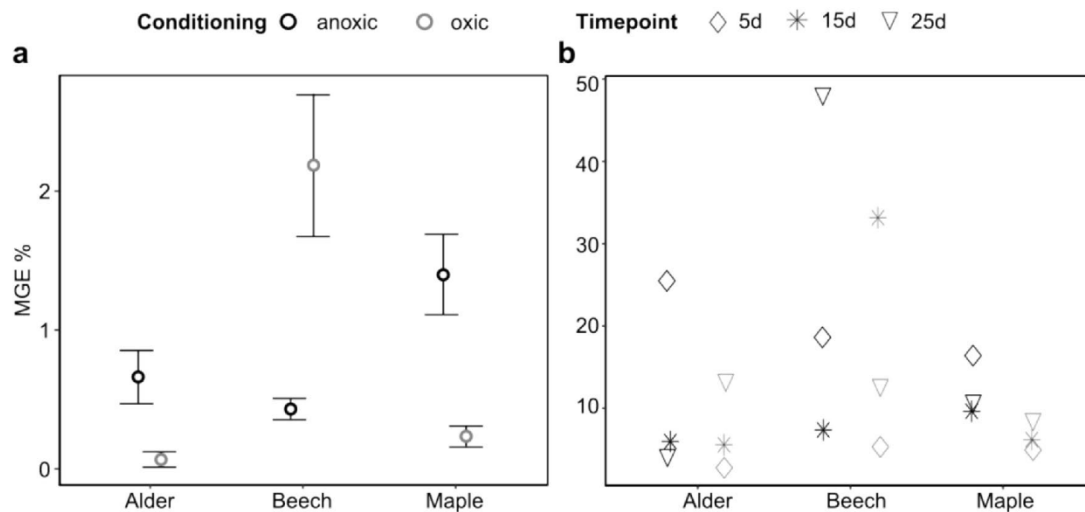


Fig. 4 Microbial growth efficiency (MGE) on shredder-produced FPOM with leaves (mean and standard error; $n=29$) (**a**) and faecal pellets ($n=18$) at different timepoints (**b**). The black and grey lines

distinguish the samples from anoxic or oxic conditioning. The three different symbols in **b** indicate three timepoints. Note the different scales of the y axes for **a** and **b**

pellets, regarding their FA composition and MGE. Our findings thus suggest that leaf conditioning and shredder activity shape FPOM chemical composition and activity of FPOM-associated microbial communities during leaf breakdown.

CPOM Quality Is Mirrored in Shredder-Produced FPOM

We predicted that different conditions during microbial colonization during leaf conditioning would influence the C/N ratio of the leaves. However, we did not find differences between anoxic- and oxic-conditioned leaves and observed decreases in C/N ratios for low-quality CPOM prior to and after conditioning. Leaching and microbial colonization independent of oxygen availability during the conditioning thus may have altered the elemental composition of beech and maple leaves as shown in other publications [37, 38]. As C/N ratios of microbial biomass are generally lower (5 to 15 for bacteria [39]) than those of leaf litter [40, 41], it is plausible that microbial biofilms on CPOM reduced the overall C/N ratios on beech and maple leaves during the conditioning phase. Additionally, the increase in the C/N ratio of beech leaves during the feeding experiment could potentially be attributed to the selective feeding preference of shredders on microbe-colonized leaf areas, as suggested by Fenoy et al. [42] and Graça [9].

CPOM-associated biofilms are considered the primary energy and nutrient resource for invertebrate shredders [8, 43], while leaves themselves are of low nutritional value [37]. Further, it is commonly assumed that the shredder activity, encompassing both shredding behaviour along with

feeding preference, and gut passage, produces FPOM with higher C/N ratios by removing N from leaf particles [9, 42, 44, 45]. However, our findings on faecal pellets challenge this concept, as we observed similar C/N ratios on CPOM and faecal pellets pointing towards no selective assimilation of certain nutrients, such as N, during gut passage. Hence, we can assume that the CPOM quality is indeed mirrored in shredder-produced FPOM. As microbes colonizing the leaves may influence the C/N ratio of the food resource for shredders [5, 25], we support the assumption that microbial colonization drives the nutritional value for aquatic consumers [8, 43] but at the same time, our results indicate that shredders do not necessarily produce low quality or nutrient-poor FPOM [44, 45].

Activity of FPOM-Associated Microbial Communities Is Mostly Influenced by Conditioning

The findings of our study uncovered that different leaf species and conditioning had significant effects on the FPOM-associated microbial metabolic processes. Additionally, leaf species alone significantly influenced the microbial activities associated with shredded leaves and faecal pellets during the feeding experiment. This may be directed towards the influence of varying nutritional value of leaf species on the microbial community composition associated with shredder-produced FPOM and subsequently, the activity of microorganisms, as evidenced by previous studies [26, 46]. Furthermore, we found, compared to the anoxic ones, significantly higher MR on oxic-conditioned shredded leaves and faecal pellets collected by feeding oxic-conditioned leaves on 5d.

Anoxic conditioning selects for anaerobes as members of the active microbial community, and anaerobic processes are typically slower in carbon turnover during the conditioning phase that was still persisting during the subsequent oxic experimental phase [47, 48]. The switch from an anoxic to oxic conditioning phase might have resulted in the availability of incompletely degraded organic matter that could now be used efficiently for growth by obligatory and facultative aerobes under oxic conditions. Moreover, the minimal turnover of primary fermentation products [49] during anoxic conditions and the revival of fungi that were inhibited under anoxic conditions [50] could have further boosted the growth of the microbial community. This could be the argument behind the higher BPP on anoxic-conditioned alder, maple leaves and faecal pellets collected on 5d. Yet, we did not perform any molecular techniques to validate the revival of fungi. Nevertheless, the observed pattern of a higher oxic MR and lower oxic BPP still holds true for the faecal pellets collected at the beginning of the experiment, indicating that the microbial metabolic processes on the produced FPOM might be preserved even after gut passage.

The distinct effect of conditioning on faecal pellet associated microbial activity with a higher MR on oxic-conditioned but higher BPP on faecal pellets obtained by feeding previously anoxic-conditioned leaves disappeared with time. This is probably attributed to a shift in activity and composition of the microbial communities on the formerly anoxic-conditioned leaves now maintained under oxic conditions during the feeding experiment. Yet, the effect of different leaf species was significant for both MR and BPP in faecal pellets over time, suggesting other factors could also play a role (e.g. organic matter quantity or quality) [25, 51]. Additionally, the senescence of the leaves over time may also contribute to a decrease in microbial activities, as the resources and quality of the leaves that the shredders were feeding on decrease in parallel. This is supported by our observation of increased C/N ratios in beech leaves from the start to the end of the feeding experiment. Hence, the large variability in the microbial metabolism observed in the shredder-produced FPOM in our experiment emphasizes the notable effects of the preceding environmental conditions during the leaf conditioning and the shredder activity in determining microbial community dynamics during LLD in streams.

Gut Passage Influences the Composition and Processing of Shredder-Produced FPOM

Our findings, based on fatty acid composition and MGE, revealed a clear difference between shredded leaves and faecal pellets, indicating differential composition and processing of organic matter by shredders and associated microbes. These differences were much more pronounced than those

observed among leaf species and conditioning. However, if this finding would hold true for faecal pellets produced by different shredder taxa remains to be investigated. Further, we showed that the MGE of the faecal pellets was markedly higher than that of the shredded leaves pointing towards competitive interactions between microbial communities on leaves [52] and the gut passage effect on faecal pellets with bacterial dominance [53]. This speculation is supported by our FA data showing significantly higher bacterial biomarkers on the faecal pellets, possibly indicating a relatively greater role of bacteria on this type of shredder-produced FPOM compared to fungi or other eukaryotic microorganisms such as algae, which may be more important on the leaves.

Leaf species influence the leaf-associated microbial community, which consists of eukaryotic and prokaryotic microorganisms as described in previous LLD studies [4, 54, 55]. It is noteworthy that our study was the first to investigate the activity and FA composition of microbial communities on shredder-produced faecal pellets from *Sericostoma* sp. after feeding on different leaf species. Additionally, our data showed that the microbial community on faecal pellets has a dominance of bacteria that apparently are very efficient in utilizing this carbon source, as evidenced by their relatively high MGEs [34]. From previous studies, it is well known that n-3 PUFAs as algal biomarkers are a high-quality food source for aquatic consumers, particularly in freshwater ecosystems [27]. Furthermore, our data showed that most of the bulk n-3 PUFA consisted of short-chain n-3 PUFA (ALA) (Table S10), which can be synthesized by both fungi and green algae. However, we did not use any other specific technique to verify whether the source of short-chain n-3 PUFA in our study was from fungi or exclusively from algae. Nevertheless, we speculate that both algal and fungal biofilms growing on leaf surfaces (Table S10) can enhance the nutritional quality of food for consumers [5, 27, 56], support their somatic growth [6] and fulfill their physiological requirements [56]. Consequently, it is possible that certain fatty acids are selectively retained during gut passage and/or others preferentially degraded [56, 57]. This can be the explanation for the higher amounts of n-3 PUFA on shredded leaves compared to faecal pellets. Therefore, this result suggests that gut passage enhances the colonization of bacteria and eventually promotes the growth of heterotrophic microbial communities in faecal FPOM.

Conclusions

Microorganisms, including heterotrophic bacteria and aquatic hyphomycetes, can affect LLD in various ways, including the colonization and decomposition of leaves and faecal pellets for aquatic organisms such as shredders,

collectors and gatherers. However, the role and activities of microorganisms on shredder-produced FPOM have been understudied so far. We showed that microbial colonization on CPOM at two oxygen levels altered the resource quality and, consequently, the microbial metabolism on the shredded leaves and faecal pellets. Our results show that faecal pellets provide good conditions for microbial communities to thrive, underscoring that faecal pellets as microbial hotspots might play a key role in accelerating nutrient cycling in aquatic ecosystems. Our study illustrates how detritivore-microbe interactions influence ecosystem functions, thus contributing to the understanding of the complexity of organic matter decomposition and how environmental and biological factors interact to shape ecosystem processes. Our results call for more studies on the identity and structure of FPOM-associated microbial communities, using advanced molecular techniques like metagenomics or meta-transcriptomics. This will further deepen our knowledge about the key microbial communities and their impact on the FPOM nutritional value along with their metabolic functions and interactions with shredders, thus advancing our understanding of FPOM dynamics in freshwater ecosystems.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00248-025-02515-2>.

Acknowledgements We thank Gertraud Steniczka, Theresa Reichenpfader, and Samuel-Karl Kämmer for providing support and help during laboratory analyses. We also thank Vojtěch Kolář for his help during macroinvertebrate shredders sampling and Libor Závorka for advises on the fatty acid analyses. Additionally, we greatly acknowledge the assistance by Hermann Hofreiter, Nobert Matschegg, and Michael Mayr for setting up the microcosm in the laboratory for the feeding experiment. We are thankful to three anonymous reviewers for their constructive feedback and comments.

Author Contribution Conceptualization and supervision: Katrin Attermeyer and Simon Vitecek; writing – original draft preparation: Pratiksha Acharya, Katrin Attermeyer, Simon Vitecek and Benjamin Misteli; writing – review and editing: Pratiksha Acharya, Mourine Yegon, Leonie Haferkemper, Benjamin Misteli, Christian Griebler, Simon Vitecek and Katrin Attermeyer; empirical analysis and sample collection: Pratiksha Acharya, Mourine Yegon and Leonie Haferkemper; data visualization: Pratiksha Acharya and Benjamin Misteli; data analysis: Pratiksha Acharya; statistical analysis: Pratiksha Acharya, Katrin Attermeyer and Simon Vitecek; fund acquisition: Katrin Attermeyer and Simon Vitecek. All authors read and approved the final manuscript.

Funding Open access funding provided by University of Vienna. This study got funded by a PhD Fellowship financially supported by WasserCluster Lunz.

Data Availability The data presented in this study are available on request from the corresponding author.

Declarations

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Fiori LF, de Cione VM, Sacramento PA, Benedito E (2016) Dynamics of leaf fall from riparian vegetation and the accumulation in benthic stock in Neotropical Streams. *Revista Árvore* 40:89–96. <https://doi.org/10.1590/0100-67622016000100010>
2. Rezende RS, Correia PR, Gonçalves JF Jr, Santos AM (2017) Organic matter dynamics in a savanna transition riparian zone: input of plant reproductive parts increases leaf breakdown process. *J Limnol* 76. <https://doi.org/10.4081/jlimnol.2017.1601>
3. Bärlocher F, Kendrick B (1975) Leaf-conditioning by microorganisms. *Oecologia* 20:359–362. <https://doi.org/10.1007/BF00345526>
4. Gonçalves S, Pollitt A, Pietz S et al (2024) Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod. *Sci Total Environ* 912:168926. <https://doi.org/10.1016/j.scitotenv.2023.168926>
5. Newman MM, Liles MR, Feminella JW (2015) Litter breakdown and microbial succession on two submerged leaf species in a small forested stream. *PLoS ONE* 10:e0130801. <https://doi.org/10.1371/journal.pone.0130801>
6. Manning DWP, Rosemond AD, Kominoski JS et al (2015) Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates. *Ecology* 96:2214–2224. <https://doi.org/10.1890/14-1582.1>
7. Funck J-A, Bec A, Perrière F, et al (2014) Aquatic hyphomycetes: a potential source of polyunsaturated fatty acids in detritus-based stream food webs. *Fungal Ecology* 13. <https://doi.org/10.1016/j.funeco.2014.09.004>
8. Cummins KW (1974) Structure and function of stream ecosystems. *Bioscience* 24:631–641. <https://doi.org/10.2307/1296676>
9. Graça MAS (2001) The role of invertebrates on leaf litter decomposition in streams - a review. *Internat Rev Hydrobiol* 86:383–393. [https://doi.org/10.1002/1522-2632\(200107\)86:4/5%3c383::AID-IROH383%3e3.0.CO;2-D](https://doi.org/10.1002/1522-2632(200107)86:4/5%3c383::AID-IROH383%3e3.0.CO;2-D)
10. Prather CM, Pelini SL, Laws A et al (2013) Invertebrates, ecosystem services and climate change: invertebrates, ecosystems and climate change. *Biol Rev* 88:327–348. <https://doi.org/10.1111/brv.12002>
11. Thomas FC, Wallace JB, Lughart GJ (1990) Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshw Biol* 23:281–299. <https://doi.org/10.1111/j.1365-2427.1990.tb00272.x>
12. Handa IT, Aerts R, Berendse F et al (2014) Consequences of biodiversity loss for litter decomposition across biomes. *Nature* 509:218–221. <https://doi.org/10.1038/nature13247>
13. García-Palacios P, McKie BG, Handa IT et al (2016) The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and

- across biomes. *Funct Ecol* 30:819–829. <https://doi.org/10.1111/1365-2435.12589>
14. Wardle DA, Bardgett RD, Klironomos JN et al (2004) Ecological linkages between aboveground and belowground biota. *Science* 304:1629–1633. <https://doi.org/10.1126/science.1094875>
 15. Battin TJ, Besemer K, Bengtsson MM et al (2016) The ecology and biogeochemistry of stream biofilms. *Nat Rev Microbiol* 14:251–263. <https://doi.org/10.1038/nrmicro.2016.15>
 16. Kristensen E, Ahmed SI, Devol AH (1995) Aerobic and anaerobic decomposition of organic matter in marine sediment: which is fastest? *Limnol Oceanogr* 40:1430–1437. <https://doi.org/10.4319/lo.1995.40.8.1430>
 17. Bastias E, Bolivar M, Ribot M et al (2020) Spatial heterogeneity in water velocity drives leaf litter dynamics in streams. *Freshw Biol* 65:435–445. <https://doi.org/10.1111/fwb.13436>
 18. Medeiros AO, Pascoal C, Graça MAS (2009) Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biol* 54:142–149. <https://doi.org/10.1111/j.1365-2427.2008.02101.x>
 19. Clivot H, Danger M, Pagnout C et al (2013) Impaired leaf litter processing in acidified streams. *Microb Ecol* 65:1–11. <https://doi.org/10.1007/s00248-012-0107-x>
 20. Maamri A, Chergui H, Pattee E (1997) Leaf litter processing in a temporary Northeastern Moroccan river. *Arch Hydrobiol* 140:513–531
 21. Li AOY, Ng LCY, Dudgeon D (2009) Effects of leaf toughness and nitrogen content on litter breakdown and macroinvertebrates in a tropical stream. *Aquat Sci* 71:80–93. <https://doi.org/10.1007/s00027-008-8117-y>
 22. Boros G, Takács P, Vanni MJ (2015) The fate of phosphorus in decomposing fish carcasses: a mesocosm experiment. *Freshw Biol* 60:479–489. <https://doi.org/10.1111/fwb.12483>
 23. Cheever BM, Webster JR, Bilger EE, Thomas SA (2013) The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology* 94:1614–1625. <https://doi.org/10.1890/12-1339.1>
 24. Tant CJ, Rosemond AD, First MR (2013) Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Sci* 32:1111–1121. <https://doi.org/10.1899/12-049.1>
 25. Stelzer RS, Heffernan J, Likens GE (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshw Biol* 48:1925–1937. <https://doi.org/10.1046/j.1365-2427.2003.01141.x>
 26. Frossard A, Gerull L, Mutz M, Gessner MO (2013) Shifts in microbial community structure and function in stream sediments during experimentally simulated riparian succession. *FEMS Microbiol Ecol* 84:398–410. <https://doi.org/10.1111/1574-6941.12072>
 27. Guo F, Kainz MJ, Valdez D et al (2016) High-quality algae attached to leaf litter boost invertebrate shredder growth. *Freshwater Sci* 35:1213–1221. <https://doi.org/10.1086/688667>
 28. Berggren M, Lapierre J-F, del Giorgio PA (2012) Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients. *ISME J* 6:984–993. <https://doi.org/10.1038/ismej.2011.157>
 29. Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* 51:201–213. <https://doi.org/10.3354/meps051201>
 30. Buesing N, Gessner MO (2003) Incorporation of Radiolabeled leucine into protein to estimate bacterial production in plant litter, sediment, epiphytic biofilms, and water samples. *Microb Ecol* 45:291–301. <https://doi.org/10.1007/s00248-002-2036-6>
 31. Wickham H (2016) *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York. <https://ggplot2.tidyverse.org>
 32. R Core Team (2023) R: a Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
 33. Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 067:1–48. <https://doi.org/10.18637/jss.v067.i01>
 34. del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. *Annu Rev Ecol Syst* 29:503–541. <https://doi.org/10.1146/annurev.ecolsys.29.1.503>
 35. Ben-Shachar M, Lüdtke D, Makowski D (2020) effectsize: Estimation of effect size indices and standardized parameters. *JOSS* 5:2815. <https://doi.org/10.21105/joss.02815>
 36. Lenth R (2025) emmeans: Estimated marginal means, aka least-squares means. R package version 1.10.7. <https://CRAN.R-project.org/package=emmeans>
 37. Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. *Oikos* 85:377–384. <https://doi.org/10.2307/3546505>
 38. Marks JC (2019) Revisiting the fates of dead leaves that fall into streams. *Annu Rev Ecol Syst* 50:547–568. <https://doi.org/10.1146/annurev-ecolsys-110218-024755>
 39. Kirchman D (2000) Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. In: Kirchman DL (ed) *Microbial ecology of the oceans*, vol 9. Wiley-Liss, New York, pp 261–288
 40. Cross WF, Benstead JP, Frost PC, Thomas SA (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshw Biol* 50:1895–1912. <https://doi.org/10.1111/j.1365-2427.2005.01458.x>
 41. Gessner MO (2010) Functional leaf traits and biodiversity effects on litter decomposition in a stream: reply. *Ecology* 91:1869–1871. <https://www.jstor.org/stable/25680427>
 42. Fenoy E, Rubio-Ríos J, González JM et al (2021) Strategies of shredders when feeding on low-quality leaf-litter: Local population adaptations or fixed species traits? *Limnol Oceanogr* 66:2063–2077. <https://doi.org/10.1002/lno.11745>
 43. Cummins KW, Klug MJ (1979) Feeding ecology of stream invertebrates. *Annu Rev Ecol Syst* 10:147–172. <https://www.jstor.org/stable/2096788>
 44. Santonja M, Pellan L, Piscart C (2018) Macroinvertebrate identity mediates the effects of litter quality and microbial conditioning on leaf litter recycling in temperate streams. *Ecol Evol* 8:2542–2553. <https://doi.org/10.1002/ece3.3790>
 45. Yoshimura C, Gessner MO, Tockner K, Furumai H (2008) Chemical properties, microbial respiration, and decomposition of coarse and fine particulate organic matter. *J N Am Benthol Soc* 27:664–673. <https://doi.org/10.1899/07-106.1>
 46. Halvorson H, Fuller C, Entrekin S, Evans-White M (2015) Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshw Biol* 60:466–478. <https://doi.org/10.1111/fwb.12462>
 47. Raczka NC, Piñeiro J, Tfaily MM et al (2021) Interactions between microbial diversity and substrate chemistry determine the fate of carbon in soil. *Sci Rep* 11:19320. <https://doi.org/10.1038/s41598-021-97942-9>
 48. Sinsabaugh RL, Findlay S (1995) Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. *Microb Ecol* 30:127–141. <https://doi.org/10.1007/BF00172569>
 49. Reith F, Drake H, Küsel K (2002) Anaerobic activities of bacteria and fungi in moderately acidic conifer and deciduous leaf litter. *FEMS Microbiol Ecol* 41(1):27–35. <https://doi.org/10.1111/j.1574-6941.2002.tb00963.x>
 50. Dieter D, Frindte K, Krüger A, Wurzbacher C (2013) Preconditioning of leaves by solar radiation and anoxia affects microbial

- colonisation and rate of leaf mass loss in an intermittent stream. *Freshw Biol* 58:1918–1931. <https://doi.org/10.1111/fwb.12180>
51. Tiegs SD, Peter FD, Robinson CT et al (2008) Leaf decomposition and invertebrate colonization responses to manipulated litter quantity in streams. *J N Am Benthol Soc* 27:321–331. <https://doi.org/10.1899/07-054.1>
 52. Silva-Sánchez A, Soares M, Rousk J (2019) Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality. *Soil Biol Biochem* 134:25–35. <https://doi.org/10.1016/j.soilbio.2019.03.008>
 53. Thimm T, Hoffmann A, Borkott H et al (1998) The gut of the soil microarthropod *Folsomia candida* (Collembola) is a frequently changeable but selective habitat and a vector for microorganisms. *Appl Environ Microbiol* 64:2660–2669. <https://doi.org/10.1128/AEM.64.7.2660-2669.1998>
 54. Bastias E, Ribot M, Romání AM et al (2018) Responses of microbially driven leaf litter decomposition to stream nutrients depend on litter quality. *Hydrobiologia* 806:333–346. <https://doi.org/10.1007/s10750-017-3372-3>
 55. Frossard A, Gerull L, Mutz M, Gessner MO (2013) Litter supply as a driver of microbial activity and community structure on decomposing leaves: a test in experimental streams. *Appl Environ Microbiol* 79:4965–4973. <https://doi.org/10.1128/AEM.00747-13>
 56. Guo F, Bunn SE, Brett MT et al (2018) Feeding strategies for the acquisition of high-quality food sources in stream macroinvertebrates: collecting, integrating, and mixed feeding. *Limnol Oceanogr* 63:1964–1978. <https://doi.org/10.1002/lno.10818>
 57. Kühmayer T, Guo F, Ehm N et al (2020) Preferential retention of algal carbon in benthic invertebrates: stable isotope and fatty acid evidence from an outdoor flume experiment. *Freshw Biol* 65:1200–1209. <https://doi.org/10.1111/fwb.13492>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

CHAPTER II:

Distinct bacterial communities affiliated with two types of shredder-produced particles in streams



Distinct bacterial communities affiliated with two types of shredder-produced particles in streams

Pratiksha Acharya^{1,2,*}, Mourine J. Yegon^{1,3}, Christian Griebler², Simon Vitecek^{3,4,†}, Katrin Attermeyer^{1,2,†}

¹WasserCluster Lunz – Biological Station, Dr. Carl Kupelwieser-Prom. 5, 3293 Lunz am See, Austria

²Department of Functional and Evolutionary Ecology, Unit Limnology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

³Institute for Hydrobiology and Water Management (IHG), University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33/DG, 1180 Vienna, Austria

⁴Department of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

*Corresponding author. WasserCluster Lunz – Biological Station, Dr. Carl Kupelwieser-Prom. 5, 3293 Lunz am See, Austria. E-mail: Pratiksha.Acharya@wcl.ac.at

Editor: [Martin Hahn]

†Shared senior authors

Abstract

Leaf litter decomposition is a vital ecosystem process in which macroinvertebrate-shredders produce substantial amounts of fine particulate organic matter (FPOM) via sloppy feeding and defecation, creating a substratum and substrate for microbial assemblages. However, microbial communities colonizing the shredder-produced FPOM are understudied compared to those in streams and on original leaves. Here, we investigated the bacterial community composition on shredder-produced FPOM in a laboratory experiment. We fed alder, beech, and maple leaves conditioned under oxic or anoxic conditions to *Sericostoma* (Insecta: Trichoptera) larvae. We collected shredded leaf particles and faecal pellets as shredder-produced FPOM at different times and examined their microbial communities using 16S rRNA amplicon sequencing. We hypothesized that shredder-produced FPOM types harbor diverse, distinct, and specialized microbial taxa in response to leaf species and conditioning. We found significantly higher alpha diversity on shredded leaves compared to faecal pellets. Microbial communities on faecal pellets differed from initial leaf communities and with anoxic and oxic conditioning. Bacterial communities developing on leaves were dominated by common leaf decomposers including *Flavobacterium* and *Pseudomonas* whereas faecal pellets harbored gut bacterial taxa including *Acinetobacter* and *Carnobacterium*. These results underline the importance of conditioning and shredder activity in shaping FPOM-attached bacterial communities, increasing bacterial diversity in stream ecosystems.

Keywords: leaf conditioning; leaf litter decomposition; microbial assemblage; microbial community composition; stream

Introduction

Microbial communities are key to the decomposition of organic matter in freshwater ecosystems (Webster and Benfield 1986, Allan et al. 2021). In headwater streams, the input of leaves and their decomposition are significant processes in carbon cycling (Vannote et al. 1980, Webster and Benfield 1986). Microorganisms including fungi and bacteria colonize and decompose leaf litter and their growth results in the accumulation of nutrients in the litter (Findlay et al. 2003, Mehring et al. 2015), a process known as “conditioning” (Bärlocher and Kendrick 1975, Gessner et al. 1999, Gonçalves et al. 2024). Over time, physical abrasion and macroinvertebrate consumers (i.e. shredders) further fragment the large leaf litter or coarse particulate organic matter (CPOM) via sloppy feeding and defecation (Wallace and Webster 1996, Graça 2001), ultimately producing different types of fine particulate organic matter (FPOM), such as shredded leaf particles and faecal pellets. FPOM is a crucial resource for collectors and deposit-feeding organisms (Bundschuh and McKie 2016) and also harbors a diverse and active microbial community (Acharya et al. 2025) that can influence in-stream leaf litter breakdown and energy cycling. Understanding the link between community composition and functions is essential for elucidating these processes and the presence of specific taxa provides insight into the biochemical and physi-

ological activities occurring during decomposition (Gessner et al. 2010).

Leaf-associated microbial communities are shaped by their surrounding environment (Gonçalves et al. 2024). Generally, microbial communities in streams respond to environmental conditions, such as hydrology (i.e. flow conditions and water retention), biotic interactions (i.e. microbial competition and predator–prey interactions), nutrient gradients, and physical and chemical parameters, such as temperature, pH, or dissolved oxygen (Cummins 1974, Petersen and Cummins 1974, Savio et al. 2015, Luo et al. 2020, Ma et al. 2020, Huang et al. 2022, Stadler and Del Giorgio 2022, Guo et al. 2023, Karačić et al. 2024, Sadeghi et al. 2024). For instance, oxygen concentrations can vary widely in stream microhabitats: Leaves in fast-flowing or turbulent oxygen-rich microhabitats are potentially decomposed quickly by aerobic microbial communities that include prokaryotes but also eukaryotes and follow a range of oxidative metabolic pathways (Brune et al. 2000, Karačić et al. 2024, Khomutovska et al. 2024). In contrast, large amounts of leaves can also accumulate in thick packs in slow-flowing sections or stagnant pools (Wantzen and Wagner 2006, Foucreau et al. 2013). These are frequently hypoxic or anoxic microhabitats dominated by specialized bacterial communities, such as anaerobic or facultative anaerobic taxa that mostly follow fermentation

Received 12 May 2025; revised 11 September 2025; accepted 11 September 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

pathways (Degelmann et al. 2009). Differences in oxygen availability affect microbial community assembly and composition. In combination with the properties of the original leaf litter, this has potential consequences for leaf litter decomposition (LLD) rates and efficiency (Gessner 2010, Dieter et al. 2011). While the effects of environmental conditions, such as oxygen availability on benthic and pelagic microbial communities are relatively well-known, their influence on leaf-associated microbial communities remains to be investigated.

Fungi are amongst the first colonizers of abscised leaves in the stream ecosystem with a predominant role at the early stages of LLD whereas bacteria usually dominate the decomposition process at a later stage (Gessner and Chauvet 1994, Gessner and Schmitt 1996, Foucreau et al. 2013, Pascoal et al. 2021). However, specific types of bacteria, including members of the genera *Pseudomonas*, *Sphingomonas*, *Pedobacter*, and *Massilia* are also found on newly submerged leaves where they support LLD by degrading recalcitrant polymers, such as lignin, cellulose, and hemicellulose (Tláškal et al. 2016, Silverman et al. 2017, Khomutovska et al. 2024). Indeed, some bacterial taxa must be considered ecological specialists, such as *Massilia* (nitrogen reducer; Hou et al. 2017), *Sphingomonas* (chitin degrader; Tláškal et al. 2016), and *Alphaproteobacteria* (phenol and tannin degrader; Wymore et al. 2016). On decomposing leaves, Wymore et al. (2016) found that *Bacteroidetes* and *Betaproteobacteria* dominated the bacterial assemblage, while *Verrucomicrobia*, along with *Alphaproteobacteria* and *Gammaproteobacteria*, became more abundant over time. The primary factor driving variation in bacterial composition was time whereas leaf species physiochemistry also significantly contributed to explaining the observed variations. These results show that the bacterial community on decomposing leaves in streams can be highly variable over time but also influenced by leaf species via differences in their chemical composition. Furthermore, shredder-produced FPOM can provide distinct ecological niches for bacterial taxa compared to the original CPOM due to changes in substrate quality or surface area via shredders gut passage or shredding. Therefore, the bacterial communities in shredder-produced FPOM may differ from those on the original leaf litter, potentially altering the nutritional value of the organic matter (Gessner et al. 1999), and playing distinct roles in organic matter turnover (Frossard et al. 2013) and nutrient cycling (Kominoski et al. 2009) within the stream's food web. Given the critical role of bacteria during LLD and associated energy cycling, understanding the presence and functional contributions of specific bacterial taxa during decomposition is essential. In this context, data on individual taxa can provide valuable insights into the biochemical and physiological processes driving litter breakdown. While substantial research targeted the initial microbial colonization and its role in LLD, the bacterial diversity and community composition of shredder-produced FPOM have received less attention.

Shredded leaves, one type of shredder-produced FPOM, may exhibit bacterial community patterns similar to those of intact leaves. However, the effects of shredder gut passage on the bacterial composition in faecal pellets, the other form of shredder-produced FPOM, remain largely unexplored. Leaf litter undergoes significant microbial transformations during gut passage and only specialist or resilient taxa typically survive the passage (Plante et al. 2023). As shredders consume and fragment leaf litter, the microbial assemblages on the ingested material are exposed to gut conditions, such as digestive enzymes, changes in pH, and potentially anoxic conditions, which can selectively promote or inhibit certain bacterial taxa. Research on various deposit-feeding taxa,

including Polychaeta (Plante and Wilde 2004), echinoids (Gao et al. 2014), hemichordates (King 2018), and crustaceans (Mongkol et al. 2018), has demonstrated that gut passage alters bacterial community composition on defecation products, likely through selective digestion (Plante et al. 1996, King 2018) or seeding from resident gut microbiomes (Lau et al. 2002, King 2018, Dale et al. 2019). This process could play a crucial role in shaping the diversity and composition of bacterial communities associated with FPOM in streams, including changes in nutritional quality and bioavailability of FPOM to other stream consumers (Bundschuh and McKie 2016) and thus influence downstream nutrient cycling (Cheever et al. 2013, Frossard et al. 2013). While numerous studies have explored the gut microbiomes of aquatic insects, including stream-dwelling species, research specifically addressing the influence of the gut passage in freshwater shredders involved in LLD is limited. Notably, there is a lack of studies examining the bacterial communities attached to the faecal pellets of shredders, highlighting a key gap in understanding their role in bacterial succession and nutrient cycling within freshwater ecosystems.

In this study, we aim to assess the variation in bacterial assemblages on leaf litter under different environmental conditions (i.e. oxygen level) and on leaf litter before ingestion and on faecal pellets after passage through the digestive tract (gut passage) of *Sericothoma* sp., using DNA metabarcoding. Specifically, our aim was to evaluate how leaf species, leaf conditioning under different oxygen levels, and gut passage affect the diversity and composition of bacterial communities associated with shredder-produced FPOM in a microcosm feeding experiment and identify indicative bacterial taxa dominating shredder-produced FPOM during litter breakdown. We hypothesized that (i) the oxic conditioned leaves have different and more diverse bacterial community than anoxic conditioned leaves and (ii) two types of shredder-produced FPOM—mechanically shredded leaves from sloppy feeding and faecal pellet FPOM—would be colonized by distinct bacterial communities due to selective digestion and microbial filtering in the gut (Plante et al. 1996, Aira et al. 2022), which we moreover expected to vary with leaf species and prior leaf conditioning.

Materials and methods

Preparation for laboratory feeding experiment

We collected naturally abscised alder, beech, and maple leaves around Lunz am See, Austria, dried and stored them in the dark at room temperature. For the experiment, we cut the leaves into 18 mm diameter circular discs prior to microbial colonization. For microbial colonization under anoxic conditions, we buried the leaf discs of each leaf species packed in mesh bags (1 mm mesh size) in a 1:1 clay (Glorex AG, Füllinsdorf, Switzerland) slurry. We mixed the clay slurry with stream water from Oberer Seebach (OSB; 47° 51' N, 15° 04' E), allowing the clay to settle and cover the discs within a short period, and leaving them undisturbed for 4 weeks. We confirmed anoxic conditions by measuring dissolved oxygen concentrations directly above the clay layer four times during the conditioning period. Leaves were washed immediately before use in experiment with water from the same stream to remove excess sediments. For microbial colonization under oxic conditions, we submerged the leaf discs in freshly collected, aerated stream water for 1 week. We confirmed the oxic conditions by regularly monitoring the dissolved oxygen concentration in the water column (WTW Oxi 315i; Xylem Analytics, Weilheim, Germany). Samples of oxic and anoxic conditioned leaves were taken right after conditioning but before the start of the feeding experiment to

assess the initial microbial communities. We refer to these leaves as mechanically shredded leaves at the initial stage of the feeding experiment ("Initial shredded leaves"). More details of the experiment preparation can be found in Acharya et al. (2025).

A day before the start of the feeding experiment, we collected the aquatic shredder species, *Sericostoma* sp. (Trichoptera; larval stage; hereafter referred to as "larvae") of similar size (head capsule width 2.0 ± 0.1 mm) from Unterer Seebach (USB; 47° 51' 29" N 15° 2' 4.74" E) and transported them to the laboratory. We acclimated them in separate food-grade plastic cups [polylactic acid (PLA), volume: 4 cl] at 12°C in filtered stream water (0.7 µm, 450°C, 4 h precombusted Whatmann GF/F filters), and they were starved for 24 h before the start of the feeding experiment.

Microcosm setup

We conducted a 25-day feeding experiment at 12°C and a light:dark cycle of 11:13 h in a full-factorial design. We set up five replicates of each of the six treatments that consisted of the three leaf species and the two oxic–anoxic conditioning treatments, resulting in a total of 30 microcosms. Each microcosm consisted of a white food grade plastic bucket (1100 ml volume and 122 mm bottom diameter) with a steel mesh (pore size 1 mm) at a height of 4.5–5 cm from the bottom to allow shredder-produced FPOM to pass through. We adapted the microcosm setup from a previous study (Rubio-Ríos et al. 2022). We supplied organic matter free sand particles (muffle furnace, 450°C, 4 h precombusted) in aluminum Petri dishes for shredders to make their cases. We added 10 shredders to each microcosm and fed them with the conditioned leaves of one of the three species. During the 25-day feeding experiment, we collected and analyzed bacterial community composition on the larval faecal pellets at three time points: on 5th, 15th, and 25th day (5 d, 15 d, and 25 d). From each treatment, we transferred each larva to a single food-grade plastic cup (polylactic acid or polylactide—PLA) filled with 20 ml of 0.7 µm-filtered stream water and left them to evacuate their gut contents for 24 h; thus, we only collected faecal pellets and no shredded leaf particles. We collected the faecal pellets from each plastic cup using a Pasteur pipette after returning the larvae to the same microcosm and after replacing the water in the microcosms with freshly collected stream water (see details of microcosms setup in Acharya et al. 2025).

At the end of the experiment, we collected the remaining leaf discs that were partly eaten by the shredders in order to assess the final microbial communities established on leaves and stored them at –80°C until further processing. We refer to these leaves as "Final shredded leaves."

DNA extraction

For DNA extraction, we initially aimed to pool faecal pellets from two larvae per microcosm. However, our tests revealed that this amount was insufficient for DNA extraction, and we thus pooled the faecal pellets from two larvae per microcosm from all five replicates per treatment (faecal pellets per 2 larvae $\times$ 5 microcosm per treatment = faecal pellets from 10 larvae). This resulted in just one sample per treatment per timepoint for the analysis (n = each treatment $\times$ 3 sampling timepoints). We stored the samples for the DNA extraction at –80°C until further processing.

We extracted total genomic DNA from freeze-dried (Virtis™ Genesis Freeze Dryer, for minimum 24 h) leaves (n = 36, 3 replicates $\times$ 6 treatment $\times$ 2 timepoints) and faecal pellets (n = 18, 6 treatment $\times$ 3 sampling timepoints) using the DNeasy Power-Soil Pro kit (Qiagen, Hilden, Germany) following the manufactur-

ers' protocol (leaves were first cut into smaller pieces with a sterile scalpel). DNA concentrations were quantified fluorometrically with the Qubit™ 1X dsDNA HS Assay Kits using an Invitrogen Qubit 4.0 Fluorometer (Thermo Fisher Scientific).

Amplicon sequencing

We used the extracted total DNA to amplify and sequence the V4 region of the 16S rRNA gene. To this end, the primer pair 515F (GTG YCA GCM GCC GCG GTA A) and 806R (GGA CTA CNV GGG TWT CTA AT) (Apprill et al. 2015, Parada et al. 2016) was used for amplification; barcoding and adapter ligation were achieved in a second step as described in Pjevac et al. (2021) for sequencing on an Illumina MiSeq platform with 600-cycle v3 chemistry (2 $\times$ 300 bp paired-end reads). Raw sequencing data were processed using a FASTQ workflow (Basespace, Illumina) with default settings, and sorted into unique amplicon sequence variants (ASVs) using the DADA2 pipeline (Callahan et al. 2016) in R (R core team 2023) using the recommended workflow. FASTQ reads were trimmed at 220/230 nt with allowed expected errors of two-fourths. The representative ASVs generated were subsequently merged and classified using the SILVA r138.1 database using default parameters (Quast et al. 2012), and taxonomy assigned using the assignTaxonomy() function in DADA2 with a confidence threshold of 0.05 for prokaryotic 16S rRNA gene amplicons.

Data filtering and statistical analysis

Before statistical analysis, we removed ASVs representing Eukaryota and Archaea, as well as ASVs classified as mitochondria or chloroplasts, as well as singleton and doubleton ASVs. Further, ASVs that did not have phylum-level information were also removed. Finally, we combined ASV read abundances, taxonomy, and sample metadata into a single dataset (function *phyloseq*; *phyloseq* package) (McMurdie and Holmes 2013) in R for subsequent analyses. All data analyses and visualizations were done in R 4.2.3 version (R core team 2023).

To test diversity and compositional differences of bacterial communities between shredded leaves and faecal pellets, we calculated alpha diversity and beta diversity. We used species accumulation curves to compare estimates of alpha diversity from each FPOM type (function *specaccum*; *vegan* package; Oksanen et al. 2022). We compared species richness (Observed and Shannon–Wiener Index) metrics on the rarefied data to the lowest sequencing effort (1000 sequences) (function *estimate_richness*; *phyloseq* package). After calculating Shannon Diversity Index, we ran a linear model where leaf species, conditioning, FPOM types, and their interactions were used as fixed factors. We then evaluated the model fit by running Analysis of Variance (Type III ANOVA) and verified the assumptions of normality and homoscedasticity by plotting residuals. Afterwards, we calculated the effect size (function *effectsize*; *effectsize* package) (Ben-Shachar et al. 2020) to identify the strongest influencing fixed factor. We did not conduct rarefaction for any other analysis, and other transformations were applied to standardize data without losing information.

We calculated beta diversity on filtered and standardized data tables using Hellinger's transformation on ASV read abundances (nonrarefied matrices) (function *decostand*; *vegan* package). We assessed the bacterial community structures in the samples using nonmetric multidimensional scaling system (NMDS; function *ordinate*; *phyloseq* package) based on Bray–Curtis distance matrix of transformed ASVs abundances (function *vegdist*; *vegan* package). The goodness of fit of the NMDS is given by stress values. Stress values above 0.2 might indicate unreliable ordinations. We

compared bacterial community composition among FPOM types, leaf species and between conditioning by permutational multivariate analysis of variance (PERMANOVA; function *adonis2*; *vegan* package) on the transformed ASVs, using “Bray–Curtis” dissimilarities as a distance measure. The assumption of homogeneous within-group dispersion was tested (function *betadisper*; *vegan* package) and was fulfilled for all groups.

For our second hypothesis regarding the differences in taxonomic composition, we did a Kruskal–Wallis test, followed by Wilcoxon rank-sum test for pairwise comparisons of mean of relative sequence abundance of specific taxonomic groups (e.g. phylum and class) among FPOM types (function *compare_means*; *ggpubr* package). *P*-values were reported after Benjamini–Hochberg (BH) correction (Benjamini and Hochberg 1995). In addition, to identify the group of samples with similar bacterial community composition, we performed a hierarchical clustering analysis on relative abundance of count taxon of ASVs using Euclidean distance and Ward’s linkage (function *dist*; *stats* package), that minimizes the sum of squares of any two clusters (function *as.dendrogram*; *stats* package). Further, to assess the differences in the specialized taxa among the distinct sample groups, ISA (function *multipatt*; *Indicspecies* package) was applied on relative sequence abundance of bacterial community composition. To retain meaningful taxa from each group, abundant taxa with a *P*-value above 0.05 were excluded after *P*-value adjustment with BH false discovery rate method. Since the number of significant species was still too big, we selected the 15 taxa with the highest association of each group.

Results

Taxonomic composition of bacterial communities in shredder-produced FPOM

The taxonomic composition of bacterial communities based on DNA extracted from initial and final shredded leaves, and faecal pellets is visualized in Fig. 1 and Fig. S1. The relative abundances of specific taxonomic groups (at phylum and class level) differed significantly among the two shredder-produced FPOM types (Kruskal–Wallis, $P < 0.05$; Tables S1 and S2). However, the majority of the abundant taxa at phylum and class level did not differ between the two conditioning and among the three leaf species (Kruskal–Wallis, $P > 0.05$; Fig. 1 and Tables S3–S6). In general, taxonomic classification of the obtained ASVs using the Silva database showed that all samples were dominated by ASV sequences assigned to the phyla Proteobacteria (on average 55% in initial shredded leaves, 51% in final shredded leaves, and 61% in faecal pellets), followed by Bacteroidetes (on average 32% in initial leaves, 29% in final leaves, and 12% in faecal pellets), Firmicutes (on average 5.9% in initial shredded leaves, 0.7% in final shredded leaves, and 10% in faecal pellets), Actinobacteria (2.5% in initial shredded leaves, 2.2% in shredded final leaves, and 7.3% in faecal pellets), and Verrucomicrobia (0.03% in initial shredded leaves, 5.1% in shredded final leaves, and 0.6% in faecal pellets) (Fig. 1 and Fig. S1; Table S7). At class level, we found that the initial shredded leaves were dominated by Gammaproteobacteria (30%), Flavobacteriia (15%), and Alphaproteobacteria (13%), whereas final shredded leaves were more dominated by Betaproteobacteria (24%), followed by Flavobacteriia (16%), and Alphaproteobacteria (14%). As with the initial communities on shredded leaves, bacterial communities on faecal pellets were dominated by Gammaproteobacteria (36%), but then followed by Betaproteobacteria (14%), Alphaproteobacteria (11%), and Bacilli (9.1%) (Fig. 1; Table S7).

Bacterial taxa richness and Shannon diversity in shredder-produced FPOM

For the quality assessment of our sequencing dataset, we rarefied the data to the lowest sequencing effort (1000 sequences). We plotted rarefied species accumulation curves for the three different types of FPOM collected during the experiment: initial shredded leaves, final shredded leaves, and faecal pellets, as a function of the sample number (Fig. 2A). Rarefied species accumulation curves indicated good coverage of richness in our samples but approached a quasi-horizontal asymptote only in the case of initial leaves (Fig. 2A). Richness estimates for the faecal pellets and especially final leaves were steeper, indicating a potentially greater richness than covered by our analyses. Overall, the final shredded leaves had the highest species richness (mean $\pm$ standard deviation; 229 ± 27 taxa) while that of faecal pellets and initial shredded leaves were considerably lower with 129 ± 26 and 136 ± 39 taxa, respectively.

Additionally, we calculated the Shannon Diversity Index on the rarefied dataset that was again highest in the final shredded leaves. The index ranged from 4.49 ± 0.24 (anoxic) and 4.81 ± 0.19 (oxic) in final shredded leaves to 3.43 ± 0.10 (anoxic) and 4.29 ± 0.20 (oxic) in initial shredded leaves and 3.60 ± 0.39 (anoxic) and 3.61 ± 0.40 (oxic) in faecal pellets (Fig. 2B). For this parameter, the FPOM type, leaf species, and the interactions between leaf species and FPOM types and between conditioning and FPOM types were significant (Table 1). Specifically, the FPOM type “Final leaves” showed a significant positive effect on Shannon diversity compared to the reference FPOM type “Faecal pellets” (linear model: Estimate = 0.36, $P < 0.05$; see Table S9 for all effect sizes), while “Initial leaves” had a significant negative effect (-0.45 , $P < 0.05$). Among leaf species, beech had a significantly reduced Shannon diversity compared to the reference alder (-0.79 , $P < 0.05$). Finally, the overall conditioning effect was not significant (Table 1). However, resulting from the significant interaction between conditioning and FPOM type, we found that for “Initial leaves” the oxic conditioning significantly increased Shannon diversity compared to anoxic conditioning (1.00 , $P < 0.05$) with ~ 1.3 times higher Shannon diversity (Table S8).

Structure of bacterial community composition in shredder-driven FPOM

For a more in-depth evaluation of bacterial community turnover relative to the initial shredded leaves, we performed a PERMANOVA using Bray–Curtis dissimilarities of Hellinger-transformed 16S rRNA sequencing data and visualized it by NMDS. The structure of bacterial community composition differed across treatments, driven by leaf species, conditioning, and among shredded leaves versus faecal pellets (Fig. 3). The PERMANOVA results based on the abundances of bacterial taxa revealed a significant difference in bacterial communities across leaf species though the proportion of explained variation was relatively small ($F_{(2,35)} = 5.51$, $R^2 = 0.045$, $P < 0.001$). In addition, we found a substantial turnover of bacterial communities from initial shredded leaves to those in final shredded leaves and faecal pellets (PERMANOVA, $F_{(2,35)} = 50.61$, $R^2 = 0.438$, $P < 0.001$) and between oxic and anoxic conditioning ($F_{(1,35)} = 52.96$, $R^2 = 0.192$, $P < 0.001$). Our NMDS plot showed the distinct clustering of bacterial communities based on conditioning and FPOM type. Bacterial communities of initial shredded leaves were markedly diverged from those in final shredded leaves and faecal pellets, along with oxic and anoxic conditioning forming well-separated clusters (Fig. 3).

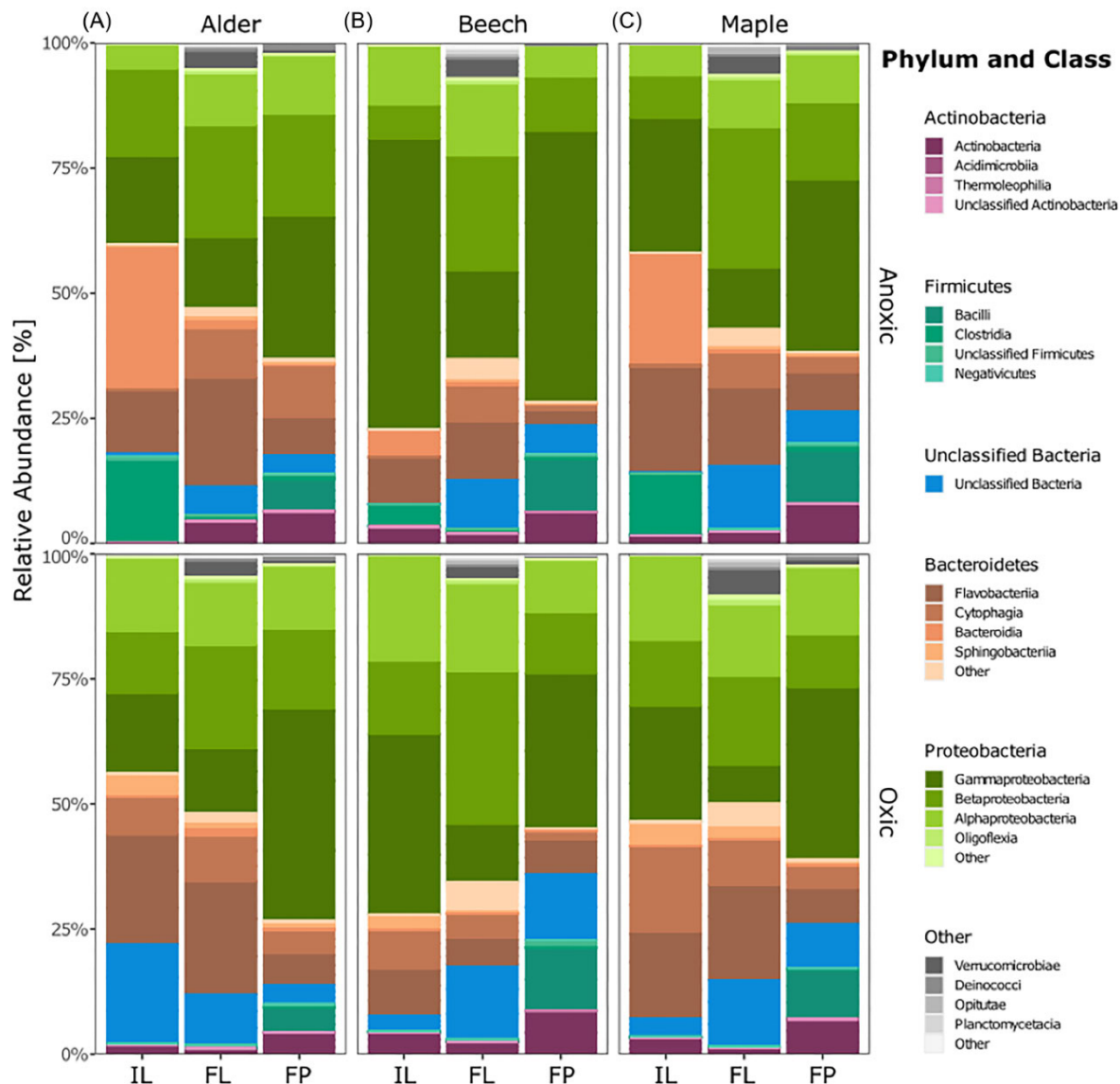


Figure 1. Taxonomic composition and relative abundance of individual taxonomic groups for each FPOM type and associated with leaf treatments: alder (A), beech (B), and maple (C). Additionally, the graph shows the data for anoxic- (top) and oxic-conditioned leaves (bottom). Each phylum is indicated by a different color and each class is indicated by a different shade of the color. Only the five most abundant phyla and four most abundant classes across phyla level for each treatment are shown whereas the remaining phyla and classes are grouped as “Other.” Abbreviations: IL; initial leaves, FL; final leaves, and FP; faecal pellets.

Indicator taxa of individual FPOM types

We performed a hierarchical clustering analysis on relative abundance of count taxon of ASVs, which showed the three clusters of taxa representing our initial anoxic shredded leaves, all other shredded leaves (oxic initial and the final shredded leaves), and faecal pellet samples (Fig. 4A). Our data showed that the bacterial taxa representing the initial anoxic shredded leaves were different from the other leaf communities including initial oxic shredded leaves and all final leaves. Indicator species analysis (ISA) on the relative abundance of the ASVs identified specialized, typical taxa associated with shredded leaves and faecal pellets and showed turnover in indicator taxa across time. Based on highest indval values obtained after ISA, the indicator taxa in initial anoxic leaves were *Alkalibacter*, *Anaerotaenia*, *Propionivibrio*, *Paludibacter*, and *Sulfurospirillum* (Table S10). *Asticcacaulis*, *Flavobacterium*, and

Pedobacter were typical for all other shredded leaves and *Acinetobacter*, *Deinococcus*, *Carnobacterium*, *Subtercola*, *Rhodococcus*, and *Vagococcus* were typical for faecal pellets (Fig. 4B; Table S10). Overall, *Paludibacter* had the highest Indval values (= 1) and thus was the most dominant taxon ($P < 0.05$) in initial anoxic leaves. Similarly, *Acinetobacter* and *Flavobacterium* were the most dominant taxa ($P < 0.05$) with indval value = 1 in faecal pellets and all other shredded leaves, respectively (Table S10).

Discussion

In this study, we aimed to provide a new perspective on diversity and composition of bacterial communities associated with shredder-produced FPOM and thereby improve our understanding on the role of microbial communities associated with terrestrial-derived organic matter processing in streams. We found

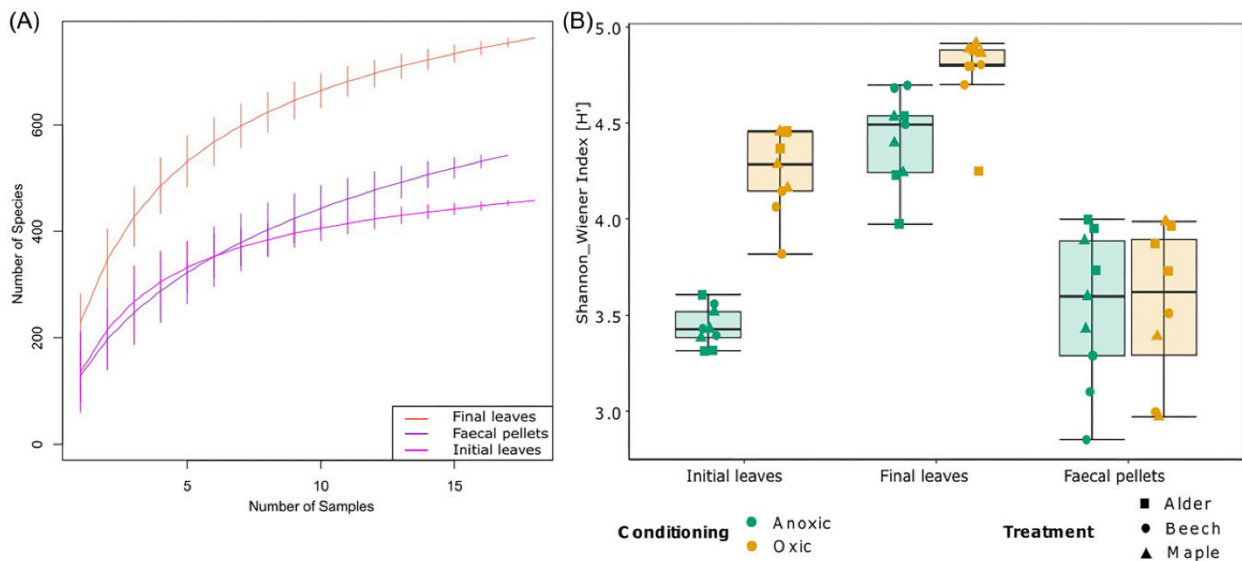


Figure 2. Species accumulation curves based on rarefaction method (A) and boxplot of Shannon bacterial diversity (B) across different FPOM types collected during 25 days of laboratory feeding experiment. In figure A, the vertical line on each curve indicates the error bar around the mean number of species observed per number of samples. The curves indicate final shredded leaves, faecal pellets, and initial shredded leaves. In figure B, two colors distinguish the samples from oxic or anoxic conditioning and three symbols indicate alder, beech, and maple treatments. The boxplots visualize the median of each FPOM type (line), the first and third quartiles (hinges), the 1.5 * interquartile ranges (whiskers).

Table 1. Effects of leaf species, conditioning, FPOM type, and their interactions on bacterial diversity of samples during feeding experiment based on linear model analysis. Abbreviations; St: Standardized, SS: sum of square, and df: degrees of freedom.

Factors	St. coefficient	SS	df	F-value	P-value
FPOM type	0.38	0.999	2	10.915	<0.001
Conditioning	0.001	0.001	1	0.031	0.860
Leaf species	0.38	0.968	2	10.583	<0.001
FPOM type * Conditioning	0.32	0.747	2	8.162	0.001
FPOM type * Leaf species	0.41	1.121	4	6.128	<0.001
Conditioning * Leaf species	0.05	0.081	2	0.888	0.421
FPOM type * Conditioning * Leaf species	0.14	0.252	4	1.380	0.261
Residuals		1.601	35		

Statistically significant P-values (< 0.05) are shown in bold.

significant differences in diversity and taxonomic composition of bacterial communities associated with different fractions of shredder-produced FPOM but also revealed that properties of terrestrial organic matter (either intrinsic or due to microbial colonization) and passage through the shredders gut are important factors in shaping bacterial turnover, and reshaping the structure of bacterial communities associated to shredder-produced FPOM.

Intrinsic leaf properties partially influence bacterial community composition

Our results show that the interaction between the leaf species and FPOM type had a significant effect on bacterial alpha diversity of shredder-produced FPOM (Table 1 and Table S9). We speculate that this difference can be attributed to the interplay between leaf properties and shredder activity since microbial colonization on leaves of varying C/N ratios may influence gut microbiota interaction and consequently influence FPOM attached microbial communities (Graça 2001, Wantzen and Wagner 2006, Villanueva et al. 2012, Ayayee et al. 2018, Gonçalves et al. 2024). Notably, we observed a higher bacterial diversity in alder compared to beech that was most prominent in the faecal pellets (Table S8). However, we did not find a strong leaf species effect on the Shannon diversity

as well as the bacterial community composition (Fig. 3). Nevertheless, our results here align with prior findings, which emphasize that the initial leaf quality is a determining factor for the microbial assemblages (Graça 2001, Rezende et al. 2017). Alder, known for its higher nutrient content and lower structural complexity (e.g. lignin concentration), appears to provide a more favorable environment for microbial colonization compared to low-quality leaves such as beech (Hladysz et al. 2009, Kominoski et al. 2009). In addition, we assume that the selective feeding of shredders on certain parts of the leaves (Fenoy et al. 2021), such as the veins versus the softer tissue, played a crucial role in modifying the leaf properties which in turn, may have resulted in the processing of leaf components of differing toughness differently. This might have sped-up or slowed down the gut passage for the ingested material, altering the substrate quality, surface area, or gut passage time for microbial colonization, thereby modulating microbial diversity in faecal pellets (Bärlocher and Kendrick 1975, Hieber and Gessner 2002, Yoshimura et al. 2008, Cheever et al. 2013). Hence, we here speculate that the combined effect of alder's intrinsic chemical properties and shredder-mediated processing may have contributed to higher bacterial diversity in alder-derived FPOM and potentially increasing the LLD rate and efficiency in streams.

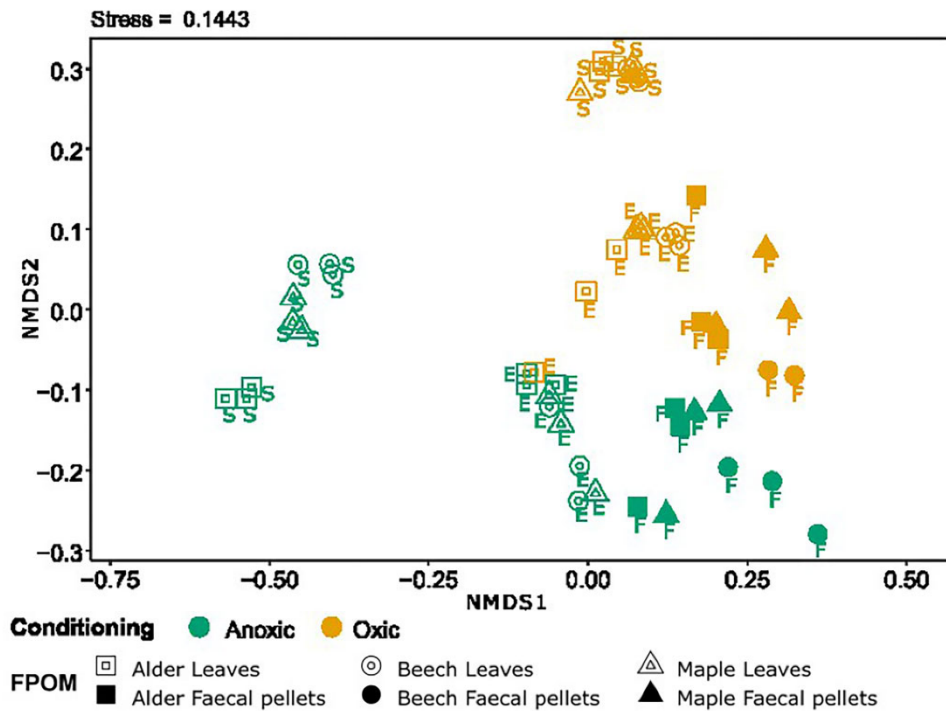


Figure 3. NMDS showing the clustering of normalized and Hellinger-transformed 16S sequencing data based on Bray-Curtis distances of bacterial communities associated with initial leaves, final leaves, and faecal pellets. Abbreviation: S; start = initial bacterial community in shredded leaves, E; end = final bacterial community in shredded leaves, and F; bacterial community in faecal pellets. The two colors distinguish the samples from oxic or anoxic conditioning. The three different empty symbols indicate three leaf species, and the three different filled symbols indicate faecal pellets obtained from shredders after feeding those three leaf species.

Moreover, we found that, over time, many ASVs that were abundant in initial shredded leaves, such as Gammaproteobacteria decreased in abundance while other ASVs such as Betaproteobacteria increased (Fig. 1) in all leaf treatments as described in other stream ecosystem studies (Newman et al. 2015, Zhao et al. 2017). Over time, alpha diversity and abundance of bacterial communities increased under aerobic conditions by following species-time relationships patterns as shown in other ecosystems (Shade et al. 2013, Karačić et al. 2022). As the biofilm matures and bacteria metabolize the cell polymers and excrete metabolites creating broader environmental niches (Karačić et al. 2024). This possibly resulted in the establishment of more diverse biofilm communities and consequently increasing diversity in final shredded leaves (Tláškal et al. 2016, Zhao et al. 2017). It is likely that more diverse communities compete or coexist within available resources and may have several metabolic functions such as carbon and nitrogen metabolism and nutrient release, potentially making a stream ecosystem resilient under different disturbances (Ramond et al. 2024). Furthermore, the increasing predominance of Betaproteobacteria in our experiments resembles bacterial communities in biofilms and surface water samples (Araya et al. 2003) and suggests that our experiments likely captured near-natural community turnover dynamics, where changes in the chemical properties of leaf litter are reflected in succession of communities (Rapoeseiro et al. 2017). In addition, introducing shredder larvae may have simply resulted in an inoculation of the water in the microcosms by gut-associated bacteria that differed from the original stream water community. The changes in light conditions during the experiment (from dark during conditioning to 11:13 h light: dark cycles) may have further resulted in algal growth, thereby increasing the resource pool for heterotrophic bacteria through

exudate production (Wyatt and Turetsky 2015, Francoeur et al. 2020, Pope et al. 2020). Altogether, we argue that changes in leaf chemical composition as well as the activity of the experimental macroinvertebrate shredders fostered community turnover and resulted in an overall higher diversity of bacterial communities in final leaves compared to initial leaves.

In contrast, the differences observed in bacterial communities among faecal pellets produced by feeding different leaf species, as compared to those found on shredded leaves (Figs 1 and 2B) may indicate that the shredders' gut selectively modifies bacterial assemblages based on the leaf properties. However, other factors such as macroinvertebrate feeding preferences and gut conditions (i.e. pH and oxygen level) may have mediated microbial communities and microbial growth efficiency could play a role in LLD (Danger et al. 2012, Foucreau et al. 2013). Our finding thus points out the differences in digestion processes during consumption of varying leaves with different qualities, suggesting the shredders' gut may selectively boost or suppress the taxa based on the nutrient content and degradability of the ingested leaves (Foucreau et al. 2013, Halvorson et al. 2014). This selection process during gut passage likely enhances both taxonomic and functional diversity of the stream microbiome and thereby may strongly influence organic matter remineralization, storage, and production.

Conditioning affects bacterial community composition

Leaf conditioning under oxic or anoxic conditions prior to the feeding experiment had a substantial impact on bacterial diversity and composition in our experiments: leaves conditioned in an oxic environment, along with the experimental leaves from the final sampling at the end of the incubation, exhibited greater

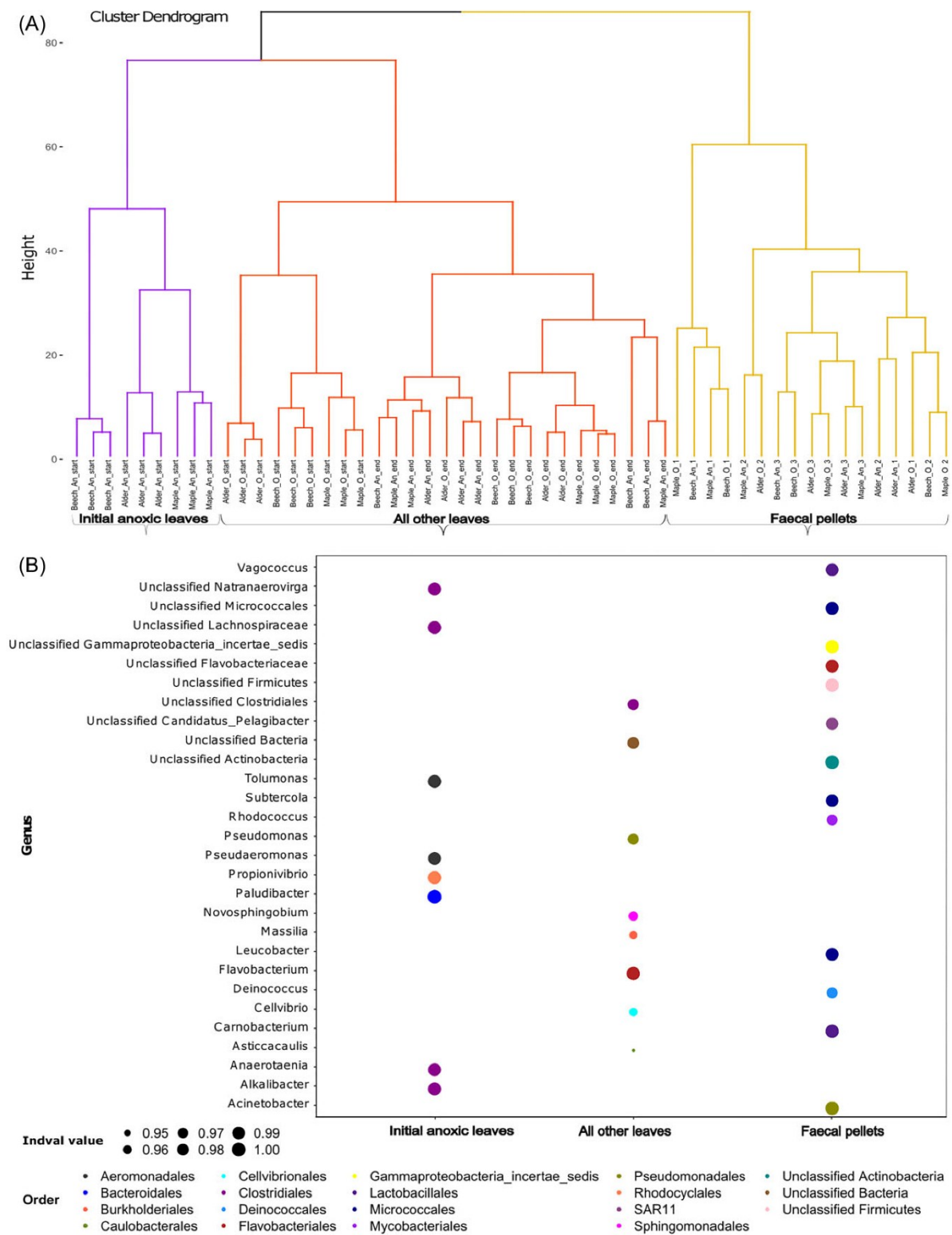


Figure 4. Representation of ISA. Hierarchical clustering (Ward's method) of Euclidean distances among ASV relative abundances returned three clusters, representing initial anoxic shredded leaves (purple), all other shredded leaves, i.e. initial oxic and final shredded leaves (red), and faecal pellet samples (yellow) (A). Indicator taxa at order and genus-level for each group are shown below (B), in a bubble plot, where size of each circle represents the Indval value, which shows the association of each taxon to specific FPOM-type. Colors of each circle represent taxa at order level.

bacterial diversity. They were dominated by aerobic taxa such as *Cellvibrio* (Order: Cellvibrionales), *Flavobacterium* (Flavobacteriales), *Sphingomonas* (Sphingomonadales), *Pseudomonas* (Pseudomonadales), and *Massilia* (Burkholderiales). These genera are commonly known leaf litter decomposers and have the ability to degrade plant-derived polymers (Rastogi et al. 2012, Tláskal et al. 2016, Silverman et al. 2017, Janssen et al. 2021, Khomutovska et al. 2024). Such aerobic and facultative aerobic taxa use oxidative metabolic pathways to break down complex organic compounds, such as lignin and cellulose (Gessner and Chauvet 1994, Ma et al. 2020). This potentially accelerates the production of FPOM by invertebrate shredders (Baldy et al. 2002, Gessner 2010, Danger et al. 2012). Indeed, we found several ASVs on the oxic-conditioned leaves corroborating this interpretation. For instance, we found *Sphingomonas* that is able to produce and use *N*-acetyl-D-glucosamine, a monomer of the fungal polymer chitin (Valášková et al. 2009) while *Pedobacter* and *Pseudomonas* have been previously well-described for chitinolytic activity (Brabcová et al. 2016), which may direct towards a fungi-bacteria interplay during LLD. In addition, *Sulfurospirillum*, another genus of bacterial leaf decomposers (Vesamäki et al. 2024), was found. The occurrences of these taxa suggest that they can utilize leaf litter as their energy source and assist the leaf breakdown process rapidly.

In contrast, ASVs assigned to the genera *Propionivibrio* (Order: Rhodocyclales), *Alkalibacter* (Clostridiales), *Paludibacter* (Bacteroidales), *Pseudaeromonas* (Gammaproteobacteria), *Tolomonus* (Gammaproteobacteria), and others were identified as indicative taxa, dominating the initial shredded leaves under anoxic conditions. These taxa are adapted to low-oxygen environments (Karačić et al. 2024), and are known for their role in anaerobic metabolism and early colonization of organic substrates (Hau and Gralnick 2007, Smeaton et al. 2009, Qiu et al. 2017, Silverman et al. 2017). We found the genus *Shewanella* on initial shredded leaves under anoxic conditions, which is known as iron-reducer and also transforms the heavy metals, such as lead and chromium into less or nontoxic forms through anaerobic respiration pathways (Hau and Gralnick 2007, Smeaton et al. 2009). Rhodocyclales, another group of versatile bacteria, are known for utilizing a wide variety of organic compounds for growth (Kittichotirat et al. 2011). These bacterial taxa, using mostly anaerobic metabolic pathways, such as fermentation, decompose organic matter somewhat slower (Degelmann et al. 2009, Qiu et al. 2017, Huang et al. 2022), yet may contribute to a pool of fermentative products upon reexposure to oxygen. Overall, our findings on the bacterial communities on initial CPOM align with previous results on the role of oxygen availability in shaping microbial decomposition strategies (Huang et al. 2022). Interestingly, the communities established under oxic and anoxic conditions persisted to some degree throughout leaf particle passage through shredders guts, suggesting that the environmental history continues to influence the microbial succession trajectories, even after gut passage.

Gut passage effects: input is not the same as output

Faecal pellets, one type of FPOM, differed substantially in their bacterial communities compared to initial CPOM and final shredded leaves, independent of the leaf species as supported by an overall shift in community structure and the ISA. These shifts in community composition from leaves to faecal pellets are possibly due to selective digestion, specific gut environments, such as pH levels, oxygen gradients, or the introduction of gut-associated bacteria (Danger et al. 2012, Wei et al. 2014, Jung and Park 2015,

Ayayee et al. 2018). In addition, ISA revealed that certain taxa were found solely in faecal pellets, suggesting that gut passage plays a crucial role in shaping the FPOM attached bacterial community composition.

Particularly, anaerobic or facultative anaerobic bacterial taxa became dominant on faecal pellets while those less adapted to gut conditions were reduced or digested in the shredders' gut. Our findings thus resemble previous results on faecal pellets of other detritivores, i.e. earthworms which showed a reduced bacterial alpha diversity after gut passage compared to the ingested soil (Aira et al. 2022) due to the homogenization of the bacterial community and the loss of rare taxa (Plante et al. 1996, Graça 2001, Yoshimura et al. 2008). Indeed, gut passage promotes the assembly of unique communities on faecal pellets suggesting that invertebrate taxa can modify the microbial community composition during gut passage, presumably, via selective digestion or preferential reduction of specific taxa (Plante et al. 1996, Graça 2001).

Our analysis also revealed a notable shift in the relative abundance of bacterial taxa, a clear decrease in Bacteroidetes and an increase in Firmicutes and Proteobacteria in the faecal pellets compared to the initial shredded leaves (Fig. S1; Table S7). Bacteroidetes are well-studied abundant surface colonizers of particulate organic matter and play a key role in their degradation and utilization (Crump et al. 1999, Riemann and Winding 2001, Kirchman 2002). Reduction of these Bacteroidetes taxa on faecal pellet may reflect a shift in metabolic pathways of communities reshaped by gut passage. Notably, Pseudomonadales (~24%) was the most frequently detected proteobacterial order in faecal pellet samples, followed by Burkholderiales (~12%) and Enterobacteriales (~10%), all of which can degrade a vast array of long-chain dicarboxylic acids and hydroxylated aromatic compounds including lignin and constituents of plant exudates (Pérez-Pantoja et al. 2012, Ghosal et al. 2016). This selective turnover and enrichment likely reflect the role of the shredders' gut as a microbial filter and bioreactor, altering bacterial diversity and community composition through selective retention or enhancement during the digestion process. Further, the faecal pellets were enriched in taxa such as *Acinetobacter*, *Carnobacterium*, and *Rhodococcus*, which are known for their ability to metabolize complex organic compounds via fermentation and survive under low-nutrient conditions (Leisner et al. 2007, Jung and Park 2015). In addition, we observed ASVs belonging to the genera *Carnobacterium* (Class: Bacilli), *Leucobacter* (Actinobacteria), *Acinetobacter* (Gammaproteobacteria), *Vagococcus* (Bacilli), and *Rhodococcus* (Actinobacteria) as indicator species in faecal pellets (Table S10), which are commonly reported as gut bacteria of Diptera larvae (Sontowski and van Dam 2020) and other detritivores such as silkworm (Gogoi et al. 2023) and green bottle fly larvae. It is likely that these taxa colonized the particles in the shredder's gut and may contribute to further processing of the egested particles.

To date, there are some studies reporting gut microbiomes of different functional feeding groups (filter feeders, grazers/collectors, predators, and omnivores) of benthic invertebrates in stream ecosystems (Ayayee et al. 2018, 2022). Yet, information on the gut microbiome of shredders, especially in the order Trichoptera, which is one of the most important functional feeding groups in headwater streams, is still missing. Our study is the first, to address the transition in structure and taxonomic composition of bacterial communities during the conversion of leaves into faecal pellets by feeding activity of *Sericostoma* sp (Insecta: Trichoptera). Hence, these findings suggest that shredders not only contribute to the physical breakdown of leaves but also change the microbial community composition on the shredder-derived

FPOM via the digestion process, thereby probably facilitating the microbial conversion of organic matter into simpler forms, which can be utilized by downstream consumers such as filter-feeders.

The prevalence of different bacterial taxonomic groups (phylum, class, and genera) in shredded leaves compared to faecal pellets revealed that the bacterial communities colonizing the leaves are not similar to the communities colonizing faecal pellets during LLD in streams. In the light of this observation, we may presume that the combination of environmental factors e.g. oxygen levels, and gut passage, which either may have digested several bacterial taxa or favored the growth of individual bacterial taxa or introduced gut residents in faecal pellets shaped the microbial communities in each FPOM type. Our data thus suggest that the bacterial assemblages on different FPOM types may employ various metabolic pathways, which possibly contribute diversely to LLD in streams with potential implications for downstream nutrient cycling, and food web dynamics.

Limitations and future directions of the study

While our microcosm study provides valuable insights related to LLD in streams, it oversimplified the complexity of natural stream ecosystems. For instance, we focused on the importance of leaf species in shaping the bacterial community composition associated with shredder-produced FPOM, where our interpretations are limited by the number of leaf species selected. Including a broader range of leaf types with a greater range of properties (e.g. evergreen versus deciduous leaves and native versus invasive leaves) could provide better insights into how CPOM substrates affect FPOM-associated microbial communities and their role in LLD in streams. Moreover, the complexities of natural stream ecosystems regarding oxygen conditions and other environmental characteristics, such as variation in flow, organic load, or temperatures, but also interactions with other microbes such as algae and fungi may modulate bacterial communities even further. These may influence diversity and composition patterns of bacterial communities associated with CPOM and shredder-produced FPOM to a much greater degree with important implications for the stability and resilience of ecosystems. Moreover, integrating fungal community dynamics, which are critical during the initial stage of LLD, and examining bacterial communities inhabiting the gut of freshwater shredders across shredder species and developmental stages could further explain the complex interactions between shredders, their food resources, and microbial dynamics. Lastly, it is crucial to link functional capacities of microbial composition associated with FPOM, such as enzyme activities, metabolism, and nutrient fluxes. All these factors will be essential to fully understand the role of microbial assemblages in stream ecosystem functioning.

Conclusion

Bacterial communities dwelling on the FPOM produced during LLD can play a pivotal role for energy flow and nutrient cycling within stream food webs. Yet, the interactions between different leaf species and their microbial assemblages under different environmental conditions, as well as how these interactions determine the shredder activity and consequently, the bacterial assembly in the shredder-produced FPOM are not yet well understood. Our study sheds light on these aspects. We found that oxic conditioning supported higher bacterial diversity, potentially enhancing terrestrial organic matter processing in streams. We also observed that the interplay between the leaf species and shredder

activity was a determinant of bacterial diversity across different FPOM types. Nevertheless, we found no clear evidence of distinct differences in alpha diversity or community composition between the three leaf species, which necessitates more targeted research on this topic. Importantly, faecal pellets exhibited distinct bacterial communities compared to that of leaf substrates, indicating that gut passage drives turnover and reshapes bacterial communities on fine particles in streams. In addition, we showed that specific bacterial communities dominated each FPOM type and discussed their potential significance during LLD. Such bacterial communities colonizing the FPOM subsequently could determine the fate of shredder-produced FPOM in streams. The variability in FPOM associated bacterial communities we detected in our study highlights how biotic interactions can shape the microbial diversity and ecosystem processes in streams. Understanding these dynamics may assist to predict how stream ecosystems respond to several environmental changes such as altered riparian inputs, oxygen dynamics, or biodiversity loss.

Acknowledgments

We thank Theresa Reichenpfader for providing support and help during laboratory analyses. Further, we want to acknowledge Petra Pjevac at the Joint Microbiome Facility (JMF) for sequencing and quality control of raw reads. We thank Vojtěch Kolář for his help during macroinvertebrate shredders sampling. We are also thankful to Luca Zoccarato, Christian Wurzbacher, and Angela Cukusic for advises on the community composition analyses. Additionally, we greatly acknowledge the assistance by Hermann Hofreiter, Nibert Matschegg, and Michael Mayr for setting up the microcosm and laboratory for the feeding experiment.

Author contributions

Pratiksha Acharya (Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing), Mourine J. Yegon (Data curation, Investigation, Methodology, Resources, Writing – review & editing), Christian Griebler (Writing – review & editing), Simon Vitecek (Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing), and Katrin Attermeyer (Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing)

Supplementary data

Supplementary data is available at [FEMSEC Journal](#) online.

Conflict of interest: All authors declare that they have no conflicts of interest.

Funding

This study got funded by a PhD Fellowship financially supported by WasserCluster Lunz.

Data availability

The 16S rRNA gene sequencing data generated in this study have been deposited in the GenBank database under the BioProject ID_PRJNA1258879. All other relevant data supporting the findings

of this study are available within the article and its supplementary information.

References

- Acharya P, Yegon MJ, Haferkemper L et al. Leaf conditioning and shredder activity shape microbial dynamics on fine particulate organic matter produced during decomposition of different leaf litter in streams. *Microb Ecol* 2025;**88**:18. <https://doi.org/10.1007/s00248-025-02515-2>.
- Aira M, Pérez-Losada M, Crandall KA et al. Composition, structure and diversity of soil bacterial communities before, during and after transit through the gut of the earthworm *Aporrectodea caliginosa*. *Microorganisms* 2022;**10**:1025. <https://doi.org/10.3390/microorganisms10051025>.
- Allan JD, Castillo MM, Capps KA. Detrital energy and the decomposition of organic matter. In: *Stream Ecology*. Cham: Springer International Publishing, 2021, 177–224. <https://doi.org/10.1007/978-3-030-61286-3>.
- Apprill A, McNally S, Parsons R et al. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* 2015;**75**:129–37. <https://doi.org/10.3354/ame01753>.
- Araya R, Tani K, Takagi T et al. Bacterial activity and community composition in stream water and biofilm from an urban river determined by fluorescent in situ hybridization and DGGE analysis. *FEMS Microbiol Ecol* 2003;**43**:111–9. <https://doi.org/10.1111/j.1574-6941.2003.tb01050.x>.
- Ayayee PA, Cosgrove CR, Beckwith A et al. Gut bacterial assemblages of freshwater macroinvertebrate functional feeding groups. *Hydrobiologia* 2018;**822**:157–72. <https://doi.org/10.1007/s10750-018-3671-3>.
- Ayayee PA, Wesner JS, Ouellette SP. Geography, taxonomy, and ecological guild: factors impacting freshwater macroinvertebrate gut microbiomes. *Ecol Evol* 2022;**12**:e9663. <https://doi.org/10.1002/ec.e3.9663>.
- Baldy V, Chauvet E, Charcosset J-Y et al. Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. *Aquat Microb Ecol* 2002;**28**:25–36. <https://doi.org/10.3354/ame028025>.
- Bärlocher F, Kendrick B. Leaf-conditioning by microorganisms. *Oecologia* 1975;**20**:359–62. <https://doi.org/10.1007/BF00345526>.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* 1995;**57**:289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
- Ben-Shachar M, Lüdtke D, Makowski D. Effectsize: estimation of effect size indices and standardized parameters. *JOSS* 2020;**5**:2815. <https://doi.org/10.21105/joss.02815>.
- Brabcová V, Nováková M, Davidová A et al. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. *New Phytol* 2016;**210**:1369–81. <https://doi.org/10.1111/nph.13849>.
- Brune A, Frenzel P, Cypionka H. Life at the oxic-anoxic interface: microbial activities and adaptations. *FEMS Microbiol Rev* 2000;**24**:691–710. [https://doi.org/10.1016/S0168-6445\(00\)00054-1](https://doi.org/10.1016/S0168-6445(00)00054-1).
- Bundschoh M, McKie BG. An ecological and ecotoxicological perspective on fine particulate organic matter in streams. *Freshwat Biol* 2016;**61**:2063–74. <https://doi.org/10.1111/fwb.12608>.
- Callahan BJ, McMurdie PJ, Rosen MJ et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3. <https://doi.org/10.1038/nmeth.3869>.
- Cheever BM, Webster JR, Bilger EE et al. The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology* 2013;**94**:1614–25. <https://doi.org/10.1890/12-1339.1>.
- Crump BC, Armbrust EV, Baross JA. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. *Appl Environ Microbiol* 1999;**65**:3192–204. <https://doi.org/10.1128/AEM.65.7.3192-3204.1999>.
- Cummins KW. Structure and function of stream ecosystems. *BioScience* 1974;**24**:631–41. <https://doi.org/10.2307/1296676>.
- Dale H, Solan M, Lam P et al. Sediment microbial assemblage structure is modified by marine polychaete gut passage. *FEMS Microbiol Ecol* 2019;**95**:fz047. <https://doi.org/10.1093/femsec/fz047>.
- Danger M, Cornut J, Elger A et al. Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa: leaf litter burial and palatability. *Freshwat Biol* 2012;**57**:1017–30. <https://doi.org/10.1111/j.1365-2427.2012.02762.x>.
- Degelmann DM, Kolb S, Dumont M et al. Enterobacteriaceae facilitate the anaerobic degradation of glucose by a forest soil. *FEMS Microbiol Ecol* 2009;**68**:312–9. <https://doi.org/10.1111/j.1574-6941.2009.00681.x>.
- Dieter D, Von Schiller D, García-Roger E et al. Preconditioning effects of flow intermittency on leaf litter decomposition. *Aquat Sci* 2011;**73**:599–609. <https://doi.org/10.1007/s00027-011-0231-6>.
- Fenoy E, Rubio-Ríos J, González JM et al. Strategies of shredders when feeding on low-quality leaf-litter: local population adaptations or fixed species traits? *Limnol Oceanogr* 2021;**66**:2063–77. <https://doi.org/10.1002/lno.11745>.
- Findlay SEG, Sinsabaugh RL, Sobczak WV et al. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnol Oceanogr* 2003;**48**:1608–17.
- Foucreau N, Puijalon S, Hervant F et al. Effect of leaf litter characteristics on leaf conditioning and on consumption by *Gammarus pulex*. *Freshwat Biol* 2013;**58**:1672–81. <https://doi.org/10.1111/fwb.12158>.
- Francoeur SN, Neely RK, Underwood S et al. Temporal and stoichiometric patterns of algal stimulation of litter-associated heterotrophic microbial activity. *Freshwat Biol* 2020;**65**:1223–38. <https://doi.org/10.1111/fwb.13442>.
- Frossard A, Gerull L, Mutz M et al. Shifts in microbial community structure and function in stream sediments during experimentally simulated riparian succession. *FEMS Microbiol Ecol* 2013;**84**:398–410. <https://doi.org/10.1111/1574-6941.12072>.
- Gao F, Li F, Tan J et al. Bacterial community composition in the gut content and ambient sediment of sea cucumber *Apostichopus japonicus* revealed by 16S rRNA gene pyrosequencing. *PLoS One* 2014;**9**:e100092. <https://doi.org/10.1371/journal.pone.0100092>.
- Gessner MO, Chauvet E, Dobson M. A perspective on leaf litter breakdown in streams. *Oikos* 1999;**85**:377–84. <https://doi.org/10.2307/3546505>.
- Gessner MO, Chauvet E. Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology* 1994;**75**:1807–17. <https://doi.org/10.2307/1939639>.
- Gessner MO, Schmitt AL. Use of solid-phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi. *Appl Environ Microbiol* 1996;**62**:415–9. <https://doi.org/10.1128/aem.62.2.415-419.1996>.
- Gessner MO, Swan CM, Dang CK et al. Diversity meets decomposition. *Trends Ecol Evol* 2010;**25**:372–80.
- Gessner MO. Functional leaf traits and biodiversity effects on litter decomposition in a stream: reply. *Ecology* 2010;**91**:1869–71. <https://doi.org/10.1890/0012-9658-91.6.1869>.

- Ghosal D, Ghosh S, Dutta TK et al. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. *Front Microbiol* 2016;7. <https://doi.org/10.3389/fmicb.2016.01369>.
- Gogoi P, Boruah JLH, Yadav A et al. Comparative seasonal analysis of Eri silkworm (*Samia ricini* Donovan) gut composition: implications for lignocellulose degradation. *Environ Sci Pollut Res* 2023;30:109198–213. <https://doi.org/10.1007/s11356-023-29893-9>.
- Gonçalves S, Pollitt A, Pietz S et al. Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod. *Sci Total Environ* 2024;912:168926. <https://doi.org/10.1016/j.scitotenv.2023.168926>.
- Graça MAS. The role of invertebrates on leaf litter decomposition in streams—a review. *Internat Rev Hydrobiol* 2001;86:383–93.
- Guo P, Li C, Liu J et al. Predation has a significant impact on the complexity and stability of microbial food webs in subalpine lakes. *Microbiol Spectr* 2023;11:e02411–23. <https://doi.org/10.1128/spectrum.02411-23>.
- Halvorson H, Fuller C, Entrekin S et al. Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwat Biol* 2015;60:466–78. <https://doi.org/10.1111/fwb.12462>.
- Hau HH, Gralnick JA. Ecology and biotechnology of the genus *Shewanella*. *Annu Rev Microbiol* 2007;61:237–58. <https://doi.org/10.1146/annurev.micro.61.080706.093257>.
- Hieber M, Gessner MO. Contribution of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. *Ecology* 2002;83:1026–38. [https://doi.org/10.1890/0012-9658\(2002\)083%5b1026::COSDFA%5d2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083%5b1026::COSDFA%5d2.0.CO;2).
- Hladysz S, Gessner MO, Giller PS et al. Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwat Biol* 2009;54:957–70. <https://doi.org/10.1111/j.1365-2427.2008.02138.x>.
- Hou D, Huang Z, Zeng S et al. Environmental factors shape water microbial community structure and function in shrimp cultural enclosure ecosystems. *Front Microbiol* 2017;8. <https://doi.org/10.3389/fmicb.2017.02359>.
- Huang W, Wang Z, Liu X et al. The microbial community and functional indicators response to flow restoration in gradient in a simulated water flume. *Front Microbiol* 2022;13:1051375. <https://doi.org/10.3389/fmicb.2022.1051375>.
- Janssen K, Low SL, Wang Y et al. Elucidating biofilm diversity on water lily leaves through 16S rRNA amplicon analysis: comparison of four DNA extraction kits. *Appl Plant Sci* 2021;9:e11444. <https://doi.org/10.1002/aps3.11444>.
- Jung J, Park W. *Acinetobacter* species as model microorganisms in environmental microbiology: current state and perspectives. *Appl Microbiol Biotechnol* 2015;99:2533–48. <https://doi.org/10.1007/s00253-015-6439-y>.
- Karačić S, Modin O, Hagelia P et al. The effect of time and surface type on the composition of biofilm communities on concrete exposed to seawater. *Int Biodeterior Biodegrad* 2022;173:105458.
- Karačić S, Palmer B, Gee CT et al. Oxygen-dependent biofilm dynamics in leaf decay: an in vitro analysis. *Sci Rep* 2024;14:6728.
- Khomutovska N, Jasser I, Sarapultseva P et al. Seasonal dynamics in leaf litter decomposing microbial communities in temperate forests: a whole-genome-sequencing-based study. *PeerJ* 2024;12:e17769. <https://doi.org/10.7717/peerj.17769>.
- King GM. Microbiomes of the enteropneust, *Saccoglossus bromophenolosus*, and associated marine intertidal sediments of Cod cove. *Front Microbiol* 2018;9. <https://doi.org/10.3389/fmicb.2018.03066>.
- Kirchman DL. The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiol Ecol* 2002;39:91–100.
- Kittichotirat W, Good NM, Hall R et al. Genome sequence of *Methyloversatilis universalis* FAM5T, a methylotrophic representative of the order Rhodocyclales. *J Bacteriol* 2011;193:4541–2. <https://doi.org/10.1128/JB.05331-11>.
- Kominoski JS, Hoellein TJ, Kelly JJ et al. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? *Oikos* 2009;118:457–63. <https://doi.org/10.1111/j.1600-0706.2008.17222.x>.
- Lau WWY, Jumars PA, Armbrust EV. Genetic diversity of attached bacteria in the hindgut of the deposit-feeding shrimp *Neotrypaea* (formerly *Callinassa*) *californiensis* (Decapoda: thalassinidae). *Microb Ecol* 2002;43:455–66. <https://doi.org/10.1007/s00248-001-1043-3>.
- Leisner JJ, Laursen BG, Prévost H et al. *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiol Rev* 2007;31:592. <https://doi.org/10.1111/j.1574-6976.2007.00080.x>.
- Luo X, Xiang X, Yang Y et al. Seasonal effects of river flow on microbial community coalescence and diversity in a riverine network. *FEMS Microbiol Ecol* 2020;96:faa132. <https://doi.org/10.1093/femsec/fiaa132>.
- Ma J, Liu H, Zhang C et al. Joint response of chemistry and functional microbial community to oxygenation of the reductive confined aquifer. *Sci Total Environ* 2020;720:137587. <https://doi.org/10.1016/j.scitotenv.2020.137587>.
- McMurdie PJ, phyloseq HS. An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;8:e61217. <https://doi.org/10.1371/journal.pone.0061217>.
- Mehring AS, Kuehn KA, Thompson A et al. Leaf litter nutrient uptake in an intermittent blackwater river: influence of tree species and associated biotic and abiotic drivers. *Funct Ecol* 2015;29:849–60. <https://doi.org/10.1111/1365-2435.12399>.
- Mongkol P, Bunphimpapha P, Rungrasamee W et al. Bacterial community composition and distribution in different segments of the gastrointestinal tract of wild-caught adult *Penaeus monodon*. *Aquac Res* 2018;49:378–92. <https://doi.org/10.1111/are.13468>.
- Newman MM, Liles MR, Feminella JW. Litter breakdown and microbial succession on two submerged leaf species in a small forested stream. *PLoS One* 2015;10:e0130801. <https://doi.org/10.1371/journal.pone.0130801>.
- Oksanen J, Simpson GL, Blanchet FG et al. *vegan: Community Ecology Package*. CRAN, 2022.
- Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* 2016;18:1403–14. <https://doi.org/10.1111/1462-2920.13023>.
- Pascoal C, Fernandes I, Seena S et al. Linking microbial decomposer diversity to plant litter decomposition and associated processes in streams. In: Swan CM, Boyero L, Canhoto C (eds), *The Ecology of Plant Litter Decomposition in Stream Ecosystems*. Cham: Springer International Publishing, 2021, 163–92. <https://doi.org/10.1007/978-3-030-72854-0>.
- Pérez-Pantoja D, Donoso R, Agulló L et al. Genomic analysis of the potential for aromatic compounds biodegradation in Burkholderiales. *Environ Microbiol* 2012;14:1091–117. <https://doi.org/10.1111/j.1462-2920.2011.02613.x>.
- Petersen RC, Cummins KW. Leaf processing in a woodland stream. *Freshwat Biol* 1974;4:343–68. <https://doi.org/10.1111/j.1365-2427.1974.tb00103.x>.
- Pjevac P, Hausmann B, Schwarz J et al. An economical and flexible dual barcoding, two-step PCR approach for highly multiplexed

- amplicon sequencing. *Front Microbiol* 2021;**12**. <https://doi.org/10.3389/fmicb.2021.669776>.
- Plante CJ, Hill-Spanik KM, Emerson R. Inputs don't equal outputs: bacterial microbiomes of the ingesta, gut, and feces of the keystone deposit feeder *Ilyanassa obsoleta*. *FEMS Microbiol Ecol* 2022;**99**:fiac152. <https://doi.org/10.1093/femsec/fiac152>.
- Plante CJ, Mayer LM, King GM. The kinetics of bacteriolysis in the gut of the deposit feeder *Arenicola marina*. *Appl Environ Microbiol* 1996;**62**:1051–7. <https://doi.org/10.1128/aem.62.3.1051-1057.1996>.
- Plante CJ, Wilde SB. Biotic disturbance, recolonization, and early succession of bacterial assemblages in intertidal sediments. *Microb Ecol* 2004;**48**:154–66. <https://doi.org/10.1007/s00248-003-1031-x>.
- Pope CA, Halvorson HM, Findlay RH et al. Light and temperature mediate algal stimulation of heterotrophic activity on decomposing leaf litter. *Freshwat Biol* 2020;**65**:1210–22. <https://doi.org/10.1111/fwb.13465>.
- Qiu Y-L, Tournelle D, Matsuura N et al. Draft genome sequence of *Paludibacter jiangxiensis* NM7 T, a propionate-producing fermentative bacterium. *Genome Announc* 2017;**5**:e00667–17. <https://doi.org/10.1128/genomeA.00667-17>.
- Quast C, Pruesse E, Yilmaz P et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2012;**41**:D590–6. <https://doi.org/10.1093/nar/gk1219>.
- R Core Team. *R: A Language and Environment for Statistical Computing*. Ames, 2023.
- Ramond P, Galand PE, Logares R. Microbial functional diversity and redundancy: moving forward. *FEMS Microbiol Rev* 2025;**49**:fuae031. <https://doi.org/10.1093/femsre/fuae031>.
- Raposeiro PM, Ferreira V, Guri R et al. Leaf litter decomposition on insular lentic systems: effects of macroinvertebrate presence, leaf species, and environmental conditions. *Hydrobiologia* 2017;**784**:65–79. <https://doi.org/10.1007/s10750-016-2852-1>.
- Rastogi G, Sbodio A, Tech JJ et al. Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. *ISME J* 2012;**6**:1812–22. <https://doi.org/10.1038/ismej.2012.32>.
- Rezende RS, Correia PR, Gonçalves JF Jr et al. Organic matter dynamics in a savanna transition riparian zone: input of plant reproductive parts increases leaf breakdown process. *J Limnol* 2017;**76**. <https://doi.org/10.4081/jlimnol.2017.1601>.
- Riemann L, Winding A. Community dynamics of free-living and particle-associated bacterial assemblages during a freshwater phytoplankton bloom. *Microb Ecol* 2001;**42**:274–85. <https://doi.org/10.1007/s00248-001-0018-8>.
- Rubio-Rios J, Pérez J, Fenoy E et al. Cross-species coprophagy in small stream detritivores counteracts low-quality litter: native versus invasive plant litter. *Aquat Sci* 2023;**85**:8. <https://doi.org/10.1007/s00027-022-00905-z>.
- Sadeghi J, Venney CJ, Wright S et al. Aquatic bacterial community connectivity: the effect of hydrological flow on community diversity and composition. *Environments* 2024;**11**:90. <https://doi.org/10.3390/environments1105090>.
- Savio D, Sinclair L, Ijaz UZ et al. Bacterial diversity along a 2600 km river continuum. *Environ Microbiol* 2015;**17**:4994–5007. <https://doi.org/10.1111/1462-2920.12886>.
- Shade A, Gregory Caporaso J, Handelsman J et al. A meta-analysis of changes in bacterial and archaeal communities with time. *ISME J* 2013;**7**:1493–506. <https://doi.org/10.1038/ismej.2013.54>.
- Silverman JD, Washburne AD, Mukherjee S et al. A phylogenetic transform enhances analysis of compositional microbiota data. *eLife* 2017;**6**:e21887. <https://doi.org/10.7554/eLife.21887>.
- Smeaton CM, Fryer BJ, Weisener CG. Intracellular precipitation of Pb by *Shewanella putrefaciens* CN32 during the reductive dissolution of Pb-jarosite. *Environ Sci Technol* 2009;**43**:8086–91. <https://doi.org/10.1021/es901629c>.
- Sontowski R, van Dam NM. Functional variation in dipteran gut bacterial communities in relation to their diet, life cycle stage and habitat. *Insects* 2020;**11**:543. <https://doi.org/10.3390/insects11080543>.
- Stadler M, Del Giorgio PA. Terrestrial connectivity, upstream aquatic history and seasonality shape bacterial community assembly within a large boreal aquatic network. *ISME J* 2022;**16**:937–47. <https://doi.org/10.1038/s41396-021-01146-y>.
- Tláškal V, Voříšková J, Baldrian P. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiol Ecol* 2016;**92**:fiw177. <https://doi.org/10.1093/femsec/fiw177>.
- Valášková V, de Boer W, Klein Gunnewiek PJ et al. Phylogenetic composition and properties of bacteria coexisting with the fungus *Hypohymenium fasciculare* in decaying wood. *ISME J* 2009;**3**:1218–21.
- Vannote RL, Minshall GW, Cummins KW et al. The river continuum concept. *Can J Fish Aquat Sci* 1980;**37**:130–7. <https://doi.org/10.1139/f80-017>.
- Vesamäki JS, Rigaud C, Litmanen JJ et al. Recycled by leaf inhabitants: terrestrial bacteria drive the mineralization of organic matter in lake water. *Ecosphere* 2024;**15**:e4871. <https://doi.org/10.1002/ecs2.4871>.
- Villanueva VD, Albariño R, Canhoto C. Positive effect of shredders on microbial biomass and decomposition in stream microcosms. *Freshwat Biol* 2012;**57**:2504–13. <https://doi.org/10.1111/fwb.12023>.
- Wallace JB, Webster JR. The role of macroinvertebrates in stream ecosystem function. *Annu Rev Entomol* 1996;**41**:115–39. <https://doi.org/10.1146/annurev.en.41.010196.000555>.
- Wantzen KM, Wagner R. Detritus processing by invertebrate shredders: a neotropical–temperate comparison. *J N Am Benthol Soc* 2006;**25**:216–32. [https://doi.org/10.1899/0887-3593\(2006\)25%5b216:DPBISA%5d2.0.CO;2](https://doi.org/10.1899/0887-3593(2006)25%5b216:DPBISA%5d2.0.CO;2).
- Webster JR, Benfield EF. Vascular plant breakdown in freshwater ecosystems. *Annu Rev Ecol Syst* 1986;**17**:567–94. <https://doi.org/10.1146/annurev.es.17.110186.003031>.
- Wei T, Ishida R, Miyanaga K et al. Seasonal variations in bacterial communities and antibiotic-resistant strains associated with green bottle flies (Diptera: calliphoridae). *Appl Microbiol Biotechnol* 2014;**98**:4197–208. <https://doi.org/10.1007/s00253-013-5498-1>.
- Wyatt KH, Turetsky MR. Algae alleviate carbon limitation of heterotrophic bacteria in a boreal peatland. *J Ecol* 2015;**103**:1165–71. <https://doi.org/10.1111/1365-2745.12455>.
- Wymore AS, Liu CM, Hungate BA et al. The influence of time and plant species on the composition of the decomposing bacterial community in a stream ecosystem. *Microb Ecol* 2016;**71**:825–34. <https://doi.org/10.1007/s00248-016-0735-7>.
- Yoshimura C, Gessner MO, Tockner K et al. Chemical properties, microbial respiration, and decomposition of coarse and fine particulate organic matter. *J N Am Benthol Soc* 2008;**27**:664–73. <https://doi.org/10.1899/07-106.1>.
- Zhao B, Xing P, Wu QL. Microbes participated in macrophyte leaf litter decomposition in freshwater habitat. *FEMS Microbiol Ecol* 2017;**93**:fix108. <https://doi.org/10.1093/femsec/fix108>.

Received 12 May 2025; revised 11 September 2025; accepted 11 September 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

CHAPTER III:

Shredder identity matters: Taxon-specific gut microbiota in aquatic shredders drives bacterial diversity during leaf breakdown in streams

Working title, “Shredder identity matters: Taxon-specific gut microbiota in aquatic shredders drives bacterial diversity during leaf breakdown in streams”

Pratiksha Acharya^{1,2}, Mourine J. Yegon^{1,3}, Luca Zoccarato^{5,6}, Christian Griebler², Simon Vitecek^{1,3,4}, Katrin Attermeyer^{1,2}

¹ WasserCluster Lunz – Biological Station, Dr. Carl Kupelwieser-Prom. 5, 3293 Lunz am See, Austria

² Department of Functional and Evolutionary Ecology, Unit Limnology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

³ Institute for Hydrobiology and Water Management (IHG), University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33/DG, 1180 Vienna, Austria

⁴ Department of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

⁵ Institute of Computational Biology, University of Natural Resources and Life Sciences, Muthgasse 18 1190 Vienna, Austria

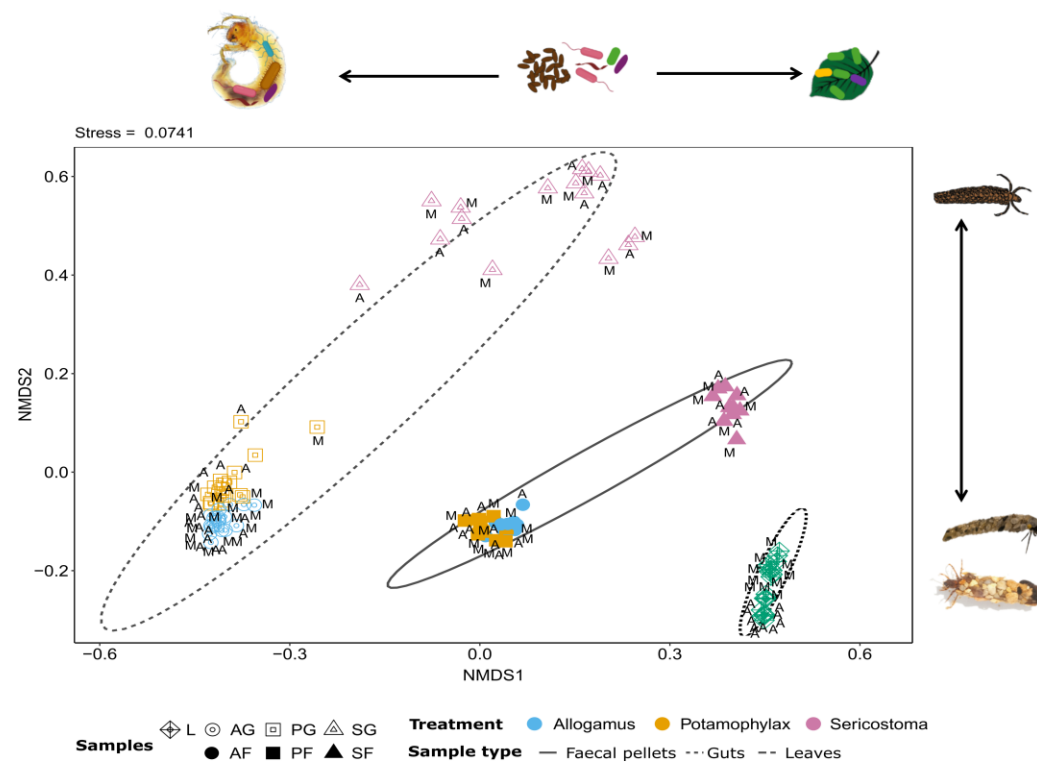
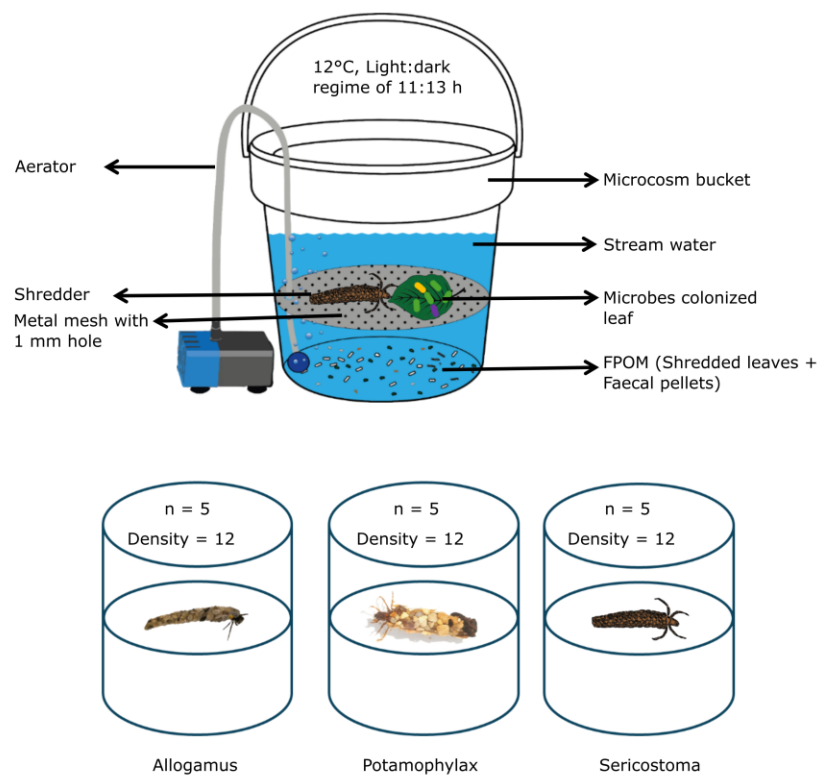
⁶ Core Facility Bioinformatics, University of Natural Resources and Life Sciences, Muthgasse 18, 1190, Vienna, Austria

Abstract

Microbial communities play a key role in organic matter decomposition and nutrient cycling in headwater streams. Macroinvertebrate shredders enhance these processes by producing large amounts of fine particles from microbe-colonized organic matter through shredding and egesta, yet how gut and faecal microbiota vary among shredder taxa remains largely unknown. Thus, we examined composition, diversity, and predicted functions of bacterial communities in leaves, guts, and faecal pellets of three Trichoptera shredders (*Allogamus*, *Potamophylax*, and *Sericostoma*) across two larval stages using 16S rRNA metabarcoding. Bacterial community composition differed significantly among the shredder taxa and between sample types, while ontogeny had only a minimal effect. *Sericostoma* showed the lowest diversity but most distinct microbiota in their gut and faecal pellets, while *Allogamus* and *Potamophylax* hosted more diverse and overlapping bacterial communities. Core bacterial taxa were *Bacteroidota*, *Proteobacteria*, and *Firmicutes*, along with *Carnobacterium* and *Tyzzellerella*, as specialized bacteria in guts. Functional predictions showed clear metabolic transitions from aromatic compound degradation in leaves to amino acid and carbohydrate metabolism in guts and continued degradation of plant-derived substrates in faecal pellets, reflecting the shift from environmental to host-associated microbial processes during gut passage. Unique pathways in *Sericostoma* guts, such as palmitate and peptidoglycan biosynthesis, suggest host-specific adaptations. Overall, shredder identity rather than ontogenetic stage was the dominant factor structuring bacterial assemblages and functions, emphasizing the taxon-specific role of shredders as mediators of microbial dynamics, linking microbial structure with ecosystem-level processes in the stream detritus-food-web.

Keywords; Leaf litter decomposition, gut microbiota, faecal pellets, shredder ontogeny, stream ecosystem.

Graphical abstract



Introduction

The stream microbiome consists of microorganisms, primarily bacteria but also archaea, fungi, protists, and viruses, distributed across distinct habitats, including the water column, epilithic biofilms on rocks, submerged leaves, suspended organic matter (OM), and streambed sediments. The composition of the stream microbiome is influenced by the environment and hydrology, which in turn affect the ecological dynamics depending on the functional roles of the selected community [1–4]. For instance, surface water often harbors Actinobacteria [5], rock-associated biofilms are enriched in Cyanobacteria and Bacteroidetes [1] while submerged leaves are colonized by common leaf decomposers such as *Pseudomonas*, *Sphingomonas*, and *Pedobacter* [6, 7]. Since different microbial taxa contribute differently to OM decomposition, nutrient cycling, and energy transfer across trophic levels [8, 9], shifts in bacterial community composition can alter biogeochemical cycles at ecosystem-level.

Connectivity with surrounding terrestrial environments also contributes to the spatial structuring of the stream microbiome through inputs of soil, groundwater, and plant OM [2, 4, 10, 11]. Such inputs are particularly important in headwater streams, where regular leaf litter supply fuels a detritus-based food web [11, 12]. Among the biotic components involved in OM processing, detritivores invertebrates, especially shredders, play a crucial role. They fragment leaf litter, i.e., coarse particulate OM (CPOM > 1mm), into fine particulate OM (FPOM < 1mm) via sloppy feeding and egestion of faecal pellets. These FPOM act as microbial hotspots [13], and harbor diverse, and specialized bacterial communities that vary depending on particle origin and processing (e.g., fragmented leaf particles vs. faecal pellets) [14]. Shredders themselves are taxonomically diverse, including species within Trichoptera (e.g., Limnephilidae), Amphipoda (e.g., Gammaridae), Plecoptera (e.g., Leuctridae), Coleoptera (e.g., Ptilodactylidae), and certain Diptera larvae (Tipulidae) [15–19], and differ in feeding behavior [20], mouthpart morphology, digestion efficiency, and habitat preference [21–24]. These traits influence both the rate and pathways of leaf litter breakdown [25], and shape the gut- and faecal pellets-associated microbial communities [13, 26]. Generally, the gut represents a highly dynamic bacterial habitat, influenced by, amongst others, diet, host gut physiology, and ontogenetic stages [17, 27–34]. The gut microbiota have a profound role in health, nutrition, immunity, and even behavior in vertebrates and invertebrates [29, 35–37]. To date, most invertebrate gut microbiome studies focused on terrestrial decomposers, such as earthworms, beetles and termites, or aquatic herbivores (i.e., filter-feeding bivalves and grazers), along with their role in the decomposition of lignocellulose, detoxification of secondary plant metabolites, or synthesis of essential nutrients [27, 31, 38–44]. Here, *Carnobacterium*, *Leucobacter*, *Acinetobacter*, *Vagococcus*, and *Rhodococcus* were commonly reported as gut bacteria in Diptera larvae [45] and other detritivores such as silkworm [44] and green bottle fly larvae [46]. Apart from a handful of studies comparing gut microbiota across

insects functional feeding groups [17, 18, 27, 39], there is still a major knowledge gap regarding community composition of gut microbiota of Trichoptera shredder taxa, which have a prominent role in processing terrestrial OM in headwater streams [22, 23, 34, 47, 48].

In addition to processing ingested OM, shredder gut microbiota can actively influence the structure and composition of bacterial communities in streams once deposited in faecal pellets [14]. During gut passage, OM-associated bacterial community composition changes likely through selective digestion [49–52] or seeding from resident gut microbes [50, 53–55], and this is reflected in the faecal pellet communities. Shredder faecal pellets, an important fraction of in-stream FPOM, thus may facilitate microbial dispersal of gut-associated microbiota into the stream ecosystem [14, 26, 39, 51, 53, 55], and possibly reshape downstream microbial assemblages and OM processing. Specifically, these faecal pellet-associated microbes can remain metabolically active [14, 26, 53, 56], continuing detrital decomposition and thereby contribute to the release of dissolved carbon, nitrogen, and phosphorus [13, 38, 57, 58]. Furthermore, they may interact with streambed biofilms to form complex microbial consortia, which are critical for nutrient retention and energy flow [9]. However, the relative persistence of leaf-associated bacteria versus the seeding of gut-associated bacteria on faecal pellets during gut passage remains poorly understood. Clarifying these bacterial linkages is essential for understanding the broader ecological role of shredders, particularly how their gut microbiota influence downstream bacterial dynamics and ecosystem functioning.

It has been demonstrated that the structure and composition of gut microbiomes can change during different developmental life stages (= ontogenetic stages) across a wide range of animal hosts including insects, amphibians, and mammals [30, 32, 34, 59]. These changes are often driven by dietary transitions, developmental modifications in digestive physiology such as pH, oxygen level independent of diet, and the maturation of the immune system [17, 27, 37, 60]. Also, freshwater invertebrates across functional feeding groups have different gut physiology [61–63], as well as gut-associated microbiota [18, 39, 64] and functions during different ontogenetic stages [65]. Many freshwater shredders change their feeding preferences and digestive capabilities during their transition from one to another larval stage, which may influence the structure and composition of their gut microbial communities [32, 33]. However, information about the shift in gut microbiota of freshwater shredders across ontogenetic stages is still missing.

In this study, we aim to elucidate how shredder-mediated leaf litter decomposition influences the particle-attached bacterial communities and how they contribute to the stream microbiome. Specifically, we investigate (i) how gut passage modifies the diversity, composition, and functional potential of bacterial communities on faecal pellets compared to those on leaf litter, (ii) to what extent the bacterial community in faecal pellets is derived from leaf-associated

bacteria and gut bacteria, and (iii) how the diversity and composition of gut-associated bacterial communities vary across ontogenetic stages. To address these questions, we performed two sequential microcosm experiments where we incubated individually three of the most abundant Trichoptera shredder taxa - *Allogamus auricollis*, *Potamophylax latipennis*, and *Sericostoma flavicorne*. [23, 48, 59, 66] with alder leaves in early April and late May. We investigated the bacterial community composition on the leaves, in the gut of each taxon, and the collected faecal pellets using DNA and RNA metabarcoding.

Methods and materials

Experimental Microcosm setup

We conducted feeding experiments in a climate-controlled chamber (12°C; 11:13 h light:dark) with alder leaves and three different shredder taxa (*Allogamus*, *Potamophylax*, *Sericostoma*). The first experiment began in early April and the second in late May 2024 (approximately two months interval) to target different larval stages of the shredder taxa. The experiments were performed with five replicates for each shredder treatment containing 12 individuals and alder leaves (dry mass: 1.0 ± 0.003 g). Five controls containing only leaves were set up to assess baseline changes in bacterial community composition during the 5-day incubation period.

Each microcosm consisted of a white food-grade plastic bucket (1100 ml volume and 122 mm bottom diameter) with a steel mesh (pore size 1 mm) suspended at a height of 4.5-5 cm from the bottom to allow shredder-derived FPOM to pass through. The shredders were fed with conditioned alder leaves for five days. Leaf and shredder gut microbiome samples were collected at the beginning (= natural baseline animals) and end (= experimental animals). After the 5-day incubation period, we transferred each larva from each treatment to a single food-grade plastic cup (Polylactic acid or polylactide - PLA) filled with 20 ml of 0.7 µm-filtered stream water and left them to evacuate their gut contents for 24 hours [19]. In this way, we made sure that we only collected faecal pellets and no shredded leaf particles. We collected the faecal pellets in the plastic cup using a Pasteur pipette after collecting and storing the experimental animals at -70°C until further processing (for more details of microcosms setup see [13]).

Preparation of conditioned alder leaves

We collected naturally fallen leaves from alder trees (*Alnus glutinosa*) around Lunz am See, Austria, in autumn 2023, dried and stored them at room temperature. Prior to microbial colonization, we cut leaves into 12-mm diameter circular discs. For the oxic microbial colonization, we submerged the leaf discs in aerated, freshly collected stream water from Oberer Seebach (OSB; 47°51' N, 15°04' E) at 12°C in the dark for one week.

Insect's sampling and preparation

A day before each feeding experiment, we collected comparably sized larvae of *Allogamus*, *Potamophylax*, and *Sericostoma* (Trichoptera) from Oberer Seebach (OSB; 47°51' N, 15°04' E) and brought them to the laboratory. We determined larval stages of targeted animals by measuring the head capsule width (HCW). In April, *Allogamus* was in early V instars (HCW = 1.57 ± 0.05 mm) [48], *Potamophylax* in IV instars (HCW = 2.18 ± 0.03 mm) [66], and *Sericostoma* in early VI instars (HCW = 1.96 ± 0.04 mm) [59, 67]. In May, the targeted larvae were mostly in the late instars; *Allogamus* late V instars (HCW = 1.68 ± 0.02) [48], *Potamophylax* V instars (HCW = 2.23 ± 0.03 mm) [66], and *Sericostoma* in late VI instars (HCW = 2.03 ± 0.05 mm) [59, 67]. Each larva was placed in a separate PLA cup (volume: 4 cl), with filtered stream water (0.7 µm, 450°C, 4 hours pre-combusted Whatmann GF/F filters) at 12°C and starved for 24 h before the start of the experiment to allow evacuation of their guts [19]. On the next day, we randomly sub-sampled 70 individuals from these freshly collected wild populations per taxon per each experiment to have a natural gut microbiota baseline.

Gut dissection

We dissected guts from 12 shredder larvae per microcosm at the end of the experiment, as well as from 12 individuals per taxon initially collected for the natural baseline. After removing larval cases, each shredder larva was dissected aseptically in a petri dish filled with 5 mL of 95% ethanol under a stereomicroscope. An incision using sterile forceps was made longitudinally along the body wall, and the entire gut, from the clitellum to anus, was removed and placed in a 1.5 mL Eppendorf tube filled with 400 µL RNA/DNA shield (Zymo Research Europe GmbH, Freiburg, Germany) for the preservation of genetic material. The samples were then stored in the refrigerator at 4°C until DNA/RNA extraction.

DNA/RNA extraction

For DNA/RNA extraction, we pooled gut content and faecal pellets from 12 larvae per microcosm. However, our tests revealed that this amount was too much for DNA extraction of *Potamophylax* gut microorganisms, and we thus pooled the guts from five larvae per microcosm in case of *Potamophylax*. Overall, we had five replicates for natural baseline animals, experimental animals, and faecal pellets from each shredder taxon for each experiment. We extracted total genomic DNA and RNA from all sample types, including alder leaves (n = 10 with 2 (initial leaves + control leaf treatments)*5 replicates), shredder guts (n = 30 with 2 (natural baseline + experimental animals)*3 shredder taxa*5 replicates) and faecal pellets produced by each shredder taxon (n = 15 with 3 shredder taxa*5 replicates) for each experiment using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research Europe GmbH, Freiburg, Germany) following the manufacturers' protocol (leaves were first cut into

smaller pieces with a sterile scalpel). To stabilize extracted RNA, we converted them into complementary DNA (cDNA) using the Reverse Transcription Kit (Qiagen, Hilden, Germany) following the manufacturers' protocol. Afterwards, concentrations were quantified fluorometrically with the Qubit™ 1X dsDNA HS Assay Kit for DNA and the Qubit™ ssDNA Assay Kit for cDNA using an Invitrogen Qubit 4.0 Fluorometer (Thermo Fisher Scientific). The replicates were the same for the cDNA datasets related to the leaves and faecal pellets, yet we do not have a cDNA dataset for the shredders' gut samples because we were not able to recover a measurable amount of material.

Amplicon sequencing

We sent the extracted total DNA and cDNA to the Joint Microbiome Facility (JMF) of the University of Vienna for amplification and sequencing the V4 region of the 16S rRNA gene. The primer pair, 515F (GTG YCA GCM GCC GCG GTA A) and 806R (GGA CTA CNV GGG TWT CTA AT) [68, 69] was used for amplification; barcoding and adapter ligation were achieved in a second step as described in [70] for sequencing on an Illumina MiSeq platform with 600-cycle v3 chemistry (2 × 300 bp paired-end reads). Raw sequencing data were processed using a FASTQ workflow (Basespace, Illumina) with default settings, and sorted into unique amplicon sequence variants (ASVs) using the DADA2 pipeline [71] in R [72] using the recommended workflow. The representative ASVs generated were subsequently merged and classified using the SILVA r138.1 database using default parameters [73], and taxonomy assigned using the assignTaxonomy() function in DADA2 with a confidence threshold of 0.05 for prokaryotic 16S rRNA gene amplicons.

Data filtering

We kept the DNA and cDNA dataset separately, and processed (filtering and stats) them identically. Before statistical analysis, we removed ASVs affiliated to Eukaryota, Archaea, mitochondria, chloroplasts (indicative of insect tissues and ingested plant material), unknown phyla as the sampling designs were not optimized for capturing these organisms. To reduce the effect of rare taxa on subsequent analyses, we excluded ASVs found in fewer than three samples, as well as singleton and doubleton except for calculations of the Chao1 index. We also removed the samples with library sizes <1500 reads prior to downstream analyses to ensure reliable community estimates. Hereafter, the leaves and gut samples represent the combined dataset from the beginning and end of the feeding experiment. We combined both datasets because we found no significant differences in the bacterial community composition of gut samples from natural baseline and experimental animals (PERMANOVA; $F_{(1,48)} = 1.565$, $R^2 = 0.333$, $p = 0.187$) and only very small yet significant variation for leaf samples ($F_{(1,18)} = 3.345$, $R^2 = 0.156$, $p = 0.022$). This combination was needed to increase the number of replicates and thus strengthen the statistical analysis. Finally, we combined ASV read

abundances, taxonomy, and sample metadata into a single dataset (function *phyloseq*; *phyloseq* package) [74] in R for subsequent analyses. All data analyses and visualisations were done in R 4.5.0 version [72].

Data visualization and statistical analyses

To visualize the bacterial taxonomic composition in the samples, we generated bar plots (function *plot_microshades*; *microshades* package, function *comp_barplot*; *microViz* package) [75, 76], aggregating ASVs (function *tax_glom*; *phyloseq* package) at the phylum, order or genus level.

To assess bacterial diversity during and after gut transit, we calculated Shannon index from the rarified (sequencing depth = 2400 for DNA samples and sequencing depth = 450 for cDNA samples) ASV tables (function *estimate_richness*; *phyloseq* package). We used unprocessed dataset for Chao1 calculation. We then tested the sample groups differences in alpha diversity metrics (Shannon and Chao1) by running a linear model (function *lm*; *stats* package) where shredder taxa, sample type (gut vs. faecal pellets), month, and their interactions were used as predictors.

To test compositional differences of bacterial communities between leaves, guts, and faecal pellets, we calculated beta diversity using Hellinger-transformed ASV abundances (function *decostand*; *vegan* package). We visualized bacterial community composition using non-metric multidimensional scaling (NMDS; function *ordinate*; *phyloseq* package) based on Bray-Curtis dissimilarities of transformed ASVs abundances (function *vegdist*; *vegan* package). We evaluated the goodness of fit of the NMDS by the stress value, with values above 0.2 indicating unreliable ordinations (<https://jkzorz.github.io/2019/06/06/NMDS.html>). We tested for differences in community composition (beta diversity) between sample types (leaves, gut, and faecal pellets) using permutational multivariate analysis of variance (PERMANOVA; function *adonis2*; *vegan* package) on the transformed ASVs. The assumption of homogeneity of within-group dispersion was tested (function *betadisper*; *vegan* package) and was fulfilled for all groups. Further we tested the differences in composition of gut-associated bacterial communities across shredder taxa and across their ontogenetic stages using analysis of similarities (ANOSIM; function *anosim*; *vegan* package).

To examine the shared (core) and unique bacterial taxa among leaves, guts, and faecal pellets across shredder taxa, we visualized taxonomic overlaps using Venn diagrams (function *ggvenn*; *ggvenn* package).

We predicted the functional potential of bacterial communities using PICRUSt2 (<https://github.com/picrust/picrust2>; Version 2.2.0) [77], focusing on the METACYC pathways, hereafter referred to as pathways, for downstream analyses. We used pathway abundance

tables as the input for defining core pathways (present in all samples of a given group) and unique pathways (>90% prevalence within a group and <50% in others).

We performed a differential abundance analysis using DESeq2 [78], applying negative binomial models to both the raw ASV count (aggregated at genus level) and the pathways abundance tables. Significance was tested using Wald tests, and we adjusted the p-values for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) correction [79]. We visualized the differentially abundant taxa and predicted pathways across leaves, guts, and faecal pellets for each shredder taxon using heatmaps (function pheatmap; pheatmap package). For the taxa, we selected and visualized the top 100 differential genera as they represented >80% of the read counts, discarding unclassified genus group. Similarly, we focused and showed pathways with a log fold change > 2.5.

Results

1. Composition of bacterial communities in leaves, shredder guts, and faecal pellets

The taxonomic composition of bacterial communities at different levels (phylum and genus) varied among leaves, guts, and faecal pellets of different shredders, but only small differences were detected (Mantel test; $r = 0.45$, $p = 0.001$) when comparing DNA (Fig. 1) and cDNA results (Fig. S1). Hereafter, we will always refer to the DNA dataset outcomes unless explicitly stated. Based on relative abundances of ASVs across months and sample type (excluding leaves), the most abundant bacterial phyla were Bacteroidota followed by Proteobacteria, Firmicutes, and Desulfobacterota. Proteobacteria was the most abundant phylum in leaves (Fig. 1A, Table S1). Within Firmicutes, *Tyzzarella*, *Spiroplasma*, and *Carnobacterium* were the most abundant genera in samples from guts of different shredders (Fig. 1A, S1). Additionally, cDNA data showed that *Bacteroides* and *Rs-D38 termite group* were abundant in the faecal pellets of shredders (Fig. S1).

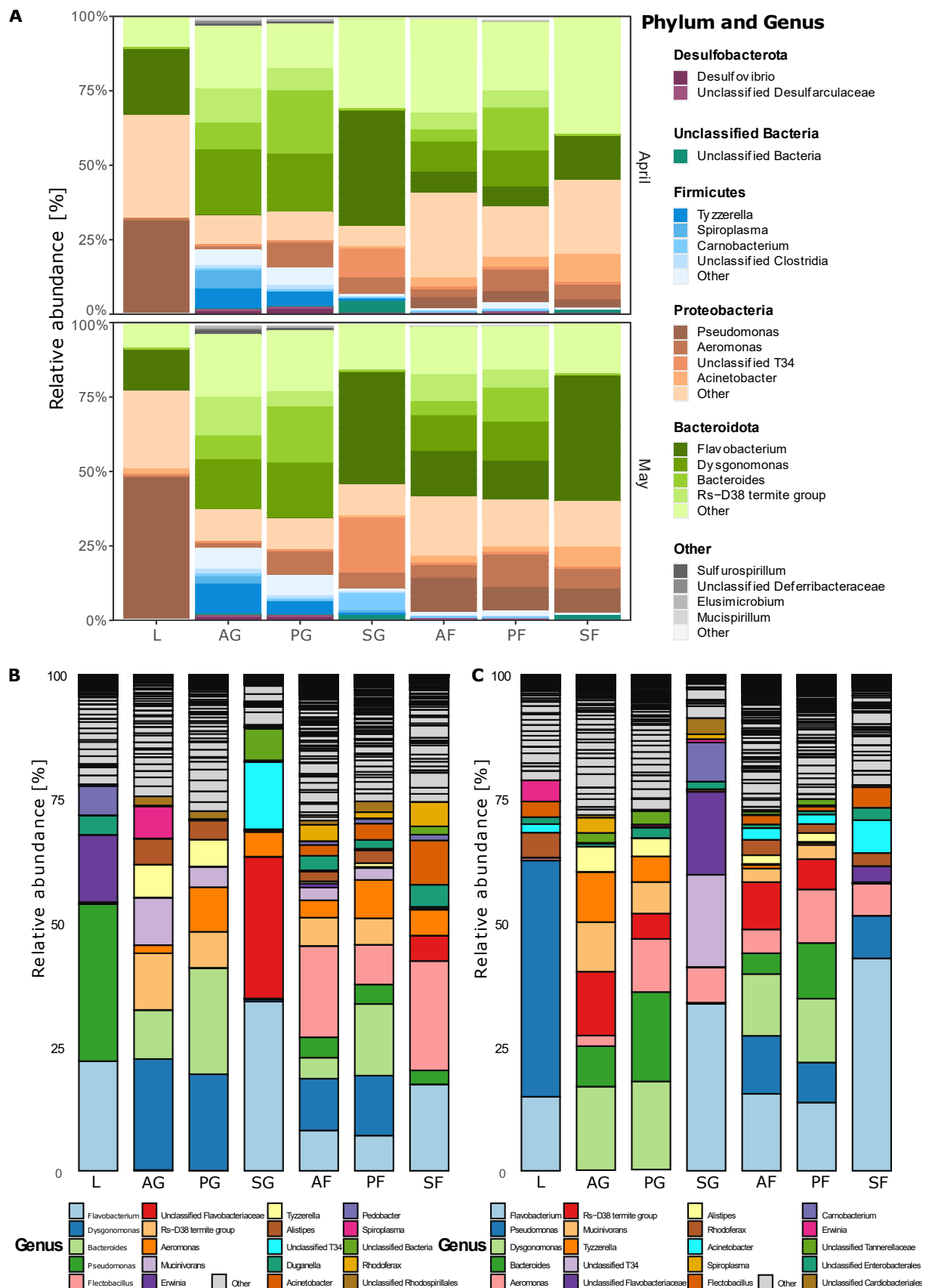


Figure 1. Taxonomic composition and relative abundance of individual taxonomic groups associated with leaves, guts of each shredder taxa, and faecal pellets produced by each shredder taxa during the feeding experiment in the months of April and May based on DNA dataset. In Fig. 1A, each phylum is indicated by a different color, and each genus is indicated by a different shade of the color. Only the five most abundant phyla and four most abundant genera across phyla level for each treatment are shown

whereas the remaining phyla and genera are grouped as “Other”. In Fig. 1B (April) and 1C (May), each genus is represented by a distinct color; the top 20 genera by relative abundance are shown, and all remaining genera are grouped as “Other”. Abbreviations; L = leaves, AG = *Allogamus* gut, AF= *Allogamus* faecal pellets, PG = *Potamophylax* gut, PF= *Potamophylax* faecal pellets, SG = *Sericostoma* gut, SF= *Sericostoma* faecal pellets.

2. Diversity of bacterial communities in leaves, shredder guts, and faecal pellets

Comparison of the bacterial alpha diversity (Shannon, Chao1) between the sample types showed that the gut and faecal pellets of *Sericostoma* had the lowest bacterial diversity followed by guts, and faecal pellets of *Allogamus* and *Potamophylax* (Table S3, Fig. 2A, 2B). Shredder taxa had the strongest effect on Shannon bacterial diversity, while sample type (gut vs. faecal pellets) had strongest effect on Chao1 bacterial diversity (Table 1). Among the three shredder taxa, *Sericostoma* had a negative significant effect on bacterial diversity compared to the reference shredder taxa “*Allogamus*” (linear model: Estimate = -0.913, $p < 0.05$ for Shannon index and -29.471, < 0.05 for Chao1 index; see Table S4 for all effect sizes). *Potamophylax* had no significant effect on both diversity indices (Table S4). In addition, there were significant differences in alpha diversity between samples from April and May for the Shannon index (Table 1, Table S4).

The NMDS ordination and PERMANOVA analysis demonstrated that the bacterial communities associated with leaves, guts, and faecal pellets were clearly distinct from one another ($F_{(2,98)} = 42.349$, $R^2 = 0.454$, $p < 0.001$). The separation along NMDS1 corresponds to the differences in the different sample types and NMDS2 separated the bacterial communities associated with the gut and faecal pellets of *Sericostoma* from those of the other two shredder taxa (Fig. 2C). We found a significant difference in gut-associated bacterial communities across shredder taxa (ANOSIM; $R = 0.7775$, $p < 0.001$) but not across months (ANOSIM; $R = 0.03222$, $p = 0.113$).

Table 1: Effects of shredder taxa (*Allogamus*, *Potamophylax*, *Sericostoma*), sample type (guts, faecal pellets), month as a proxy for ontogenetic stage, and their interactions on bacterial diversity of samples during feeding experiment based on linear model analysis. Abbreviations; St: Standardized, SS: sum of square, df: degrees of freedom.

Factors	Shannon1					Chao1				
	Standard coefficient	SS	df	F-value	p-value	Standard coefficient	SS	df	F-value	p-value
Sample type	0.03	0.238	1	2.038	0.158	0.88	1585833	1	513.211	<0.05
Shredder taxa	0.28	3.216	2	13.794	<0.05	0.55	260357	2	42.129	<0.05
Month	0.06	0.513	1	4.402	0.040	0.03	7437	1	2.407	0.125
Sample type: Shredder taxa	0.25	2.792	2	11.973	<0.05	0.13	32582	2	5.272	<0.05
Sample type: Month	0.01	0.088	1	0.757	0.387	0.03	7353	1	2.380	0.127
Shredder taxa:Month	0.01	0.115	2	0.494	0.613	0.19	50761	2	8.214	<0.05
Sample type: Shredder taxa: Month	0.006	0.051	2	0.217	0.805	0.18	48305	2	7.816	<0.05
Residuals		8.161	70				216302	70		

Statistically significant p-values (< 0.05) are shown in bold.

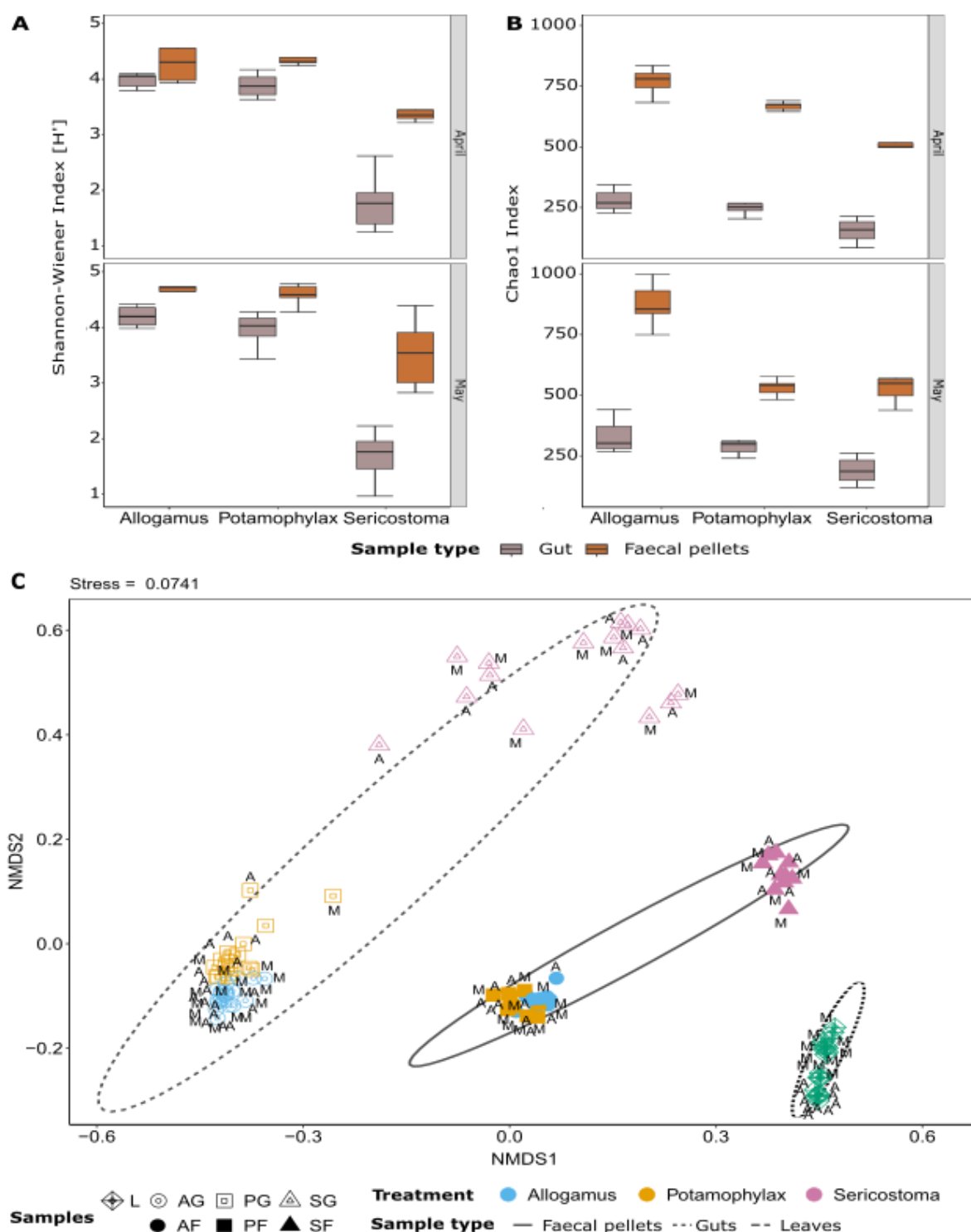


Figure 2. Alpha and beta diversity of bacterial communities in different samples. Shannon (A) and Chao1 (B) metrics for samples collected in the months of April and May. Non-metric dimensional scaling (NMDS) showing the clustering of normalized and Hellinger-transformed 16S sequencing data based on Bray-Curtis distances of bacterial communities associated with leaves, gut, and faecal pellets of three different shredder taxa (C). Here the bacterial communities in L = leaves, AG = *Allogamus* gut, AF = *Allogamus* faecal pellets, PG = *Potamophylax* gut, PF = *Potamophylax* faecal pellets, SG = *Sericostoma* gut, SF = *Sericostoma* faecal pellets are shown by different symbols, and four colors represent the three shredder taxa and the leaves. Abbreviations; A = April and M = May distinguish the two different months where samples were collected from the feeding experiments. The dotted, solid, and semi-dotted line of ellipses (99% confidence interval) encircle the shredder guts, faecal pellets, and leaves collected during the experiment, respectively. The 2-dimensional NMDS solution had a stress value of 0.0741, indicating a good representation of multivariate distances in reduced space.

3. Core and unique bacterial communities and the predicted pathways between leaves, guts, and faecal pellets

The Venn diagrams showed that a large proportion of taxa are shared across leaves, guts, and faecal pellets for all three shredder taxa and both months (Fig. 3). In April, a comparison of bacterial communities of the resource (alder leaves) with the guts and faecal pellets at family level showed 30.4%, 30.6% and 34.2% shared (core) ASVs for *Allogamus*, *Potamophylax*, and *Sericostoma*, respectively (Fig. 3). Furthermore, in April, we observed a similar proportion of shared ASVs between the gut and faecal pellets with 30.4% for *Allogamus* and 34.7% for *Potamophylax*. *Sericostoma*, however, showed a lower proportion of shared ASVs between its gut and faecal pellets with 12.6% but a larger proportion of unique gut ASVs (26.1%) compared to *Allogamus* (6.4%) and *Potamophylax* (4.0%). The proportions of shared and unique ASVs among alder leaves, guts, and faecal pellets for each shredder taxon in May were broadly similar to those observed in April (Fig. 3).

Analysis at genus level revealed that ASVs occurring uniquely in gut samples corresponded to genera *Dysgonomonas*, *Mucinivorans*, *Carnobacterium*, *Tyzzelerella*, and *Rs-D38 termite group* (Fig. 4A, S1). The shared genera between gut and faecal pellets were quite similar in the months May and April for each shredder taxa (Fig 4, S1). Based on prevalence percentage, superpathway of N-acetylglucosamine, N-acetylmannosamine and N-acetylneuraminate degradation, lysine synthesis, and pyruvate fermentation were unique pathways in the guts while degradation of toluene, propanoate, and catechol were unique pathways in faecal pellets of shredders (see Table S7).

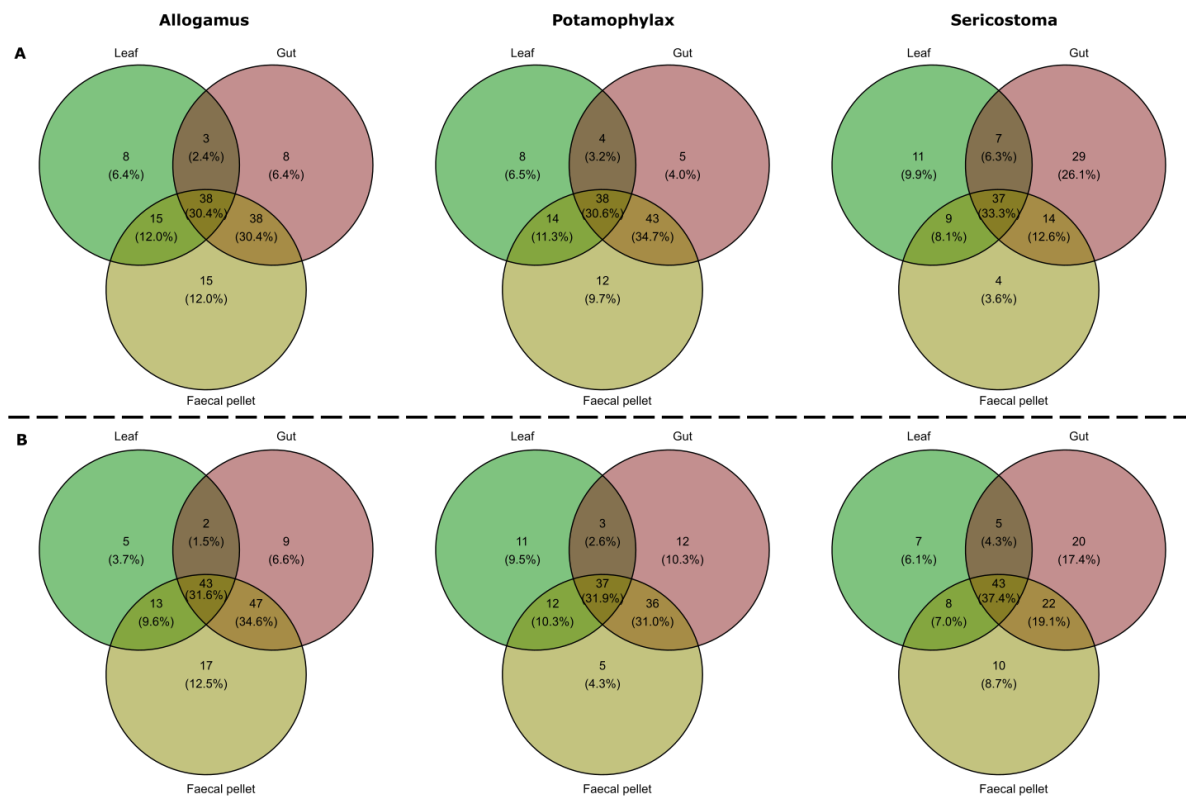


Figure 3. Venn diagrams showing the number and percentages of shared and unique ASVs in leaves, guts, and faecal pellets of *Allogamus*, *Potamophylax*, and *Sericostoma* at family level for the months of April (A) and May (B).

4. Differentially abundant genera and pathways in leaves, shredder guts, and faecal pellets

The hierarchical clustering of differentially abundant genera and differential pathways showed coherent pattern among sample type, i.e., leaves, guts, and faecal pellets of each shredder taxon (Fig. 4). *Dyadobacter*, *Pseudomonas*, *Flavobacterium*, *Pedobacter*, *Novosphingobium* and *Sphingobium* were differentially abundant genera in the leaf samples (Fig. 4A, see Fig. S2A for cDNA dataset) while genera such as *Carnobacterium*, and *Tyzzereella* were enriched in the guts, and *Flavobacterium*, *Pseudomonas*, *Bacteroides*, *Dysgonomonas*, *Acinetobacter*, *Elusimicrobium* Rs–D38 termite group, *Mucinivorans* in faecal pellets samples (Fig. 4A). Furthermore, cDNA data showed *Acinetobacter*, *Duganella*, *Flectobacillus*, *Polaromonas*, and *Undibacterium* were active and enriched in faecal pellets of *Sericostoma* while *Bacteroides*, *Elusimicrobium*, and *Tyzzereella* were enriched in faecal pellets of *Allogamus* and *Potamophylax* (Fig. S2A).

The differential pathways in leaves were the degradation of aromatic compounds, toluene, catechol, syringate, gallate and vanillin and vanillate (Fig. 4B, Table S5, see Fig. S2B, Table S6 for cDNA dataset). L-lysine biosynthesis II and superpathway of N-acetylneuraminate degradation were enriched in guts of *Allogamus* and *Sericostoma*, (Fig. 4B, Table S5), and nitrate reduction VI (assimilatory), degradation of 2-nitrobenzoate and L-tryptophan were

abundant in faecal pellets of *Allogamus* and *Sericostoma* (Fig. 4B, S2B, Table S5, S6). Furthermore, superpathway of demethylmenaquinol-6 biosynthesis II and menaquinol-8 biosynthesis II were enriched in gut of *Allogamus* and *Potamophylax* (Fig. 4B, Table S5). Interestingly, palmitate and peptidoglycan biosynthesis were differential pathways in the gut of *Sericostoma* only (Fig. 4B, Table S5).

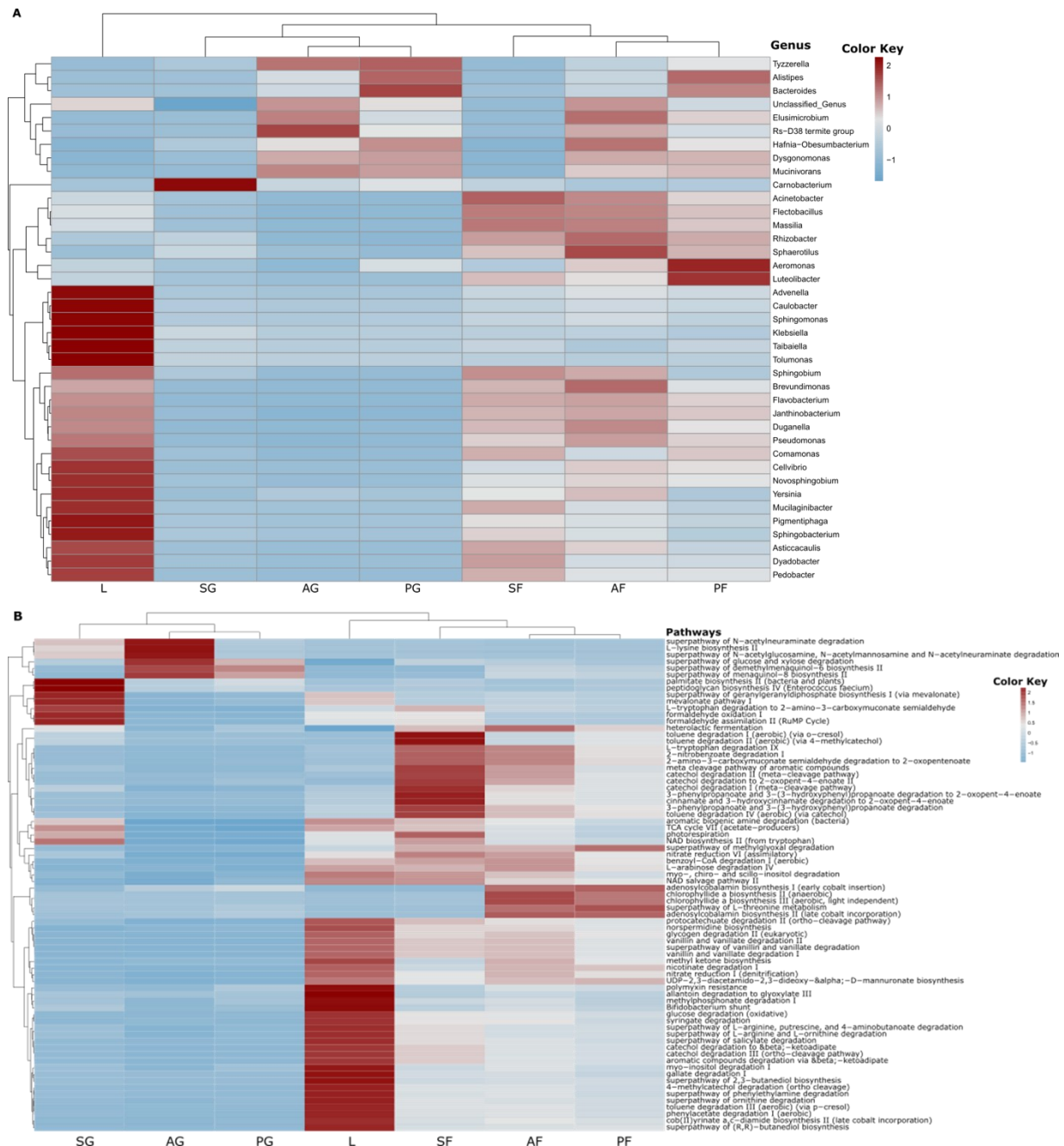


Figure 4. Hierarchical heat map of differentially abundant taxa (A) and pathways (B), showing the log2 transformed abundant values for leaves as well as the guts, and faecal pellets of each shredder taxa. The differentially abundant individual taxa and pathways across the leaves (L), guts (AG = *Allogamus* gut, PG = *Potamophylax* gut SG = *Sericostoma* gut), and faecal pellets of each shredder taxa (AF= *Allogamus* faecal pellets, PF= *Potamophylax* faecal pellets, SF= *Sericostoma* faecal pellets) are shown horizontally. The color key reflects normalized log-transformed counts with skyblue for lower abundance and red color for higher abundance.

Discussion

By comparing the leaf biofilm bacteria with the gut- and faecal-pellet-associated microbiota of different shredder taxa, we assessed how shredder-mediated leaf litter decomposition shape particle-attached bacterial communities, and thereby influence the structure, diversity, and ecological functions of the stream microbiome. Our findings revealed significant differences in bacterial communities and predicted functions associated with leaf particles, shredder guts, and faecal pellets. These differences were dependent on Trichoptera shredder identity, indicating that the composition and activity of stream bacterial communities is influenced by which shredders are present. In contrast, ontogeny (using sampling months as a proxy, validated by head capsule width) had only a minimal effect. Together, these findings showed that shredder identity rather than ontogenetic stage, is a primary factor shaping bacterial assemblages in the gut and in shredder-produced faecal pellets. This underscores shredders as key drivers of the stream microbiome, not only through leaf fragmentation but also through the gut mediated microbial filtration and inoculation.

1. Shredder identity driving the bacterial community structure

Among the three Trichoptera shredders we studied, *Sericostoma* guts and faecal pellets had the lowest bacterial diversity (Fig. 2A), and different community composition compared to the guts and faecal pellets of *Allogamus* and *Potamophylax* (Fig. 2C). Other insect studies have also shown species-specific differences in gut microbiomes [17, 27, 29, 31, 43, 80]. So far, most of the studies assessing insect gut microbiomes focused on differences between different feeding types [17, 37, 39] with fewer studies talking about those differences within the same feeding types [43, 80, 81]. For instance, a study from Lange et al. (2023) has advocated that the host species can have unique internal gut filtering process and physio-chemical conditions in their gut, such as gut pH, oxygen levels, and immune system activity, which select for specific microbial communities. This aligns with our results suggesting that there are more species-specific traits than just the food the species feed on that determines its gut microbiome [27, 43, 80].

The consistently lower bacterial diversity in *Sericostoma* suggests that its gut may be more selective for specific bacteria, possibly due to digestive physiology, enzyme activity, or narrower feeding preferences allowing only certain bacterial groups to persist. In contrast, *Allogamus* and *Potamophylax* hosted more diverse microbiota, implying a less restrictive gut habitat. Yet, we did not test actual gut microenvironment parameters across the taxa. These insects mostly feed on leaf fragments and detritus [22, 34, 48, 59]. However, *Allogamus* is a facultative filter-feeder/grazer and shredder [22, 48] and may potentially encounter more diverse microbial sources while switching their ecological roles [82]. Furthermore, *Allogamus*

and *Potamophylax* are found more in the uppermost sediment layers while *Sericostoma* dwells deeper (up to 1 m) and feeds on fine detritus and mosses along with leaf litter [23, 34, 59, 83]. These differences in habitat and feeding preferences likely expose shredders to distinct environmental microbial pools, and consequently, have contributed to the establishment of species-specific core gut microbiomes that persisted throughout the experiment.

Taxonomic closeness of *Allogamus* and *Potamophylax* (Limnephelidae) as opposed to *Sericostoma* (Sericostomatidae) suggests their evolutionary differences, which in turn might have an effect on their gut physiology and microbial filtering. This may explain the observed variation in *Sericostoma* gut microbiota compared to the other two taxa, despite their classification within the same functional feeding group. Noteworthy, these taxonomic patterns were further evident in the predicted functional profiles (Fig. 4B). For instance, *Sericostoma* gut microbiota showed several unique functional pathways including palmitate biosynthesis II and peptidoglycan biosynthesis IV, that can be linked to differentially abundant taxa - *Carnobacterium* (Phyla: Firmicutes) [84]. Together with the scarcity of *Carnobacterium* in the *Sericostoma* faecal samples, our evidence supports that the gut microbiome of *Sericostoma* is highly specialized and strongly linked to their host physiology, as shown for other organisms [23, 43, 63, 81]. Such host-specific differences in gut microbiota may have ecosystem-level implications as each shredder taxon introduces distinct microbial and functional signatures associated with faecal pellets into the stream, which possibly influences the downstream microbial activity and, consequently, nutrient cycling, and food web dynamics.

To sum up, this interspecies variability in both taxonomic and predicted functions among the shredder gut microbiota suggests that shredders not only contribute to the physical fragmentation of leaves, but also play a key role in the functional recycling of microbial communities within stream detritus, acting as biotic connectors that bridge microbial processing across habitats [20, 21].

2. Gut passage functions as a filter and a vector of microbial dispersal

Food transit through the gut has a dual effect on the bacterial community. First, it acts as a filter, selectively promoting or inhibiting certain bacterial taxa already present on the ingested material [26, 49, 50, 53, 85]. Second, it acts as a microbial dispersal vector by introducing gut-derived bacteria onto the digested material, as well as by re-distributing ingested material associated microbes back into the environment through egestion [26, 50, 51, 53, 55]. The lower bacterial diversity in guts compared to faecal pellets, as indicated by the Shannon and Chao1 richness index in our data, shows that faecal pellets contain a higher number of different bacterial taxa than the gut. This pattern suggests that the gut functions as a selective environment, retaining only certain taxa. Faecal pellets, in contrast, contain both gut-resident

bacteria and additional leaf-associated bacteria that pass through the gut, but did not establish there, resulting in higher diversity [26, 51, 53, 85]. Therefore, gut passage contributes to overall bacterial diversity in the stream microbiome, and this could be crucial for the resilience of stream functions and health. Furthermore, our findings align with the idea that faecal pellets contribute to the seeding of downstream microbial assemblages, and influence the processing of OM beyond the site of initial ingestion [49, 51, 53].

We found a larger proportion of shared bacterial taxa between guts and faecal pellets (12-35%) than between leaves and faecal pellets (8-15%, see Fig. 3). This suggests that the gut may exert a stronger influence on the bacterial composition of faecal pellets than the leaf material itself. Venn diagram (Fig. 3) showing certain proportion of unique bacterial taxa in faecal pellets further supported that gut passage facilitates the enrichment of certain taxa not previously detected in the ingested material. Yet, presence of unique taxa in faecal pellets cannot be exclusively attributed to gut passage, as larval cases may also serve as favorable microhabitats for microorganisms, and could have also contributed to faecal pellets-associated microbial inoculation during the 24-h gut-emptying period. cDNA data further showed substantial numbers of active and unique bacterial taxa in both leaves and faecal pellets (Fig. S2A). Unique taxa, particularly those affiliated with *Bacteroides*, *Elusimicrobium*, *Tyzzelerella* (in *Allogamus* and *Potamophylax*), were differentially abundant in guts (see Fig. 4A) and also present as active members in faecal pellets (see Fig. S2A). This indicates that the alive gut-resident bacterial taxa were introduced to faecal pellets [41, 52–55], and possibly further contribute to the processing of the egested particles. Enrichment of *Acinetobacter* and *Pseudomonas* in faecal pellets known for their active role in aromatic compound degradation such as polysaccharides, might point to the inoculation of these functions into the stream and thereby accelerating OM turnover [86–88]. Furthermore, leaf-associated communities, such as several variants of *Flavobacterium*, *Pseudomonas*, and *Sphingobium* were consistently dominant in leaves as well as appearing in faecal samples. This implies that these bacteria possibly continue to degrade plant-derived polymers even after gut passage [89, 90]. Additionally, active genera including *Aeromonas*, *Flavobacterium*, and *Pseudomonas* (Fig. S2A) in faecal pellets of different shredders likely facilitates the leaf litter decomposition by reducing either nitrate or nitrite and producing N₂O as these are known bacteria involved in the nitrogen cycle [91]. Together, these findings suggest that shredders not only passively redistribute microbes through their guts but also actively shape functional roles of bacteria associated with faecal pellets, which continue to further contribute to detrital degradation.

We further showed clear transitions from a dominance of aromatic compound degradation in leaves to a dominance of amino acid and carbohydrate metabolism in gut and faecal pellets (Table S5). Our results thus highlight the active involvement of bacteria in breakdown of lignin-derived substrates and polyphenols on leaves [44, 86, 88]. Gut microbiota were enriched in

biosynthesis and host-substrate metabolism, such as L-lysine biosynthesis, N-acetylneuraminate degradation (mucin), and menaquinol biosynthesis, indicating that gut bacteria specialize in rapid breakdown of easily digestible aromatic compounds and nitrogen-rich materials. This likely reflects shifts in bacterial metabolism favorable for the hosts' digestive processes. Furthermore, the enrichment of specialized pathways such as tryptophan degradation and aromatic compound meta-cleavage pathways in faecal pellets associated with active bacteria (see Fig. S2B) indicate the continuity of bacterial breakdown of plant-derived aromatic compounds even after egestion inside the stream. These findings emphasize the pivotal role of shredders in enhancing the stream's microbiome functions by introducing faecal pellets enriched with active and functionally specialized bacteria, and thereby supporting complex metabolic processes downstream.

3. Minimal ontogenetic influence

Studies of terrestrial insects and other aquatic macroinvertebrates showed that gut microbiota and their functions differed between ontogenetic stages on their life history [37, 45, 65, 92]. In our study, ontogenetic stage had a small but significant effect on Shannon diversity, but overall bacterial community structure remained stable across months. This suggests that once established, shredder gut microbiota remains relatively consistent, despite slight changes in diet, microhabitat, and environmental microbial inputs. A similar stability of the gut microbiome during later larval instars was observed in other holometabolous insects [30, 80]. Such stability may offer similar niches for shredder gut microbiota across two months, April and May, and thereby consistently contributing to OM processing. However, it is possible that substantial shifts may occur during later life stages, particularly during pupation and metamorphosis, as seen in other terrestrial insects [32, 33, 93]. For instance, honey bee larval guts are dominated by Firmicutes and adults by Proteobacteria, including probiotics taxa, suggesting the importance of specific bacteria at each life stage for improving host's health [92, 93]. Moreover, a seasonal analysis of the *Samia ricini* silkworm gut revealed changes in bacterial community composition between summer and winter, as well as between early and late instars, which is linked to the silkworm's development and has implications for lignocellulose degradation [44]. However, we did not find clear ontogenetic influence on the gut microbiota or associated predicted pathways in our shredders. Therefore, future studies could trace the influence of ontogeny on gut microbiota of aquatic shredders across the early-to-late larval stages.

Conclusion

Overall, our findings show that shredder identity is a key factor shaping the stream microbiome. Different shredder taxa host distinct gut and faecal pellet associated bacterial communities, likely driven by taxon-specific traits such as gut morphology, feeding preferences, and microhabitat. These taxonomic differences are supported by predicted functions, indicating faecal pellet from different shredders have distinct impacts on bacterial assemblages, diversity, and the potential functions of the stream microbiome. In particular, *Sericostoma* showed a highly selective and specialized gut microbiome, emphasizing that not all shredders contribute equally to bacterial diversity or potential decomposition pathways. Gut passage appeared as a major driver of bacteria redistribution where shredder guts act as both a filter, selectively promoting or inhibiting bacterial taxa and potential functions, and a microbial dispersal vector, redistributing gut-and leaf-associated bacteria into the environment via faecal pellets. In contrast, ontogenetic stage (as measured by months) had a minimal effect on bacterial community structure, indicating a strong funder effect and relative stability of gut microbiota once established. While metagenomic and metatranscriptomic approaches are essential to resolve active metabolic networks and microbial interactions during leaf litter decomposition, our results demonstrated that shredders are important mediators linking bacterial structure with ecosystem-level processes in detritus-based stream food-webs. Understanding these host-bacteria linkages is critical for predicting how environmental change or biodiversity loss might affect shredder communities and their gut microbiota, thereby affecting overall stream ecosystem functioning and health.

Acknowledgments

We thank Gertraud Steniczka, Theresa Reichenpfader, Benjamin Misteli, Hermann Hofreiter, and Norbert Matschegg (WasserCluster Lunz) for assisting with microcosm experimental set-up and laboratory analysis. Further, we want to acknowledge Petra Pjevac at the Joint Microbiome Facility (JMF) for sequencing and quality control of raw reads. We are thankful to Anna-Maria Gschwadner for her help during macroinvertebrate shredder sampling.

Author Contributions

Conceptualization and Supervision: Katrin Attermeyer and Simon Vitecek; Methodology: Pratiksha Acharya, Simon Vitecek and Luca Zoccarato; Investigation: Pratiksha Acharya, Simon Vitecek, Mourine Yegon; Formal analysis: Pratiksha Acharya and Luca Zoccarato; Writing – original draft: Pratiksha Acharya and Katrin Attermeyer; Writing – review & editing: Pratiksha Acharya, Mourine Yegon, Luca Zoccarato, Christian Griebler, Simon Vitecek and Katrin Attermeyer; Funding acquisition: Katrin Attermeyer and Simon Vitecek; Project administration: Katrin Attermeyer. All authors read and approved the final manuscript.

Conflict of Interest

We have no competing interests.

Funding

This study is part of the outcomes of the Biodiversity-Ecosystem Functioning Relationships Project funded by WasserCluster Lunz.

Availability of data and materials

The sequence datasets generated and analyzed during the current study are available in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under BioProject ID - PRJNA1321319.

References:

1. Zeglin LH. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Front Microbiol* 2015;6:454. <https://doi.org/10.3389/fmicb.2015.00454>
2. Ren Z, Gao H. Ecological networks reveal contrasting patterns of bacterial and fungal communities in glacier-fed streams in Central Asia. *PeerJ* 2019;7:e7715. <https://doi.org/10.7717/peerj.7715>
3. Thorpe AC et al. National-scale biogeography and function of river and stream bacterial biofilm communities. 2025. *Ecology*, 2025.
4. Lupon A et al. Groundwater-stream connections shape the spatial pattern and rates of aquatic metabolism. *Limnology and Oceanography Letters* 2023;8:350–358. <https://doi.org/10.1002/lol2.10305>
5. Besemer K et al. Unraveling assembly of stream biofilm communities. *ISME J* 2012;6:1459–1468. <https://doi.org/10.1038/ismej.2011.205>
6. Tláškal V, Voříšková J, Baldrian P. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiol Ecol* 2016;92:fiw177. <https://doi.org/10.1093/femsec/fiw177>
7. Silverman JD et al. A phylogenetic transform enhances analysis of compositional microbiota data. *eLife* 2017;6:e21887. <https://doi.org/10.7554/eLife.21887>
8. Battin TJ et al. The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology* 2016;14:251–263.
9. Grossart H-P et al. Linking metagenomics to aquatic microbial ecology and biogeochemical cycles. *Limnology and Oceanography* 2020;65:S2–S20. <https://doi.org/10.1002/lno.11382>
10. Stegen JC et al. Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nat Commun* 2016;7:11237. <https://doi.org/10.1038/ncomms11237>
11. Wallace JB et al. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 1997;277:102–104.
12. Allan JD, Castillo MM, Capps KA. Energy Flow and Nutrient Cycling in Aquatic Communities. In: Allan JD, Castillo MM, Capps KA (eds.), *Stream Ecology : Structure and Function of Running Waters*. Cham: Springer International Publishing, 2021, 357–381.
13. Acharya P et al. Leaf Conditioning and Shredder Activity Shape Microbial Dynamics on Fine Particulate Organic Matter Produced During Decomposition of Different Leaf Litter in Streams. *Microb Ecol* 2025;88:18. <https://doi.org/10.1007/s00248-025-02515-2>
14. Acharya P et al. Distinct bacterial communities affiliated with two types of shredder-produced particles in streams. *FEMS Microbiol Ecol* 2025;101:fiaf091. <https://doi.org/10.1093/femsec/fiaf091>
15. Graça MAS, Ferreira RCF, Coimbra CN. Litter processing along a stream gradient: the role of invertebrates and decomposers. *Journal of the North American Benthological Society* 2001;20:408–420. <https://doi.org/10.2307/1468038>
16. López-Rodríguez MJ et al. Living Apart Together: On the Biology of two Sympatric Leuctra Species (Plecoptera, Leuctridae) in an Apenninic Stream, Italy. *International Review of Hydrobiology* 2012;97:117–123. <https://doi.org/10.1002/iroh.201111413>
17. Ayayee PA, Wesner JS, Ouellette SP. Geography, taxonomy, and ecological guild: Factors impacting freshwater macroinvertebrate gut microbiomes. *Ecology and Evolution* 2022;12:e9663. <https://doi.org/10.1002/ece3.9663>
18. Receveur JP, Fenoglio S, Benbow ME. Insect-associated bacterial communities in an alpine stream. *Hydrobiologia* 2020;847:331–344. <https://doi.org/10.1007/s10750-019-04097-w>
19. Rubio-Ríos J et al. Cross-species coprophagy in small stream detritivores counteracts low-quality litter: native versus invasive plant litter. *Aquat Sci* 2022;85:8. <https://doi.org/10.1007/s00027-022-00905-z>
20. Dekanová V et al. Functional diversity of shredders, not species richness, drives the decomposition rate of leaf litter in ponds. *Front Ecol Evol* 2023;11. <https://doi.org/10.3389/fevo.2023.1286672>
21. Thomsen AG, Friberg N. Growth and emergence of the stonefly *Leuctra nigra* in coniferous forest streams with contrasting pH. *Freshwater Biology* 2002;47:1159–1172. <https://doi.org/10.1046/j.1365-2427.2002.00827.x>
22. Grasser U, Wolfram G, Moog O. The role of *Allogamus auricollis* (Trichoptera: Limnephilidae) in benthic communities of a 4th-order crystalline mountain stream with some ecological notes. 1992.

23. Waringer J, Malicky H. The larvae of *Sericostoma bergeri* Malicky, 1973 and *Sericostoma herakles* Malicky, 1999 (Trichoptera, Sericostomatidae). *Zookeys* 2017;123:133. <https://doi.org/10.3897/zookeys.695.14531>
24. Urbanič G, Toman MJ, Krušnik C. Microhabitat type selection of caddisfly larvae (Insecta: Trichoptera) in a shallow lowland stream. *Hydrobiologia* 2005;541:1–12. <https://doi.org/10.1007/s10750-004-4314-4>
25. Boyero L et al. Global Patterns of Plant Litter Decomposition in Streams. In: Swan CM, Boyero L, Canhoto C (eds.), *The Ecology of Plant Litter Decomposition in Stream Ecosystems*. Cham: Springer International Publishing, 2021, 51–71.
26. Plante CJ, Hill-Spanik KM, Emerson R. Inputs don't equal outputs: bacterial microbiomes of the ingesta, gut, and feces of the keystone deposit feeder *Ilyanassa obsoleta*. *FEMS Microbiology Ecology* 2023;99:fiac152.
27. Kroetsch SA et al. The effects of taxonomy, diet, and ecology on the microbiota of riverine macroinvertebrates. *Ecology and Evolution* 2020;10:14000–14019. <https://doi.org/10.1002/ece3.6993>
28. Ayayee PA et al. Role of the gut microbiome in mediating standard metabolic rate after dietary shifts in the viviparous cockroach, *Diploptera punctata*. *Journal of Experimental Biology* 2020;223:jeb218271. <https://doi.org/10.1242/jeb.218271>
29. Dai W et al. The role of host traits and geography in shaping the gut microbiome of insectivorous bats. *mSphere* 2024;9:e00087-24. <https://doi.org/10.1128/msphere.00087-24>
30. Hammer TJ, Moran NA. Links between metamorphosis and symbiosis in holometabolous insects. *Phil Trans R Soc B* 2019;374:20190068. <https://doi.org/10.1098/rstb.2019.0068>
31. Huang K et al. Host phylogeny and diet shape gut microbial communities within bamboo-feeding insects. *Frontiers in Microbiology* 2021;12:633075.
32. Chen L et al. The role of gut microbiota at different developmental stages in the adaptation of the *Etiella zinckenella* to a plant host. *Sci Rep* 2025;15:4971. <https://doi.org/10.1038/s41598-025-86359-3>
33. Yun J-H et al. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Appl Environ Microbiol* 2014;80:5254–5264. <https://doi.org/10.1128/AEM.01226-14>
34. Basaguren A, Riaño P, Pozo J. Life history patterns and dietary changes of several caddisfly (Trichoptera) species in a northern Spain stream. *Archiv fur Hydrobiologie* 2002;155:23–41. <https://doi.org/10.1127/archiv-hydrobiol/155/2002/23>
35. Engel P, Moran NA. The gut microbiota of insects - diversity in structure and function. *FEMS Microbiol Rev* 2013;37:699–735. <https://doi.org/10.1111/1574-6976.12025>
36. Cryan JF et al. The Microbiota-Gut-Brain Axis. *Physiological Reviews* 2019;99:1877–2013. <https://doi.org/10.1152/physrev.00018.2018>
37. Receveur J. *Influence of Aquatic Microbes on Mosquito Oviposition Behavior and Life History*. Michigan State University, 2021.
38. Bhattacharyya S. *The Role of Macroinvertebrates and Gut Microbiomes in Freshwater Ecosystem Biogeochemistry and Bacterial Community Composition*. 2022. PhD Thesis, Kent State University.
39. Ayayee PA et al. Gut bacterial assemblages of freshwater macroinvertebrate functional feeding groups. *Hydrobiologia* 2018;822:157–172. <https://doi.org/10.1007/s10750-018-3671-3>
40. Gong X et al. Gut microbiome reflect adaptation of earthworms to cave and surface environments. *Animal Microbiome* 2022;4:47. <https://doi.org/10.1186/s42523-022-00200-0>
41. König H, Varma A. *Intestinal Microorganisms of Termites and Other Invertebrates*. 2006.
42. Moran NancyA, Ochman H, Hammer TJ. Evolutionary and ecological consequences of gut microbial communities. *Annu Rev Ecol Evol Syst* 2019;50:451–475. <https://doi.org/10.1146/annurev-ecolsys-110617-062453>
43. Kolasa M et al. How Hosts Taxonomy, Trophic, and Endosymbionts Shape Microbiome Diversity in Beetles. *Microb Ecol* 2019;78:995–1013. <https://doi.org/10.1007/s00248-019-01358-y>
44. Gogoi P et al. Comparative seasonal analysis of Eri silkworm (*Samia ricini* Donovan) gut composition: implications for lignocellulose degradation. *Environ Sci Pollut Res* 2023;30:109198–109213. <https://doi.org/10.1007/s11356-023-29893-9>
45. Sontowski R, van Dam NM. Functional variation in dipteran gut bacterial communities in relation to their diet, life cycle stage and habitat. *Insects* 2020;11:543.
46. Wei T et al. Seasonal variations in bacterial communities and antibiotic-resistant strains associated with green bottle flies (Diptera: Calliphoridae). *Appl Microbiol Biotechnol* 2014;98:4197–4208. <https://doi.org/10.1007/s00253-013-5498-1>

47. Carvalho EM, Graça MAS. A laboratory study on feeding plasticity of the shredder *Sericostoma vittatum* Rambur (Sericostomatidae). *Hydrobiologia* 2007;575:353–359. <https://doi.org/10.1007/s10750-006-0383-x>
48. Waringer JA. Life cycle, horizontal microdistribution and current resistance of *Allogamus auricollis* Trichoptera: Limnephilidae in an Austrian mountain brook. *Freshwater Biology* 1989;22:177–188. <https://doi.org/10.1111/j.1365-2427.1989.tb01094.x>
49. Plante CJ, Mayer LM, King GM. The Kinetics of Bacteriolysis in the Gut of the Deposit Feeder *Arenicola marina*. *Appl Environ Microbiol* 1996;62:1051–1057. <https://doi.org/10.1128/aem.62.3.1051-1057.1996>
50. King GM. Microbiomes of the Enteropneust, *Saccoglossus bromophenolosus*, and Associated Marine Intertidal Sediments of Cod Cove, Maine. *Front Microbiol* 2018;9. <https://doi.org/10.3389/fmicb.2018.03066>
51. Plante CJ, Wilde SB. Biotic Disturbance, Recolonization, and Early Succession of Bacterial Assemblages in Intertidal Sediments. *Microb Ecol* 2004;48:154–166. <https://doi.org/10.1007/s00248-003-1031-x>
52. Aira M et al. Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. *Soil Biology and Biochemistry* 2008;40:2511–2516. <https://doi.org/10.1016/j.soilbio.2008.06.010>
53. Aira M et al. Composition, Structure and Diversity of Soil Bacterial Communities before, during and after Transit through the Gut of the Earthworm *Aporrectodea caliginosa*. *Microorganisms* 2022;10:1025. <https://doi.org/10.3390/microorganisms10051025>
54. Lau WWY, Jumars PA, Armbrust EV. Genetic Diversity of Attached Bacteria in the Hindgut of the Deposit-Feeding Shrimp *Neotrypaea* (Formerly *Callinassa*) *californiensis* (Decapoda: Thalassinidae). *Microbial Ecology* 2002;43:455–466.
55. Dale H et al. Sediment microbial assemblage structure is modified by marine polychaete gut passage. *FEMS Microbiology Ecology* 2019;95:fiz047. <https://doi.org/10.1093/femsec/fiz047>
56. Crump BC, Armbrust EV, Baross JA. Phylogenetic Analysis of Particle-Attached and Free-Living Bacterial Communities in the Columbia River, Its Estuary, and the Adjacent Coastal Ocean. *Appl Environ Microbiol* 1999;65:3192–3204. <https://doi.org/10.1128/AEM.65.7.3192-3204.1999>
57. Catalán N et al. Behind the Scenes: Mechanisms Regulating Climatic Patterns of Dissolved Organic Carbon Uptake in Headwater Streams. *Global Biogeochemical Cycles* 2018;32:1528–1541. <https://doi.org/10.1029/2018GB005919>
58. Torres-Ruiz M, Wehr JD. Changes in the nutritional quality of decaying leaf litter in a stream based on fatty acid content. *Hydrobiologia* 2010;651:265–278. <https://doi.org/10.1007/s10750-010-0305-9>
59. Elliott JM. Life History and Biology of *Sericostoma personatum* Spence (Trichoptera). *Oikos* 1969;20:110. <https://doi.org/10.2307/3543750>
60. Ayayee PA, Currie A, Peterson JA. Different Gut Microbiomes of Developmental Stages of Field-Collected Native and Invasive Western Bean Cutworm, *Striacosta albicosta*, in Western Nebraska. *Microorganisms* 2022;10:1828. <https://doi.org/10.3390/microorganisms10091828>
61. Cummins KW, Klug MJ. Feeding Ecology of Stream Invertebrates. *Annual Review of Ecology and Systematics* 1979;10:147–172.
62. Austin DA, Baker JH. Fate of bacteria ingested by larvae of the freshwater mayfly, *Ephemera danica*. *Microb Ecol* 1988;15:323–332. <https://doi.org/10.1007/BF02012645>
63. Tierno de Figueroa JM et al. Digestive enzyme activity of two stonefly species (Insecta, Plecoptera) and their feeding habits. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 2011;160:426–430. <https://doi.org/10.1016/j.cbpa.2011.07.014>
64. Pechal JL, Benbow ME. Microbial ecology of the salmon necrobiome: evidence salmon carrion decomposition influences aquatic and terrestrial insect microbiomes. *Environmental Microbiology* 2016;18:1511–1522. <https://doi.org/10.1111/1462-2920.13187>
65. Chen B et al. Biodiversity and Activity of the Gut Microbiota across the Life History of the Insect Herbivore *Spodoptera littoralis*. *Sci Rep* 2016;6:29505. <https://doi.org/10.1038/srep29505>
66. Otto C. Growth and Population Movements of *Potamophylax cingulatus* (Trichoptera) Larvae in a South Swedish Stream. *Oikos* 1971;22:292–301. <https://doi.org/10.2307/3543853>
67. Iversen. Life Cycle and Growth of *Sericostoma personatum* Spence (Trichoptera, Sericostomatidae) in a Danish Spring. 1973. <https://doi.org/10.1163/187631273X00282>
68. Apprill A et al. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* 2015;75:129–137. <https://doi.org/10.3354/ame01753>

69. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology* 2016;18:1403–1414. <https://doi.org/10.1111/1462-2920.13023>
70. Pjevac P et al. An Economical and Flexible Dual Barcoding, Two-Step PCR Approach for Highly Multiplexed Amplicon Sequencing. *Front Microbiol* 2021;12. <https://doi.org/10.3389/fmicb.2021.669776>
71. Callahan BJ et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13:581–583. <https://doi.org/10.1038/nmeth.3869>
72. R core team. R: A Language and Environment for Statistical Computing. 2023. Vienna, Austria: R Foundation for Statistical Computing, 2023.
73. Quast C et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 2012;41:D590–D596. <https://doi.org/10.1093/nar/gks1219>
74. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* 2013;8:e61217. <https://doi.org/10.1371/journal.pone.0061217>
75. Dahl EM et al. microshades: An R Package for Improving Color Accessibility and Organization of Microbiome Data. *Microbiol Resour Announc* ;11:e00795-22. <https://doi.org/10.1128/mra.00795-22>
76. Barnett DJ m, Arts IC w, Penders J. microViz: an R package for microbiome data visualization and statistics. *Journal of Open Source Software* 2021;6:3201. <https://doi.org/10.21105/joss.03201>
77. Douglas GM et al. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 2020;38:685–688. <https://doi.org/10.1038/s41587-020-0548-6>
78. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 2014;15:550. <https://doi.org/10.1186/s13059-014-0550-8>
79. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)* 1995;57:289–300.
80. Malacrinò A. Host species identity shapes the diversity and structure of insect microbiota. *Molecular Ecology* 2022;31:723–735. <https://doi.org/10.1111/mec.16285>
81. Lange C et al. Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. *ISME J* 2023;17:1798–1807. <https://doi.org/10.1038/s41396-023-01500-2>
82. Graf W, Lubini V, Pauls S. Larval description of *Drusus muelleri* McLachlan, 1868 (Trichoptera : Limnephilidae) with some notes on its ecology and systematic position within the genus *Drusus*. *Ann Limnol - Int J Lim* 2005;41:93–98. <https://doi.org/10.1051/limn/2005012>
83. Waringer JA. Spatial distribution of Trichoptera larvae in the sediments of an Austrian mountain brook. *Freshwater Biology* 1987;18:469–482. <https://doi.org/10.1111/j.1365-2427.1987.tb01332.x>
84. Walter A, Mayer C. Peptidoglycan Structure, Biosynthesis, and Dynamics During Bacterial Growth. 2019, 237–299.
85. Frouz J et al. Changes in amount of bacteria during gut passage of leaf litter and during coprophagy in three species of Bibionidae (Diptera) larvae. *Folia Microbiol* 2003;48:535–542. <https://doi.org/10.1007/BF02931337>
86. Frouz J, Jandová K. Soluble phenols in litter are reduced during passage through the soil macrofauna gut due to the formation of insoluble complexes with proteins: A case study with isopods and Diptera larvae. *Soil Biology and Biochemistry* 2023;187:109191.
87. Wang X et al. Polysaccharide preferred minority-dominant community assembly and exoenzyme enrichment in transparent exopolymer particles: Implication for global carbon cycle in water. *Science of The Total Environment* 2024;914:169976. <https://doi.org/10.1016/j.scitotenv.2024.169976>
88. Gu J et al. Bacterial transformation of lignin: key enzymes and high-value products. *Biotechnol Biofuels* 2024;17:2. <https://doi.org/10.1186/s13068-023-02447-4>
89. Rastogi G et al. Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. *The ISME Journal* 2012;6:1812–1822. <https://doi.org/10.1038/ismej.2012.32>
90. Khomutovska N et al. Seasonal dynamics in leaf litter decomposing microbial communities in temperate forests: a whole-genome- sequencing-based study. *PeerJ* 2024;12:e17769. <https://doi.org/10.7717/peerj.17769>
91. Wüst PK, Horn MA, Drake HL. Clostridiaceae and Enterobacteriaceae as active fermenters in earthworm gut content. *The ISME Journal* 2011;5:92–106. <https://doi.org/10.1038/ismej.2010.99>

92. Dong Z-X et al. The Succession of the Gut Microbiota in Insects: A Dynamic Alteration of the Gut Microbiota During the Whole Life Cycle of Honey Bees (*Apis cerana*). *Front Microbiol* 2021;12. <https://doi.org/10.3389/fmicb.2021.513962>
93. Lanh PT et al. The Gut Microbiota at Different Developmental Stages of *Apis cerana* Reveals Potential Bacteria for Improving Honeybee Health. *Microorganisms* 2022;10:1938. <https://doi.org/10.3390/microorganisms10101938>

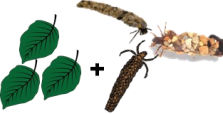
DISCUSSION

Despite the well-recognized importance of fine particulate organic matter (FPOM) as the predominant pool of carbon and nutrients in streams, there remains a major knowledge gap regarding the dynamics of FPOM produced during leaf litter decomposition (LLD) and the FPOM-associated microbial ecology. Usually, FPOM produced during LLD is considered as a chemically and biologically uniform pool, with its microbial ecology largely understudied. With this thesis, I aimed to fill this gap by explicitly linking the origin, quality, and microbial processing of shredder-produced FPOM, thereby providing a more mechanistic understanding of how shredder activity integrates detrital and microbial food webs in streams. To this end, I conducted two controlled laboratory feeding experiments that manipulated leaf resources under varying oxygen levels (Experiment I) and shredder identity (Experiment II). At a finer scale, this thesis sheds light on the mechanisms driven by resources and consumers that shape microbial dynamics within shredder-produced FPOM (see Table 1 for key findings).

I found that FPOM quality and associated microbial activity depend on leaf species traits and the leaf conditioning (Chapter I), revealing persistent chemical and microbial legacies of oxic versus anoxic decomposition pathways. By experimentally disentangling mechanical fragmentation from gut passage, my work further resolved how shredder feeding behaviour and digestion produce distinct FPOM types with divergent biochemical composition (Chapter I), microbial activity (Chapter I), and bacterial community structure (Chapter II). Using 16S rRNA amplicon sequencing, I presented novel findings on bacterial community composition associated with two types of shredder-produced FPOM (Chapter II). Finally, the thesis addressed the largely unexplored role of shredder gut microbiomes in FPOM formation and stream microbial ecology. By characterizing taxon-specific gut bacterial communities and their signatures in faecal pellets using DNA- and RNA-based metabarcoding, I showed that shredders are active microbial filters and vectors rather than passive producers of fine particles (Chapter III).

Together, these mechanistic insights close a key gap in stream ecology by showing how resource (i.e., leaf species under oxic-anoxic conditioning) and consumer diversity (i.e., shredder identity) can influence not only FPOM production but also the FPOM-associated biochemical composition, as well as the composition, function, and persistence of particle-associated microbial communities. The following sections will synthesize the results of the three previous chapters and put them into a broader ecological context, with particular emphasis on microbial legacy effects, FPOM heterogeneity, host filtration, and microbial assembly and implications for the stream microbiome, carbon, and nutrient cycling across connected stream ecosystems.

Table 1. Schematic overview of the experimental framework (Experiments I and II), and key findings correspondence to Chapters (Papers I–III). Here, diversity simply implies Shannon bacterial diversity, and MGE stands for microbial growth efficiency; the findings presented in this table are based solely on the DNA dataset.

Experiments	Manipulated factors	Chapters	Samples	Findings
I	Resource diversity  Includes microbial colonization of leaves under oxic/anoxic condition	I	 Shredded leaves	Bacterial fatty acids: low MGE: low
			 Faecal pellets	Bacterial fatty acids: high MGE: high
		II	 Shredded leaves	Diversity : low Indicator taxa: Propionivibrio, Alkalibacter (Anoxic) Diversity : high Indicator taxa: Flavobacterium, Pseudomonas (Oxic)
			 Faecal pellets	Diversity: low Indicator taxa: Acinetobacter, Carnobacterium
II	Shredder diversity  Includes microbial of leaves colonization under oxic condition	III	 Shredded leaves	Differentially abundant taxa: Flavobacterium, Pseudomonas, Pedobacter
			 Allogamus	Diversity: high
			 Gut	Differentially abundant taxa: Tyzzerella, Elusimicrobium, Rs-D38 termite group, Dysgonomonas, Mucinivorans (Gut)
			 Faecal pellets	Diversity: high Differentially abundant taxa: Elusimicrobium, Rs-D38 termite group, Dysgonomonas, Mucinivorans, Flavobacterium, Pseudomonas (Faecal pellets)
			 Potamophylax	Diversity: high
			 Gut	Differentially abundant taxa: Bacteroides, Elusimicrobium, Dysgonomonas (Gut)
			 Faecal pellets	Diversity: high Differentially abundant taxa: Bacteroides, Elusimicrobium, Dysgonomonas, Flavobacterium, Pseudomonas (Faecal pellets)
			 Sericostoma	Diversity: low
			 Gut	Differentially abundant taxa: Carnobacterium (Gut)
			 Faecal pellets	Diversity: low Differentially abundant taxa: Flavobacterium, Pseudomonas, Pedobacter (Faecal pellets)

Resource quality and environmental conditions shape the FPOM microbial dynamics

When dead leaves from riparian trees fall into a stream, all of them do not break down in the same way: some drift into fast-flowing, well-oxygenated sections, while others end up in stagnant pools and in hyporheic sediments, where oxygen may be limited. Depending on their C/N ratios, leaves undergo different microbial-mediated processing either in oxic or anoxic conditions, which strongly influences their subsequent fate. Oxygen regimes and the leaf itself therefore form microhabitats that host several microbial communities, collectively influencing the stream microbiome (Zeglin et al., 2015) and LLD pathways (Bani et al., 2018; Gruppuso et al., 2023).

Even though most of the bacteria colonizing the leaves originate from the water column, each leaf species under varying oxygen availability serves as a distinct microhabitat and can have its own bacterial community composition and diversity (Hayer et al., 2022), potentially with specific functional roles as well. This echoes the findings of my thesis, in which I show that leaf species strongly influence FPOM-associated microbial growth efficiency (MGE; Chapter I) and bacterial Shannon diversity (Chapter II), thereby resulting in heterogeneity in the stream microbiome. These results also align with other LLD studies, which suggest that substrate quality and environmental conditions play pivotal roles in shaping the composition of microbial communities and their activity during CPOM breakdown (Bani et al., 2018; Frossard et al., 2013; Gruppuso et al., 2023).

In this thesis, I further show that initial microbial colonization under both oxic-anoxic conditions decreases the C/N ratios of low-quality coarse particulate organic matter (CPOM), i.e., beech and maple leaves (Chapter I). This result corroborates other LLD studies showing that leaf conditioning improves CPOM quality through fungal and bacterial biomass, thereby shapes their later decomposition trajectories (Baldy et al., 1995; Danger et al., 2012; Newman et al., 2015). Usually, it is assumed that detritivores produce low-quality (i.e., higher C/N ratios) or nutrient-poor FPOM by removing N from leaf particles during gut passage (Yoshimura et al., 2008; Santonja et al., 2018). However, the findings of this thesis challenge this concept, as I observe similar C/N ratios in CPOM and faecal pellets (Chapter I). These results therefore emphasize the need to re-evaluate the traditional interpretation of FPOM quality produced during LLD. Here, gut passage alone does not determine FPOM nutrient stoichiometry; rather, prior microbial colonization of leaves under oxic and anoxic conditions seems to play a key role in shaping FPOM properties. Consistent with this interpretation, I demonstrate higher microbial respiration in oxic-conditioned shredded leaves and in faecal pellets produced on the fifth day (5d) after feeding oxic-conditioned leaves compared to anoxic ones (Chapter I). In addition, faecal pellets have a distinct clustering of bacterial communities based on

conditioning history, mirroring the patterns observed in shredded leaves (Chapter II). Together, these findings reveal consistent chemical and microbial legacies across oxic-anoxic decomposition pathways. This matches the finding of a recent study, showing the conditioning history of leaf litter, including the timing and order of exposure to stream and riparian environments, creates persistent microbial legacies that regulate decomposition dynamics and carbon processing across ecosystems (Simões et al., 2024).

Oxygen availability is a key driver for microbial biofilm formation during leaves decaying, where oxic conditions support a wider range of microbial metabolic strategies and thereby boosting the richness and diversity of aerobic microbial communities (Karačić et al., 2024). This aligns with my finding in which I show for “Initial leaves,” oxic conditioning contribute to higher Shannon diversity compared to anoxic conditioning (Chapter II). Furthermore, I demonstrate that under oxic conditions, leaves are dominated by well-known leaf litter decomposers such as *Flavobacterium*, *Pseudomonas*, and *Massilia* (Chapter II), consistent with previous studies (Karačić et al., 2024; Khomutovska et al., 2024). These aerobic and facultative aerobic genera can degrade plant-derived polymers, including lignin and cellulose, using oxidative metabolic pathways (Baldy et al., 1995; Janssen et al., 2021; Khomutovska et al., 2024; Ma et al., 2020; Rastogi et al., 2012; Tláškal et al., 2016). Such ability of these bacterial taxa potentially accelerates the rate and efficiency of FPOM production by invertebrate shredders (Baldy et al., 2002; Danger et al., 2012; Gessner, 2010). In simple words, in an oxygen-rich environment, leaves quickly enter the active “recycling loop” of the stream. Nevertheless, not all leaves are that lucky. Some end up in sediments or stagnant pools with anoxic conditions. In such cases, leaves are colonized by anaerobic bacteria such as *Paludibacter* and *Propionivibrio* (Chapter II), consistent with findings from another study on organic matter decomposition under low oxygen availability (Karačić et al., 2024). These bacterial taxa, using mostly anaerobic and fermentative metabolic pathways, may decompose organic matter more slowly (Degelmann et al., 2009; Huang et al., 2022; Qiu et al., 2017). Consequently, leaf particles may stay longer in these environments, thereby extending their residence time and potentially influencing the timing and form of carbon release. Such partially decomposed leaf particles may be transported further before being fully decomposed, and their decomposition may accelerate, specially once they re-enter oxygenated sections of the streams through reoxygenation or hydrological mixing, as shown in other studies (Janssen et al., 2021; Ma et al., 2020).

Indeed, the distinct bacterial communities in different microhabitats play an essential role in in-stream processes, energy fluxes, and biogeochemical cycles. Shifts in oxygen regimes and riparian vegetation could be more frequent under changing environments, such as climate-induced low-flow events or changes in canopy cover, which may substantially affect organic matter processing in streams. To conclude, the studies included in this thesis strongly suggest considering leaf origin (i.e., initial resource quality) and history (i.e., initial environmental

conditions where the leaves were fallen) while predicting the fate and pathways of LLD in streams, as they can have long-lasting influence over metabolic responses and composition of microbial communities, and consequently shape decomposition and carbon processing within the stream detrital food web.

Shredder-produced FPOM is a chemically and functionally diverse organic matter pool

Traditionally, FPOM produced during leaf breakdown has always been treated as a single pool of organic matter (Cummins & Klug, 1979). However, the findings of this thesis challenge that assumption. Across all chapters of this thesis, it is evidenced that there are large differences among shredder-produced FPOM types, such as shredded leaves and faecal pellets, as a function of original resource quality, environmental conditions, and biotic agents, with different consequences for biotic and abiotic processes in streams.

Differences in C/N ratios of leaf species used in the experiment created the initial variability in FPOM quality, which was further facilitated by microbial colonization under different environmental conditions, as discussed before (Chapter I). These findings are consistent with other LLD studies, showing microbial conditioning contribute to differences in the biochemical signatures and physical properties of detritus before it is processed by detritivore-shredders. (Danger et al., 2012; Gessner et al., 1999; Tláškal et al., 2016). Taking this into account, in this thesis, I demonstrate clear differences in MGE (Chapter I), fatty acid profiles (Chapter I), and bacterial composition (Chapter II) between two types of shredder-produced FPOM during LLD. In addition, faecal pellets produced by different shredder taxa harbour distinct bacterial communities and bacterial functional potentials (Chapter III). Together, these results indicate that shredder-produced FPOM are chemically and functionally different rather than interchangeable fractions of organic matter.

FPOM produced through microbe- and shredder-mediated modifications can have varying particle density, surface chemistry, microbial extracellular polymeric substances (EPS) coating, hydrophobicity, and aggregation potential, which, in turn, affect particle transport dynamics in running waters (Battin et al., 2003; Pan et al., 2022). For instance, FPOM enriched in bacterial fatty acids may have higher adhesion capacity and greater potential for floc formation, which may affect sedimentation velocity and deposition patterns (Battin et al., 2003; Zita & Hermansson, 1994). Furthermore, higher MGE in faecal pellets than in shredded leaves (Chapter I) may directly point towards the differences in microbial activity and biomass accumulation between these two FPOM types that may, in turn, alter densities, sinking rates, and adhesion properties (Fettweis et al., 2022; Ho et al., 2022; Wiedmann, 2015). Such differences will determine FPOM fate, i.e., whether it is retained locally, settles into benthic

sediments, or is suspended and transported downstream. This has implications for longitudinal carbon transport: while high microbial activity usually accelerates FPOM decomposition, it may also promote short-term retention of a larger fraction of FPOM locally than previously thought through biofilm formation and microbial biomass accumulation, thereby altering organic matter mineralization and in situ CO₂ production at the local scale (Giorgio & Cole, 1998). However, natural events, including floods and high flow velocities, may trigger the transport of FPOM over longer distances, thereby serving as an important food resource for downstream consumers. The transport of FPOM far from where it was produced is an excellent example of ecosystem connectivity. Indeed, such shredder-produced FPOM can act as a connector to the downstream ecosystem, consistent with a broader ecological concept: meta-ecosystem theory (Harvey et al., 2023).

Overall, the studies presented in this thesis show that shredder-produced FPOM is highly diverse and may contribute differently to carbon cycling processes, including gaseous emissions, carbon storage in sediments, and downstream carbon export. Integrating these aspects with advances in riverine particle transport and carbon cycling modeling clearly requires a shift from treating FPOM as a uniform pool to including particle-level biochemical and microbial variability into hydrodynamic and geomorphological frameworks (Gurnell et al., 2016; Vázquez-Tarrío et al., 2019). Without capturing this variability, predictions of FPOM retention, breakdown, and export remain incomplete. Ultimately, FPOM is the organic matter fraction that has traditionally been included in stream ecosystem concepts, such as the river continuum concept, as a homogeneous pool (Vannote et al., 1978). Therefore, as a propitious side effect, the results presented in this thesis indicate that shredder-produced FPOM is highly heterogeneous based on particle origin, microbial activity, and bacterial community composition. Incorporating such FPOM-level heterogeneity into stream ecosystem models not only advances mechanistic understanding of microbes-mediated and shredder-mediated decomposition processes but also offers valuable inputs for more comprehensive and quantitative predictions of ecosystem-scale carbon and nutrient cycling.

Gut passage and shredder identity control the FPOM microbiome

One important insight of this thesis is that shredders not only physically fragment leaves into smaller pieces but also act as biological processors of organic matter. Their gut microbiota, feeding physiology, and taxon-specific traits strongly shape the biogeochemical properties of FPOM produced during LLD.

Transit through the gut of shredders not simply breaks down the coarse leaf litter into fine particles but also biochemically transforms it, as observed before (Chapter I) and supported by another study, showing gut passage selectively alters biochemicals of egested material

relative to ingested ones via preferential degradation and/or retention of certain biochemicals (Kühmayer et al., 2020). In addition, the gut environment of invertebrates alters the bacterial community composition of ingested organic matter through processes such as enzymatic digestion, microbial inoculation, and selective retention or removal of taxa (Dale et al., 2019; King, 2018; Lau et al., 2002; Plante & Wilde, 2004). Past studies have shown similar gut-mediated transformations of organic matter in Polychaeta (Plante & Wilde, 2004), echinoids (Gao et al., 2014), hemichordates (King, 2018), and crustaceans (Mongkol et al., 2018). Consistent with these studies, the results from Chapters II and III of this thesis show distinct bacterial communities on leaves and faecal pellets, indicating that gut passage modifies the bacterial communities in defecated particles relative to those in ingested particles.

I also found that faecal pellets are enriched with bacterial fatty acid signatures (Chapter I) and are dominated by bacterial communities, such as *Acinetobacter* and *Carnobacterium* (Chapter II), suggesting faecal pellets provide good conditions for microbial communities to thrive. These faecal pellets can therefore act as microbial hotspots, possibly accelerating nutrient cycling and organic matter turnover within the detritus-based food web, as evidenced in other detritus-based systems (Aira et al., 2022; Battin et al., 2003; Joly et al., 2020; Plante et al., 2023). Across shredder taxa, faecal pellets consistently harbour unique bacterial communities and have distinct bacterial predicted metabolic pathways, which are derived from the gut and are different from those on leaves, despite sharing some leaf-associated taxa (Chapter III). This result has been reflected in earthworms and other deposit feeders' studies as well, where the invertebrates' gut acts as a microbial dispersal vector by introducing gut microbiota into defecated particles, as well as by re-distributing ingested particles associated microbes back into the environment through their faeces (Aira et al., 2022; Plante et al., 2023). Such ingested and defecated particles, harboring diverse microbial communities and potential functions, are part of the stream microbiome (Zeglin et al., 2015), which may contribute to the functioning and resiliency of the stream under a changing environment.

Notably, my thesis highlights that shredder identity, rather than ontogenetic shifts, is an important factor shaping bacterial assemblages and bacterial functional potentials in the gut and in shredder-produced faecal pellets (Chapter III). I show that each shredder taxon has distinct gut- and faecal pellets-associated microbiota and bacterial functional potentials (Chapter III). This result is in line with broader principles of microbial ecology: strong host filtration and gut-driven microbial assembly (Kolasa et al., 2019; Lange et al., 2023; Plante et al., 2023). Shredder-taxon-specific guts therefore represent selective microhabitats in which physicochemical conditions and digestive processes favor specific microbial taxa and functional traits (Lange et al., 2023; Plante et al., 2023). These species-specific microbial signatures in FPOM, as shown previously (Chapter III), may influence not only the biochemical composition and physical properties of particles but also microbial colonization potential (Dale

et al., 2019; Malacrino et al., 2020; Plante et al., 2023). Indeed, shredder-mediated microbial filtering during the gut-passage may have direct implications for the fate of FPOM produced by different shredders, influencing decomposition rates, retention times, and downstream transport (Gessner, 2010; Tláškal et al., 2016). In fact, such FPOM can also act as an active component of the global carbon cycle, as it is the main form of organic matter exported from forested headwaters to downstream ecosystems.

As shredder assemblages change across landscapes due to global warming, land-use change, or invasive species, shifts in LLD pathways, FPOM quality, residence time, and even cross-ecosystem carbon subsidies could occur. Consequently, such dynamics can influence food-web connectivity, microbial metabolism, and carbon fluxes across river networks. In sum, the studies included in this thesis show that shredder diversity contributes to high microbial diversity and microbial functional potential associated with FPOM, with implications for the structure and functioning of the stream microbiome and downstream biogeochemical processes. Therefore, maintaining shredder biodiversity in forested headwaters is crucial, as these areas are the primary source of FPOM for entire fluvial systems. Additionally, my thesis highlights the importance of protecting and conserving shredder diversity in order to safeguard ecosystem functions related to carbon cycling and trophic connectivity (Hieber & Gessner, 2002; Vannote et al., 1980).

Conclusion and Outlook

In this thesis, I investigated the conversion of CPOM into FPOM along the leaf-gut-FPOM continuum, including shredder feeding and shredder-taxon-specific gut passage effects mediated by microbially complex processes, and faecal pellets as hotspots of microbial communities with high functional potential. Collectively, the studies included in this thesis integrate microbial ecology, detrital food-web theory, and biogeochemistry, with implications for the meta-ecosystem concept. A key insight of my thesis is that FPOM is not only a by-product of shredder processing during LLD but also a microbially enriched, mobile substrate that links local decomposition processes with longitudinal transport and ecosystem connectivity across river networks. This warrants greater attention to consider FPOM heterogeneity, microbial legacies, and shredder identity in ecological theory, river-network modeling, and global change research. As climate change, biodiversity loss, and land-use change alter freshwater environments worldwide, it is crucial to understand the microbial ecology of shredder-produced FPOM in order to predict the resilience and functioning of detritus-based stream ecosystems.

While my thesis provides novel insights into bacterial dynamics associated with shredder-produced FPOM during LLD, it also has several limitations that should be acknowledged. First, it focuses solely on bacteria, without including fungi, which play a crucial role in leaf

conditioning and decomposition in streams. Although the exclusion of fungi extends beyond the original scope of this study, the prominent bacterial patterns identified here provide a valuable foundation for future research. Investigating how fungal and bacterial communities work together to influence FPOM properties and decomposition processes would be worthwhile, particularly in shredder-produced fecal pellets, where fungal ecology remains largely unexplored. Second, I am aware that studies of bacterial communities based solely on metabarcoding do not allow us to assess the active functional aspects of these communities, which may indeed influence the ecosystem processes studied. Here, I used metabarcoding approaches that are subject to primer specificity and PCR-related biases and were restricted to bacteria, thereby excluding Archaea and limiting inferences about microbial activity and functional expression. Future work should focus on integrating shotgun (meta)genomics, (meta)transcriptomics, and, potentially, proteomics to better resolve the functional potential and active metabolic processes of FPOM-associated microbial communities. Enzymatic activity assessment could complement molecular approaches by revealing how microbial functional capacities are distributed across leaf, gut, and FPOM microhabitats, independent of integrative ecosystem-scale processes such as respiration and decomposition rates. Finally, it would be interesting to replicate this experiment under field or mesocosm settings that incorporate natural hydrology and spatial heterogeneity. Further, integrating compound-specific isotope analysis and microbial trait-profiling approaches across contrasting environments would better understand the effects of human-induced land-use change on microbial-mediated organic matter processing in streams.

References

- Aira, M., Pérez-Losada, M., Crandall, K. A., & Domínguez, J. (2022). Composition, Structure and Diversity of Soil Bacterial Communities before, during and after Transit through the Gut of the Earthworm *Aporrectodea caliginosa*. *Microorganisms*, 10(5), Article 5. <https://doi.org/10.3390/microorganisms10051025>
- Baldy, V., Chauvet, E., Charcosset, J.-Y., & Gessner, M. O. (2002). Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. *Aquatic Microbial Ecology*, 28(1), 25–36.
- Baldy, V., Gessner, M. O., & Chauvet, E. (1995). Bacteria, Fungi and the Breakdown of Leaf Litter in a Large River. *Oikos*, 74(1), 93. <https://doi.org/10.2307/3545678>
- Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., ... & Brusetti, L. (2018). The role of microbial community in the decomposition of leaf litter and deadwood. *Applied soil ecology*, 126, 75–84.
- Battin, T. J., Kaplan, L. A., Denis Newbold, J., & Hansen, C. M. E. (2003). Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*, 426(6965), 439–442. <https://doi.org/10.1038/nature02152>
- Cummins, K. W., & Klug, M. J. (1979). Feeding Ecology of Stream Invertebrates. *Annual Review of Ecology and Systematics*, 10, 147–172.
- Dale, H., Solan, M., Lam, P., & Cunliffe, M. (2019). Sediment microbial assemblage structure is modified by marine polychaete gut passage. *FEMS Microbiology Ecology*, 95(5), fiz047. <https://doi.org/10.1093/femsec/fiz047>
- Danger, M., Cornut, J., Elger, A., & Chauvet, E. (2012). Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. *Freshwater Biology*, 57(5), 1017–1030. <https://doi.org/10.1111/j.1365-2427.2012.02762.x>
- Degelmann, D. M., Kolb, S., Dumont, M., Murrell, J. C., & Drake, H. L. (2009). Enterobacteriaceae facilitate the anaerobic degradation of glucose by a forest soil: Glucose-assimilating Enterobacteriaceae in forest soil. *FEMS Microbiology Ecology*, 68(3), 312–319. <https://doi.org/10.1111/j.1574-6941.2009.00681.x>
- Fettweis, M., Schartau, M., Desmit, X., Lee, B. J., Terseleer, N., Van der Zande, D., Parmentier, K., & Riethmüller, R. (2022). Organic Matter Composition of Biomineral Flocs and Its Influence on Suspended Particulate Matter Dynamics Along a Nearshore to Offshore Transect. *Journal of Geophysical Research: Biogeosciences*, 127. <https://doi.org/10.1029/2021JG006332>
- Frossard, A., Gerull, L., Mutz, M., & Gessner, M. O. (2013). Shifts in microbial community structure and function in stream sediments during experimentally simulated riparian succession. *FEMS microbiology ecology*, 84(2), 398–410.
- Gao, F., Li, F., Tan, J., Yan, J., & Sun, H. (2014). Bacterial Community Composition in the Gut Content and Ambient Sediment of Sea Cucumber *Apostichopus japonicus* Revealed by 16S rRNA Gene Pyrosequencing. *PLOS ONE*, 9(6), e100092. <https://doi.org/10.1371/journal.pone.0100092>
- Gessner, M. O. (2010). Functional leaf traits and biodiversity effects on litter decomposition in a stream: Reply. *Ecology*, 91(6), 1869–1871.
- Gessner, M. O., Chauvet, E., & Dobson, M. (1999). A Perspective on Leaf Litter Breakdown in Streams. *Oikos*, 85(2), 377–384. <https://doi.org/10.2307/3546505>
- Giorgio, P. A. del, & Cole, J. J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology, Evolution, and Systematics*, 29(Volume 29, 1998), 503–541. <https://doi.org/10.1146/annurev.ecolsys.29.1.503>
- Gruppuso, L., Receveur, J. P., Fenoglio, S., Bona, F., & Benbow, M. E. (2023). Hidden decomposers: The role of bacteria and fungi in recently intermittent Alpine streams heterotrophic pathways. *Microbial Ecology*, 86(3), 1499–1512.
- Gurnell, A. M., Rinaldi, M., Buijse, A. D., Brierley, G., & Piégay, H. (2016). Hydromorphological frameworks: Emerging trajectories. *Aquatic Sciences*, 78(1), 135–138. <https://doi.org/10.1007/s00027-015-0436-1>
- Harvey, E., Marleau, J. N., Gounand, I., Leroux, S. J., Firkowski, C. R., Altermatt, F., ... & Fortin, M. J. (2023). A general meta-ecosystem model to predict ecosystem functions at landscape extents. *Ecography*, 2023(11), e06790.
- Hayer, M., Wymore, A. S., Hungate, B. A., Schwartz, E., Koch, B. J., & Marks, J. C. (2022). Microbes on decomposing litter in streams: entering on the leaf or colonizing in the water?. *The ISME Journal*, 16(3), 717–725.
- Hieber, M., & Gessner, M. O. (2002). Contribution of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83(4), 1026–1038. [https://doi.org/10.1890/0012-9658\(2002\)083\[1026:COSDFA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1026:COSDFA]2.0.CO;2)

- Ho, Q., Fettweis, M., Hur, J., Desmit, X., Kim, J., Jung, D., Lee, S. D., Lee, S., Choi, Y., & Lee, B. J. (2022). Flocculation kinetics and mechanisms of microalgae- and clay-containing suspensions in different microalgal growth phases. *Water Research*, 226, 119300. <https://doi.org/10.1016/j.watres.2022.119300>
- Huang, W., Wang, Z., Liu, X., Zhu, D., Wang, Y., & Wu, L. (2022). The microbial community and functional indicators response to flow restoration in gradient in a simulated water flume. *Frontiers in Microbiology*, 13, 1051375. <https://doi.org/10.3389/fmicb.2022.1051375>
- Janssen, K., Low, S. L., Wang, Y., Mu, Q.-Y., Bierbaum, G., & Gee, C. T. (2021). Elucidating biofilm diversity on water lily leaves through 16S rRNA amplicon analysis: Comparison of four DNA extraction kits. *Applications in Plant Sciences*, 9(8), e11444. <https://doi.org/10.1002/aps3.11444>
- Joly, F. X., Coq, S., Coulis, M., David, J. F., Hättenschwiler, S., Mueller, C. W., ... & Subke, J. A. (2020). *Detritivore conversion of litter into faeces accelerates organic matter turnover*. *Commun Biol* 3: 660.
- Karačić, S., Palmer, B., Gee, C. T., & Bierbaum, G. (2024). Oxygen-dependent biofilm dynamics in leaf decay: An in vitro analysis. *Scientific Reports*, 14(1), 6728.
- Khomutovska, N., Jasser, I., Sarapultseva, P., Spirina, V., Zaitsev, A., Masłowiecka, J., & Isidorov, V. A. (2024). Seasonal dynamics in leaf litter decomposing microbial communities in temperate forests: A whole-genome-sequencing-based study. *PeerJ*, 12, e17769. <https://doi.org/10.7717/peerj.17769>
- King, G. M. (2018). Microbiomes of the Enteropneust, *Saccoglossus bromophenolosus*, and Associated Marine Intertidal Sediments of Cod Cove, Maine. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.03066>
- Kolasa, M., Ścibior, R., Mazur, M. A., Kubisz, D., Dudek, K., & Kajtoch, Ł. (2019). How hosts taxonomy, trophic, and endosymbionts shape microbiome diversity in beetles. *Microbial ecology*, 78(4), 995-1013.
- Kühmayer, T., Guo, F., Ebn, N., Battin, T. J., Brett, M. T., Bunn, S. E., ... & Kainz, M. J. (2020). Preferential retention of algal carbon in benthic invertebrates: stable isotope and fatty acid evidence from an outdoor flume experiment. *Freshwater Biology*, 65(7), 1200-1209.
- Lange, C., Boyer, S., Bezemer, T. M., Lefort, M. C., Dhami, M. K., Biggs, E., ... & Kaltenpoth, M. (2023). Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. *The ISME journal*, 17(11), 1798-1807.
- Lau, W. W. Y., Jumars, P. A., & Armbrust, E. V. (2002). Genetic Diversity of Attached Bacteria in the Hindgut of the Deposit-Feeding Shrimp *Neotrypaea* (Formerly *Callinassa*) *californiensis* (Decapoda: Thalassinidae). *Microbial Ecology*, 43(4), 455-466.
- Ma, J., Liu, H., Zhang, C., Ding, K., Chen, R., & Liu, S. (2020). Joint response of chemistry and functional microbial community to oxygenation of the reductive confined aquifer. *Science of The Total Environment*, 720, 137587. <https://doi.org/10.1016/j.scitotenv.2020.137587>
- Malacrinò, A. (2022). Host species identity shapes the diversity and structure of insect microbiota. *Molecular Ecology*, 31(3), 723-735.
- Mongkol, P., Bunphimpapha, P., Rungrasamee, W., Arayamethakorn, S., Klinbunga, S., Menasveta, P., & Chaiyapechara, S. (2018). Bacterial community composition and distribution in different segments of the gastrointestinal tract of wild-caught adult *Penaeus monodon*. *Aquaculture Research*, 49(1), 378-392. <https://doi.org/10.1111/are.13468>
- Newman, M. M., Liles, M. R., & Feminella, J. W. (2015). Litter Breakdown and Microbial Succession on Two Submerged Leaf Species in a Small Forested Stream. *PLOS ONE*, 10(6), e0130801. <https://doi.org/10.1371/journal.pone.0130801>
- Pan, M., Li, H., Han, X., Ma, W., Li, X., Guo, Q., Yang, B., Ding, C., & Ma, Y. (2022). Effects of hydrodynamic conditions on the composition, spatiotemporal distribution of different extracellular polymeric substances and the architecture of biofilms. *Chemosphere*, 307, 135965. <https://doi.org/10.1016/j.chemosphere.2022.135965>
- Plante, C. J., Hill-Spanik, K. M., & Emerson, R. (2023). Inputs don't equal outputs: Bacterial microbiomes of the ingesta, gut, and feces of the keystone deposit feeder *Ilyanassa obsoleta*. *FEMS Microbiology Ecology*, 99(1), fiac152.
- Plante, C. J., & Wilde, S. B. (2004). Biotic Disturbance, Recolonization, and Early Succession of Bacterial Assemblages in Intertidal Sediments. *Microbial Ecology*, 48(2), 154-166. <https://doi.org/10.1007/s00248-003-1031-x>
- Qiu, Y.-L., Tourlousse, D., Matsuura, N., Ohashi, A., & Sekiguchi, Y. (2017). Draft Genome Sequence of *Paludibacter jiangxiensis* NM7 T, a Propionate-Producing Fermentative Bacterium. *Genome Announcements*, 5, e00667-17. <https://doi.org/10.1128/genomeA.00667-17>
- Rastogi, G., Sbodio, A., Tech, J. J., Suslow, T. V., Coaker, G. L., & Leveau, J. H. J. (2012). Leaf microbiota in an agroecosystem: Spatiotemporal variation in bacterial community composition on field-grown lettuce. *The ISME Journal*, 6(10), 1812-1822. <https://doi.org/10.1038/ismej.2012.32>

- Santonja, M., L. Pellan, and C. Piscart. 2018. Macroinvertebrate identity mediates the effects of litter quality and microbial conditioning on leaf litter recycling in temperate streams. *Ecology and Evolution* 8:2542–2553.
- Silva-Sánchez, A., Soares, M., & Rousk, J. (2019). Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality. *Soil Biology and Biochemistry*, 134, 25-35.
- Simões, S., Gonçalves, A. L., Jones, T. H., Sousa, J. P., & Canhoto, C. (2024). Reciprocal stream–riparian fluxes: effects of distinct exposure patterns on litter decomposition. *Aquatic Ecology*, 58(2), 313-322.
- Tláškal, V., Voříšková, J., & Baldrian, P. (2016). Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiology Ecology*, 92(11), fiw177. <https://doi.org/10.1093/femsec/fiw177>
- Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(1), 130–137. <https://doi.org/10.1139/f80-017>
- Vázquez-Tarrio, D., Recking, A., Liébault, F., Tal, M., & Menéndez-Duarte, R. (2019). Particle transport in gravel-bed rivers: Revisiting passive tracer data. *Earth Surface Processes and Landforms*, 44(1), 112–128. <https://doi.org/10.1002/esp.4484>
- Wiedmann, I. (2015). Potential drivers of the downward carbon and particle flux in Arctic marine ecosystems under contrasting hydrographical and ecological situations.
- Yoshimura, C., M. O. Gessner, K. Tockner, and H. Furumai. 2008. Chemical properties, microbial respiration, and decomposition of coarse and fine particulate organic matter. *Journal of the North American Benthological Society* 27:664–673.
- Zeglin, L. H. (2015). Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Frontiers in microbiology*, 6, 454.
- Zita, A., & Hermansson, M. (1994). Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system. *Applied and Environmental Microbiology*, 60(9), 3041–3048. <https://doi.org/10.1128/aem.60.9.3041-3048.1994>

APPENDIX

Supplementary material Chapter I-III

Supplementary material Chapter I

Materials and Methods

Table S1. POC and PN concentrations of different leaf species used in this study. Data show mean $\pm$ standard deviation and the sample size for each analysis was $n = 3$.

Leaf species	C (mol g ⁻¹)	N (mol g ⁻¹)	Molar C/N
Alder	34.91 $\pm$ 0.74	1.82 $\pm$ 0.02	19.12 $\pm$ 0.27
Beech	29.64 $\pm$ 0.18	0.51 $\pm$ 0.03	58.92 $\pm$ 3.37
Maple	33.87 $\pm$ 0.54	0.62 $\pm$ 0.04	54.60 $\pm$ 2.26

Detailed description of experimental setup

The feeding experiment was conducted in microcosms in a climate chamber at 12°C and a light:dark regime of 11:13 hrs. The experiment lasted for a total of 25 days (14th October - 7th November 2022). We set up six treatments with three leaf species conditioned under either oxic or anoxic conditions (i.e., Alder_anoxic, Alder_oxic, Beech_anoxic, Beech_oxic, Maple_anoxic, Maple_oxic) with five replicates resulting in 30 microcosms. Each microcosm consisted of a food-grade white plastic bucket with a volume of 1100 ml and a bottom diameter of 122 mm, in which we installed a steel mesh (pore size 1 mm) at a height of 4.5-5 cm from the bottom to allow the FPOM produced during leaf processing to pass through. We supplied 3.0 ± 0.2 g muffled sand particles (0.7-1.2 mm size) in aluminium petri-dishes to each microcosm for the shredders to build their cases. We filled each microcosm with 750 ml of stream water, which was bubbled with an aquarium pump connected to a tube and bubbling stone to sustain aerobic conditions throughout the whole experimental phase. We collected fresh stream water every four days to exchange the water in the microcosms. The water quality parameters dissolved organic carbon (DOC), nitrate (NO₃⁻), nitrite (NO₂⁻), ammonium (NH₄⁺), and phosphorous (soluble reactive phosphorous = SRP) of the stream water were analysed before refilling the microcosms upon removal every fifth day.

We added ten shredders to each microcosm and fed them with the different conditioned leaves of one of the three species. After feeding for four days, each larva was transferred to a single food-grade plastic cup (Polylactic acid or polylactide (PLA), volume: 4 cl) filled with 20 ml of filtered stream water (0.7 μ m, 450°C for 4 hrs pre-combusted Whatmann GF/F filters) and allowed to empty its gut for 24 hrs for faecal pellet collection. During the 24-hour period, the mesh and leaves were taken out and the water in the microcosms was replaced by freshly collected stream water to prevent the leaves from drying out. On the next day after 24 hrs, the faecal pellets in each cup were collected using a Pasteur pipette after the larvae were returned to their original microcosms. During the 25-day feeding experiment, we collected and analysed

the larvae's faecal pellets three times: on the fifth, 15th, and 25th day (5d, 15d, and 25d). We collected the faecal pellets from each larva separately but pooled pellets from two larvae to have enough material for our measurements, providing us with five samples per microcosm and sampling. We measured bacterial protein production (BPP) and microbial respiration (MR) immediately, while samples for particulate organic carbon (POC) and particulate nitrogen (PN) content, and fatty acids (FA) were stored at -80 °C until further processing. At the end of the experiment, we additionally collected the leaf discs not consumed by the shredders and stored them at -80°C until further processing.

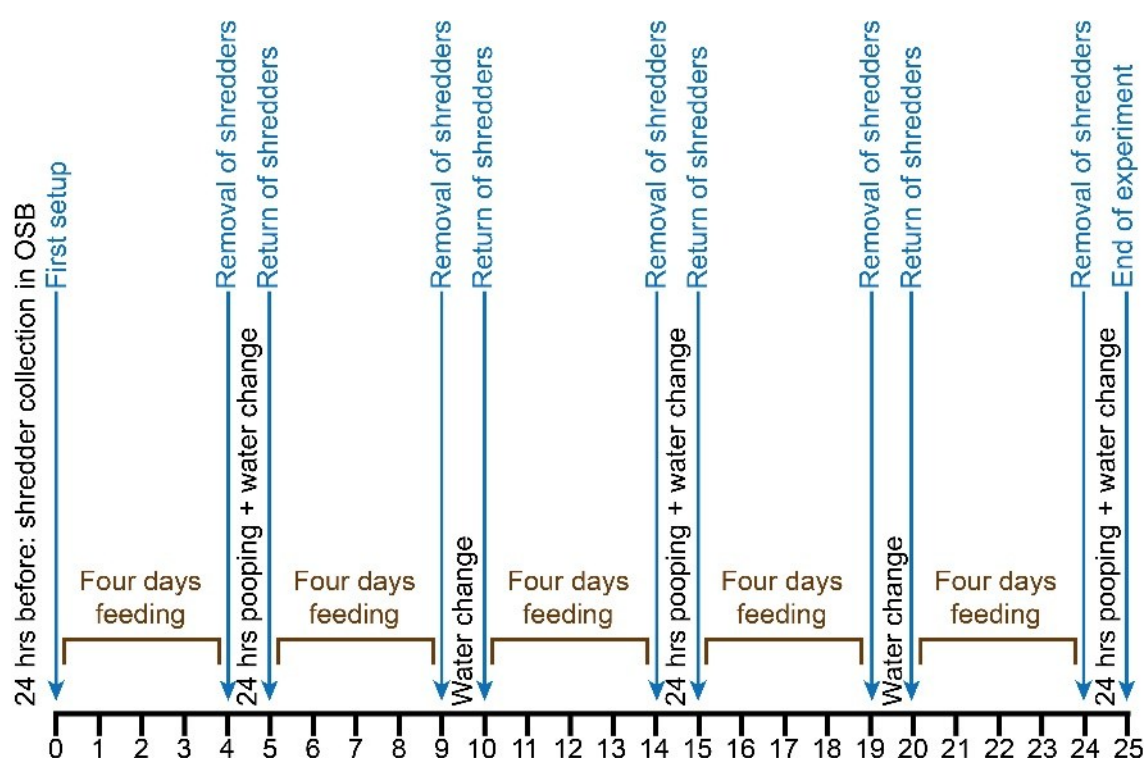


Figure S1. Timeline of the 25d-experiment with the references to the days when faecal pellets were collected.

Physio-chemical characterization of stream water

We characterised the water quality of the stream water before refilling the microcosms and upon removal after four days by measuring different physical and chemical water parameters. We measured pH using a pH meter (WTW pH 3310; Xylem Analytics, Weilheim, Germany) and conductivity using a conductivity meter (WTW Cond 3310; Xylem Analytics, Weilheim, Germany). Water temperature and dissolved oxygen concentrations were measured using an Oximeter (WTW Oxi 315i; Xylem Analytics, Weilheim, Germany). The concentrations of dissolved nutrients (ammonium, $\text{NH}_4\text{-N}$; nitrate, $\text{NO}_3\text{-N}$, nitrite, $\text{NO}_2\text{-N}$, and soluble reactive phosphorus, SRP) were measured using a continuous flow nutrient analyser (CFA, Alliance Instruments GmbH, Salzburg, Austria). We also analysed the DOC concentrations on a total organic carbon (TOC) analyser (Shimadzu Corporation, Kyoto, Japan) through combustion catalytic oxidation of the sample and NDIR-detection.

Table S2. Physical and chemical characteristics of the sampled water from the stream and six microcosm treatments: water temperature (T), pH, dissolved oxygen (dO), specific electrical conductivity (Cond.), and dissolved organic carbon (DOC). Dissolved nutrient concentrations (ammonium, N-NH₄, nitrate, N-NO₃, and soluble reactive phosphorus, SRP) are shown as mean values $\pm$ standard deviation (SD). Physical parameters were measured for each microcosm for each water replacement i.e., n = 25, while chemical parameters were measured in one microcosm from each treatment for each water replacement i.e., n = 5.

Samples	T [°C]	pH	dO [mg/L]	Cond. [μ S/cm]	DOC [mg/L]	N-NH ₄ [μ g/L]	N-NO ₂ [μ g/L]	N-NO ₃ [μ g/L]	SRP [μ g/L]
Stream	11.9 $\pm$ 0.1	8.3 $\pm$ 0.1	9.8 $\pm$ 1.7	257.6 $\pm$ 3.7	2.2 $\pm$ 0.4	4.1 $\pm$ 2.2	2.4 $\pm$ 0.4	584 $\pm$ 64	0.84 $\pm$ 0.5
Alder_anoxic	11 $\pm$ 0.8	8.3 $\pm$ 0.1	11.9 $\pm$ 2.4	281 $\pm$ 18	4.6 $\pm$ 0.8	287 $\pm$ 148	11.9 $\pm$ 2.2	606 $\pm$ 70	0.33 $\pm$ 0.12
Beech_anoxic	11.1 $\pm$ 0.4	8.2 $\pm$ 0.1	11.8 $\pm$ 2.2	267 $\pm$ 8	3.1 $\pm$ 0.8	104 $\pm$ 94	8.2 $\pm$ 3.5	564 $\pm$ 59	19.1 $\pm$ 18. 6
Maple_anoxic	10.4 $\pm$ 0.8	8.3 $\pm$ 0.1	12.2 $\pm$ 2.2	279 $\pm$ 22	4.17 $\pm$ 0.3	33 $\pm$ 26	12.1 $\pm$ 2.3	474 $\pm$ 78	0.53 $\pm$ 0.12
Alder_oxic	11.1 $\pm$ 0.3	8.3 $\pm$ 0.1	12.1 $\pm$ 2.7	277 $\pm$ 16	6.6 $\pm$ 2.3	426 $\pm$ 183	11.4 $\pm$ 3.5	516 $\pm$ 90	0.5 $\pm$ 0.2
Beech_oxic	10.5 $\pm$ 0.3	8.3 $\pm$ 0.1	12.8 $\pm$ 2.9	276 $\pm$ 15	3.5 $\pm$ 0.5	64.74 $\pm$ 104	6.2 $\pm$ 1.6	518 $\pm$ 56	5.4 $\pm$ 8.5
Maple_oxic	10.9 $\pm$ 0.3	8.2 $\pm$ 0.1	12.1 $\pm$ 3.1	267 $\pm$ 6	5.2 $\pm$ 0.9	70 $\pm$ 58	8.1 $\pm$ 1.9	349 $\pm$ 120	1.65 $\pm$ 0.94

FPOM elemental and fatty acid composition

For elemental and fatty acid composition analyses, the amount of faecal pellets from two larvae per microcosms were insufficient. Hence, we pooled the five samples per treatment (faecal pellets per 2 larvae *5 microcosm per treatment = faecal pellets from 10 larvae), which gives us only one sample per treatment.

For the analyses of the elements organic C and N, we first freeze dried (Virtis™ Genesis Freeze Dryer) the collected leaf samples from start and end, and faecal pellets from different time points for 2 days. For POC and PN analyses, we grounded and homogenized all samples and weighed about 1-1.2 mg into tin capsules (5 mm x 9 mm; IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany) using a microbalance (Sartorius CPA2P, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). For determining the POC and PN content of the samples, we used Algae standards (Bladderwrack; 1-1.2 mg DW, IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany). We measured the elemental composition of all prepared samples on a Flash HT Plus CHNS/O elemental analyser (Thermo Fisher Scientific, Bremen, Germany) and the C/N ratios reported here are expressed as molar ratios.

We conducted FA analysis following the procedures described in Guo et al. (2016) as a three-step process consisting of lipid extraction, derivatisation to fatty acid methyl esters (FAMES), and quantification of FAMES using gas chromatography (GC). Briefly, we mixed freeze-dried samples with chloroform:methanol (2:1 V/V) following the addition of 0.9% NaCl, sonication, vortexing, and centrifuging three times to remove non-lipid materials. We evaporated pooled organic phases to a final volume of 1.5 mL under N₂ gas flow. For FAME formation, we incubated a known volume of lipid extracts with sulfuric acid:methanol (1:100 V/V) for 16 h at 50°C, followed by the addition of toluene, KHCO₃, and hexane. Samples were shaken, vortexed, CO₂ released and centrifuged for three minutes at 4°C and 1500 rpm, and the upper organic layers were collected twice, pooled, and concentrated under N₂ to obtain a defined volume for the analysis. We quantified FAME on a gas chromatograph (Trace GC with TriPlus RHS Autosampler, Thermo Fisher Scientific, Waltham, MA, USA, Detector: FID 260 °C, Carrier gas: He: 1 mL/min, Detector gases: H₂: 40 mL/min, N₂: 45 mL/min, air: 450 mL/min) with an HP-88 column (length: 100 m, inner diameter: 0.25 mm, film thickness: 0.2 µm, format: 7 inch; Agilent Technologies, Inc., Santa Clara, CA, USA) for FAME separation. FAMES were identified by comparing their retention time with known reference standards (Supelco 37 Component FAME Mix, CRM47885; and Bacterial Acid Methyl Ester (BAME) Mix, 47080-U; both obtained from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and two in-house standards for stearidonic acid (18:4n-3) and eicosatetraenoic acid (20:4n-3). In total, these standards allowed to identify 47 single FAs. We used the software Chromeleon 7™ (Thermo Fisher Scientific, Waltham, MA, USA) for manual peak adjustment and calculated concentrations of

FA based on individual calibration curves. Results were expressed as mass fractions (μg FAME/mg DW) and reported as mass percentages (% total FAME).

FPOM-associated microbial activities

At the start of the feeding experiment, we finely chopped five to six conditioned leaf discs from each leaf species (= 6 samples*5 replicates) with a knife. This process was mimicking the sloppy feeding of the shredders when eating the leaves and we refer to this type of FPOM as “shredded leaves” throughout the manuscript. We immediately incubated a known amount of shredded leaves for measurements of MR and BPP. In addition, we incubated the faecal pellets pooled from two larvae per microcosm on the fifth, 15th, and 25th day (5d, 15d, and 25d) of the feeding experiment for MR and BPP measurements.

We quantified respiration via oxygen consumption over time with a needle-type O_2 microsensor (Microx 4, Presens GmbH, Regensburg, Germany). We weighed about 0.099 ± 0.074 g of shredded leaves into 10 mL precombusted glass vials and filled each vial to the rim with filtered stream water ($0.7 \mu\text{m}$, precombusted Whatmann GF/F filters). We closed the vials with a lid with butyl rubber septum through which the O_2 microsensor was inserted. For each treatment, we filled five vials with the shredded leaves (= 30 samples). We also incubated five vials filled with filtered stream water as blank. For faecal pellets, we transferred the faecal pellets pooled from two larvae into 10-ml precombusted glass vials and filled the vials with the stream water from the plastic cups after collection of the faecal pellets. Additionally, we incubated the water from the plastic cups without faecal pellets as blank. For each treatment, we had five replicates (= 30 samples + 5 blanks). All samples were incubated for 72 hrs at in situ temperatures in the dark and O_2 concentrations were measured at regular intervals (every 12 hrs). Before each measurement, the vials were carefully mixed by turning them upside down. We calculated MR from linear regressions of oxygen depletion over time. We converted the amount of consumed O_2 to $\mu\text{g C L}^{-1} \text{ d}^{-1}$ by using a conversion factor of 1 (Berggren et al., 2012). We here express MR for shredded leaves and faecal pellets as $\mu\text{g C h}^{-1} \text{ gDW}^{-1}$ and $\text{ng C h}^{-1} 2 \text{ animals}^{-1}$, respectively. We converted the MR values from L to gDW by determining the dry weight of the weighed wet leaves after the MR measurement for each replicate. To do this, we dried the leaves at 60°C for 48 hours.

We determined BPP via incorporation of L-Leucine [4,5- ^3H] into the protein fraction using the protocol of Simon and Azam (1989) and Buesing and Gessner (2003). We incubated a volume of 1 mL for FPOM types in 2 mL Eppendorf tubes. For shredded leaves, it was 0.058 ± 0.018 g of shredded leaves with 1 mL filtered stream water ($0.22 \mu\text{m}$ cellulose acetate syringe filters (VWR)). For faecal pellets, it was 0.5 mL faecal pellets as we always

collected small amounts of water when picking the faecal pellets, which was mixed with 0.5 mL filtered stream water. We added 32 μL working solution of leucine (Hartmann Analytic, Braunschweig, Germany, specific activity 39.90 Ci mmol^{-1}) at a final concentration of 125 nmol L^{-1} . Prior to incubation, we added 50 μL 100% trichloroacetic acid (TCA) (5% final concentration) to the respective blanks and at the end of the incubation after one hour to each sample. All incubations were run in four replicates and one blank per treatment. For optimal coagulation of the proteins after TCA addition, we left the samples for 10 min at room temperature in the dark before centrifugation. After centrifugation at 14,000 rpm (15,800 $\times g$) for 10 min, we decanted the supernatant and washed the pellets with 1 mL ice-cold 5% TCA. We washed the faecal pellets again with 1 mL ice-cold 80% ethanol, each time alternating with a vigorous vortexing and 10-min centrifugation. Afterwards, we added 1 mL scintillation cocktail (Ultima Gold; PerkinElmer, Inc., Waltham, MA, USA) and let the samples sit overnight. The next day, we counted disintegrations per minute (DPM) on a liquid scintillation counter (LS6500; Beckman Coulter, Brea, CA, USA). Net disintegrations per minute were converted to $\text{pmol leucine L}^{-1} \text{ d}^{-1}$ according to Simon and Azam (1989), and an isotope dilution factor of 2 was applied (Kirchman, 1993). We report BPP as $\mu\text{g h}^{-1} \text{ gDW}^{-1}$ for shredded leaves and $\text{ng h}^{-1} 2 \text{ animals}^{-1}$ for faecal pellet samples.

Due to some negative numbers after blank subtraction for MR and BPP measurements on faecal pellets, we removed 22 samples (15 MR + 7 BPP) from the data analysis leading to unequal observations/replication as for each treatment (this is referred to as an unbalanced dataset here). This issue may have emerged due to insufficient amounts of faecal pellets obtained from two larvae. Small sample size, therefore, confined us to run just a linear model instead of a linear mixed model for BPP in faecal pellets. Afterwards we evaluated the model fit by running a Type III ANOVA (function *Anova*; *car* package) and reported the significant factors after calculating the estimated marginal means and the upper and lower limits of the 95% confidence interval (*emmeans* package) and applying multivariate t-distribution adjustment approach for pairwise comparisons among the groups.

Table S3. MR and BPP measured in leaves (MR; $\mu\text{g C h}^{-1} \text{gDW}^{-1}$, BPP; $\mu\text{g h}^{-1} \text{gDW}^{-1}$) at start and in faecal pellets (MR; $\text{ng C h}^{-1} 2 \text{ animals}^{-1}$, BPP; $\text{ng h}^{-1} 2 \text{ animals}^{-1}$) at different timepoints during 25 days of feeding experiment. Median, mean, and standard deviation (SD) are shown.

FPOM type	Time	Leaf species	Conditioning	MR			BPP		
				Mean	Median	SD	Mean	Median	SD
Leaves	start	Alder	Anoxic	36.93	36.88	4.79	0.246	0.28	0.05
		Beech	Anoxic	24.66	25.85	2.79	0.112	0.13	0.02
		Maple	Anoxic	30.10	34.93	3.37	0.53	0.62	0.10
		Alder	Oxic	39.43	40.59	5.57	0.03	0.33	0.02
		Beech	Oxic	31.14	29.6	2.78	0.69	0.79	0.12
		Maple	Oxic	61.95	63.71	8.28	0.156	0.17	0.04
Faecal pellets	5d	Alder	Anoxic	46.79	29.48	41.69	20.04	19.26	5.72
		Beech	Anoxic	35.86	19.82	29.42	9.87	9.88	0.61
		Maple	Anoxic	98.99	90.87	22.25	22.93	23.39	7.32
		Alder	Oxic	105.46	109.72	51.70	3.83	3.80	0.92
		Beech	Oxic	64.28	69.12	16.21	4.28	4.43	1.18
		Maple	Oxic	80.56	110.68	57.56	5.52	5.44	0.35
	15d	Alder	Anoxic	132.05	119.38	43.29	9.06	10.25	2.90
		Beech	Anoxic	59.06	56.07	16.54	5.72	5.72	1.39
		Maple	Anoxic	76.37	76.61	14.32	9.64	9.88	2.18
		Alder	Oxic	85.74	92.32	21.87	6.73	6.19	0.82
		Beech	Oxic	54.33	59.93	11.41	30.01	32.47	5.59
		Maple	Oxic	99.37	81.68	39.76	7.60	8.02	1.21
	25d	Alder	Anoxic	76.75	61.38	31.53	4.96	5.43	1.41
		Beech	Anoxic	4.06	1.93	4.40	4.56	4.55	0.43
		Maple	Anoxic	69.50	67.67	31.94	9.56	9.69	1.74
		Alder	Oxic	24.97	26.58	19.76	2.75	2.64	0.31
		Beech	Oxic	47.753	62.35	26.98	4.01	4.43	2.17
		Maple	Oxic	17.24	1.45	23.36	4.76	4.91	1.66

Table S4. Result of the linear model to observe the effects of leaf species, conditioning, and their combined effects on MR of shredded leaves. The model structure is MR ~ leaf species*conditioning. Abbreviations; SE: Standard error.

Factors	Estimate	SE	t-value	p-value
ConditioningOxic	0.064	0.077	0.828	0.415
Leaf speciesBeech	-0.402	0.077	-5.211	< 0.001
Leaf speciesMaple	0.022	0.082	0.275	0.785
ConditioningOxic:Leaf speciesBeech	0.171	0.109	1.571	0.130
ConditioningOxic:Leaf speciesMaple	0.429	0.113	3.820	< 0.001

Statistically significant p-values (< 0.05) are shown in bold

Table S5. Results of the linear mixed model for microbial respiration (MR) measured on faecal pellets collected at different sampling times during the feeding experiment. Leaf species, conditioning and time were included as fixed factors, and Microcosms ID as the random factor. The model structure is MR ~ leaf species*conditioning*time, (1 | mesocosm ID). Abbreviations; SE: Standard error, df: degrees of freedom.

Fixed effects	Estimate	SE	df	t-value	p-value
Leaf speciesBeech	-72.983	21.133	54	-3.453	0.001
Leaf speciesMaple	-53.910	22.295	55	-2.418	0.019
ConditioningOxic	-46.303	21.133	54	-2.191	0.033
time25d	-55.293	18.361	36	-3.011	0.005
time5d	-85.260	18.361	36	-4.643	<0.001
Leaf speciesBeech:ConditioningOxic	41.567	29.887	54	1.391	0.170
Leaf speciesMaple:ConditioningOxic	67.540	30.719	55	2.199	0.032
Leaf speciesBeech:time25d	8.561	31.327	42	0.273	0.786
Leaf speciesMaple:time25d	46.660	26.920	38	1.733	0.091
Leaf speciesBeech:time5d	69.891	26.934	38	2.595	0.013
Leaf speciesMaple:time5d	106.110	26.920	38	3.942	<0.001
ConditioningOxic:time25d	3.961	31.256	41	0.127	0.900
ConditioningOxic:time5d	104.980	25.967	36	4.043	<0.001
Leaf speciesBeech:ConditioningOxic:time25d	27.416	47.549	43	0.577	0.567
Leaf speciesMaple:ConditioningOxic:time25d	-46.947	41.251	40	-1.138	0.262
Leaf speciesBeech:ConditioningOxic:time5d	-79.654	37.413	37	-2.129	0.040
Leaf speciesMaple:ConditioningOxic:time5d	-104.998	41.251	40	-2.545	0.015
Random effects	Variance	SD			
Microcosms_ID	273.7	16.54			
Residual	842.8	29.03			

Statistically significant p-values (< 0.05) are shown in bold

Table S6. Result of the linear model to observe the effects of leaf species, conditioning, and their interactions in BPP of shredded leaves and faecal pellets collected at different sampling times during the feeding experiment. The model structure is $BPP \sim \text{leaf species} * \text{conditioning}$. Abbreviations; SE: Standard error.

FPOM type	Time	Factors	Estimate	SE	t-value	p-value
Shredded leaves	start	ConditioningOxic	-0.355	0.040	-8.941	<0.001
		Leaf speciesBeech	-0.176	0.040	-4.419	<0.001
		Leaf speciesMaple	0.257	0.040	6.481	<0.001
		ConditioningOxic:Leaf speciesBeech	0.895	0.056	15.931	<0.001
		ConditioningOxic:Leaf speciesMaple	-0.022	0.056	-0.384	0.704
Faecal pellets	5d	ConditioningOxic	-1.604	0.204	-7.851	<0.001
		Leaf speciesBeech	-0.621	0.204	-3.037	0.008
		Leaf speciesMaple	0.191	0.204	0.936	0.363
		ConditioningOxic:Leaf speciesBeech	0.768	0.301	2.554	0.021
		ConditioningOxic:Leaf speciesMaple	0.197	0.301	0.653	0.523
	15d	ConditioningOxic	-0.477	0.186	-2.567	0.021
		Leaf speciesBeech	-0.578	0.172	-3.357	0.004
		Leaf speciesMaple	-0.026	0.186	-0.140	0.890
		ConditioningOxic:Leaf speciesBeech	2.230	0.263	8.478	<0.001
		ConditioningOxic:Leaf speciesMaple	0.283	0.263	1.077	0.299
	25d	ConditioningOxic	-2.793	1.322	-2.113	0.05
		Leaf speciesBeech	-0.874	1.224	-0.714	0.485
		Leaf speciesMaple	4.265	1.224	3.485	0.003
		ConditioningOxic:Leaf speciesBeech	2.665	1.801	1.48	0.157
		ConditioningOxic:Leaf speciesMaple	-1.988	1.801	-1.103	0.285

Statistically significant p-values (< 0.05) are shown in bold

Table S7. Result of the contrast test to observe pairwise comparisons among the groups based on marginal mean values of MR and BPP. The p-values were obtained after applying multivariate *t*-distribution adjustment. Abbreviations; SE: Standard error, df: degrees of freedom.

FPOM type	Time	Contrast	MR					BPP				
			<i>Estimate</i>	<i>SE</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>	<i>Estimate</i>	<i>SE</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
Shredded leaves	start	Alder Anoxic - Alder Oxic	-0.064	0.077	23	-0.828	0.959	0.355	0.040	24	8.941	<0.001
		Alder Anoxic - Beech Anoxic	0.402	0.077	23	5.211	<0.001	0.176	0.040	24	4.419	0.002
		Alder Anoxic - Beech Oxic	0.167	0.077	23	2.161	0.293	-0.364	0.040	24	-9.170	<0.001
		Alder Anoxic - Maple Anoxic	-0.023	0.082	23	-0.275	1.000	-0.257	0.040	24	-6.481	<0.001
		Alder Anoxic - Maple Oxic	-0.516	0.077	23	-6.689	<0.001	0.119	0.040	24	3.003	0.061
		Alder Oxic - Beech Oxic	0.231	0.077	23	2.989	0.063	-0.720	0.040	24	-18.111	<0.001
		Alder Oxic - Maple Oxic	-0.452	0.077	23	-5.861	<0.001	-0.236	0.040	24	-5.938	<0.001
		Beech Anoxic - Alder Oxic	-0.466	0.077	23	-6.040	<0.001	0.180	0.040	24	4.522	0.002
		Beech Anoxic - Beech Oxic	-0.236	0.077	23	-3.051	0.056	-0.540	0.040	24	-13.589	<0.001
		Beech Anoxic - Maple Anoxic	-0.425	0.082	23	-5.188	<0.001	-0.433	0.040	24	-10.900	<0.001
		Beech Anoxic - Maple Oxic	-0.919	0.077	23	-11.901	<0.001	-0.056	0.040	24	-1.416	0.718
		Beech Oxic - Maple Oxic	-0.683	0.077	23	-8.850	<0.001	0.484	0.040	24	12.173	<0.001
		Maple Anoxic - Alder Oxic	-0.041	0.082	23	-0.506	0.995	0.613	0.040	24	15.422	<0.001
		Maple Anoxic - Beech Oxic	0.189	0.082	23	2.312	0.229	-0.107	0.040	24	-2.689	0.115
		Maple Anoxic - Maple Oxic	-0.494	0.082	23	-6.032	<0.001	0.377	0.040	24	9.484	<0.001
Faecal pellets	5d	Alder Anoxic - Alder Oxic	-58.677	21.133	53	-2.776	0.344	1.604	0.204	16	7.851	<0.001
		Alder Anoxic - Beech Anoxic	3.093	22.377	55	0.138	1	0.621	0.204	16	3.037	0.071
		Alder Anoxic - Beech Oxic	-17.497	21.133	53	-0.828	1	1.457	0.221	16	6.600	<0.001
		Alder Anoxic - Maple Anoxic	-52.2	21.133	53	-2.47	0.545	-0.191	0.204	16	-0.936	0.931
		Alder Anoxic - Maple Oxic	-73.419	27.689	58	-2.652	0.42	1.217	0.221	16	5.512	0.001
		Alder Oxic - Beech Oxic	41.18	21.133	53	1.949	0.87	-0.148	0.221	16	-0.669	0.983
		Alder Oxic - Maple Oxic	-14.742	27.689	58	-0.532	1	-0.388	0.221	16	-1.757	0.517
		Beech Anoxic - Alder Oxic	-61.769	22.377	55	-2.76	0.352	0.984	0.204	16	4.814	0.002
		Beech Anoxic - Beech Oxic	-20.589	22.377	55	-0.92	1	0.836	0.221	16	3.788	0.017

Appendix Chapter I

	Beech Anoxic - Maple Anoxic	-55.293	22.377	55	-2.471	0.546	-0.812	0.204	16	-3.973	0.012
	Beech Anoxic - Maple Oxid	-76.511	28.649	58	-2.671	0.408	0.596	0.221	16	2.700	0.129
	Beech Oxid - Maple Oxid	-55.922	27.689	58	-2.02	0.836	-0.240	0.236	16	-1.018	0.905
	Maple Anoxic - Alder Oxid	-6.477	21.133	53	-0.306	1	1.796	0.204	16	8.786	<0.001
	Maple Anoxic - Beech Oxid	34.703	21.133	53	1.642	0.966	1.648	0.221	16	7.466	<0.001
	Maple Anoxic - Maple Oxid	-21.219	27.689	58	-0.766	1	1.408	0.221	16	6.378	<0.001
15d	Alder Anoxic - Alder Oxid	46.303	21.133	53	2.191	0.736	0.477	0.186	15	2.567	0.166
	Alder Anoxic - Beech Anoxic	72.983	21.133	53	3.453	0.08	0.578	0.172	15	3.357	0.041
	Alder Anoxic - Beech Oxid	77.72	21.133	53	3.678	0.046	-1.175	0.186	15	-6.316	<0.001
	Alder Anoxic - Maple Anoxic	53.91	22.36	55	2.411	0.587	0.026	0.186	15	0.140	1.000
	Alder Anoxic - Maple Oxid	32.673	21.133	53	1.546	0.98	0.220	0.172	15	1.279	0.791
	Alder Oxid - Beech Oxid	31.417	21.133	53	1.487	0.987	-1.652	0.199	15	-8.309	<0.001
	Alder Oxid - Maple Oxid	-13.63	21.133	53	-0.645	1	-0.257	0.186	15	-1.382	0.736
	Beech Anoxic - Alder Oxid	-26.68	21.133	53	-1.262	0.998	-0.101	0.186	15	-0.541	0.993
	Beech Anoxic - Beech Oxid	4.737	21.133	53	0.224	1	-1.753	0.186	15	-9.424	<0.001
	Beech Anoxic - Maple Anoxic	-19.073	22.36	55	-0.853	1	-0.552	0.186	15	-2.968	0.083
	Beech Anoxic - Maple Oxid	-40.31	21.133	53	-1.907	0.888	-0.358	0.172	15	-2.078	0.348
	Beech Oxid - Maple Oxid	-45.047	21.133	53	-2.132	0.774	1.395	0.186	15	7.500	<0.001
	Maple Anoxic - Alder Oxid	-7.607	22.36	55	-0.34	1	0.451	0.199	15	2.270	0.264
	Maple Anoxic - Beech Oxid	23.81	22.36	55	1.065	1	-1.201	0.199	15	-6.039	<0.001
	Maple Anoxic - Maple Oxid	-21.237	22.36	55	-0.95	1	0.194	0.186	15	1.044	0.895
25d	Alder Anoxic - Alder Oxid	42.343	27.689	58	1.529	0.983	2.793	1.322	17	2.113	0.326
	Alder Anoxic - Beech Anoxic	64.422	27.77	58	2.32	0.651	0.874	1.224	17	0.714	0.977
	Alder Anoxic - Beech Oxid	37.782	27.689	58	1.365	0.995	1.002	1.224	17	0.818	0.960
	Alder Anoxic - Maple Anoxic	7.25	21.133	53	0.343	1	-4.265	1.224	17	-3.485	0.028
	Alder Anoxic - Maple Oxid	29	21.133	53	1.372	0.994	0.515	1.224	17	0.421	0.998
	Alder Oxid - Beech Oxid	-4.561	32.966	58	-0.138	1	-1.791	1.322	17	-1.355	0.751
	Alder Oxid - Maple Oxid	-13.343	27.689	58	-0.482	1	-2.277	1.322	17	-1.723	0.536
	Beech Anoxic - Alder Oxid	-22.079	33.034	58	-0.668	1	1.918	1.322	17	1.451	0.697

Appendix Chapter I

Beech Anoxic - Beech Oxic	-26.64	33.034	58	-0.806	1	0.127	1.224	17	0.104	1.000
Beech Anoxic - Maple Anoxic	-57.172	27.77	58	-2.059	0.816	-5.139	1.224	17	-4.200	0.007
Beech Anoxic - Maple Oxic	-35.422	27.77	58	-1.276	0.997	-0.359	1.224	17	-0.293	1.000
Beech Oxic - Maple Oxic	-8.782	27.689	58	-0.317	1	-0.486	1.224	17	-0.397	0.998
Maple Anoxic - Alder Oxic	35.093	27.689	58	1.267	0.998	7.057	1.322	17	5.339	0.001
Maple Anoxic - Beech Oxic	30.532	27.689	58	1.103	1	5.266	1.224	17	4.304	0.005
Maple Anoxic - Maple Oxic	21.75	21.133	53	1.029	1	4.780	1.224	17	3.906	0.012

Statistically significant p-values (< 0.05) are shown in bold

Table S8. Result of the linear model to observe the effects of FPOM type, leaf species, and conditioning on microbial growth efficiency (MGE). The model structure is $MGE \sim FPOM\ type * leaf\ species * conditioning$. Abbreviations; SE: Standard error.

Factors	Estimate	SE	t-value	p-value
FPOM typeLeaves	-12.048	5.043	-2.389	0.022
ConditioningOxic	-7.305	5.638	-1.296	0.204
Leaf speciesBeech	12.670	5.638	2.247	0.031
Leaf speciesMaple	-0.175	5.638	-0.031	0.975
FPOM typeLeaves:ConditioningOxic	6.710	7.132	0.941	0.353
FPOM typeLeaves:Leaf speciesBeech	-12.901	7.132	-1.809	0.079
FPOM typeLeaves:Leaf speciesMaple	0.910	7.297	0.125	0.902
ConditioningOxic:Leaf speciesBeech	-0.485	7.973	-0.061	0.952
ConditioningOxic:Leaf speciesMaple	1.472	7.973	0.185	0.855
FPOM typeLeaves:ConditioningOxic:Leaf speciesBeech	2.833	10.086	0.281	0.780
FPOM typeLeaves:ConditioningOxic:Leaf speciesMaple	-2.039	10.203	-0.200	0.843

Statistically significant p-values (< 0.05) are shown in bold

Table S9. Microbial growth efficiency (MGE) measured in leaves at start (n = 29) and in faecal pellets collected at different time points (n = 18) during 25 days of feeding experiment. Mean and standard deviation (SD) are shown.

FPOM type	Leaf species	Conditioning	Mean	SD
Faecal pellets	Alder	Anoxic	12.71	11.64
	Alder	Oxic	5.41	2.21
	Beech	Anoxic	25.38	21.49
	Beech	Oxic	17.59	14.67
	Maple	Anoxic	12.54	3.79
	Maple	Oxic	6.70	1.33
Leaves	Alder	Anoxic	0.66	0.19
	Alder	Oxic	0.07	0.05
	Beech	Anoxic	0.43	0.07
	Beech	Oxic	2.19	0.51
	Maple	Anoxic	1.40	0.29
	Maple	Oxic	0.24	0.07

Table S10. Proportions of different fatty acids measured in leaves at start (n = 12) and in faecal pellets collected (n = 17) during 25 days of feeding experiment. Mean and standard deviation (SD) are shown. Abbreviations; ALA: α -linolenic acid, BFA: sum of all bacterial fatty acids, n-3 PUFA: omega-3 polyunsaturated fatty acids, n-3 SC-PUFA: omega-3 short chain polyunsaturated fatty acids.

FPOM type	FA_type	Conditioning	Mean	SD
Leaves	ALA	Anoxic	9.23	3.92
		Oxic	9.54	4.72
	BFA	Anoxic	5.94	4.18
		Oxic	1.99	0.47
	n-3 PUFA	Anoxic	10.54	5.05
		Oxic	10.00	4.69
	n-3 SC_PUFA	Anoxic	9.30	3.99
		Oxic	9.67	4.79
Faecal pellets	ALA	Anoxic	1.73	1.91
		Oxic	1.11	0.85
	BFA	Anoxic	11.22	6.34
		Oxic	12.37	8.01
	n3PUFA	Anoxic	1.99	1.78
		Oxic	1.58	1.23
	n-3 SC_PUFA	Anoxic	1.73	1.91
		Oxic	1.14	0.79

Supplementary material Chapter II

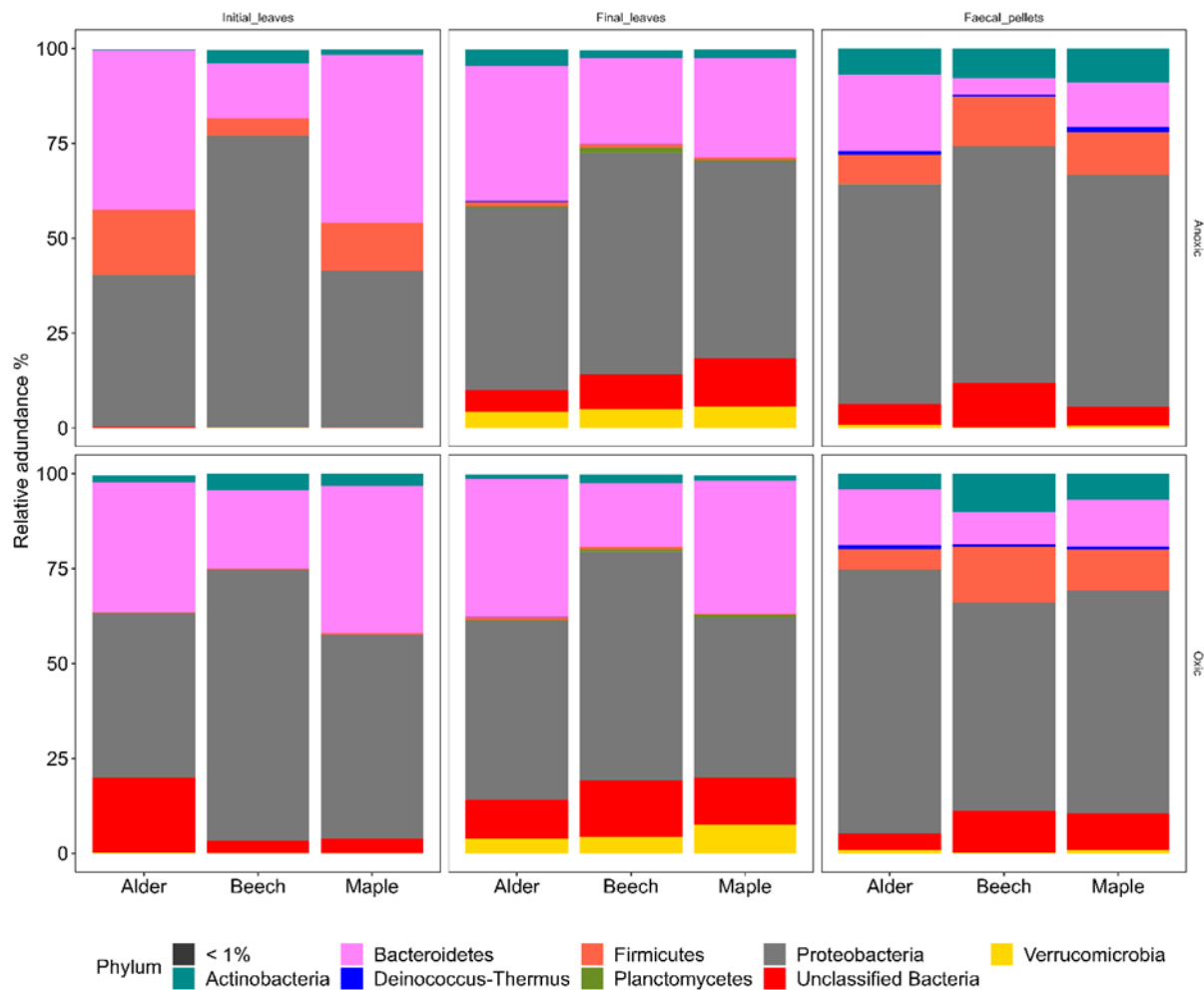


Figure S1. Relative abundance of ASVs belonging to different FPOM types at a phylum level are shown. ASVs that were present in less than 1% were grouped in one as “< 1%”.

Table S1. Differences in bacterial phylum level relative abundance among FPOM types i.e., initial leaves, final leaves and faecal pellets. A Kruskal-Wallis test with a Benjamini-Hochberg (BH) correction was used to compare among the three groups. Only bacterial phyla with greater than 1% of the total relative abundance across all samples are shown. Abundances are given as mean $\pm$ SE. Abbreviations; p: p-values, p.adj: p-adjusted values.

Phylum	p	p.adj	Initial leaves Abundance (N=18)	Final leaves Abundance (N=18)	Faecal pellets Abundance (N=17)
Actinobacteria	0.006	0.011	2.46 $\pm$ 0.37	2.21 $\pm$ 0.39	7.28 $\pm$ 1.03
Bacteroidetes	0.008	0.012	32.32 $\pm$ 2.81	28.782 $\pm$ 2.21	12.12 $\pm$ 1.67
Deinococcus-Thermus	0.002	0.006	0.015 $\pm$ 0.004	0.069 $\pm$ 0.02	0.95 $\pm$ 0.23
Firmicutes	0.042	0.05	5.87 $\pm$ 1.66	0.69 $\pm$ 0.09	10.14 $\pm$ 1.11
Planctomycetes	<0.001	0.003	0.004 $\pm$ 0.002	0.65 $\pm$ 0.11	0.05 $\pm$ 0.007
Proteobacteria	0.198	0.200	54.46 $\pm$ 3.71	51.26 $\pm$ 2.42	61.11 $\pm$ 3.03
Unclassified Bacteria	0.045	0.05	4.58 $\pm$ 1.72	10.93 $\pm$ 1.29	7.68 $\pm$ 2.47
Verrucomicrobia	<0.001	0.003	0.03 $\pm$ 0.007	5.03 $\pm$ 0.48	0.59 $\pm$ 0.17

Statistically significant p-values (< 0.05) are shown in bold

Table S2. Differences in bacterial class level relative abundance among FPOM types i.e., initial leaves, final leaves and faecal pellets. A Kruskal-Wallis test with a Benjamini-Hochberg (BH) correction was used to compare among the three groups. Only bacterial class with greater than 1% of the total relative abundance across all samples are shown. Abundances are given as mean $\pm$ SE. Abbreviations; p: p-values, p.adj: p-adjusted values.

Class	p	p.adj	Initial leaves Abundance (N=18)	Final leaves Abundance (N=18)	Faecal pellets Abundance (N=17)
Actinobacteria	0.006	0.009	2.45 $\pm$ 0.37	2.13 $\pm$ 0.38	7.22 $\pm$ 1.03
Alphaproteobacteria	0.691	0.730	12.90 $\pm$ 1.48	13.46 $\pm$ 0.99	11.49 $\pm$ 1.78
Bacilli	0.003	0.006	0.12 $\pm$ 0.03	0.064 $\pm$ 0.02	9.09 $\pm$ 1.05
Bacteroidia	0.777	0.780	9.38 $\pm$ 2.86	0.93 $\pm$ 0.23	0.22 $\pm$ 0.04
Betaproteobacteria	0.006	0.009	12.02 $\pm$ 0.98	24.39 $\pm$ 1.39	13.80 $\pm$ 1.32
Chitinophagia	<0.001	0.002	0.007 $\pm$ 0.002	0.91 $\pm$ 0.08	0.22 $\pm$ 0.05
Clostridia	0.117	0.150	5.611 $\pm$ 1.61	0.60 $\pm$ 0.08	0.27 $\pm$ 0.08
Cytophagia	0.281	0.330	5.56 $\pm$ 1.55	7.84 $\pm$ 0.59	4.62 $\pm$ 1.01
Deinococci	0.002	0.005	0.015 $\pm$ 0.003	0.07 $\pm$ 0.023	0.96 $\pm$ 0.23
Flavobacteriia	0.015	0.021	15.29 $\pm$ 1.42	15.46 $\pm$ 1.79	6.34 $\pm$ 0.87
Gammaproteobacteria	0.004	0.006	29.51 $\pm$ 3.65	12.37 $\pm$ 1.12	35.53 $\pm$ 2.43
Opitutae	<0.001	0.0015	0.004 $\pm$ 0.001	0.85 $\pm$ 0.16	0.12 $\pm$ 0.03
Planctomycetacia	<0.001	0.0015	0.004 $\pm$ 0.002	0.65 $\pm$ 0.11	0.05 $\pm$ 0.01
Sphingobacteriia	0.324	0.360	2.07 $\pm$ 0.52	0.72 $\pm$ 0.21	0.33 $\pm$ 0.23
Unclassified Bacteria	0.045	0.059	4.58 $\pm$ 1.73	10.93 $\pm$ 1.29	7.68 $\pm$ 2.48
Verrucomicrobiae	<0.001	0.0015	0.005 $\pm$ 0.001	3.79 $\pm$ 0.37	0.42 $\pm$ 0.13

Statistically significant p-values (< 0.05) are shown in bold

Table S3. Differences in bacterial phylum level relative abundance between conditioning i.e., anoxic and oxic. A Kruskal-Wallis test with a Benjamini-Hochberg (BH) correction was used to compare between the two groups. Only bacterial phyla with greater than 1% of the total relative abundance across all samples are shown. Abundances are given as mean $\pm$ SE. Abbreviations; p: p-values, p.adj: p-adjusted values.

Phylum	p	p.adj	Anoxic Abundance (N=27)	Oxic Abundance (N=26)
Actinobacteria	0.825	0.89	4.15 $\pm$ 0.73	3.69 $\pm$ 0.65
Bacteroidetes	0.895	0.89	24.59 $\pm$ 2.73	24.70 $\pm$ 2.31
Deinococcus-Thermus	0.690	0.89	0.35 $\pm$ 0.13	0.32 $\pm$ 0.14
Firmicutes	0.058	0.52	7.63 $\pm$ 1.20	3.24 $\pm$ 1.04
Planctomycetes	0.757	0.89	0.29 $\pm$ 0.10	0.19 $\pm$ 0.05
Proteobacteria	0.895	0.89	55.28 $\pm$ 2.81	55.74 $\pm$ 2.43
Unclassified Bacteria	0.171	0.77	5.65 $\pm$ 1.57	9.89 $\pm$ 1.51
Verrucomicrobia	0.566	0.89	1.80 $\pm$ 0.49	2.02 $\pm$ 0.52

Table S4. Differences in bacterial phylum level relative abundance among treatment i.e., alder, beech and maple. A Kruskal-Wallis test with a Benjamini-Hochberg (BH) correction was used to compare among the three groups. Only bacterial phyla with greater than 1% of the total relative abundance across all samples are shown. Abundances are given as mean $\pm$ SE. Abbreviations; p: p-values, p.adj: p-adjusted values.

Phylum	p	p.adj	Alder Abundance (N=18)	Beech Abundance (N=17)	Maple Abundance (N=18)
Actinobacteria	0.385	0.98	3.07 $\pm$ 0.75	4.73 $\pm$ 0.95	4.01 $\pm$ 0.84
Bacteroidetes	0.091	0.41	30.45 $\pm$ 2.54	14.87 $\pm$ 1.99	28.06 $\pm$ 3.24
Deinococcus-Thermus	0.927	0.98	0.41 $\pm$ 0.19	0.19 $\pm$ 0.10	0.39 $\pm$ 0.18
Firmicutes	0.960	0.98	5.39 $\pm$ 1.45	5.11 $\pm$ 1.46	5.91 $\pm$ 1.55
Planctomycetes	0.894	0.98	0.15 $\pm$ 0.05	0.36 $\pm$ 0.14	0.21 $\pm$ 0.07
Proteobacteria	0.050	0.41	50.97 $\pm$ 2.72	64.57 $\pm$ 3.10	51.47 $\pm$ 2.82
Unclassified Bacteria	0.983	0.98	7.66 $\pm$ 1.68	8.24 $\pm$ 2.52	7.33 $\pm$ 1.66
Verrucomicrobia	0.884	0.98	1.63 $\pm$ 0.47	1.66 $\pm$ 0.55	2.41 $\pm$ 0.79

Table S5. Differences in bacterial class level relative abundance among treatment i.e., alder, beech and maple. A Kruskal-Wallis test with a Benjamini-Hochberg (BH) correction was used to compare among the three groups. Only bacterial class with greater than 1% of the total relative abundance across all samples are shown. Abundances are given as mean $\pm$ SE. Abbreviations; p: p-values, p.adj: p-adjusted values.

Class	p	p.adj	Alder Abundance (N=18)	Beech Abundance (N=17)	Maple Abundance (N=18)
Actinobacteria	0.444	0.99	3.04 $\pm$ 0.74	4.67 $\pm$ 0.95	3.96 $\pm$ 0.84
Alphaproteobacteria	0.368	0.99	11.07 $\pm$ 1.09	14.83 $\pm$ 1.67	12.15 $\pm$ 1.43
Bacilli	0.884	0.99	1.90 $\pm$ 0.64	3.66 $\pm$ 1.41	3.42 $\pm$ 1.32
Bacteroidia	0.423	0.99	5.45 $\pm$ 2.55	1.19 $\pm$ 0.51	3.96 $\pm$ 2.01
Betaproteobacteria	0.529	0.99	18.21 $\pm$ 1.09	16.61 $\pm$ 2.28	15.55 $\pm$ 1.92
Chitinophagia	0.834	0.99	0.30 $\pm$ 0.07	0.41 $\pm$ 0.13	0.43 $\pm$ 0.12
Clostridia	0.692	0.99	3.17 $\pm$ 1.45	1.05 $\pm$ 0.41	2.30 $\pm$ 1.11
Cytophagia	0.331	0.99	7.03 $\pm$ 1.04	3.97 $\pm$ 0.88	6.99 $\pm$ 1.36
Deinococci	0.927	0.99	0.41 $\pm$ 0.18	0.19 $\pm$ 0.09	0.39 $\pm$ 0.18
Flavobacteriia	0.069	0.99	15.77 $\pm$ 1.71	7.11 $\pm$ 0.97	14.25 $\pm$ 1.68
Gammaproteobacteria	0.421	0.99	21.28 $\pm$ 2.54	32.75 $\pm$ 4.37	23.21 $\pm$ 2.97
Opitutae	0.805	0.99	0.21 $\pm$ 0.05	0.26 $\pm$ 0.09	0.51 $\pm$ 0.19
Planctomycetacia	0.894	0.99	0.15 $\pm$ 0.04	0.36 $\pm$ 0.15	0.21 $\pm$ 0.07
Saprospiria	0.994	0.99	0.21 $\pm$ 0.05	0.37 $\pm$ 0.12	0.36 $\pm$ 0.12
Sphingobacteriia	0.587	0.99	1.26 $\pm$ 0.42	0.65 $\pm$ 0.29	1.23 $\pm$ 0.43
Unclassified Bacteria	0.983	0.99	7.66 $\pm$ 1.68	8.24 $\pm$ 2.52	7.33 $\pm$ 1.66
Unclassified Bacteroidetes	0.982	0.99	0.43 $\pm$ 0.11	1.17 $\pm$ 0.41	0.84 $\pm$ 0.26
Unclassified Firmicutes	0.729	0.99	0.21 $\pm$ 0.09	0.39 $\pm$ 0.16	0.18 $\pm$ 0.07
Verrucomicrobiae	0.750	0.99	1.34 $\pm$ 0.41	1.15 $\pm$ 0.39	1.77 $\pm$ 0.59

Table S6. Differences in bacterial class level relative abundance between conditioning i.e., anoxic and oxic. A Kruskal-Wallis test with a Benjamini-Hochberg (BH) correction was used to compare between the two groups. Only bacterial class with greater than 1% of the total relative abundance across all samples are shown. Abundances are given as mean $\pm$ SE. Abbreviations; p: p-values, p.adj: p-adjusted values.

Class	p	p.adj	Anoxic Abundance (N=27)	Oxic Abundance (N=26)
Actinobacteria	0.895	0.94	4.09 $\pm$ 0.73	3.65 $\pm$ 0.65
Alphaproteobacteria	0.002	0.017	9.95 $\pm$ 0.99	15.43 $\pm$ 1.09
Bacilli	0.310	0.89	3.17 $\pm$ 0.92	2.78 $\pm$ 0.99
Bacteroidia	0.009	0.046	6.77 $\pm$ 2.03	0.25 $\pm$ 0.11
Betaproteobacteria	0.691	0.94	17.20 $\pm$ 1.63	16.38 $\pm$ 1.31
Chitinophagia	0.825	0.94	0.37 $\pm$ 0.09	0.39 $\pm$ 0.09
Clostridia	0.005	0.036	4.08 $\pm$ 1.14	0.24 $\pm$ 0.05
Cytophagia	0.171	0.57	4.32 $\pm$ 0.88	7.81 $\pm$ 0.89
Deinococci	0.690	0.94	0.35 $\pm$ 0.13	0.32 $\pm$ 0.14
Flavobacteriia	0.965	0.96	12.08 $\pm$ 1.41	12.89 $\pm$ 1.46
Gammaproteobacteria	0.566	0.94	27.72 $\pm$ 3.05	23.44 $\pm$ 2.61
Opitutae	0.690	0.94	0.31 $\pm$ 0.12	0.36 $\pm$ 0.09
Planctomycetacia	0.757	0.94	0.29 $\pm$ 0.10	0.19 $\pm$ 0.05
Saprospiria	0.787	0.94	0.34 $\pm$ 0.09	0.29 $\pm$ 0.07
Sphingobacteriia	0.001	0.017	0.07 $\pm$ 0.02	2.08 $\pm$ 0.37
Unclassified Bacteria	0.171	0.57	5.65 $\pm$ 1.57	9.89 $\pm$ 1.51
Unclassified Bacteroidetes	0.688	0.94	0.63 $\pm$ 0.17	0.99 $\pm$ 0.28
Unclassified Firmicutes	0.857	0.94	0.30 $\pm$ 0.08	0.22 $\pm$ 0.11
Verrucomicrobiae	0.626	0.94	1.36 $\pm$ 0.37	1.49 $\pm$ 0.40

Statistically significant p-values (< 0.05) are shown in bold

Table S7. Taxonomic classification of bacterial communities with more than 5% relative abundances present in different treatments are shown here.

Sample_type	Leaf species	Conditioning	Phylum	Class	Order	Family	Genus	Relative abundance [%]
Initial_leaves	Alder	Anoxic	Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	Paludibacter	27.585
	Alder	Oxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	21.487
			Unclassified		Unclassified		Unclassified	
	Alder	Oxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	19.791
	Alder	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	13.236
	Alder	Anoxic	Proteobacteria	Betaproteobacteria	Rhodocyclales	Zoogloeaceae	Uliginosibacterium	12.469
	Alder	Oxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	10.363
							Unclassified	
	Alder	Anoxic	Firmicutes	Clostridia	Clostridiales	Natranaerovirga	Natranaerovirga	5.663
							Unclassified	
	Alder	Anoxic	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae	5.428
	Alder	Anoxic	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	5.055
	Beech	Anoxic	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	22.701
	Beech	Oxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	22.341
	Beech	Anoxic	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Tolumonas	18.507
	Beech	Oxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	8.907
	Beech	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	8.849
	Beech	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	7.852
	Beech	Oxic	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	Pantoea	7.316
	Beech	Oxic	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Arcicella	6.423
	Beech	Oxic	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	6.127
	Beech	Anoxic	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella	5.320
	Maple	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	21.242

Appendix Chapter II

	Maple	Oxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	16.539
	Maple	Anoxic	Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	Paludibacter	16.203
	Maple	Anoxic	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Tolumonas	14.602
	Maple	Oxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	12.805
	Maple	Oxic	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Arcicella	9.751
							Unclassified	
	Maple	Anoxic	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae	6.116
	Maple	Anoxic	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Breznakibacter	5.548
	Maple	Anoxic	Proteobacteria	Betaproteobacteria	Rhodocyclales	Zoogloeaceae	Uliginosibacterium	5.107
	Maple	Anoxic	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	5.003
Final_leaves	Alder	Oxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	22.468
	Alder	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	21.634
			Unclassified		Unclassified		Unclassified	
	Alder	Oxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	10.225
			Unclassified		Unclassified		Unclassified	
	Alder	Anoxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	5.790
	Alder	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	5.739
							Unclassified	
	Alder	Oxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	5.051
			Unclassified		Unclassified		Unclassified	
	Beech	Oxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	14.972
	Beech	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	12.234
	Beech	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	9.974
			Unclassified		Unclassified		Unclassified	
	Beech	Anoxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	9.311
							Unclassified	
	Beech	Oxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	8.660

Appendix Chapter II

Unclassified

	Beech	Anoxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	5.848
	Beech	Oxic	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Novosphingobium	5.773
	Beech	Anoxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Aquabacterium	5.707
	Beech	Oxic	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Methylophilaceae	Methylophilus	5.659
	Maple	Oxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	18.650
	Maple	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	14.390
			Unclassified		Unclassified		Unclassified	
	Maple	Anoxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	12.819
			Unclassified		Unclassified		Unclassified	
	Maple	Oxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	12.475
	Maple	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	6.760
							Unclassified	
	Maple	Anoxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	6.111
							Unclassified	
	Maple	Oxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	5.922
Faecal_pellets	Alder	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	16.048
	Alder	Oxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	13.495
	Alder	Oxic	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	Pantoea	12.704
	Alder	Oxic	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella	9.286
							Unclassified	
	Alder	Anoxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	8.387
							Unclassified	
	Alder	Oxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	5.912
			Unclassified		Unclassified		Unclassified	
	Alder	Anoxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	5.394
	Alder	Anoxic	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	5.302

Appendix Chapter II

Beech	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	34.836
Beech	Oxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	19.049
Beech	Oxic	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	12.678
		Unclassified		Unclassified		Unclassified	
Beech	Anoxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	11.787
Beech	Anoxic	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	11.125
		Unclassified		Unclassified		Unclassified	
Beech	Oxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	11.031
Beech	Oxic	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	6.529
						Unclassified	
Beech	Oxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	5.439
Maple	Anoxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	25.815
Maple	Oxic	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	18.772
Maple	Oxic	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	9.961
		Unclassified		Unclassified		Unclassified	
Maple	Oxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	9.529
Maple	Anoxic	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	9.446
Maple	Oxic	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	Pantoea	8.211
						Unclassified	
Maple	Anoxic	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	6.569
Maple	Oxic	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella	6.393
						Unclassified	
Maple	Anoxic	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	5.655
		Unclassified		Unclassified		Unclassified	
Maple	Anoxic	Bacteria	Unclassified Bacteria	Bacteria	Unclassified Bacteria	Bacteria	5.162

Table S8. Alpha diversity indices are based on rarefaction method for FPOM types collected during feeding experiment. Mean and standard deviation for species richness (Observed), Chao1 and Shannon diversity are shown.

FPOM_type	Leaf species	Conditioning	Observed	Chao1	Shannon
Initial_leaves	Alder	Anoxic	86±9	123±30	3.4±0.2
		Oxic	180±11	277±27	4.4±0.1
	Beech	Anoxic	117±8	148±17	3.5±0.1
		Oxic	166±4	276±26	4.0±0.2
	Maple	Anoxic	96±6	130±28	3.4±0.1
		Oxic	169±13	223±25	4.3±0.1
Final_leaves	Alder	Anoxic	199±36	328±88	4.2±0.3
		Oxic	236±33	380±56	4.6±0.3
	Beech	Anoxic	238±8	379±27	4.6±0.1
		Oxic	240±6	392±70	4.8±0.1
	Maple	Anoxic	205±7	335±51	4.4±0.1
		Oxic	258±7	417±23	4.9±0.1
Faecal_pellets	Alder	Anoxic	146±11	256±37	3.9±0.2
		Oxic	153±11	235±19	3.8±0.1
	Beech	Anoxic	97±7	155±34	3.1±0.2
		Oxic	116±4	240±49	3.3±0.3
	Maple	Anoxic	134±7	250±13	3.6±0.2
		Oxic	124±42	206±62	3.5±0.5

Table S9. Results of the linear model to observe the effects of leaf species, conditioning, FPOM type and their interactions on bacterial diversity associated to shredder-produced FPOM. The model structure is $H' \sim \text{leaf species} * \text{conditioning} * \text{FPOM type}$ where alder treatment and faecal pellet for FPOM type was used as reference. Abbreviations; SE: Standard error, H' : Shannon-Wiener Index.

Factors	Estimate	SE	t-value	p-values
FPOM typeFinal_leaves	0.36	0.17	2.06	<0.05
FPOM typeInitial_leaves	-0.45	0.17	-2.60	<0.05
ConditioningOxic	-0.03	0.17	-0.18	0.860
TreatmentBeech	-0.79	0.17	-4.51	<0.05
TreatmentMaple	-0.26	0.17	-1.47	0.151
FPOM typeFinal_leaves:ConditioningOxic	0.44	0.25	1.78	0.084
FPOM typeInitial_leaves:ConditioningOxic	1.00	0.25	4.03	<0.05
FPOM typeFinal_leaves:TreatmentBeech	1.17	0.25	4.73	<0.05
FPOM typeInitial_leaves:TreatmentBeech	0.83	0.25	3.36	<0.05
FPOM typeFinal_leaves:TreatmentMaple	0.40	0.25	1.63	0.112
FPOM typeInitial_leaves:TreatmentMaple	0.28	0.25	1.12	0.270
ConditioningOxic:TreatmentBeech	0.21	0.26	0.81	0.423
ConditioningOxic:TreatmentMaple	-0.14	0.25	-0.55	0.586
FPOM typeFinal_leaves:ConditioningOxic:TreatmentBeech	-0.47	0.36	-1.31	0.197
FPOM typeInitial_leaves:ConditioningOxic:TreatmentBeech	-0.63	0.36	-1.75	0.089
FPOM typeFinal_leaves:ConditioningOxic:TreatmentMaple	0.23	0.35	0.66	0.511
FPOM typeInitial_leaves:ConditioningOxic:TreatmentMaple	0.02	0.35	0.06	0.950

Statistically significant p-values (< 0.05) are shown in bold

Table S10. Taxonomic classification of bacterial communities present in all sample types characterized by Indicator Species Analysis. Taxa associated to different groups with stat value above 0.90 are shown here. All stat values shown here are statistically significant ($p < 0.05$).

Sample type	Phylum	Class	Order	Family	Genus	stat
Initial anoxic						
leaf	Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	Paludibacter	1.000
	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Propionivibrio	0.995
	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Alkalibacter	0.994
					Unclassified	
	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae	0.993
					Unclassified	
	Firmicutes	Clostridia	Clostridiales	Natranaerovirga	Natranaerovirga	0.993
	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerotaenia	0.992
	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Tolumonas	0.992
	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Pseudaeromonas	0.992
	Campilobacterota	Campylobacteria	Campylobacterales	Sulfurospirillaceae	Sulfurospirillum	0.988
	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	Sporomusa	0.988
	Proteobacteria	Betaproteobacteria	Neisseriales	Chromobacteriaceae	Formivibrio	0.987
	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	0.980
	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erwiniaceae	Unclassified Erwiniaceae	0.978
	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Trichococcus	0.977
	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Pectobacteriaceae	Pectobacterium	0.972
					Unclassified	
	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae	0.959
	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	0.958
	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto	0.955
	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Acetobacterium	0.952

Appendix Chapter II

				Unclassified	
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Beijerinckiaceae 0.945
	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas 0.942
				Unclassified	
	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Marinilabiliaceae 0.942
	Proteobacteria	Betaproteobacteria	Neisseriales	Chromobacteriaceae	Iodobacter 0.935
				Unclassified	
	Firmicutes	Clostridia	Clostridiales	Clostridiales	Unclassified Clostridiales 0.929
	Proteobacteria	Betaproteobacteria	Rhodocyclales	Zoogloeaceae	Uliginosibacterium 0.926
				Unclassified	
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales	Unclassified Rhizobiales 0.924
	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	Pelosinus 0.917
	Proteobacteria	Gammaproteobacteria	Enterobacterales	Pectobacteriaceae	Brenneria 0.917
	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Buttiauxella 0.915
				Unclassified	
	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acetobacteraceae 0.914
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium 0.908
All other leaves	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium 1.00
	Unclassified				
	Bacteria	Unclassified Bacteria	Unclassified Bacteria	Unclassified Bacteria	Unclassified Bacteria 0.981
				Unclassified	
	Firmicutes	Clostridia	Clostridiales	Clostridiales	Unclassified Clostridiales 0.975
	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas 0.974
	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Novosphingobium 0.963
	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	Cellvibrio 0.958
	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia 0.955
	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis 0.944

	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.935
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Neorhizobium	0.918
	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	0.910
	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.909
	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhizobacter	0.907
	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	0.902
Faecal pellets			Unclassified	Unclassified	Unclassified	
	Actinobacteria	Actinobacteria	Actinobacteria	Actinobacteria	Actinobacteria	1.000
	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.999
		Unclassified		Unclassified		
	Firmicutes	Firmicutes	Unclassified Firmicutes	Firmicutes	Unclassified Firmicutes	0.997
				Unclassified	Unclassified	
			Gammaproteobacteria	Gammaproteobacteria	Gammaproteobacteria_in	
	Proteobacteria	Gammaproteobacteria	_incertae_sedis	_incertae_sedis	certae_sedis	0.996
	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium	0.995
					Unclassified	
	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	0.992
				Unclassified	Unclassified	
	Actinobacteria	Actinobacteria	Micrococcales	Micrococcales	Micrococcales	0.992
	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leucobacter	0.992
	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus	0.990
	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Subtercola	0.987
				Candidatus_Pelagibac	Unclassified	
	Proteobacteria	Alphaproteobacteria	SAR11	ter	Candidatus_Pelagibacter	0.987
	Deinococcus-					
	Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	0.974
	Actinobacteria	Actinobacteria	Mycobacteriales	Nocardiaceae	Rhodococcus	0.973

Appendix Chapter II

Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Formosimonas	0.968
				Unclassified	
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseriaceae	0.968
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	0.967
			Unclassified	Unclassified	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales	Bacteroidales	0.966
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Cryobacterium	0.965
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	0.964
		Gammaproteobacteria		Unclassified	
Proteobacteria	Gammaproteobacteria	_incertae_sedis	Ignatzschineria	Ignatzschineria	0.957
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.956
		Unclassified	Unclassified	Unclassified	
Proteobacteria	Betaproteobacteria	Betaproteobacteria	Betaproteobacteria	Betaproteobacteria	0.940
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Alkanindiges	0.934
				Unclassified	
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	0.934
Acidobacteria	Blastocatellia	Blastocatellales	Blastocatellaceae	Stenotrophobacter	0.925
Unclassified					
Bacteria	Unclassified Bacteria	Unclassified Bacteria	Unclassified Bacteria	Unclassified Bacteria	0.920
				Unclassified	
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacteriaceae	0.907

Supplementary material Chapter III

Results:

After removing all the undesirable taxa, we used 23,509 reads to identify 1370 taxa with a median of 8964 per DNA sample and 32,262 reads to identify 1370 taxa with a median of 5620 per cDNA sample. After we filtered out low abundance reads, 1248 taxa remained for DNA samples and 1085 taxa for cDNA samples, which we agglomerated into 20 phyla, 77 order, 141 family, and 239 genera for DNA samples and 19 phyla, 61 order, 125 family, and 208 genera for cDNA samples. Comparing the bacterial community composition between DNA and cDNA, we found that there were only small differences. Thus, we mostly focus on the DNA here if not explicitly stated.

Table S1. Taxonomic classification of bacterial communities with more than 2% relative abundances present in different samples are shown here. Here data includes whole genomic DNA.

Month	Sample_type	Treatment	Phylum	Order	Family	Genus	Relative abundance [%]
April	Leaf	Leaves	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	31.63
			Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	22.08
			Proteobacteria	Enterobacterales	Erwiniaceae	Erwinia	13.58
			Bacteroidota	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	5.89
			Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	3.88
	Gut	Allogamus	Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	22.35
			Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	11.41
			Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	9.85
			Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	9.54
			Firmicutes	Lachnospirales	Lachnospiraceae	Tyzzereella	6.62
			Firmicutes	Entomoplasmatales	Spiroplasmataceae	Spiroplasma	6.44
			Bacteroidota	Bacteroidales	Rikenellaceae	Alistipes	5.2
			Bacteroidota	Bacteroidales	Tannerellaceae	Tannerellaceae	2.02
		Potamophylax	Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	21.38

		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	19.4
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	8.99
		Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	7.25
		Firmicutes	Lachnospirales	Lachnospiraceae	Tyzzera	5.4
		Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	4.07
		Bacteroidota	Bacteroidales	Rikenellaceae	Alistipes	3.85
				Unclassified	Unclassified	
		Bacteroidota	Bacteroidales	Bacteroidales	Bacteroidales	2.21
		Desulfobacterota	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	2.17
		Proteobacteria	Burkholderiales	Rhodocyclaceae	Propionivibrio	2.17
	Sericostoma	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	34.11
					Unclassified	
		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	28.5
		Proteobacteria	Burkholderiales	T34	Unclassified T34	13.54
		Unclassified	Unclassified			
		Bacteria	Bacteria	Unclassified Bacteria	Unclassified Bacteria	6.37
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	4.96
				Unclassified	Unclassified	
		Proteobacteria	Cardiobacteriales	Cardiobacteriales	Cardiobacteriales	2.52
Faecal pellets	Allogamus	Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	18.39
		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	10.43
		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	8.13
		Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	5.7
		Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	4.23
		Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	4.12
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	3.49
		Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	3.28
		Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	2.92
		Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	2.57
		Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	2.19
		Proteobacteria	Burkholderiales	Methylophilaceae	Methylothera	2.12
		Proteobacteria	Caulobacteriales	Caulobacteraceae	Brevundimonas	2.03
	Potamophylax	Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	14.44
		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	12.07

			Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	7.99
			Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	7.73
			Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	7.09
			Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	5.33
			Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	3.92
			Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	3.3
			Bacteroidota	Bacteroidales	Rikenellaceae	Alistipes	2.59
			Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	2.40
					Unclassified	Unclassified	
			Proteobacteria	Rhodospirillales	Rhodospirillales	Rhodospirillales	2.17
		Sericostoma	Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	22.03
			Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	17.42
			Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	8.92
			Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	5.23
					Unclassified	Unclassified	
			Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	5.13
			Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	4.95
			Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	4.45
			Bacteroidota	Cytophagales	Spirosomaceae	Arcicella	2.98
			Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	2.8
May	Leaf	Leaves	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	47.59
			Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	14.92
			Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	5.01
			Proteobacteria	Enterobacterales	Erwiniaceae	Erwinia	4.26
			Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	3.16
	Gut	Allogamus	Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	16.83
			Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	12.86
			Firmicutes	Lachnospirales	Lachnospiraceae	Tyzzera	10.09
			Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	9.98
			Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	8.16
			Bacteroidota	Bacteroidales	Rikenellaceae	Alistipes	5.04
			Firmicutes	Entomoplasmatales	Spiroplasmataceae	Spiroplasma	3.07

		Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified Burkholderiaceae	2.66
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	2.13
	Potamophylax	Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	18.01
		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	17.75
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	10.7
		Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	6.35
		Firmicutes	Lachnospirales	Lachnospiraceae	Tyzzera	5.15
		Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	5.11
		Bacteroidota	Bacteroidales	Rikenellaceae	Alistipes	3.67
				Unclassified		
		Bacteroidota	Bacteroidales	Tannerellaceae	Tannerellaceae	2.67
				Unclassified		
		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonadaceae	2.11
				Unclassified		
	Sericostoma	Proteobacteria	Enterobacterales	Enterobacterales	Enterobacterales	2.07
		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	33.64
		Proteobacteria	Burkholderiales	T34	Unclassified T34	18.57
				Unclassified		
		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	16.72
		Firmicutes	Lactobacillales	Carnobacteriaceae	Carnobacterium	7.85
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	7.06
				Unclassified	Unclassified	
		Proteobacteria	Cardiobacteriales	Cardiobacteriales	Cardiobacteriales	3.27
		Unclassified	Unclassified			
		Bacteria	Bacteria	Unclassified Bacteria	Unclassified Bacteria	2.36
		Proteobacteria	Rickettsiales	Rickettsiaceae	Rickettsia	2.11
Faecal pellets	Allogamus	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	15.5
		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	12.46
		Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	11.68
		Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	9.53
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	4.8
		Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	4.18
		Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	3.1
		Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	2.74

Potamophylax	Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	2.35
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	2.08
	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	13.75
	Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	12.86
	Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	11.21
	Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	10.79
	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	8.06
	Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	6.11
Sericostoma	Bacteroidota	Bacteroidales	Rikenellaceae	Mucinivorans	2.86
	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	42.79
	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	8.59
	Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	6.63
	Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	6.53
	Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	4.15
				Unclassified	
	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	3.23
	Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	2.64
			Unclassified	Unclassified	
	Proteobacteria	Enterobacterales	Enterobacterales	Enterobacterales	2.48
			Unclassified	Unclassified	
	Bacteroidota	Chitinophagales	Chitinophagales	Chitinophagales	2.28
	Unclassified	Unclassified			
	Bacteria	Bacteria	Unclassified Bacteria	Unclassified Bacteria	2.08
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	2.02

Table S2. Taxonomic classification of active bacterial communities with more than 2% relative abundances present in different samples are shown here. Here data includes complementary DNA.

Month	Sample_type	Treatment	Phylum	Order	Family	Genus	Relative Abundance [%]
April	Leaves	Leaves	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	31.91
			Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	16.92
			Proteobacteria	Burkholderiales	Comamonadaceae	Inhella	6.05
			Proteobacteria	Enterobacterales	Erwinaceae	Erwinia	5.22
			Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	4.22
			Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	3.49
			Proteobacteria	Pseudomonadales	Cellvibrionaceae	Cellvibrio	2.43
			Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	2.38
	Faecal pellets	Allogamus	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	21.05
			Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	6.27
			Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	5.76
			Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	5.49
			Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	5.00
						Unclassified	
			Proteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	4.26
			Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	3.83
			Proteobacteria	Burkholderiales	Comamonadaceae	Roseateles	2.96
			Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	2.39
			Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	2.32
			Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	2.28
	Potamophylax		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	15.30
			Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	7.54
			Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	6.80
			Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	6.21
			Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	5.79
			Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	4.44

		Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	4.17
		Firmicutes	Lachnospirales	Lachnospiraceae	Tyzzera	3.94
				Unclassified	Unclassified	
		Proteobacteria	Rhodospirillales	Rhodospirillales	Rhodospirillales	2.84
		Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	2.67
		Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	2.51
		Bacteroidota	Bacteroidales	Rikenellaceae	Alistipes	2.07
	Sericostoma	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	22.74
		Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	10.45
		Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	10.10
		Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	7.48
		Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	4.70
		Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	4.65
		Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	3.83
		Proteobacteria	Burkholderiales	Oxalobacteraceae	Undibacterium	3.32
		Proteobacteria	Burkholderiales	Comamonadaceae	Roseateles	3.02
					Unclassified	
		Proteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	2.94
		Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	2.48
					Unclassified	
		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	2.11
May	Leaves	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	43.14
		Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	9.30
		Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	7.69
		Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	5.01
		Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	2.76
					Unclassified	
		Proteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	2.33
		Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	2.21
		Proteobacteria	Enterobacterales	Erwiniaceae	Erwinia	2.18
	Faecal pellets	Allogamus	Bacteroidota	Flavobacteriales	Flavobacteriaceae	10.40
			Proteobacteria	Pseudomonadales	Pseudomonadaceae	9.55
			Bacteroidota	Bacteroidales	Williamwhitmaniaceae	8.29
			Proteobacteria	Burkholderiales	Comamonadaceae	6.66

	Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	4.29
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	3.74
	Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	3.09
	Proteobacteria	Burkholderiales	Comamonadaceae	Roseateles	2.93
				Unclassified	
	Proteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	2.75
	Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	2.58
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	2.54
	Bacteroidota	Sphingobacteriales	env.OPS 17	Unclassified env.OPS 17	2.30
Potamophylax	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	12.83
	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	11.75
	Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	11.00
	Bacteroidota	Bacteroidales	Bacteroidaceae	Bacteroides	6.29
	Bacteroidota	Bacteroidales	Williamwhitmaniaceae	Rs-D38 termite group	6.17
	Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	4.62
	Bacteroidota	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	3.59
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	2.90
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	2.21
	Firmicutes	Lachnospirales	Lachnospiraceae	Tyzzera	2.09
	Proteobacteria	Burkholderiales	Comamonadaceae	Roseateles	2.00
Sericostoma	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacterium	23.46
	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	12.66
	Proteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	7.56
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	6.13
	Proteobacteria	Enterobacterales	Aeromonadaceae	Aeromonas	5.60
				Unclassified	
	Bacteroidota	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae	5.28
				Unclassified	
	Proteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae	3.50
	Bacteroidota	Cytophagales	Spirosomaceae	Flectobacillus	3.46
	Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	2.84
	Proteobacteria	Burkholderiales	Comamonadaceae	Roseateles	2.70

Table S3. Alpha diversity indices are based on rarefaction method for different samples collected during feeding experiment. Mean and standard deviation for species richness (Observed), Chao1 and Shannon diversity based on whole genomic DNA dataset are shown. Abbreviations; L: Leaves, AG: *Allogamous* gut, PG: *Potamophylax* gut, SG: *Sericostoma* gut, AF: *Allogamus* Faecal pellets, PF: *Potamophylax* Faecal pellets and SF: *Sericostoma* Faecal pellets.

Samples	Sample_type	Treatment	Month	Observed	Chao1	Shannon
AF	Faecal pellets	Allogamus	April	306.4±36.85	768.93±58.29	4.26±0.30
AF	Faecal pellets	Allogamus	May	332.2±24.52	874.08±94.97	4.71±0.16
PF	Faecal pellets	Potamophylax	April	303±30.30	668.32±17.68	4.39±0.19
PF	Faecal pellets	Potamophylax	May	304.6±18.30	531.95±36.37	4.58±0.20
SF	Faecal pellets	Sericostoma	April	212.2±10.52	492.90±74.90	3.35±0.10
SF	Faecal pellets	Sericostoma	May	233.2±37.32	524.45±55.93	3.53±0.64
AG	Gut	Allogamus	April	162.44±16.78	284.44±58.96	3.99±0.12
AG	Gut	Allogamus	May	194.6±16.95	328.43±61.12	4.21±0.17
PG	Gut	Potamophylax	April	149.2±21.68	254.20±40.02	3.76±0.43
PG	Gut	Potamophylax	May	175.33±13.70	293.51±44.84	3.92±0.39
SG	Gut	Sericostoma	April	66.28±33.68	154.38±49.59	1.76±0.48
SG	Gut	Sericostoma	May	59.5±20.70	189.66±53.54	1.68±0.42
L	Leaves	Leaves	April	196.3±10.33	418.72±75.24	3.55±0.17
L	Leaves	Leaves	May	190.5±14.62	382.81±57.01	3.59±0.27

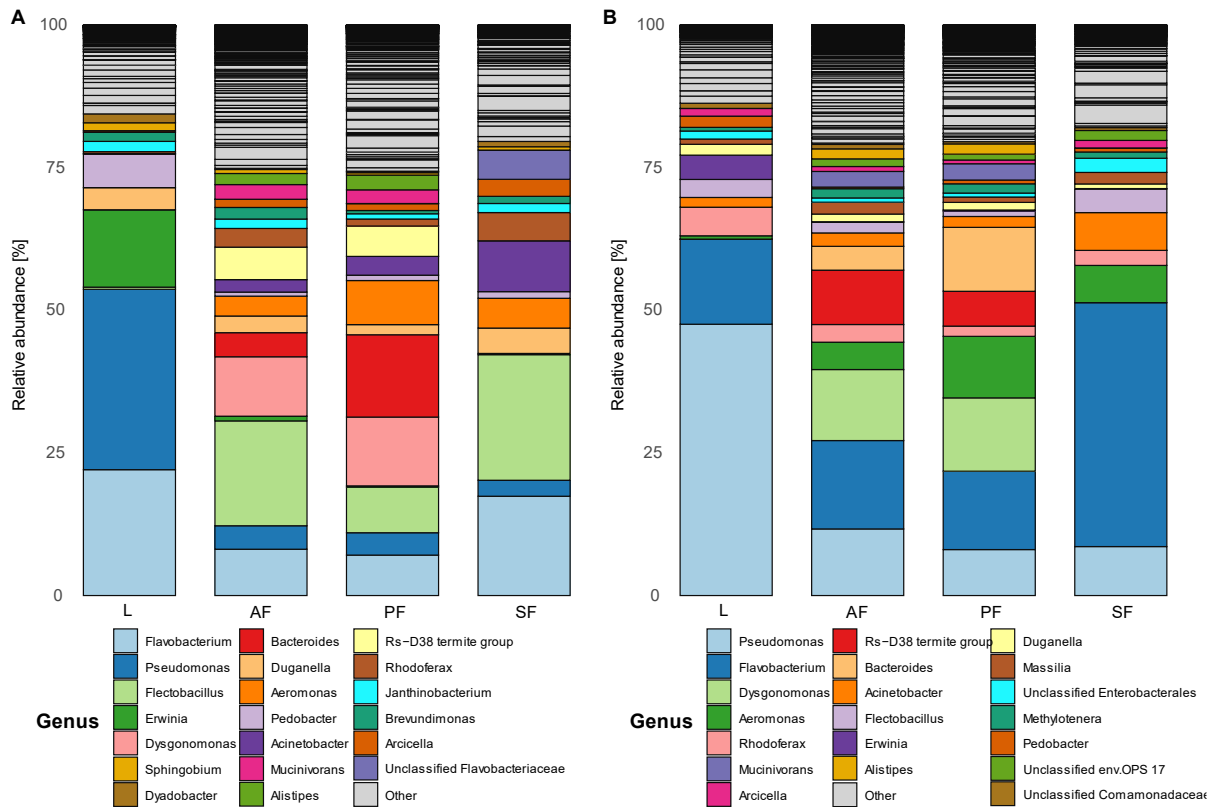


Figure S1. Taxonomic composition and relative abundance of individual taxonomic groups associated with leaves, and Faecal pellets produced by each shredder taxon during the feeding experiment in the month of April (A) and May (B) based on complementary DNA (cDNA) dataset. In Fig. each genus is represented by distinct color and top 20 of in relative abundance of bacterial composition at the genus level are shown whereas the remaining order are grouped as “Other”. Abbreviations; L: Leaves, AF: *Allogamus* Faecal pellets, PF: *Potamophylax* Faecal pellets and SF: *Sericostoma* Faecal pellets.

Table S4. Results of the linear model testing the effects of shredder taxa (treatment = *Allogamus*, *Potamophylax*. and *Sericostoma*.), sample type (Gut vs Faecal pellets), month and their interaction on bacterial diversity. The model structures are $H' \sim \text{Sample_type} * \text{Treatment} * \text{Month}$ and $\text{Chao1} \sim \text{Sample_type} * \text{Treatment} * \text{Month}$ for Shannon and Chao metrics respectively. Abbreviations: SE, standard error; H' : Shannon–Wiener index, Chao1: Chao index. Data used here was whole genomic DNA dataset.

Factors	Shannon				Chao1			
	Estimate	Std. Error	t value	Pr(> t)	Estimate	Std. Error	t value	Pr(> t)
Sample_typeGut	-0.272	0.190	-1.428	0.158	291.591	12.871	22.654	<0.05
TreatmentPotamophylax	0.127	0.216	0.588	0.559	-143.574	15.893	-9.034	0.316
TreatmentSericostoma	-0.913	0.216	-4.226	<0.05	-29.471	15.634	-1.885	<0.05
MonthMay	0.453	0.216	2.098	0.040	19.818	12.774	1.551	0.096
Sample_typeGut:TreatmentPotamophylax	-0.355	0.269	-1.317	0.192	-72.983	22.477	-3.247	0.153
Sample_typeGut:TreatmentSericostoma	-1.313	0.276	-4.757	<0.05	-1.513	22.110	-0.068	0.404
Sample_typeGut:MonthMay	-0.232	0.267	-0.870	0.387	-27.868	18.066	-1.543	0.379
TreatmentPotamophylax:MonthMay	-0.258	0.305	-0.845	0.401	-29.102	22.226	-1.309	0.015
TreatmentSericostoma:MonthMay	-0.267	0.305	-0.875	0.385	83.710	22.025	3.801	0.647
Sample_typeGut:TreatmentPotamophylax:MonthMay	0.198	0.379	0.522	0.603	-32.454	31.433	-1.032	0.013
Sample_typeGut:TreatmentSericostoma:MonthMay	-0.038	0.386	-0.098	0.923	118.010	31.149	3.789	0.969

Statistically significant p-values (< 0.05) are shown in bold

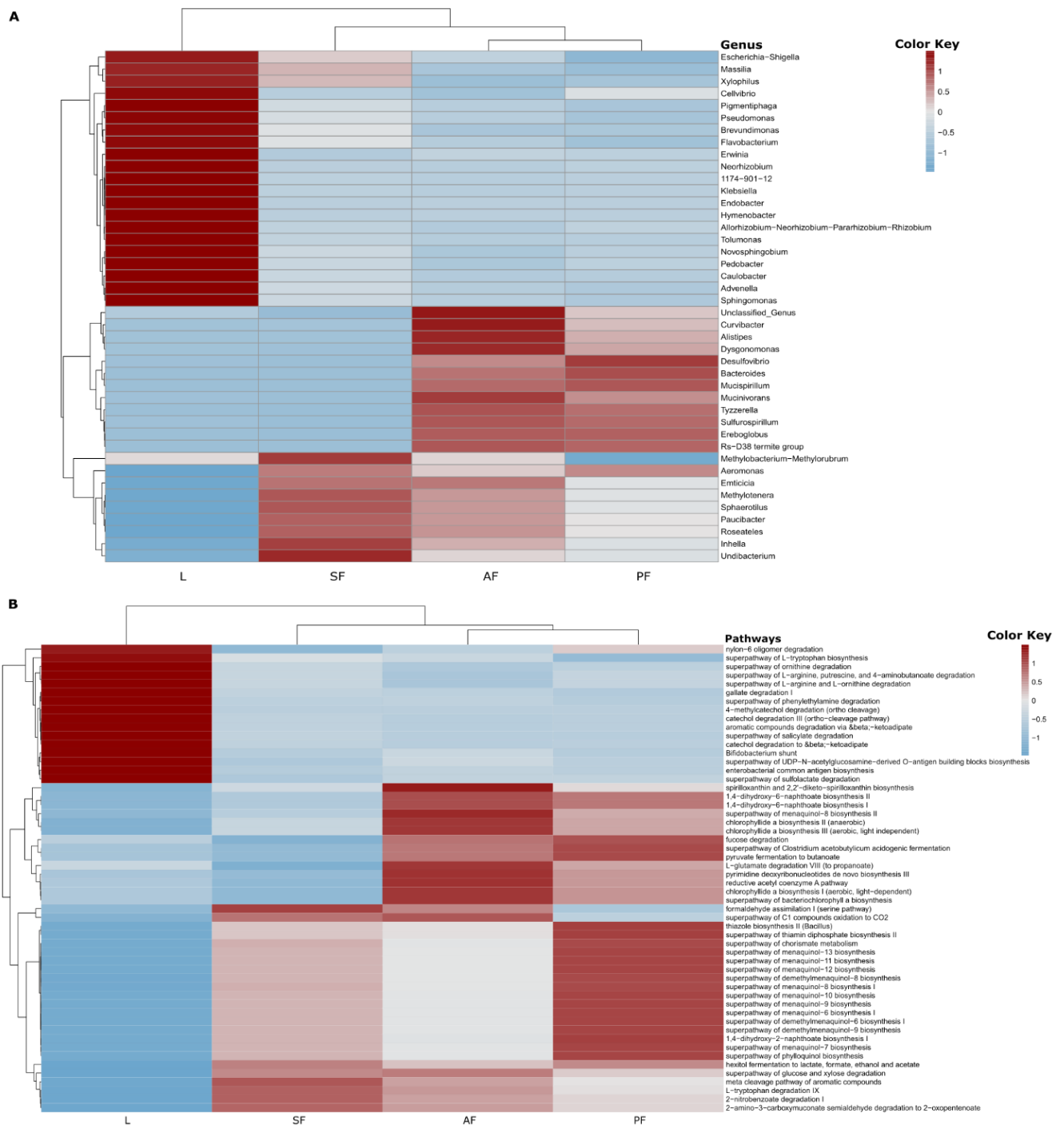


Figure S2. Hierarchical heat map of differentially abundant taxa (A) and pathways (B) in leaves, guts and Faecal pellets of each shredder taxa are shown. Data used here is cDNA dataset. A hierarchical cluster heat map showing the log2 transformed abundant values for leaves, guts and Faecal pellets of each shredder taxa. The differentially abundant individual taxa across the leaves (L) and Faecal pellets of each shredder taxa (AF= *Allogamus* Faecal pellets, PF= *Potamophylax* Faecal pellets, SF= *Sericostoma* Faecal pellets) are shown horizontally. Here color key reflects normalized log2-transformed counts. Skyblue shows lower abundance and red color shows a higher abundance.

Table S5. Summary of the differential pathways identified using DESeq2 analysis. All the significant pathways with a log fold change > 2.5 were listed here. *log2FoldChange (log2FC)* represents the estimated log2-scale effect size for the comparison across the samples. *abs_logFC* is the absolute effect size and reflects the magnitude of change regardless of direction. *baseMean* corresponds to the mean of normalized counts for each pathway across all samples. Data used here was whole genomic DNA dataset.

Pathway	Description	baseMean	log2FoldChange	abs_log2FC
PWY-2941	L-lysine biosynthesis II	349.64	11.27	11.27
P441-PWY	superpathway of N-acetylneuraminate degradation	367.09	10.94	10.94
GLCMANNANAUT-PWY	superpathway of N-acetylglucosamine, N-acetylmannosamine and N-acetylneuraminate degradation	169.17	10.18	10.18
PWY-7431	aromatic biogenic amine degradation (bacteria)	1707.13	-8.03	8.03
CATECHOL-ORTHO-CLEAVAGE-PWY	catechol degradation to β-ketoadipate	593.11	-7.70	7.70
PWY-5417	catechol degradation III (ortho-cleavage pathway)	647.79	-7.68	7.68
PWY-5431	aromatic compounds degradation via β-ketoadipate	647.79	-7.68	7.68
PWY-6071	superpathway of phenylethylamine degradation	697.87	-7.59	7.59
PWY0-321	phenylacetate degradation I (aerobic)	808.74	-7.58	7.58
PWY-6185	4-methylcatechol degradation (ortho cleavage)	606.47	-7.46	7.46

PWY-6182	superpathway of salicylate degradation	606.63	-7.45	7.45
PWY-5181	toluene degradation III (aerobic) (via p-cresol)	768.35	-7.43	7.43
DHGLUCONATE-PYR-CAT-PWY	glucose degradation (oxidative)	777.84	-7.36	7.36
PWY-181	photorespiration	378.30	-7.28	7.28
PROTocatechuate-ORTHO-CLEAVAGE-PWY	protocatechuate degradation II (ortho-cleavage pathway)	1167.44	-7.14	7.14
METHGLYUT-PWY	superpathway of methylglyoxal degradation	70.84	-6.86	6.86
DENITRIFICATION-PWY	nitrate reduction I (denitrification)	82.37	-6.77	6.77
GALLATE-DEGRADATION-II-PWY	gallate degradation I	444.60	-6.75	6.75
PWY490-3	nitrate reduction VI (assimilatory)	677.46	-6.72	6.72
PWY-7376	cob(II)yrinate a,c-diamide biosynthesis II (late cobalt incorporation)	679.65	-6.61	6.61
PWY-7098	vanillin and vanillate degradation II	162.22	-6.19	6.19
PWY-6338	superpathway of vanillin and vanillate degradation	149.66	-6.16	6.16
PWY-7097	vanillin and vanillate degradation I	149.66	-6.16	6.16
PWY-1361	benzoyl-CoA degradation I (aerobic)	95.80	-6.09	6.09
PWY-5655	L-tryptophan degradation IX	22.81	-5.82	5.82
PWY-7007	methyl ketone biosynthesis	320.61	-5.74	5.74
P125-PWY	superpathway of (R,R)-butanediol biosynthesis	89.26	-5.56	5.56

P381-PWY	adenosylcobalamin biosynthesis II (late cobalt incorporation)	70.15	-5.52	5.52
PWY-7237	myo-, chiro- and scillo-inositol degradation	1087.26	-5.34	5.34
PWY-6396	superpathway of 2,3-butanediol biosynthesis	119.87	-5.31	5.31
THREOCAT-PWY	superpathway of L-threonine metabolism	26.16	-5.28	5.28
PWY-7090	UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate biosynthesis	24.58	-5.14	5.14
PWY-5941	glycogen degradation II (eukaryotic)	30.57	-5.13	5.13
PWY-5651	L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde	1437.39	-5.13	5.13
PWY-5647	2-nitrobenzoate degradation I	12.16	-5.01	5.01
NADSYN-PWY	NAD biosynthesis II (from tryptophan)	1107.82	-4.98	4.98
PWY-5531	chlorophyllide a biosynthesis II (anaerobic)	7.06	-4.78	4.78
PWY-7159	chlorophyllide a biosynthesis III (aerobic, light independent)	7.06	-4.78	4.78
PWY-5654	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate	10.10	-4.73	4.73
ORNDEG-PWY	superpathway of ornithine degradation	874.87	-4.65	4.65
PWY-5420	catechol degradation II (meta-cleavage pathway)	23.01	-4.65	4.65
PWY-722	nicotinate degradation I	16.96	-4.63	4.63
PWY-1861	formaldehyde assimilation II (RuMP Cycle)	1137.35	-4.38	4.38
RUMP-PWY	formaldehyde oxidation I	756.86	-4.32	4.32

PWY-5705	allantoin degradation to glyoxylate III	162.09	-4.30	4.30
PWY-5178	toluene degradation IV (aerobic) (via catechol)	14.61	-4.25	4.25
PWY-5419	catechol degradation to 2-oxopent-4-enoate II	17.85	-4.16	4.16
PWY0-1277	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation	13.26	-4.15	4.15
PWY-6562	norspermidine biosynthesis	597.89	-4.14	4.14
PWY-6339	syringate degradation	623.83	-4.06	4.06
ARGDEG-PWY	superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation	420.49	-4.00	4.00
ORNARGDEG-PWY	superpathway of L-arginine and L-ornithine degradation	420.49	-4.00	4.00
PWY-7295	L-arabinose degradation IV	53.42	-3.96	3.96
PWY-5971	palmitate biosynthesis II (bacteria and plants)	500.26	3.94	3.94
PWY-5430	meta cleavage pathway of aromatic compounds	7.70	-3.90	3.90
NAD-BIOSYNTHESIS-II	NAD salvage pathway II	768.49	-3.76	3.76
P562-PWY	myo-inositol degradation I	362.90	-3.49	3.49
PWY-5180	toluene degradation I (aerobic) (via o-cresol)	31.94	-3.31	3.31
PWY-5182	toluene degradation II (aerobic) (via 4-methylcatechol)	31.94	-3.31	3.31
PWY-7373	superpathway of demethylmenaquinol-6 biosynthesis II	120.04	3.13	3.13

P124-PWY	Bifidobacterium shunt	437.07	-3.11	3.11
PWY-6471	peptidoglycan biosynthesis IV (Enterococcus faecium)	66.26	3.08	3.08
PWY-5507	adenosylcobalamin biosynthesis I (early cobalt insertion)	65.15	-2.99	2.99
PWY0-1338	polymyxin resistance	279.39	-2.89	2.89
PWY-6901	superpathway of glucose and xylose degradation	1461.17	2.86	2.86
PWY-6263	superpathway of menaquinol-8 biosynthesis II	647.46	2.80	2.80
PWY-7254	TCA cycle VII (acetate-producers)	3859.53	-2.72	2.72
HCAMHPDEG-PWY	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate	5.53	-2.67	2.67
PWY-6690	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate	5.53	-2.67	2.67
PWY-5415	catechol degradation I (meta-cleavage pathway)	11.30	-2.61	2.61
P122-PWY	heterolactic fermentation	75.15	-2.59	2.59
PWY-5910	superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)	55.24	-2.59	2.59
PWY0-1533	methylphosphonate degradation I	351.26	-2.58	2.58
PWY-922	mevalonate pathway I	40.50	-2.57	2.57

Table S6. Summary of the differential pathways identified using DESeq2 analysis. All the significant pathways with a log fold change > 2 were listed here. *log2FoldChange* (*log2FC*) represents the estimated log2-scale effect size for the comparison across the samples. *abs_logFC* is the absolute effect size and reflects the magnitude of change regardless of direction. *baseMean* corresponds to the mean of normalized counts for each pathway across all samples. Data used here was cDNA dataset.

Pathway	Description	baseMean	log2FoldChange	abs_log2FC
PWY-6545	pyrimidine deoxyribonucleotides de novo biosynthesis III	43.94	-7.76	7.76
CODH-PWY	reductive acetyl coenzyme A pathway	26.03	-6.66	6.66
PWY-7371	1,4-dihydroxy-6-naphthoate biosynthesis II	62.27	-3.52	3.52
PWY-7374	1,4-dihydroxy-6-naphthoate biosynthesis I	62.07	-3.52	3.52
CHLOROPHYLL-SYN	chlorophyllide a biosynthesis I (aerobic, light-dependent)	56.17	-6.47	6.47
CENTFERM-PWY	pyruvate fermentation to butanoate	199.81	-6.88	6.88
PWY-6590	superpathway of Clostridium acetobutylicum acidogenic fermentation	240.31	-6.91	6.91
PWY-5529	superpathway of bacteriochlorophyll a biosynthesis	67.19	-6.22	6.22
PWY-6182	superpathway of salicylate degradation	693.30	2.17	2.17
PWY-6185	4-methylcatechol degradation (ortho cleavage)	720.03	2.35	2.35
PWY-5417	catechol degradation III (ortho-cleavage pathway)	707.14	2.28	2.28
PWY-5431	aromatic compounds degradation via β-ketoadipate	707.14	2.28	2.28
CATECHOL-ORTHO-CLEAVAGE-PWY	catechol degradation to β-ketoadipate	652.44	2.25	2.25
GALLATE-DEGRADATION-II-PWY	gallate degradation I	523.98	2.80	2.80
PWY-6071	superpathway of phenylethylamine degradation	796.60	2.39	2.39
PWY-5531	chlorophyllide a biosynthesis II (anaerobic)	91.44	-4.53	4.53
PWY-7159	chlorophyllide a biosynthesis III (aerobic, light independent)	91.44	-4.53	4.53
PWY-6581	spirilloxanthin and 2,2'-diketo-spirilloxanthin biosynthesis	43.69	-4.24	4.24
P124-PWY	Bifidobacterium shunt	275.16	2.50	2.50
ECASYN-PWY	enterobacterial common antigen biosynthesis	100.36	4.28	4.28
PWY-6901	superpathway of glucose and xylose degradation	655.94	-3.46	3.46
P461-PWY	hexitol fermentation to lactate, formate, ethanol and acetate	172.32	-2.10	2.10
PWY-5088	L-glutamate degradation VIII (to propanoate)	14.17	-4.11	4.11
PWY-6891	thiazole biosynthesis II (Bacillus)	348.42	-3.11	3.11
PWY-1622	formaldehyde assimilation I (serine pathway)	105.83	-5.58	5.58

ARGDEG-PWY	superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation	244.65	2.66	2.66
ORNARGDEG-PWY	superpathway of L-arginine and L-ornithine degradation	244.65	2.66	2.66
PWY-5840	superpathway of menaquinol-7 biosynthesis	596.25	-2.59	2.59
PWY-5430	meta cleavage pathway of aromatic compounds	68.89	-2.95	2.95
PWY-5654	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate	33.93	-2.95	2.95
PWY-5647	2-nitrobenzoate degradation I	38.61	-2.95	2.95
PWY-5861	superpathway of demethylmenaquinol-8 biosynthesis	544.80	-2.64	2.64
PWY-5860	superpathway of demethylmenaquinol-6 biosynthesis I	454.13	-2.72	2.72
PWY-5862	superpathway of demethylmenaquinol-9 biosynthesis	454.13	-2.72	2.72
ORNDEG-PWY	superpathway of ornithine degradation	916.25	2.47	2.47
PWY-5838	superpathway of menaquinol-8 biosynthesis I	702.28	-2.52	2.52
PWY-5897	superpathway of menaquinol-11 biosynthesis	655.84	-2.54	2.54
PWY-5898	superpathway of menaquinol-12 biosynthesis	655.84	-2.54	2.54
PWY-5899	superpathway of menaquinol-13 biosynthesis	655.84	-2.54	2.54
PWY-5655	L-tryptophan degradation IX	66.81	-2.83	2.83
PWY-5845	superpathway of menaquinol-9 biosynthesis	595.45	-2.61	2.61
PWY-5850	superpathway of menaquinol-6 biosynthesis I	595.45	-2.61	2.61
PWY-5896	superpathway of menaquinol-10 biosynthesis	595.45	-2.61	2.61
PWY-5837	1,4-dihydroxy-2-naphthoate biosynthesis I	340.81	-2.68	2.68
PWY-5863	superpathway of phylloquinol biosynthesis	369.64	-2.65	2.65
FUCCAT-PWY	fucose degradation	95.79	-2.88	2.88
PWY-7332	superpathway of UDP-N-acetylglucosamine-derived O-antigen building blocks biosynthesis	162.00	2.22	2.22
PWY-6263	superpathway of menaquinol-8 biosynthesis II	65.50	-3.11	3.11
PWY-6895	superpathway of thiamin diphosphate biosynthesis II	978.25	-2.52	2.52
ALL-CHORISMATE-PWY	superpathway of chorismate metabolism	694.62	-2.20	2.20
PWY-1882	superpathway of C1 compounds oxidation to CO ₂	47.05	-2.91	2.91
PWY-6641	superpathway of sulfolactate degradation	29.49	2.03	2.03
P621-PWY	nylon-6 oligomer degradation	18.16	2.17	2.17
PWY-6629	superpathway of L-tryptophan biosynthesis	112.23	2.17	2.17

Table S7. Table showing the unique pathways predicted for different sample groups based on whole genomic DNA dataset.

Sample_Group	Pathway	Group_Prevalence	Other_Prevalence	Description
Allogamus gut	PWY-7373	1	0.48	superpathway of demethylmenaquinol-6 biosynthesis II
Sericoctoma gut	GOLPDLCAT-PWY	0.93	0.47	superpathway of glycerol degradation to 1,3-propanediol
Sericoctoma gut	PWY-5971	1	0.47	palmitate biosynthesis II (bacteria and plants)
Sericoctoma gut	PWY-6471	1	0.47	peptidoglycan biosynthesis IV (Enterococcus faecium)
Potamophylax gut	PWY-7373	1	0.49	superpathway of demethylmenaquinol-6 biosynthesis II
Leaves	PWY-5941	1	0.43	glycogen degradation II (eukaryotic)
Allogamus Faecal pellets	HCAMHPDEG-PWY	0.9	0.42	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate
Allogamus Faecal pellets	P381-PWY	1	0.38	adenosylcobalamin biosynthesis II (late cobalt incorporation)
Allogamus Faecal pellets	PWY-5178	0.9	0.48	toluene degradation IV (aerobic) (via catechol)
Allogamus Faecal pellets	PWY-5430	0.9	0.41	meta cleavage pathway of aromatic compounds
Allogamus Faecal pellets	PWY-5507	1	0.46	adenosylcobalamin biosynthesis I (early cobalt insertion)
Allogamus Faecal pellets	PWY-5531	1	0.27	chlorophyllide a biosynthesis II (anaerobic)
Allogamus Faecal pellets	PWY-5647	1	0.4	2-nitrobenzoate degradation I
Allogamus Faecal pellets	PWY-5654	1	0.4	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate
Allogamus Faecal pellets	PWY-5655	1	0.4	L-tryptophan degradation IX
Allogamus Faecal pellets	PWY-5941	1	0.49	glycogen degradation II (eukaryotic)
Allogamus Faecal pellets	PWY-6690	0.9	0.42	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate

Appendix Chapter III

Allogamus Faecal pellets	PWY-7159	1	0.27	chlorophyllide a biosynthesis III (aerobic, light independent)
Allogamus Faecal pellets	PWY0-1277	0.9	0.42	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation
Allogamus Faecal pellets	THREOCAT-PWY	1	0.28	superpathway of L-threonine metabolism
Sericostoma Faecal pellets	HCAMHPDEG-PWY	1	0.41	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate
Sericostoma Faecal pellets	PWY-5178	1	0.47	toluene degradation IV (aerobic) (via catechol)
Sericostoma Faecal pellets	PWY-5180	0.9	0.35	toluene degradation I (aerobic) (via o-cresol)
Sericostoma Faecal pellets	PWY-5182	0.9	0.35	toluene degradation II (aerobic) (via 4-methylcatechol)
Sericostoma Faecal pellets	PWY-5183	0.9	0.18	superpathway of aerobic toluene degradation
Sericostoma Faecal pellets	PWY-5430	1	0.4	meta cleavage pathway of aromatic compounds
Sericostoma Faecal pellets	PWY-5531	0.9	0.28	chlorophyllide a biosynthesis II (anaerobic)
Sericostoma Faecal pellets	PWY-5647	1	0.4	2-nitrobenzoate degradation I
Sericostoma Faecal pellets	PWY-5654	1	0.4	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate
Sericostoma Faecal pellets	PWY-5655	1	0.4	L-tryptophan degradation IX
Sericostoma Faecal pellets	PWY-5941	1	0.49	glycogen degradation II (eukaryotic)
Sericostoma Faecal pellets	PWY-6690	1	0.41	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate
Sericostoma Faecal pellets	PWY-7159	0.9	0.28	chlorophyllide a biosynthesis III (aerobic, light independent)
Sericostoma Faecal pellets	PWY-7391	0.9	0.15	isoprene biosynthesis II (engineered)
Sericostoma Faecal pellets	PWY0-1277	1	0.41	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation

Potamophylax Faecal pellets	HCAMHPDEG-PWY	0.9	0.42	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate
Potamophylax Faecal pellets	P381-PWY	1	0.38	adenosylcobalamin biosynthesis II (late cobalt incorporation)
Potamophylax Faecal pellets	PWY-5178	1	0.47	toluene degradation IV (aerobic) (via catechol)
Potamophylax Faecal pellets	PWY-5430	1	0.4	meta cleavage pathway of aromatic compounds
Potamophylax Faecal pellets	PWY-5507	1	0.46	adenosylcobalamin biosynthesis I (early cobalt insertion)
Potamophylax Faecal pellets	PWY-5531	1	0.27	chlorophyllide a biosynthesis II (anaerobic)
Potamophylax Faecal pellets	PWY-5647	0.9	0.41	2-nitrobenzoate degradation I
Potamophylax Faecal pellets	PWY-5654	0.9	0.41	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate
Potamophylax Faecal pellets	PWY-5655	0.9	0.41	L-tryptophan degradation IX
Potamophylax Faecal pellets	PWY-5941	1	0.49	glycogen degradation II (eukaryotic)
Potamophylax Faecal pellets	PWY-6690	0.9	0.42	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate
Potamophylax Faecal pellets	PWY-7159	1	0.27	chlorophyllide a biosynthesis III (aerobic, light independent)
Potamophylax Faecal pellets	PWY0-1277	0.9	0.42	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation
Potamophylax Faecal pellets	THREOCAT-PWY	1	0.28	superpathway of L-threonine metabolism