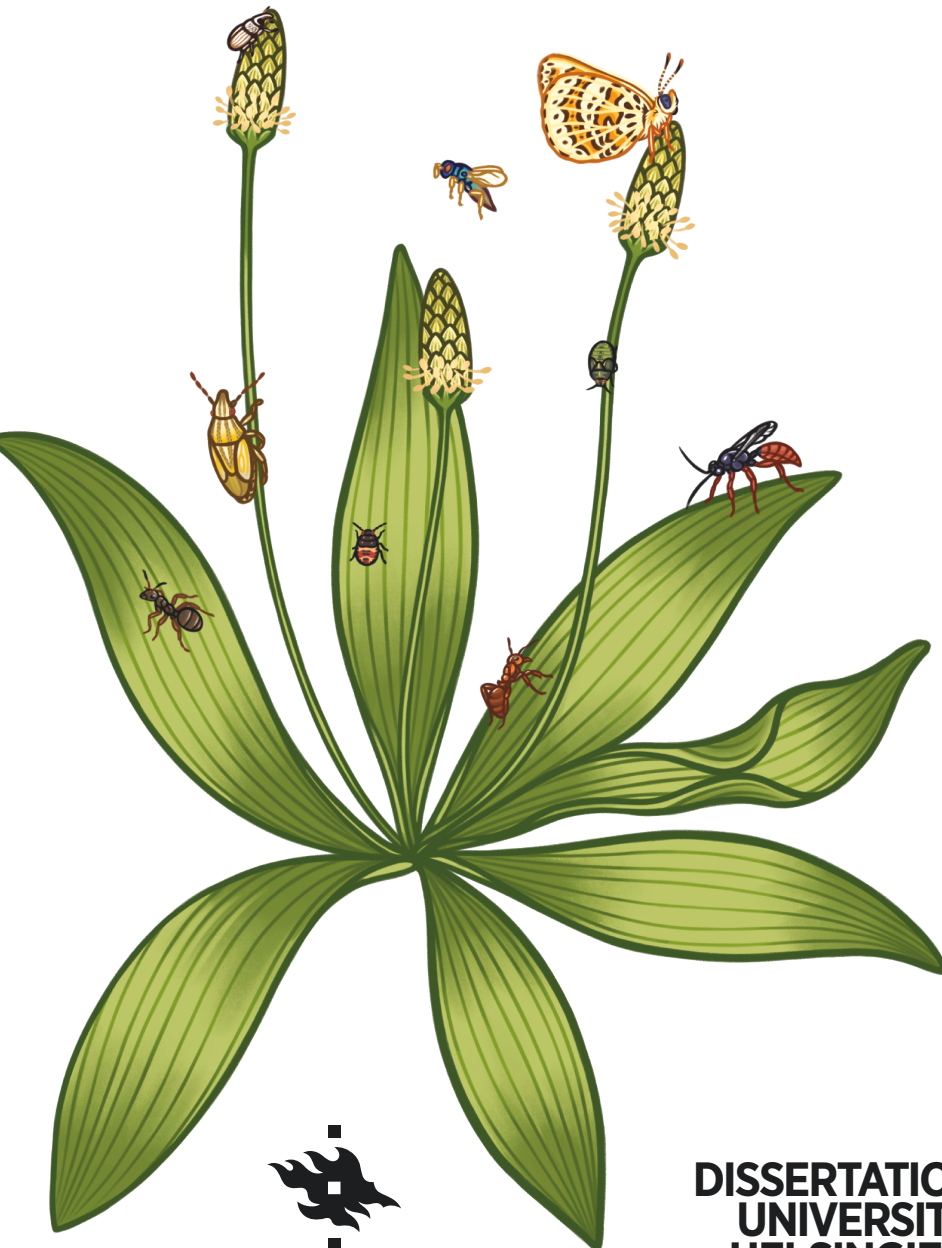


Communities within communities:

ecological interactions and environmental drivers
of microbiota in insect communities

CAMILA SOUZA BERALDO



University of Helsinki
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*ecological interactions and environmental drivers of microbiota in
insect communities*

Camila Souza Beraldo

Doctoral Programme in Wildlife Biology (LUOVA)
Faculty of Biological and Environmental Sciences
University of Helsinki
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Supervised by	Lecturer Anne Duplouy Faculty of Biological and Environmental Sciences Organismal and Evolutionary Biology Research Programme University of Helsinki
	Associate Professor Saskya van Nouhuys Centre for Ecological Sciences Indian Institute of Science
Thesis committee	Assistant Professor Jonna Kulmuni Institute of Biodiversity and Ecosystem Dynamics University of Amsterdam
	Lecturer Juan Galarza Faculty of Science University of Oulu
Pre-examiners	Professor Phillip Watts Faculty of Mathematics and Science Department of Biological and Environmental Science University of Jyväskylä
	Dr. Stefanie Schulz Helmholtz Zentrum München
Opponent	Associate Professor Suvi Ruuskanen Faculty of Mathematics and Science Department of Biological and Environmental Science University of Jyväskylä
Custos	Professor Jarno Vanhatalo Faculty of Biological and Environmental Sciences Organismal and Evolutionary Biology Research Programme University of Helsinki

To Vovó Tonha (1944 - 2023)
In a world spinning ever faster, thank you for being patience

À Vovó Tonha (1944 - 2023)
Em um mundo que gira cada vez mais veloz, obrigada por ser paciência

O apanhador de desperdícios

Uso a palavra para compor meus silêncios.
Não gosto das palavras
fatigadas de informar.
Dou mais respeito
às que vivem de barriga no chão
tipo água pedra sapo.
Entendo bem o sotaque das águas
Dou respeito às coisas desimportantes
e aos seres desimportantes.
Prezo insetos mais que aviões.
Prezo a velocidade
das tartarugas mais que a dos mísseis.
Tenho em mim um atraso de nascença.
Eu fui aparelhado
para gostar de passarinhos.
Tenho abundância de ser feliz por isso.
Meu quintal é maior do que o mundo.
Sou um apanhador de desperdícios:
Amo os restos
como as boas moscas.
Queria que a minha voz tivesse um formato
de canto.
Porque eu não sou da informática:
eu sou da invencionática.
Só uso a palavra para compor meus silêncios.

Manoel de Barros

Abstract

In nature, all organisms are connected by a web of ecological interactions. Among these are the associations between hosts and their microbial partners, or microbiota, which include bacteria, archaea, fungi, protozoa, and microalgae. In insects, one of the most diverse animal groups, microbiota enhances host fitness and are considered key contributors to their evolutionary success. Insect species and communities, along with their microbiota, are influenced by environmental changes, and because microbes play vital roles in insect biology, shifts in microbial communities can have significant consequences for host health and offspring fitness. Yet, the generality of these patterns is only beginning to be understood, especially in wild systems. In addition, effectively communicating these complex and invisible interactions to broader audiences remains a challenge. Bridging this gap requires creative and accessible approaches of science communication to make host–microbe relationships more tangible and engaging. In this thesis, I used the insect community associated with *Plantago lanceolata* (Plantaginaceae) in the Åland Islands, Finland, to examine how ecological interactions and environmental factors influence insect microbiota. I focused on three types of ecological interactions (host–microbe, microbe–parasite, and microbe–microbe) and three environmental drivers (seasonality, habitat quality, and temperature). Finally, I reflected on how to effectively communicate these findings to broader audiences.

I first expanded the known network of insect species associated with *P. lanceolata* and characterised their microbiota, adding a novel layer of diversity that includes interactions with symbionts (**Chapter I**). In **Chapters II** and **III**, I focused on how host species influence the composition and diversity of insect-associated microbial communities. Using 10 insect species, I found that microbiota is primarily structured by host identity, with each species harbouring a distinct microbial community. In **Chapter I**, I also investigated whether infection by a trypanosomatid parasite alters the microbiota of a true bug species. No significant effect was detected, indicating no effect of parasite infection in the microbiota composition or diversity. In **Chapter III**, I examined the microbiota of a parasitoid wasp infected with a widely spread bacterium among arthropods, *Wolbachia*. I observed that individuals carrying *Wolbachia* exhibited markedly different microbiota composition compared to uninfected individuals and hosted fewer bacterial taxa overall.

To evaluate the effects of environmental factors on insect microbiota, I started by exploring how abiotic conditions influence the transfer of microbes among host species (**Chapter IV**). I concluded that environmental factors can both hinder and facilitate novel host–microbe associations. In **Chapter II**, I investigated how seasonality and habitat quality affect bacterial community composition and diversity in eight sympatric insect species. Despite sharing the same environment, insect species responded differently: some showed variation in response to seasonality or habitat quality, while others remained unaffected. Then, I tested the effects of temperature on the microbiota of a butterfly host and its specialist parasitoid (**Chapter III**). Although these species interact closely, they also exhibited distinct microbial responses to temperature. In the parasitoid wasp, this response was further modulated by the presence or absence of *Wolbachia*.

Finally, I developed an art-based activity to help students visualise the complexity of host–microbe–environment interactions (**Chapter V**). Using simple materials as analogies, participants created visual representations of these relationships. The session ended with a mini exhibition

and group discussion to reflect on emerging patterns and the meanings behind the artworks. Taken together, this thesis highlights the central role of host–microbe relationships in shaping insect microbiota, while also showing that microbial variation is influenced by both environmental context and interactions among microbes. Furthermore, this work demonstrates that art can deepen public understanding of complex ecological relationships and exemplifies how participatory approaches can make science more inclusive, engaging, and impactful.

Tiivistelmä

Luonnossa kaikki eliöt ovat yhteydessä toisiinsa ekologisten vuorovaikutusten verkoston kautta. Näihin kuuluvat myös isäntien ja niiden mikrobikumppaneiden eli mikrobiotan väliset suhteet, joihin lukeutuvat bakteerit, arkeonit, sienet, alkueläimet ja mikrolevät. Hyönteisillä, jotka muodostavat yhden eläinkunnan monimuotoisimmista ryhmistä, mikrobiota lisää isännän elinkelpoisuutta ja sitä pidetään keskeisenä tekijänä niiden evolutiivisessa menestyksessä. Sekä hyönteislajit että niiden yhteisöt – mikrobiota mukaan lukien – muotoutuvat ympäristön muutosten vaikutuksesta, koska mikrobit näyttelevät tärkeää roolia hyönteisten biologiassa, mikrobiyhteisöjen muutoksilla voi olla merkittäviä seurauksia isännän terveydelle ja jälkeläisten menestykselle. Kuitenkin näiden ilmiöiden yleistettävyyttä vasta aletaan ymmärtää, erityisesti luonnonvaraisissa järjestelmissä. Näiden monimutkaisten ja näkymättömien vuorovaikutusten viestiminen laajalle yleisölle on edelleen haastavaa. Tämän kuilun kuominen umpeen vaatii luovia ja saavutettavia tieteellisen viestinnän keinoja, jotka tekevät isännän ja mikrobien suhteista käsin koskettavampia ja kiinnostavampia. Tässä väitöskirjassa tutkin, miten ekologiset vuorovaikutukset ja ympäristötekijät vaikuttavat hyönteisten mikrobiotaan käyttäen esimerkkinä *Plantago lanceolata*-kasviin (Plantaginaceae) liittyvää hyönteisyhteisöä Ahvenanmaalla. Keskityin kolmeen ekologiseen vuorovaikutukseen (isäntä–mikrobi, mikrobi–loinen ja mikrobi–mikrobi) ja kolmeen ympäristötekijään (vuodenaikojen vaihtelu, elinympäristön laatu ja lämpötila). Lopuksi pohdin, miten tutkimustuloksia voidaan tehokkaasti viestiä laajemmalle yleisölle.

Laajensin ensin tunnettua *P. lanceolata*-kasviin liittyvien hyönteislajien verkostoa ja kuvasin niiden mikrobiotan, lisäen uudenlaisen monimuotoisuuden tason, johon sisältyy vuorovaikutuksia symbionttien kanssa (**Luku I**). **Luvuissa II ja III** tarkastelin, miten isäntälaji vaikuttaa hyönteisten mikrobiyhteisöjen koostumukseen ja monimuotoisuuteen. Käyttäen kymmentä hyönteislajia havaitsin, että mikrobiota määräytyy ensisijaisesti isännän mukaan, ja jokaisella lajilla oli sille ominainen mikrobiyhteisö. **Luvussa I** tutkin, muuttaako trypanosomaattiloisen aiheuttama infektio erään ludelajin mikrobiotaa. Infektion vaikutusta ei havaittu, mikä viittaa siihen, ettei loinen vaikuttanut mikrobiotan koostumukseen tai monimuotoisuuteen. **Luvussa III** tarkastelin loispistiäisen mikrobiotaa, kun isäntänä oli yleisesti niveljalkaisilla esiintyvä *Wolbachia*-bakteeri. Huomasin, että *Wolbachia*-infektoiduilla yksilöillä mikrobiotan koostumus poikkesi selvästi muista, ja näillä yksilöillä esiintyi vähemmän bakteerilajeja.

Ympäristötekijöiden vaikutusten arvioimiseksi aloitin tutkimalla, miten abioottiset olosuhteet vaikuttavat mikrobien siirtymiseen eri isäntälajien välillä (**Luku IV**). Päätelmäni oli, että ympäristötekijät voivat sekä estää että helpottaa uusien isäntä–mikrobi-suhteiden muodostumista. **Luvussa II** tutkin, miten vuodenaikojen vaihtelu ja elinympäristön laatu vaikuttavat bakteeriyhteisöjen koostumukseen ja monimuotoisuuteen kahdeksalla sympatrisella hyönteislajilla. Vaikka lajit elivät samassa ympäristössä, ne reagoivat eri tavoin: jotkut lajit osoittivat vaihtelua vuodenajan tai elinympäristön laadun mukaan, toiset eivät. Seuraavaksi testasin lämpötilan vaikutusta perhosisännän ja sen erikoistuneen loispistiäisen mikrobiotaan (**Luku III**). Vaikka lajit olivat läheisessä vuorovaikutuksessa, niiden mikrobivasteet lämpötilaan erosivat toisistaan. Pistiäisellä tätä vaikutusta sääтели lisäksi *Wolbachia*-infektion esiintyminen tai puuttuminen.

Lopuksi kehitin taidepohjaisen aktiviteetin, jonka avulla oppilaat saivat mahdollisuuden havainnollistaa isännän, mikrobien ja ympäristön välisten suhteiden monimutkaisuutta (**Luku V**). Yksinkertaisia materiaaleja hyödyntäen osallistujat loivat visuaalisia esityksiä näistä suhteista. Työpaja päättyi pienimuotoiseen näyttelyyn ja yhteiseen keskusteluun, jossa pohdittiin esiin

nousseita teemoja ja teosten merkityksiä. Yhteenvedona väitöskirjani tuo esiin isäntä–mikrobisuhteiden keskeisen roolin hyönteisten mikrobiotän muotoutumisessa sekä osoittaa, että mikrobien vaihtelua säätelevät niin ympäristön olosuhteet kuin mikrobien väliset vuorovaikutukset. Lisäksi työni osoittaa, että taide voi syventää yleisön ymmärrystä monimutkaisista ekologisista suhteista ja että osallistavat lähestymistavat voivat tehdä tieteestä saavutettavampaa, kiinnostavampaa ja vaikuttavampaa.

Resumo

Na natureza, todos os organismos estão conectados por uma rede de interações ecológicas. Entre essas interações estão as associações entre hospedeiros e seus parceiros microbianos, ou microbiota, que incluem bactérias, arqueias, fungos, protozoários e microalgas. Nos insetos, um dos grupos animais mais diversos, a microbiota melhora a aptidão dos hospedeiros e é considerada um dos principais fatores que contribuíram para seu sucesso evolutivo. As espécies e comunidades de insetos, juntamente com suas microbiotas, são influenciadas por mudanças ambientais e, como os microrganismos desempenham papéis essenciais na biologia dos insetos, alterações nas comunidades microbianas podem ter consequências significativas para a saúde dos hospedeiros e a aptidão de sua prole. No entanto, a generalidade desses padrões ainda está começando a ser compreendida, especialmente em sistemas naturais. Além disso, comunicar essas interações complexas e invisíveis a públicos mais amplos continua sendo um desafio. Preencher essa lacuna exige abordagens criativas e acessíveis de comunicação científica, que tornem as relações entre hospedeiros e microrganismos mais tangíveis e envolventes. Nesta tese, utilizei a comunidade de insetos associada à *Plantago lanceolata* (Plantaginaceae) nas Ilhas Åland, Finlândia, para investigar como interações ecológicas e fatores ambientais influenciam a microbiota de insetos. Foquei em três tipos de interações ecológicas (hospedeiro–microrganismo, microrganismo–parasita e microrganismo–microrganismo) e em três fatores ambientais (sazonalidade, qualidade do habitat e temperatura). Por fim, refleti sobre como comunicar tais resultados de forma eficaz a públicos mais amplos.

Primeiramente, ampliei a rede conhecida de espécies de insetos associadas à *P. lanceolata* e caracterizei suas microbiotas, adicionando uma nova camada de diversidade que inclui interações com simbios (Capítulo I). Nos Capítulos II e III, explorei como a identidade do hospedeiro influencia a composição e diversidade das comunidades microbianas associadas aos insetos. Utilizando dez espécies de insetos, constatei que a microbiota é estruturada principalmente pela identidade do hospedeiro, com cada espécie abrigando uma comunidade microbiana distinta. No Capítulo I, também investiguei como a infecção por um parasita tripanossomatídeo altera a microbiota de uma espécie de percevejo. Nenhum efeito significativo foi detectado, indicando que a infecção parasitária não influenciou a composição ou diversidade da microbiota. No Capítulo III, examinei a microbiota de uma vespa parasitoide infectada com uma bactéria amplamente disseminada entre artrópodes, a *Wolbachia*. Observei que indivíduos portadores de *Wolbachia* apresentaram composição microbiana diferente dos indivíduos não infectados, além de abrigarem um número menor de táxons bacterianos.

Para avaliar os efeitos de fatores ambientais sobre a microbiota de insetos, comecei explorando como condições abióticas influenciam a transferência de microrganismos entre espécies hospedeiras (Capítulo IV). Concluí que fatores ambientais podem tanto dificultar quanto facilitar associações novas entre hospedeiros e microrganismos. No Capítulo II, investiguei como a sazonalidade e a qualidade do habitat afetam a composição e a diversidade das comunidades bacterianas em oito espécies de insetos simpátricas. Apesar de compartilharem o mesmo ambiente, as espécies responderam de forma distinta: algumas apresentaram variações em resposta à sazonalidade ou à qualidade do habitat, enquanto outras não foram afetadas. Em seguida, testei os efeitos da temperatura sobre a microbiota de uma borboleta hospedeira e de seu parasitoide especialista (Capítulo III). Embora essas espécies interajam intimamente, elas

também apresentaram respostas microbianas distintas à temperatura. Na vespa parasitoide, essa resposta foi modulada pela presença ou ausência de *Wolbachia*.

Por fim, desenvolvi uma atividade artística para ajudar estudantes a visualizarem a complexidade das interações entre hospedeiros, microrganismos e ambiente (**Capítulo V**). Utilizando materiais simples e analogias, os participantes criaram representações visuais dessas relações. A sessão terminou com uma mini exposição e uma discussão em grupo para refletir sobre os padrões emergentes e os significados das obras. Em conjunto, esta tese destaca o papel central das relações hospedeiro–microrganismo na formação da microbiota de insetos, ao mesmo tempo em que demonstra que a variação da microbiota é influenciada tanto pelo contexto ambiental quanto pelas interações entre microrganismos. Além disso, este trabalho mostra que a arte pode aprofundar a compreensão pública de relações ecológicas complexas e exemplifica como abordagens participativas podem tornar a ciência mais inclusiva, envolvente e impactante.

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“(...) Tudo o que é ruim de passar, é bom de contar. E tudo o que é bom de passar, é ruim de contar.”

Ariano Suassuna

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Preface

“Art is something unpredictable, it’s discovery, it’s an invention of life. (...) Art exists because life is not enough”.
Ferreira Gullar

I grew up in a small countryside town in the state of São Paulo, Brazil. Being surrounded by tropical forests sparked my fascination and curiosity about life sciences. At the same time, raised by artistic parents, paints, crayons and all sorts of papers were always available to my sassy creativity. As a child, these two sides of me, science and art, coexisted in harmony. But as a teenager, when the time to choose a university degree came, one side took the lead. I made a practical decision: *“I’ll do what I consider the most difficult first, Biological Sciences. Art will come second”*. Once I entered university, however, art had to step aside completely so I could keep up with the demands of the hard sciences.

As the scientific life progressed, art received little attention in my routine. I pursued a Master’s degree focused on host–pathogen interactions in model organisms. Motivated to expand my research interests, I moved to Finland to pursue a project that aligned perfectly with my goals: studying host–symbiont interactions in wild populations and communities. However, it was not until the onset of the COVID-19 pandemic, during a time of personal questioning and struggle for meaning (Souza Beraldo, 2024), that I realized science alone would not sustain me. At that point, I became more involved with science communication, and I saw in it the space to merge science and art, an attempt to rescue a piece of my old self. I began to explore how my artistic side could enrich my scientific work. Yet, I found it challenging to translate the invisible world of microbes into something tangible for non-specialist audiences. How could I represent microscopic relationships in ways that would resonate more deeply with a broader public? This question followed me throughout my PhD and ultimately helped shape the structure of this thesis.

Here, I begin by exploring the nature of symbiosis, the diversity of host–microbe associations, and how ecological interactions and environmental factors influence insect-associated microbial communities. After examining the scientific questions that drive my research, I return to the question that connects both sides of me: how can we use art to communicate the invisible, making microbial life more accessible, engaging, and inspiring? This thesis reflects a journey that has been both challenging and full of discoveries — scientifically and personally. I hope I have captured at least a glimpse of that experience in the pages that follow.

Table of contents

List of original publications	1
Author contributions	1
List of abbreviations	2
I. Introduction	3
(1) Microbial transmission modes and host-microbe associations	3
(2) Factors influencing microbiota composition and diversity	4
(3) The insect community of <i>Plantago lanceolata</i>	5
(4) Science communication on host-microbe interactions	6
II. Aims of the thesis	8
III. Material and methods	11
(1) Systematic search of the literature	17
(2) Insect sampling in the Åland Islands	11
(3) Temperature experimental design	12
(4) Molecular methods, bioinformatics and statistical analyses	14
(a) DNA extraction	14
(b) Library preparation and sequencing	15
(c) Amplicon sequencing data curation and taxonomic assignment	15
(d) Statistical analyses	16
(5) MicrobiART activity preparation	18
IV. Results and discussion	19
(1) Beyond the <i>Plantago lanceolata</i> insect community	19
(2) Effects of ecological interactions on insect microbiota composition and diversity	22
(a) Host-microbe interaction	22
(b) Microbe-parasite interaction	24
(c) Microbe-microbe interaction	24
(3) Effects of environmental factors on insect microbiota composition and diversity	25
(a) Seasonality and habitat quality	25
(b) Temperature	28
(4) Effects of environmental factors on host shifts	29
(5) Art as a tool to communicate my PhD thesis	32
V. Conclusions and future directions	34
References	36

List of original publications

The thesis is based on the following articles, referred to by their roman numerals in the text:

I

Beraldo, C.S., Buczek, M., Franco, D.C., Michalik, A., Łukasik, P., van Nouhuys, S., Duplouy, A. (2025). *COI* barcoding reveals ecological interactions within the insect community associated with *Plantago lanceolata*. *Manuscript*.

II

Beraldo, C.S., Buczek, M., Franco, D.C., Łukasik, P., van Nouhuys, S., Duplouy, A. (2025). The microbiota of an insect community is structured by host species, while the effects of environmental factors are species-specific. *Manuscript*.

III

Beraldo, C.S., Franco, D.C., van Nouhuys, S., Duplouy, A. (2025). Temperature at parental generation affects microbial communities associated with offspring for both host and parasitoid. *Manuscript*.

IV

Beraldo, C.S., van Nouhuys, S., Duplouy, A. (2025). How environmental conditions affect the acquisition, establishment, and persistence of microbial endosymbiosis in insects. *Manuscript*.

V

Beraldo, C.S. (2025). An interactive art activity to promote student reflection and learning about host-microbe interactions. *Journal of Microbiology and Biology Education*. 0:e00063-25. <https://doi.org/10.1128/jmbe.00063-25>

Author contributions

The thesis includes co-authored publications. Table 1 outlines the division of labour and the author’s and coauthors’ contributions to each manuscript.

Table 1. Division of labour for the manuscripts included in the doctoral dissertation (Chapters I–V). Co-author contributions are indicated by abbreviations. AD: Anne Duplouy, AM: Anna Michalik, **CSB: Camila Souza Beraldo**, DCF: Diego Castillo Franco, MB: Mateusz Buczek, PŁ: Piotr Łukasik, SvN: Saskya van Nouhuys.

	I	II	III	IV	V
Conceptualization	PŁ, CSB , AD, SvN	CSB , AD, SvN	CSB , AD, SvN	CSB , AD, SvN	CSB
Sample/data collection	CSB	CSB	CSB	CSB , AD, SvN	CSB
Laboratory work	CSB , MB, AM	CSB , MB	CSB , AD	-	-
Data analysis	CSB , DCF	CSB , DCF	CSB , DCF	CSB	-
Writing and editing	CSB , AD, SvN, PŁ, AM, DCF, MB	CSB , AD, SvN, PŁ, DCF, MB	CSB , AD, SvN, DCF	CSB , AD, SvN	CSB

List of abbreviations

16S rRNA	16S ribosomal RNA gene
AIC	Akaike Information Criterion
COI	Cytochrome c oxidase subunit I
F0	parental generation
F1	first filial generation/offspring generation
GLMM	Generalized Linear Mixed Model
PCR	Polymerase Chain Reaction
PCoA	Principal Coordinates Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
SPRI	Solid Phase Reversible Immobilization
TE	Tris-EDTA
zOTU	zero-radius Operational Taxonomic Unit

I. Introduction

Species interactions are foundational to shaping ecological systems, biodiversity, evolutionary trajectories, and ecosystem functioning. Among these interactions, symbiosis — a long-term relationship between dissimilar species, encompassing a spectrum that ranges from mutualism and commensalism to parasitism (de Bary, 1878; Douglas & Smith, 1989) — plays a key role. One type of symbiosis is the association between hosts and the microbial communities that live in or on them, known as the microbiota (Shapira, 2016). Microbiota refers to the microbial community, including bacteria, archaea, fungi, protozoa, and microalgal symbionts, inhabiting a specific environment. (Berg *et al.*, 2020). When microbial communities associate with host communities, they collectively form what is generally referred to as “communities of communities” (Brown *et al.*, 2020). Insects, one of the most diverse animal groups (Eggleton, 2020), harbour microbiota that play essential roles in their digestion, reproduction, and development, ultimately influencing their life history traits and evolution (Zheng *et al.*, 2017; Walters *et al.*, 2020; Cornwallis *et al.*, 2023; Rombaut *et al.*, 2023; Łukasik & Kolasa, 2024). Thus, changes in host microbial communities can have significant implications for host fitness and their offspring (Łukasik & Kolasa, 2024). However, most studies on insect microbiota focus on single populations or species, often using lab-reared specimens (Moghadam *et al.*, 2018; Walters *et al.*, 2020; Wang *et al.*, 2024), limiting our understanding of microbial interactions in natural settings. Investigating microbiota composition and diversity in wild insects not only deepens our understanding of these associations but also reveals broader ecological and evolutionary implications for hosts populations and communities. Effectively conveying these complex interactions to broader audiences is a challenge (McGenity *et al.*, 2020), as microbiota are invisible and their functions often abstract. To bridge this gap, exploring alternative approaches to science communication is essential for making host-microbe interactions more accessible and engaging.

(1) *Microbial transmission modes and host-microbe associations*

The colonization of an insect host by microbial partners occurs through vertical transmission and/or horizontal transfer. Vertical transmission refers to the inheritance of symbionts from parents to offspring, either through direct germline transmission (Moran & Telang, 1998; Moran, McCutcheon & Nakabachi, 2008) or extracellular mechanisms such as the intentional ingestion of symbiont-infected parental secretions (Hosokawa *et al.*, 2005; Salem *et al.*, 2015). However, many symbionts also undergo horizontal transfer, moving between unrelated individuals of the same or different species (Bright & Bulgheresi, 2010; Turelli *et al.*, 2018). A notable example is the bacterium *Wolbachia*, which is primarily transmitted vertically but occasionally transfers horizontally among species (Haine, Pickup & Cook, 2005; Werren, Baldo & Clark, 2008; Stahlhut *et al.*, 2010). When symbionts successfully colonize and establish long-term in a naïve host lineage or species, this process is called a host shift (Sanaei, Charlat & Engelstädter, 2021). Successful host shifts require four key steps: (i) acquisition from the environment or a donor host, (ii) proliferation within the naïve host, (iii) persistence through vertical transmission, and (iv) spread within the host population (Bright & Bulgheresi, 2010; Chrostek *et al.*, 2017; McCutcheon, Boyd & Dale, 2019; Sanaei *et al.*, 2021). Host shifts can reshape host-symbiont interactions, expanding host ranges, altering ecological networks, and introducing novel microbial functions or diseases (Pimentel, Beraldo & Cogni, 2020; Sanaei *et al.*, 2021, 2023).

Microbial associations with hosts can be classified as resident or transient, depending on the duration of their relationship. Resident symbionts persist within a host for extended periods,

often forming stable associations and being maintained across generations (Bennett & Moran, 2015; N pflin & Schmid-Hempel, 2018). These microbes have typically coevolved with their insect hosts, playing a crucial role in key fitness traits such as nutrition (Moran, 2001; Moran *et al.*, 2003; Garc a-Lozano *et al.*, 2024), development, fecundity (Lewis *et al.*, 2014; Walters *et al.*, 2020), and behaviour (Rombaut *et al.*, 2023). For instance, in aphids, the resident endosymbiont *Buchnera aphidicola* is an obligate mutualist essential for host nutrition (Chen, Lai & Kuo, 2009). In contrast, transient symbionts associate with a host temporarily, and are often influenced by external environmental conditions or diet (Bright & Bulgheresi, 2010; Hammer *et al.*, 2017; Hannula *et al.*, 2019). The interplay between these symbiont groups shapes the microbiota's profile within host species and populations.

(2) Factors influencing microbiota composition and diversity

Anthropogenic activities are profoundly altering ecosystems (Calvin *et al.*, 2023), disrupting ecological and evolutionary processes that sustain insect biodiversity and impacting ecosystem stability (Hautier *et al.*, 2015; Jordano, 2016). These shifts affect not only macro-community dynamics but also microbial interactions, as the composition and diversity of microbial communities result from both neutral and selective processes driven by ecological interactions and environmental conditions (N pflin & Schmid-Hempel, 2018; D  az-S  nchez *et al.*, 2018; Magoga *et al.*, 2023; Chen *et al.*, 2024; Maritan *et al.*, 2024).

In this thesis, the effects of three key ecological interactions on microbiota composition and diversity are examined: host-microbe, microbe-parasite, and microbe-microbe interactions (Table 2). Host species differ in cellular and physiological characteristics, as well as immune responses, which influence microbial colonization and shape microbiota diversity and composition (Malacrin , 2022). Within a host species, microbial communities also vary across insect developmental stages, particularly in holometabolous insects that undergo complete metamorphosis. Each stage can impose unique physiological and nutritional demands, resulting in shifts in microbial assemblies (Kingsolver & Buckley, 2020; English & Barreaux, 2020; Brueggemann, Singh & M  ller, 2025). In addition, microbiota composition and diversity are affected by interactions with other microbes and organisms colonizing the same host. For example, the symbiont *Wolbachia* has been shown to disrupt host microbiota, reducing the abundance of Spiroplasmataceae, Lactobacillaceae, and Acetobacteraceae bacterial families (Simhadri *et al.*, 2017; Fromont, Adair & Douglas, 2019). Furthermore, multicellular parasites can influence microbiota composition. For instance, infections by the (sterilised) tapeworm *Hymenolepis diminuta* in the grain beetle (*Tenebrio molitor*) resulted in significant shifts in bacterial communities, with infected beetles showing a higher relative abundance of *Proteobacteria* and a lower relative abundance of *Firmicutes* compared to uninfected controls (Fredensborg *et al.*, 2020).

Environmental factors, in this study, are defined as both abiotic conditions (e.g., temperature) and host-environment characteristics, such as host plant abundance and landscapes with varying degrees of anthropogenic influence (Table 2). These factors can profoundly shape microbial communities in insect hosts. Host resource availability, pollutant exposure, and temperature fluctuations, for instance, all influence microbial presence and abundance (Sepulveda & Moeller, 2020; Pajarillo, Lee & Kang, 2021; Li *et al.*, 2023; Martins, C  sar & Cogni, 2023). Among these, temperature plays a particularly significant role. Shifts in temperature can alter microbial diversity, stability, and function, ultimately impacting host fitness (Toby Kiers *et al.*, 2010; Wernegreen, 2012; Moghadam *et al.*, 2018; Sepulveda & Moeller, 2020; Iltis *et al.*, 2022). For

example, *Drosophila melanogaster* reared at 13 °C exhibit greater microbial diversity than those raised at 31 °C, with potential consequences for reproduction and survival of the fly host (Moghadam *et al.*, 2018). Certain microbes, such as *Wolbachia*, are especially sensitive to temperature variations (Charlesworth *et al.*, 2019; Hague *et al.*, 2022). In *Drosophila*, elevated temperature reduces *Wolbachia* titer, which in turn increases the abundance of other microbial taxa, including *Spiroplasmataceae*, *Lactobacillaceae*, and *Acetobacteraceae* (Simhadri *et al.*, 2017; Fromont *et al.*, 2019).

(3) *The insect community of Plantago lanceolata*

The insect community associated with the ribwort plantain (*Plantago lanceolata* L., Plantaginaceae) in the Åland Islands, Finland, provides a valuable model for studying the influence of ecological interactions and environmental factors on microbiota. This well-documented system benefits from extensive long-term ecological and evolutionary data (Ojanen *et al.*, 2013; Nieminen & Vikberg, 2015; Duploux *et al.*, 2015; Minard *et al.*, 2019). The *P. lanceolata* population is mapped across approximately 4,400 meadow sites, surrounded by various land-use types, ranging from human-modified landscapes to more natural habitats (Ojanen *et al.*, 2013; Schulz, Vanhatalo & Saastamoinen, 2019). Studying this single, well-characterized ecological community offers the opportunity to investigate both host plant–insect associations and interactions among insect species. This approach minimizes variation in host plant identity while enabling the sampling of sympatric insect species across a gradient of shared environmental conditions. The *P. lanceolata* insect community is taxonomically and functionally diverse, comprising herbivores, predators, pollinators, and their parasitoids (Nieminen, Siljander & Hanski, 2004; Nieminen & Vikberg, 2015). Additionally, several insect species in this system, as well as the host plant, have been shown to harbour symbionts and pathogens (Jousimo *et al.*, 2014; Duploux *et al.*, 2015; Minard *et al.*, 2019).

Previous descriptions of the insect community associated with *P. lanceolata* in Åland have relied on taxonomic identification (Nieminen *et al.*, 2004; Nieminen & Vikberg, 2015). DNA barcoding offers a powerful and efficient alternative for species identification by targeting short, conserved genetic sequences, such as the mitochondrial *COI* gene (Hebert *et al.*, 2003). This approach has enhanced biodiversity assessments at both local and global scales (Savolainen *et al.*, 2005; Vernooij *et al.*, 2010) and facilitated the characterization of complex ecological networks, such as plant-pollinator, host-parasite, predator-prey, and seed-dispersal interactions (González-Varo, Arroyo & Jordano, 2014; Abrego *et al.*, 2021; Gostel & Kress, 2022; Ficetola & Taberlet, 2023). However, barcoding can be complicated by the presence of non-target DNA from other organisms associated with a sample. For instance, when analysing predators, *COI* sequences from tissues of their prey remaining in their guts may be amplified alongside the predator’s DNA (Jurado-Rivera *et al.*, 2009; Unger *et al.*, 2020). Similarly, DNA from parasites, parasitoids, or symbiotic bacteria is also co-amplified with host DNA (Santos, Besnard & Quicke, 2011; Smith *et al.*, 2012; Stahlhut *et al.*, 2013; Tay *et al.*, 2017). While such non-target sequences are often discarded as contaminants, they can provide valuable insights into hidden ecological interactions that shape host ecology and evolution. Although the *P. lanceolata* insect community in Åland is well-studied, unidentified species and interactions likely remain, highlighting the potential of molecular approaches to refine our understanding of the system.

Within this insect community, the Glanville fritillary butterfly, *Melitaea cinxia* (Linnaeus 1758, Lepidoptera: Nymphalidae) and its specialist parasitoid *Hyposoter horticola* (Gravenhorst)

(Ichneumonidae: Campopleginae) have been the focus of extensive ecological and evolutionary research (Ehrlich & Hanski, 2004; Duploux *et al.*, 2015; Opedal *et al.*, 2020). Both the butterfly and the parasitoid are univoltine in the Åland Islands, with parasitism occurring in the summer. The parasitoid larva develops inside the caterpillar host through winter and emerges as an adult wasp in the spring (van Nouhuys & Hanski, 2004). Both species are known to carry symbionts, which may influence their physiology, interactions, or adaptation to environmental conditions (van Nouhuys, Kohonen & Duploux, 2016; Rosa *et al.*, 2019; Duploux, Minard & Saastamoinen, 2020). The microbiota of *M. cinxia* caterpillars appears to be largely transient, primarily acquired from the environment, with its composition varying depending on host plant conditions (Rosa *et al.*, 2019; Minard *et al.*, 2019). In contrast, the microbiota of *H. horticola* remains largely uncharacterized, although the bacterium *Wolbachia* is known to infect about half of the parasitoid population in Åland (Duploux *et al.*, 2015, 2021), and to affect its susceptibility to hyperparasitism (Duploux *et al.*, 2015, 2021). *Wolbachia* is reported as absent in the butterfly host *M. cinxia* (Jones *et al.*, 2025).

Although the temperate maritime climate of the Åland Islands is characterized by cool summers and mild winters, recent years have seen increasing temperatures and more frequent droughts (Tack, Mononen & Hanski, 2015). These climatic changes have been shown to impact *P. lanceolata*, *M. cinxia* populations (Nieminen *et al.*, 2004; Salgado, DiLeo & Saastamoinen, 2020; Kahilainen *et al.*, 2022) and some the parasitoids (Nair, Nonaka & van Nouhuys, 2018). However, their effects on other insect species of the *P. lanceolata* community, as well as on their associated microbiota, remain unexplored.

(4) Science communication on host-microbe interactions

Science communication practices are organised actions aiming to communicate scientific knowledge, methodology or processes in settings where non-specialists are a considerable part of the audience (Burns, O'Connor & Stocklmayer, 2003; Davies & Horst, 2016). Science communication is not a simple transfer of information from “those who know” to “those who do not know”. Rather, it is a dynamic, ongoing process through which scientific knowledge becomes embedded in broader cultural and societal contexts. According to Davies & Horst (2016), science communication holds three key dimensions of social importance: (i) it promotes the well-being of individuals, institutions, and nations; (ii) it fosters democracy by strengthening the connection between citizens and scientific knowledge; and (iii) it functions as a cultural and identity-shaping force, intertwining with several aspects of social life, such as organisations, spaces, values, and emotions. In an ecological context, science communication is fundamental to environmental stewardship, enabling informed decision-making and fostering public engagement in conservation efforts.

Effective science communication about microbes has become increasingly important, particularly in the wake of emerging infectious diseases such as COVID-19 (Rocha *et al.*, 2021). Although microbes play essential roles in ecosystems — from producing atmospheric oxygen to decomposing organic matter — they are primarily perceived as associated with diseases, despite fewer than 1% of microbial taxa being pathogenic (Anon., 2011). This misconception can lead to uncertainty and negative emotions, such as fear and anxiety (Timmis *et al.*, 2019; Zaelzer, 2020). Additionally, the microscopic and complex nature of microbes presents further obstacles, hindering public engagement with microbial science (McGenity *et al.*, 2020). Recent research suggests that integrating microbiology with art can enhance understanding of complex and

abstract concepts across diverse age groups (Segarra *et al.*, 2018; Parks & White, 2021), providing means to process negative emotions associated with microbes.

Artistic approaches to microbiology education range from digital tools, such as games (Nicholls, Merkel & Cordts, 1996; Farrell *et al.*, 2011), to hands-on activities like agar painting with fluorescent bacteria (Adkins-Jablonsky *et al.*, 2021). However, despite their relatively low cost, these methods often remain inaccessible in many schools, particularly in countries in the Global South, due to limited access to electronic devices and laboratory resources (Murillo & Román, 2011). This highlights the need for alternative, more accessible strategies. One promising approach is the use of analogies, which have been shown to make abstract concepts more relatable and easier to understand (Treagust, Harrison & Venville, 1998; Brown & Salter, 2010). By integrating these frameworks into science communication, informal learning environments can be created to spark curiosity and encourage meaningful, participatory discussions about microbes, especially their roles and interactions with hosts.

II. Aims of the thesis

In this thesis, I explore the diversity of the insect community associated with *Plantago lanceolata* and investigate how ecological interactions and environmental factors affect its microbiota. Additionally, I consider effective ways to communicate these findings to the public. Specifically, I address the following questions:

- a. What insect species are associated with *P. lanceolata* in the Åland Islands, and which symbionts, parasites, and parasitoids do they host?
- b. How do ecological interactions affect microbiota composition and diversity in insect communities?
- c. How do environmental conditions affect microbiota composition and diversity and symbiotic host shifts in insect communities?
- d. How can art be used to communicate the influence of environmental and ecological factors on host-microbe interactions to the public?

My research is structured into five chapters (I–V below). Table 2 provides an overview of which thesis questions are addressed in each chapter.

In **Chapter I**, I describe the insect community associated with ribwort plantain (*P. lanceolata*) in the Åland Islands, extending previous descriptions (Nieminen *et al.*, 2004; Nieminen & Vikberg, 2015). I argue that sequencing *COI* along with microbiota studies allows for *COI* barcoding, which is essential not only to identify host species but also to detect *COI* "contamination" in the samples. Using the "contamination" data, I present a network of previously unknown interactions within the study system, demonstrating the value of the method. Additionally, I investigate whether the microbiota of parasitized individuals of *Carpocoris purpureipennis* (Hemiptera) differs from that of unparasitized individuals, thus exploring the impact of microbe-parasite interactions on microbial communities.

In **Chapter II**, I examine how ecological interactions — host-microbe relationships — and environmental factors — seasonality and habitat quality (i.e., connectivity, landscape type and host plant abundance) — influence bacterial community composition and diversity in eight sympatric insect species. This study focuses on the insect community associated with *P. lanceolata* in the Åland Islands. Based on the long-term evolutionary relationships between insects and their symbionts, I hypothesize that host species would primarily influence microbiota variation. However, I also expect that species sharing similar feeding resources or engaging in ecological interactions (e.g., parasitoidism) would share microbial species. Furthermore, considering that insects often acquire transient microbes from their environment, I anticipate that environmental factors, including seasonality and habitat quality, would contribute to microbial community variation.

In **Chapter III**, I use an experimental approach to examine how environmental temperature and host-microbe interactions shape the bacterial community composition and diversity of two insect species: the butterfly *Melitaea cinxia* and its specialist parasitoid *Hyposoter horticola*. Here, I investigate two main aspects: first, the differences in microbiota between a butterfly host and its specialist parasitoid, and second, how temperature conditions experienced by the parental generation and across developmental stages of the offspring (pre-diapause vs. post-diapause caterpillar stages) affect the microbiota of adult offspring in these species. Since *H. horticola*

larvae exclusively feed on *M. cinxia* caterpillars, I expect the microbiota of the two species to be highly similar. In addition, I hypothesize that temperature conditions would similarly affect the microbiota of adult offspring from both species.

In **Chapter IV**, I present a literature review synthesizing the current knowledge on how environmental factors influence symbiotic host shifts in insects. The chapter examines two key aspects: the dominant biological systems studied in this field and the environmental conditions that affect each stage of a successful microbial host shift — acquisition, establishment, and persistence. Specifically, I present the results of a systematic search for published studies on endosymbionts, insect hosts, and abiotic factors and then summarize key findings, biases, and knowledge gaps. The review focuses on non-pathogenic endosymbionts and terrestrial abiotic factors known to influence insects (Wagner, 2020), including atmospheric gases (e.g., O₃), environmental toxins (e.g., pesticides, antibiotics), inorganic nutrients (e.g., nitrogen), precipitation, relative humidity, seasonality, temperature, and water stress (e.g., drought). Finally, I discuss how environmental factors impact each step of the host shift process, emphasizing the potential effects of changing environments on host shift success.

Finally, in **Chapter V**, I describe an art-based activity designed to engage the public in discussions about how environmental and ecological factors affect host-microbe interactions. The activity, called *MicrobiART*, invites participants to use papers (representing hosts), toy balls (representing microbes), and paint (representing environmental conditions) to create visual representations of these interactions. Participants are encouraged to observe all the paintings done during the activity and to reflect on the patterns created, discussing how different features of hosts, microbes and environmental conditions may contribute for the emergence of such interactions.

Table 2. Overview of the thesis questions (a-d) addressed in each chapter (I-V), along with a description of the manuscript type, host species studied, ecological interactions, environmental factors, and traits examined. Although **Chapter I** discusses the entire *P. lanceolata* insect community, there is a focus on *Carpocoris purpureipennis* (Hemiptera). The host species used in **Chapter II** are *Mecinus pascuorum* (Coleoptera), *Aelia acuminata* (Hemiptera), *Carpocoris purpureipennis* (Hemiptera), *Palomena prasina* (Hemiptera), *Formica aquilonia* (Hymenoptera), *Formica fusca* (Hymenoptera), *Mesopolobus incultus* (Hymenoptera), and *Aspilapteryx tringipennella* (Lepidoptera). **Chapter III** studies the host species *Melitaea cinxia* (Lepidoptera) and *Hypsoater horticola* (Hymenoptera).

Chapter	Manuscript type	Question	Host species	Factors		Trait
				Ecological interaction	Environmental	
I	method article	a, b	Insecta ()	microbe-parasite	-	microbiota composition and diversity
II	research article	a, b, c		host-microbe	seasonality and habitat quality	microbiota composition and diversity
III	research article	a, b, c		host-microbe and microbe-microbe	temperature	microbiota composition and diversity
IV	literature review	c	Insecta	-	atmospheric gases, environmental toxin, inorganic nutrient, precipitation, relative humidity, seasonality, temperature, water stress	host shift
V	teaching report	d	any	any	any	environment-host-microbe interaction

III. Material and methods

This thesis work is based on fieldwork collections (**Chapters I and II**), a laboratory experiment (**Chapter III**), a systematic literature review (**Chapter IV**), and a science communication activity (**Chapter V**). Insect samples collected from the field and the experiment (**Chapters I, II, and III**) were processed through DNA extraction, sequencing, and subsequent bioinformatics and statistical analyses.

(1) Insect sampling in the Åland Islands

For **Chapters I and II**, insects were collected from meadow sites colonized by *Plantago lanceolata* in the Åland Islands, Finland (Figure 2A, D-E), areas that are surveyed annually (Ojanen *et al.*, 2013). In **Chapter II**, I used environmental data from the Åland annual surveys, including site connectivity and *P. lanceolata* density (Moilanen & Nieminen, 2002; Ojanen *et al.*, 2013). Additionally, land-use metrics from buffer areas surrounding each site (Schulz *et al.*, 2019) were employed to classify the meadow sites into two types: (1) those with high anthropogenic influence due to nearby agricultural fields, buildings, and roads, and (2) those with low anthropogenic influence, primarily characterized by meadows, lakes, exposed rocks, marshes, forests, and streams (Figure 2A-C). A total of 30 sites, distributed across the Åland Islands, were selected, with 12 sites classified as having high anthropogenic influence and 18 with low influence (Figure 2A).

Sites were visited monthly in late spring (May), early summer (June), and mid-to-late summer (July) of 2021 to capture seasonal variations in insect activity. Sampling (hand collection) occurred between 10:00 and 18:00 on clear days, with different sites in each commune visited on separate days to minimize daily weather variation. Insects were collected from one to four 1 m² quadrats on *P. lanceolata*, with quadrats varying in number depending on site size. Leaf-miners and leaf-rollers were also collected, and inflorescences were incubated in the lab to monitor emerging insects. Insect specimens were preserved in 99% ethanol, labelled, and stored at -20 °C. Insects were categorized into morphotypes, and the most abundant ones were selected for DNA extraction (Figure 2F-M) and microbiota analyses in **Chapter II**. Among them, *Carpocoris purpureipennis* (Hemiptera) (Figure 2H) was also used in **Chapter I**.

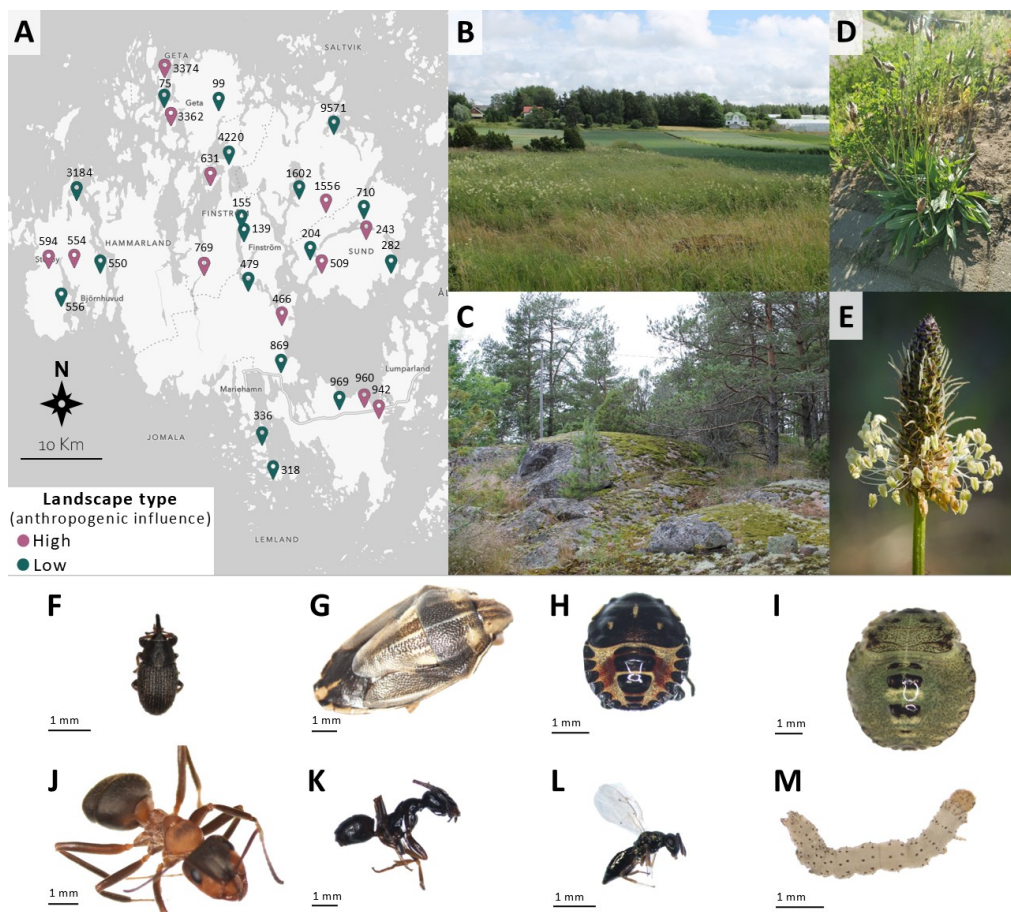


Figure 2. Sample sites and the eight most common insect species collected in the Åland Islands. A. Geographical position of 30 sites sampled during the summer of 2021. Sites surrounded by human disturbed land-uses are marked in pink, and sites surrounded by natural landscapes are marked in green. B. Site (ID 554) surrounded by landscape with high anthropogenic influence, which presents agricultural fields and buildings. C. Site (ID 719) surrounded by landscape with low anthropogenic influence, which has forests and rocks. D. The host plant *Plantago lanceolata*, with a close-up of a flower head (E.). F-M. Insect species: F. *Mecinus pascuorum* (Coleoptera) (N = 84), G. *Aelia acuminata* (Hemiptera) (N = 17), H. *Carpocoris purpureipennis* nymph (Hemiptera) (N = 33), I. *Palomena prasina* nymph (Hemiptera) (N = 11), J. *Formica aquilonia* (Hymenoptera) (N = 39), K. *Formica fusca* (Hymenoptera) (N = 13), L. *Mesopolobus incultus* (Hymenoptera) (N = 69), and M. *Aspilapteryx tringipennella* caterpillar (Lepidoptera) (N = 30). Retrieved from **Chapter II**.

(2) Temperature experimental design

For **Chapter III**, I reared two generations of the butterfly *Melitaea cinxia* and its parasitoid *Hyposoter horticola* under different temperature conditions in the laboratory. Host plants (*P. lanceolata*) were cultivated in a greenhouse at 25 °C. Caterpillars were fed fresh *P. lanceolata* leaves *ad libitum*, while adult insects were provided with 30% honey water on a sponge daily.

In the fall of 2020, *M. cinxia* caterpillars at the early diapause stage, representing the parental generation (F0), were collected from the Åland Islands by the yearly meadow survey. They diapaused in lab conditions, being kept in an incubator at 5 °C until the spring of 2021. Post-diapause caterpillars were reared individually at 28:18 °C (day:night temperatures), with approximately one-third naturally parasitized by *H. horticola*. Once caterpillars or parasitoid larvae pupated, pupae were assigned to one of three temperature treatments (26:18 °C, 28:18 °C, or 31:18 °C) until adult emergence (Figure 3). Butterflies mated in a greenhouse at ~25 °C, and mating pairs were recorded. Mated females were provided with potted *P. lanceolata* plants for egg-laying. Eggs were exposed to *H. horticola* females for parasitisation in the laboratory. Because the female wasps were unmated, they laid only haploid eggs, producing male offspring (F1). No wasps emerged from the 31 °C treatment, leading to its discontinuation in subsequent experiments.

In the offspring generation (F1), egg clutches were randomly assigned to either 26 °C or 28 °C. After diapause ended in the fall of 2021, post-diapause caterpillars were divided into smaller groups (15–25 individuals) and maintained at either 26 °C or 28 °C until pupation and emergence of either *M. cinxia* butterflies or *H. horticola* wasps. Upon emergence, adults were killed within 24 hours by freezing at -20 °C. Specimens were kept in 99% ethanol.

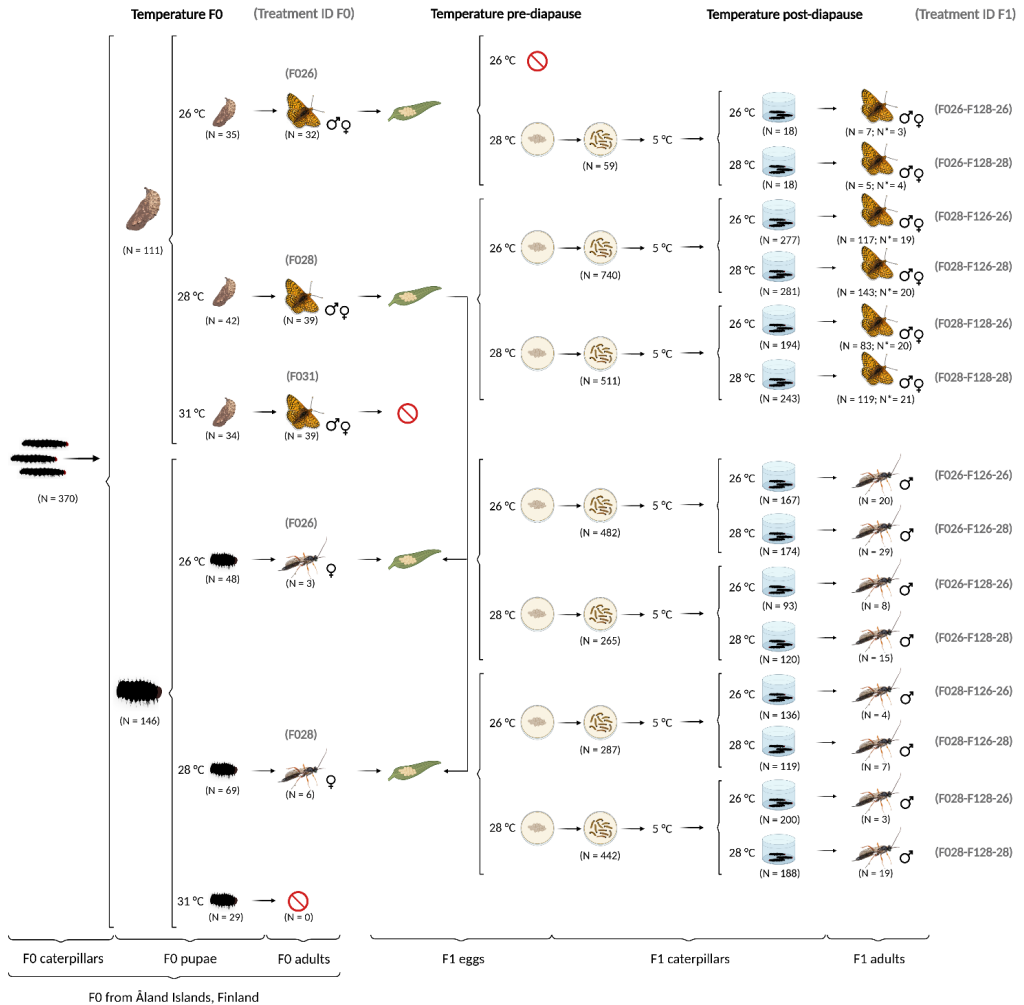


Figure 3. Cross-sectional experimental design used over the parental (F0) and offspring (F1) generations. F0 caterpillars were collected in the Åland Islands, Finland, in fall 2020, and the F1 generation was born and reared under laboratory conditions. N represents the total number of individuals that survived at each developmental stage, while N* represents the number of individuals selected for DNA extraction. All adult *Hyposoter horticola* specimens were used for DNA extraction (N = N*). Retrieved from **Chapter III**.

(3) Molecular methods, bioinformatics and statistical analyses

(a) DNA extraction

Due to laboratory-specific constraints and projects requirements, in this thesis, I employed two different DNA extraction methods to analyse the microbial communities associated with the insect samples. For **Chapters I** and **II**, individual whole-body insect specimens (N = 403) were homogenized in Vesterinen lysis buffer with proteinase K and incubated at 55 °C. A 1/3 fraction of the total homogenate was used for DNA extraction following the protocol by Iwaszkiewicz-Eggebrecht (2023). To quantify absolute microbial abundance, a linearized plasmid carrying an

artificial *16S rRNA* target (Ec5502) (Tourlousse *et al.*, 2016) was added to each homogenate. DNA was extracted using SPRI magnetic beads in deep-well plates, followed by ethanol washes and elution in TE buffer. Extractions were performed at the Institute of Environmental Sciences, Jagiellonian University, Poland. Each 96-well plate included a positive control (*Pyrops candellaria*) and two negative controls (DNA extraction and PCR TE blanks). For **Chapter III**, the abdomen of each specimen (N *M. cinxia* = 87; N *H. horticola* = 105) was aseptically removed with a sterile blade and air-dried under a sterile hood for 2–4 hours. DNA extraction was then carried out using a Tissue kit on the Chemagic™ 360 Nucleic Acid Extractor (PerkinElmer, United States) at the Molecular Ecology and Systematics (MES) Laboratory, University of Helsinki. Each extraction plate also included two negative controls (DNA extraction and PCR TE blanks).

(b) Library preparation and sequencing

In **Chapters I** and **II**, a two-step PCR protocol was used to identify both the insect species and their associated bacterial communities. The mitochondrial *cytochrome c oxidase I (COI)* gene and the V4 region of the bacterial *16S rRNA* gene were amplified using primers (Apprill *et al.*, 2015; Parada, Needham & Fuhrman, 2016; Elbrecht *et al.*, 2019) modified with Illumina adapter tails. Bead-purified PCR products were used for indexing PCR, and the *COI* and *16S* libraries were pooled for sequencing on an Illumina MiSeq platform at the Institute of Environmental Sciences, Jagiellonian University, Poland. In **Chapter III**, the V5-V6 region of the *16S rRNA* gene was amplified (Toft & Andersson, 2010), with each specimen amplified in duplicate. PCR products were pooled and sent to the Genomics NGS Sequencing unit at the Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, for library preparation and sequencing on an Illumina MiSeq platform.

(c) Amplicon sequencing data curation and taxonomic assignment

In **Chapters I**, **II**, and **III**, *16S* amplicons were curated using a custom pipeline based on USEARCH/VSEARCH. The amplicon datasets were initially split into bins corresponding to the target genes based on primer sequences. Quality-filtered forward and reverse reads were assembled into contigs using PEAR (Zhang *et al.*, 2014). Contigs were dereplicated and denoised (Edgar, 2016; Rognes *et al.*, 2016), and chimeras were detected and removed using USEARCH (Edgar, 2010). Exact sequence variants (zOTUs) for bacterial *16S* were clustered using the UPARSE-OTU algorithm implemented in USEARCH, and bacterial taxonomy was assigned using the SINTAX algorithm and the SILVA database (version 138 SSU). The bacterial *16S* sequences were screened for putative contaminants from DNA extraction and PCR reagents using negative controls (TE buffer blanks) as a reference. In **Chapters I** and **II**, after removing contamination from the bacterial data, microbiota absolute abundance was calculated using spike-in reads as a reference, while in **Chapter III**, the data was normalized using Total Sum Scaling (TSS), and zOTUs with a read count lower than 10 per specimen were removed.

In **Chapters I** and **II**, *COI* amplicons were curated following the same pipeline as described above. Insect host taxonomy was assigned using MIDORI (version GB 239). The identity of each insect host was verified by cross-checking species names obtained from barcoding with morphotype images. In **Chapter I**, I used the *COI* barcode to identify target and non-target species, generating a table of *COI* zOTUs for each sample. Species identification for the target species was determined based on the most abundant *COI* zOTU, with validation through morphotype images. Non-target species were identified based on the presence of additional *COI* zOTUs, and their

ecological roles and interactions were inferred from the literature. Based on the identified species, in **Chapter II**, I selected eight species with a widespread distribution for further analysis of their associated microbial communities (Figure 2F-M).

(d) Statistical analyses

In **Chapters I, II, and III**, bacterial community composition was analysed using distance matrices, including Bray-Curtis and both unweighted and weighted UniFrac distances, in the phyloseq R package. In summary, the Bray-Curtis distance matrix measures the dissimilarity between two or more microbial communities based on the presence and abundance of shared species, without considering their phylogenetic relationships (Bray & Curtis, 1957). It calculates dissimilarity by comparing the relative abundance of species across samples, giving more weight to abundant species (Bray & Curtis, 1957). The unweighted UniFrac distance matrix compares microbial communities based solely on the presence or absence of taxa, ignoring their relative abundances (Lozupone & Knight, 2005). It emphasizes the phylogenetic distance between communities, meaning that two communities that share species but differ phylogenetically will appear more dissimilar than those that share the same phylogenetic groups but differ in species composition (Lozupone & Knight, 2005). The weighted UniFrac distance matrix, on the other hand, incorporates both the phylogenetic relatedness of species and their relative abundance in each community (Lozupone *et al.*, 2011). This provides a more nuanced measure of dissimilarity, reflecting both the evolutionary distance between species and their abundance in the samples (Lozupone *et al.*, 2011). These matrices were calculated to compare microbiota across insect species and different treatments, and PERMANOVA tests (adonis2 function) were applied to assess differences in microbial community structure, with 10,000 permutations. Generalized linear mixed models (GLMMs) were employed to compare bacterial species richness (zOTU richness) using a Poisson distribution and the Shannon index with a Gaussian distribution across different groups. PERMANOVA and GLMM model selection followed a hierarchical approach, with model performance assessed using the Akaike Information Criterion (AIC). Data visualisation was conducted using the ggplot2 package in R.

In **Chapter I**, the effect of parasitism on microbiota composition was investigated, specifically looking at how the presence of *Blastocrithidia* sp. in *C. purpureipennis* affected its microbial community. The presence of the parasite was included as a fixed effect in the PERMANOVA and GLMM analyses. In **Chapter II**, to compare the bacterial communities associated with the *P. lanceolata* insect species, I added insect host species, seasons, and habitat quality indicators (site connectivity, landscape types, and *P. lanceolata* host plant density) as fixed effects in both PERMANOVA and GLMM models. I also included interactions between host species and these factors, as well as the interaction between season and site connectivity. Site ID was included as random effects in both PERMANOVA and GLMM analyses to account for potential confounding factors. Since host species accounted for most of the variation in bacterial community composition, I performed individual analyses for each insect species. Due to insufficient seasonal data for most species, seasonality and its interactions were excluded from models for all but *F. aquilonia*, *F. fusca*, and *M. pascuorum*. Given that a few bacterial symbionts accounted for more than 50% of the total reads in each insect species' microbiota (here considered dominant taxa), I also investigated the effects of seasonality and habitat quality on the composition of the non-dominant microbiota.

Finally, in **Chapter III**, I compared the bacterial communities of *M. cinxia* and *H. horticola* by testing the effects of insect species and generation as fixed explanatory variables in both PERMANOVA and GLMM models. I then focused on the effect of temperature treatments on the F1 generation, analysing each species separately with temperature as a nested variable across pre- and post-diapause stages, and temperature experienced by the F0 mother. For *M. cinxia*, sex was included as a fixed effect, while for *H. horticola*, *Wolbachia* infection status was considered. Analyses for *H. horticola* were conducted separately for *Wolbachia*-infected and uninfected individuals. IDs of the mother butterflies or mother wasps were included as random effects in both PERMANOVA and GLMM analyses.

(4) Systematic search of the literature

To gather the data discussed in **Chapter IV**, I searched the Institute for Scientific Information Web of Science database for studies investigating the effect of abiotic conditions on microbial host shifts in insects. The search was done using a search string, which included variations of the keywords ‘*abiotic factor*,’ ‘*host shift*,’ ‘*symbiont*,’ and ‘*insect*’. The initial search returned 2,664 papers, which were sorted through three stages (Figure 1). First, abstracts were reviewed, and studies mentioning at least one abiotic factor involved in any of the four steps of a symbiotic host shift were selected. This narrowed the list to 568 papers. In the second stage, full texts were reviewed to extract the number of *endosymbiont-insect host-abiotic factor* combinations investigated, resulting in 142 papers and 602 combinations. In the final sorting stage, protozoa and nematode symbionts, as well as pathogenic symbionts, were excluded, along with abiotic factors appearing in fewer than three combinations. Ultimately, 50 studies published between 1994 and 2022 were included, contributing 177 combinations of *endosymbiont-insect host-abiotic factor* (Figure 1).

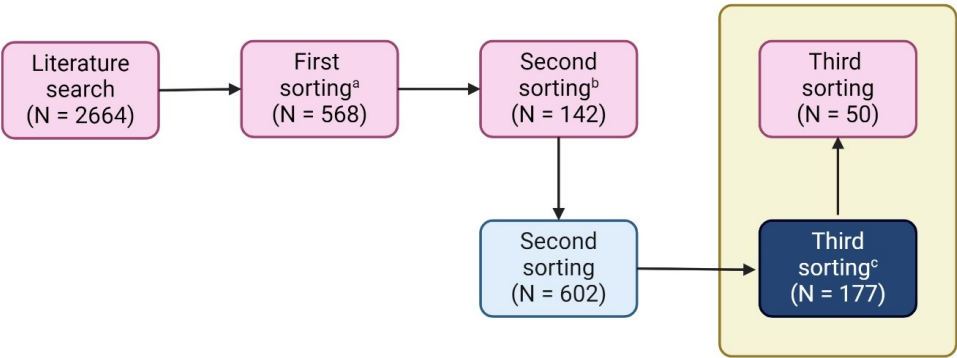


Figure 1. Flow chart illustrating the literature sorting process. The upper row (red) indicates the sorting of studies, which were screened (a) to select those relevant for the literature review and (b) to extract the combinations “endosymbiont-insect host-abiotic factor”. The lower row (blue) indicates the sorting of combinations, which were screened (c) to remove combinations that included protozoa and nematodes symbionts, pathogenic symbionts, and rare abiotic factors (pH and nuclear radiation). The final data used in this study (yellow) comprises 177 combinations (dark blue) from total of 50 studies. Retrieved from **Chapter IV**.

(5) MicrobiART activity preparation

In **Chapter V**, the *MicrobiART* activity setup involved arranging two tables in the room, each labelled as “Environment A” and “Environment B.” Each table contained various types of paper differing in colour, texture, and weight, as well as different toy balls (e.g., marbles, crumpled paper balls, and table tennis balls). Additionally, warm-coloured paints were provided at one table, while cool-coloured paints were available at the other. Some paper types, toy balls, and paint colours were shared between both environments, while others were exclusive to a specific table.

At the beginning of the activity, I introduced the students to the *MicrobiART* project, explaining its goals and the key analogies used. Each participant was then asked to choose an environment (A or B) and received a tray to simulate host-microbe interactions under different environmental conditions. The students followed a structured process to complete their artistic representations:

1. *Host selection.* Participants selected a paper sheet (representing a host) from their chosen environment and secured it to the tray with tape.
2. *Abiotic factor selection.* Up to four drops of paint (representing abiotic factors) were added to the tray, using only the colours available in that environment.
3. *Microbe selection.* Participants chose between two and five toy balls (representing microbes) from the available options and placed them onto the tray.
4. *Interaction duration.* Each participant determined how long the host-microbe interaction would last, selecting a time between 30 seconds and three minutes.
5. *Host-microbe interaction.* Holding the tray, participants gently moved it to allow the toy balls to roll through the paint, spreading it across the paper to create unique patterns.
6. *Finalization.* At the end of the interaction period, the paper was removed from the tray and set aside to dry.

To conclude the activity, I collected the completed artworks and displayed them on the wall, creating a small exhibition.

IV. Results and discussion

(1) *Beyond the Plantago lanceolata insect community*

In **Chapter I**, I identified 32 insect species associated with *Plantago lanceolata* in the Åland Islands, including three species not previously recorded in Finland (*Eupelmus confusus*, *Temnothorax* sp. and *Formica rufibarbis* sensu lato) and 19 not included in previous descriptions of the diversity of this insect community (Nieminen *et al.*, 2004; Nieminen & Vikberg, 2015) (Figure 4). Previous research on *P. lanceolata* focused on insects associated to flower heads during summer (Nieminen & Vikberg, 2015), whereas the current study sampled insects from the entire host plant over a broader seasonal window. These methodological differences likely explain the reported differences. For instance, Formicidae species were detected as part of the *P. lanceolata* insect community, likely because ants primarily forage at ground level (Cosens & Toussaint, 1985; Domisch *et al.*, 2009), and thus their occurrence was not registered in previous surveys (Nieminen & Vikberg, 2015).

The complexity of this insect community was further highlighted by the analysis of *COI* reads, which revealed non-target species ("contaminants") in approximately 4% of the 403 specimens analysed. Most of the inferred interactions had also not been previously documented within the *P. lanceolata* insect community in the Åland Islands, but they align with and expand upon existing literature. Parasitism and parasitoidism were the most common inferred relationships (Figure 4). The trypanosomatid *Blastocrithidia* sp., a protist parasite, was detected in several pentatomid bugs (Figure 4), marking its first report in Finnish insects.

While *COI* barcoding is a widely used and effective tool for species identification, relying on a single mitochondrial gene comes with limitations. The *COI* gene may lack the resolution to distinguish between recently diverged or closely related species (Collins & Cruickshank, 2013), leading to under- or overestimation of diversity in some cases (Meier, Zhang & Ali, 2008; Collins & Cruickshank, 2013). Additionally, mitochondrial introgression, incomplete lineage sorting, and the presence of nuclear mitochondrial pseudogenes can confound species-level assignments (Ballard & Whitlock, 2004; Song *et al.*, 2008). These issues are especially relevant in taxa where *COI* reference databases remain incomplete or where taxonomic revisions are ongoing. In the present study, species-level identifications were supported by morphological traits (e.g., morphotyping) as well as ecological data, but future work could benefit from multilocus barcoding approaches to improve taxonomic resolution and accuracy.

In **Chapters II** and **III**, I explored the microbial communities associated with some of the insect species of the *P. lanceolata* community. I identified that in most of the insect species analysed, although the insect species is associated with a diverse microbial community, a few bacteria dominate (Figure 4), a result consistent with the available literature (Duploux *et al.*, 2015; Duron & Noël, 2016; Minard *et al.*, 2019; Koga *et al.*, 2022; Jackson *et al.*, 2023; Hanhimäki *et al.*, 2024). In **Chapter II**, *M. pascuorum* and *A. tringipennella* were found to carry a similar *Wolbachia* strain, suggesting horizontal transfer between phylogenetically distinct hosts (Figure 4). Both insects are specialist herbivores of *P. lanceolata* (E. A. Dickason, 1968; Hamid *et al.*, 1999; Nieminen & Vikberg, 2015; Poulton, Logan & Barraclough, 2021), which may have facilitated bacterial transmission, as observed in other systems (Sintupachee *et al.*, 2006; Stahlhut *et al.*, 2010; Chrostek *et al.*, 2017; Miraldo & Duploux, 2019). Similarly, the weevil *M. pascuorum* and its specialist parasitoid *M. incultus* share *Rickettsia* and *Wolbachia* strains (Figure 4), reinforcing

the role of parasitoid-host interactions in symbiont transfer (Heath *et al.*, 1999; Ahmed *et al.*, 2015). However, our results, based on *16S* and *COI* sequences, lack the genomic resolution to confirm whether hosts carry identical strains (Merhej & Raoult, 2011), and should be interpreted with caution.

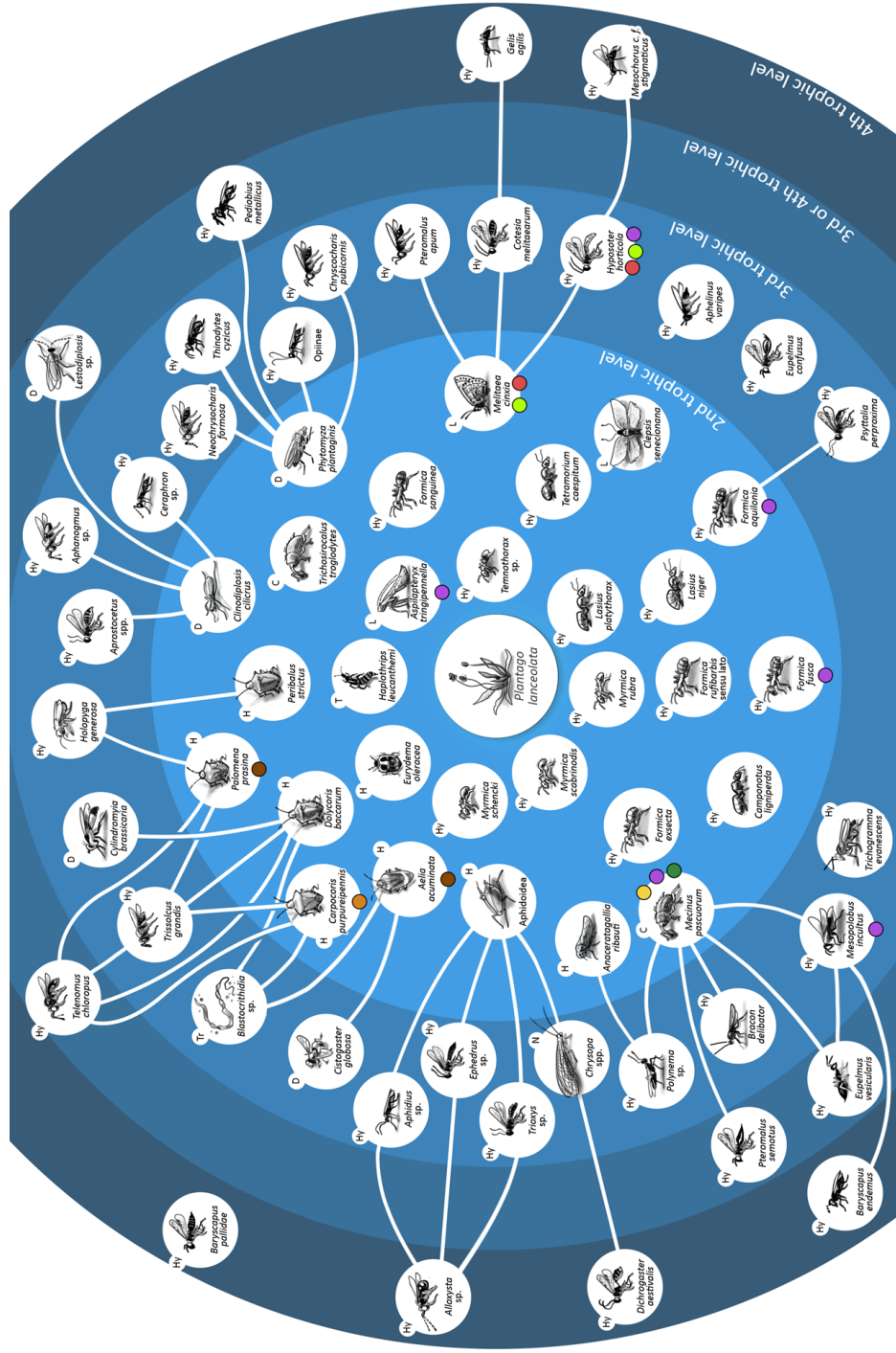


Figure 4. Insect community associated with *Plantago lanceolata* in the Åland Islands, Finland. The diagram includes insect species identified in **Chapter I** of this thesis as well as in previous literature (Nieminen *et al.*, 2004; Nieminen & Vikberg, 2015). Species are arranged by trophic level (indicated by different shades of blue): herbivores at the second trophic level, parasitoids and predators at the third trophic level, and hyperparasitoids at the fourth trophic level. Orders are indicated by letters. Lines indicate ecological interactions and were retrieved from Nieminen & Vikberg (2015). Bacterial species identified in **Chapters II** and **III** of this thesis are indicated by colours. Image credits: Lukera Andrade.

(2) Effects of ecological interactions on insect microbiota composition and diversity

(a) Host-microbe interaction

In **Chapters II** and **III**, I found that each insect species harboured a distinct dominant bacterial species, with microbial community composition consistently differing between species (Figures 5-6; see **Chapters II** and **III** for Bray-Curtis results). Although the eight insect species were sympatric within the Åland meadow sites and *H. horticola* larvae feed exclusively on *M. cinxia* caterpillars, the composition and richness of their microbiota still differed. This is perhaps unsurprising given the marked ecological and dietary differences among the insect species (Nieminen & Vikberg, 2015; Iakovlev *et al.*, 2017; Nieminen *et al.*, 2018; Aymamí *et al.*, 2023). However, the fact that microbial community composition also varied between species within the same trophic level or for species that exclusively feed on their hosts suggests that factors beyond feeding guild, such as host-specific traits or immune filtering (Schmidt & Engel, 2021), play a key role in determining microbiota composition and diversity. The strong host species effect is consistent with previous research across multiple insect taxa (Malacrinò, 2022; Maritan *et al.*, 2024). Malacrinò (2022) conducted a meta-analysis across five insect orders and found that host species explained the largest part of the variance, while diet, sex, and life stage had minimal influence on microbial communities. This raises the question of whether microbiota composition reflects the evolutionary history of their insect hosts. Interestingly, Malacrinò's study (2022) also tested for phylosymbiosis — the relationship between host evolutionary history and microbiota structure (Lim & Bordenstein, 2020) —, and did not find such pattern for Insecta. This suggests that each insect species harbours a unique microbiota shaped by a unique combination of host's traits, rather than being determined by host phylogenetic relationships.

Some microbial associations observed in **Chapter II** aligned with prior research. *Lactobacillus* and *Wolbachia*, the dominant bacteria in *Formica* ants in the Åland Islands, have been documented in other Finnish (Lindström *et al.*, 2019; Jackson *et al.*, 2023) and non-Finnish *Formica* populations (Viljakainen, Reuter & Pamilo, 2008; Russell, 2012). While *Lactobacillus*, a gut-associated bacterium, is not typically vertically transmitted, it may provide nutritional or defensive benefits, aiding in sugar metabolism from aphid honeydew (Zheng *et al.*, 2022; Jackson *et al.*, 2023). Similarly, *Pantoea* was found in two of three Pentatomidae stinkbug species (*Aelia acuminata* and *Palomena prasina*), consistent with its known role in stinkbug survival and fecundity (Hosokawa *et al.*, 2016a, 2016b; Duron & Noël, 2016). In *Carpocoris purpureipennis*, an infection by *Escherichia/Shigella*, a closely related to *E. coli*, was identified. Notably, *Plautia stali*, a closely related stinkbug, can experimentally associate with *E. coli* after losing its native *Pantoea* symbionts (Koga *et al.*, 2022; Sugiyama *et al.*, 2024). This natural *Escherichia/Shigella* infection in *C. purpureipennis* suggests a potential susceptibility to *Escherichia* group bacteria.

In **Chapter III**, I observed that the microbiota of the parasitoid *H. horticola* was often dominated by *Wolbachia*, whereas its host, *M. cinxia*, did not carry this symbiont. This difference likely contributes to the distinct microbial communities observed between the two species. These findings contrast with those of Hu and coauthors (2024), who reported similar bacterial composition between the larvae of the parasitoid *Cotesia vestalis* and their host, the moth caterpillar *Plutella xylostella*. Importantly, previous studies focused on larval stages, while my analyses were based on adult individuals.

While the analyses in **Chapters II** and **III** provide valuable insights into the microbiota of insect species in the *P. lanceolata* community, some methodological aspects should be considered. In **Chapter II**, DNA was extracted from unwashed whole insect bodies, which may have captured both external and internal microbial DNA. In contrast, in **Chapter III** used washed abdomens for DNA extraction, targeting internal microbiota, particularly from gut and reproductive tissues. Since different bacterial taxa can colonize distinct tissues across host species (see section III.1.c in **Chapter IV**), the resulting microbial profiles are not directly comparable. Therefore, differences in microbiota composition and diversity between **Chapters II** and **III** should be interpreted with caution.

Despite considerable sampling efforts, the number of samples varied greatly among insect species in **Chapter II** and among temperature treatments in **Chapter III**. This uneven sampling may have influenced the observed patterns, potentially contributing to the horseshoe-shaped clustering (Novembre & Stephens, 2008; François & Jay, 2020) in Weighted UniFrac (Figure 5) and Bray–Curtis analyses (see **Chapter II**). Although small sample sizes for some species may have limited the statistical power of certain analyses, the results still provide strong evidence that host identity is a primary determinant of microbiota composition.

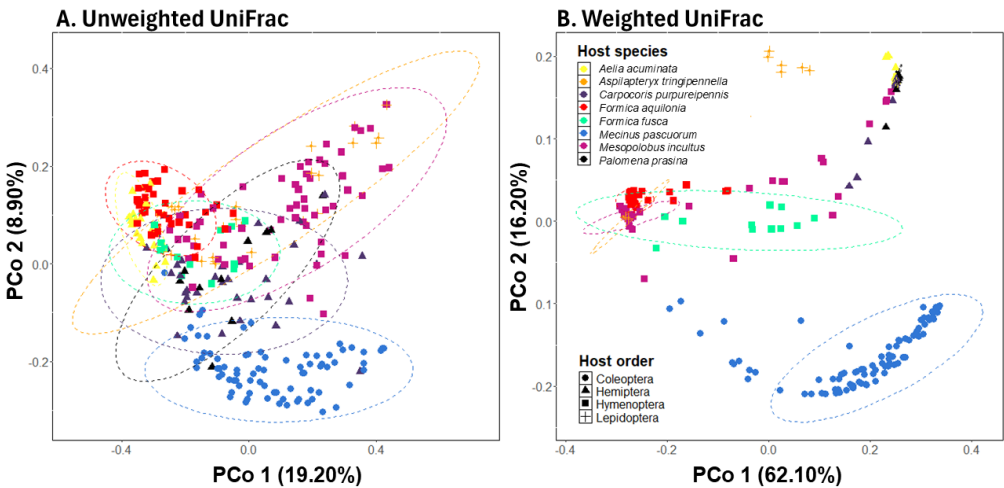


Figure 5. Microbiota composition for the eight common insect species associated with *Plantago lanceolata* from the Åland Islands (N specimens = 296). A-B. PCoA plot using (A) Unweighted and (B) Weighted UniFrac distances. Colours indicate insect species, and shapes indicate insect Orders. Elipses indicate 95% confidence limits for each host species. Retrieved from **Chapter II**.

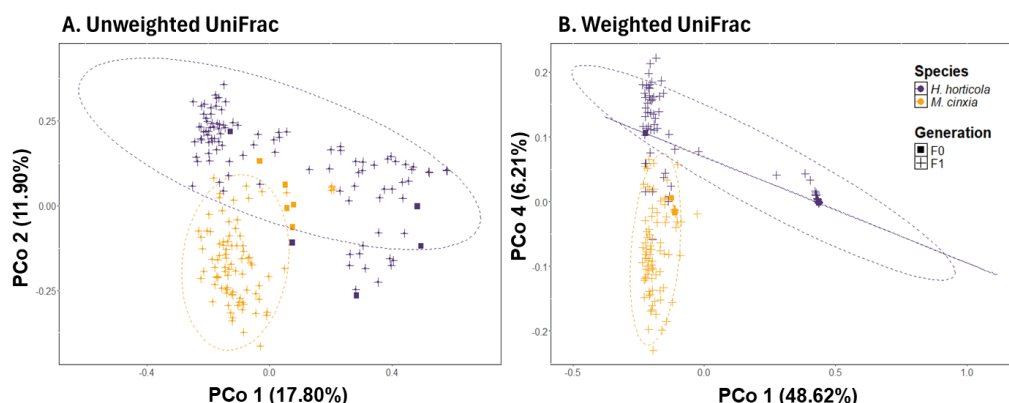


Figure 6. Microbiota composition of *Melitaea cinxia* and *Hyposoter horticola*. A–B. PCoA plots based on (A) Unweighted UniFrac and (B) Weighted UniFrac distances, showing the microbiota of parental (F0; squares) and offspring (F1; crosses) generations of *M. cinxia* (yellow) and *H. horticola* (purple). In panel B, axes 1 and 4 are displayed to better illustrate differences between the insect species. Ellipses represent 95% confidence intervals for each host species. Figure retrieved from **Chapter III**.

(b) Microbe-parasite interaction

In **Chapter I**, I examined the effect of the parasite *Blastocrithidia* sp. (Euglenozoa: Trypanosomatida) on the microbiota of *Carpocoris purpureipennis* (Hemiptera). Of the 33 specimens analysed, five carried the parasite. However, its presence did not significantly alter host microbiota composition, as no differences were detected in relative and absolute zOTU abundance, zOTU richness, or Shannon index. This suggests that *Blastocrithidia* sp. may have minimal impact on the host microbiota, potentially due to its low titer or small size relative to the host. These findings contrast with studies on other trypanosomatids, such as *Trypanosoma cruzi*, which have been shown to modify their host's microbiota (Díaz *et al.*, 2016; Guarneri & Schaub, 2021; Omond, Caner & Arserim, 2024). It is also possible that more specimens were infected but went undetected due to the limited specificity of the *COI* marker used.

(c) Microbe-microbe interaction

In the parasitoid wasp *H. horticola*, bacterial communities were distinctly structured based on the presence or absence of the symbiont *Wolbachia* (**Chapter III**) (Figure 9A–B; see **Chapter III** for Bray-Curtis results). In the Åland Islands, *Wolbachia* is estimated to infect approximately half of the *H. horticola* population (Duploux *et al.*, 2015). While the bacterium has been shown to increase susceptibility of the *H. horticola* larvae to hyperparasitism by *Mesochorus cf. stigmaticus* (van Nouhuys *et al.*, 2016), its broader effects on parasitoid host population dynamics (Duploux *et al.*, 2021) remain largely unexplored. In **Chapter III**, it was further demonstrated that *H. horticola* individuals infected with *Wolbachia* exhibited lower bacterial richness, but a higher Shannon index compared to uninfected individuals (Figure 9C–D). This pattern has been observed in other bacterial communities (Li *et al.*, 2022) and may be explained by *Wolbachia*'s high abundance, which limits niche space for other bacterial taxa. As a result, fewer rare taxa persist, leading to a more even distribution of abundances among bacterial species (Li *et al.*, 2022).

(3) Effects of environmental factors on insect microbiota composition and diversity

(a) Seasonality and habitat quality

In **Chapter II**, season and habitat quality indicators (site connectivity, landscape type, and *P. lanceolata* density) were found to have little effect on the microbial community composition of the eight insect species analysed, with a few exceptions. The weevil *M. pascuorum* showed significant seasonal shifts in microbiota composition and diversity (Figure 7A-B, 8A-B; see **Chapter II** for Bray-Curtis results). Seasonal microbiota variations can reflect host life cycle changes, developmental stages, or generational shifts (Yang *et al.*, 2022; Wang *et al.*, 2024). In Åland, *M. pascuorum* is multivoltine, with a spring generation emerging from winter diapause and reproducing in June, followed by a summer generation active from July to August (E. A. Dickason, 1968; Nieminen & Vikberg, 2015). Specimens collected in June or July may belong to different generations, potentially explaining observed microbiota differences. Seasonality also significantly affected the microbial community of the ant *F. aquilonia*, increasing its bacterial richness from spring to late summer (Figure 8A-B). *Formica aquilonia* workers obtain carbohydrates primarily by collecting honeydew from aphids that feed on tree sap, while protein is acquired through the consumption of invertebrate prey (Lamb & Ollason, 1993; Iakovlev *et al.*, 2017). The species has been reported to shift from a predominantly honeydew-based diet in spring to a more varied diet in summer (Iakovlev *et al.*, 2017), which may explain the seasonal variation in its microbiota.

Dominant zOTUs can influence overall community structure through interactions with the host or with other microbes, potentially masking subtler patterns in the rest of the community (Simhadri *et al.*, 2017; Detcharoen *et al.*, 2023; Flores *et al.*, 2023). In some cases, highly abundant taxa may also bias diversity metrics or compositional analyses (McKnight *et al.*, 2019). When analysing only the non-dominant microbial community (i.e., excluding the most abundant bacteria), five of the eight insect species showed no significant response to environmental variables. In *M. pascuorum*, seasonal effects disappeared, although the microbiota composition formed two distinct clusters in the PCoA plot (Figure 7C–D; see **Chapter II** for Bray-Curtis results), possibly due to the presence of another symbiont, the bacterium *Rickettsia*. This suggests that the previously observed seasonal variation was likely driven by dominant bacterial taxa (Morganellaceae). These findings highlight how different types of interactions — both host–microbe and microbe–microbe — can influence microbial responses to environmental conditions (Nemergut *et al.*, 2013).

Landscape type significantly influenced microbial richness in six of the eight insect species (*A. acuminata*, *A. tringipennella*, *F. aquilonia*, *M. pascuorum*, *M. incultus*, and *P. prasina*). Landscapes with high anthropogenic activity are often assumed to reduce host-associated microbial diversity (Magura *et al.*, 2024a, 2024b), and this was indeed the case for most insect species. However, *F. aquilonia* and *M. pascuorum* exhibited the opposite effect, with greater microbial richness in environments with higher anthropogenic influence (Figure 8C–D). This finding may be due to increased microbial exposure in environments with high anthropogenic influence, stress-induced microbiota shifts, or dietary changes resulting from altered plant communities (Antonelli *et al.*, 2022). These species-specific responses highlight the role of host traits — such as dispersal, feeding behaviour, and immune function — in shaping microbial communities (Jones *et al.*, 2018; Paddock *et al.*, 2022; Shell & Rehan, 2022).

Overall, higher site connectivity was generally associated with increased microbial richness, likely because well-connected habitats promote insect movement and exposure to diverse environmental microbes (Sessitsch *et al.*, 2023). This pattern aligns with findings in plant-pathogen systems, where connectivity has been associated with higher pathogen diversity and infection rates (Laine, 2023), suggesting that similar dynamics may govern both symbionts and pathogens across host populations. Similarly, higher host plant density correlated with greater microbiota richness in several species, potentially due to larger host population sizes, increased availability of microbial reservoirs on plant surfaces or more stable feeding resources (Yang *et al.*, 2023; Ge *et al.*, 2025).

When analysing the effects of environmental factors on insect-associated microbiota, the small sample size for a few insect species may have contributed to undetected or weak patterns. Limited representation can reduce statistical power, making it more difficult to detect subtle but biologically meaningful responses to environmental variation.

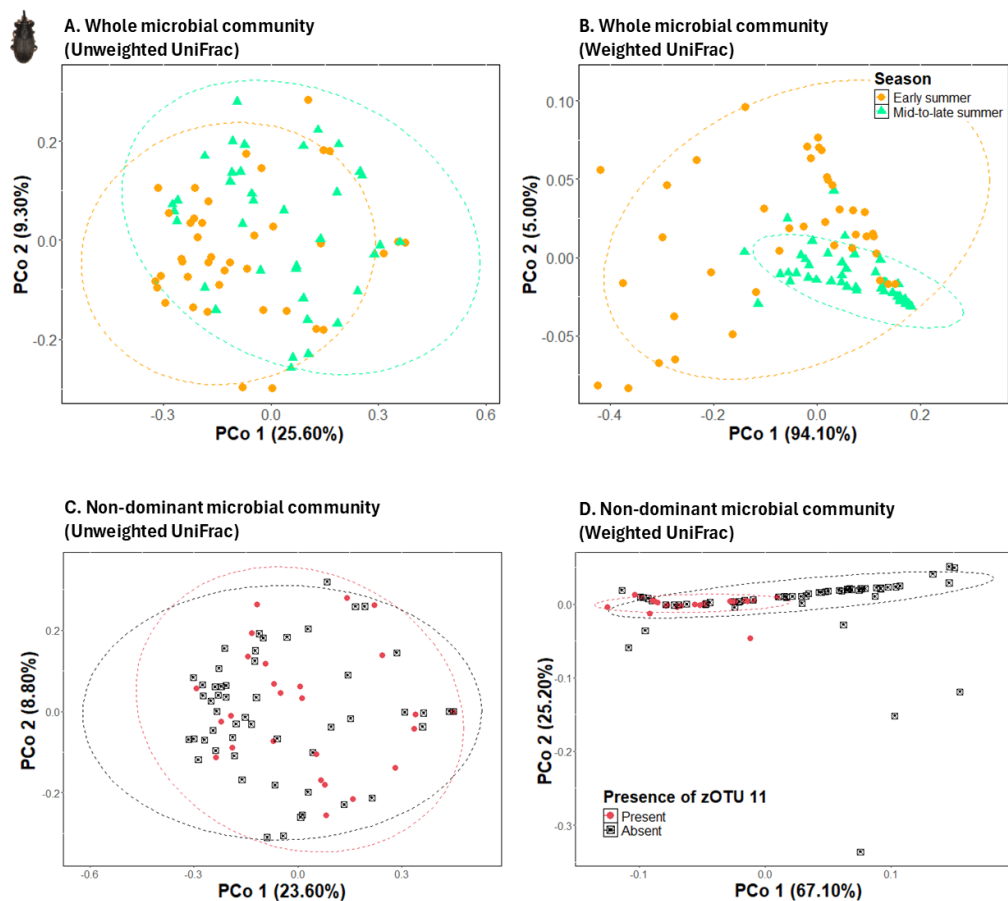


Figure 7. Whole and non-dominant microbiota composition for *Mecinus pascuorum*. A-B. PCoA plots based on (A) Unweighted UniFrac and (B) Weighted UniFrac distances for the whole microbial community coloured by season. Seasons are represented by yellow circles (early summer) and green triangles (mid-to-late summer). C-D. PCoA plots based on (C) Unweighted UniFrac and (D) Weighted UniFrac distances for the non-dominant microbial community (after

removing the most abundant zOTU (zOTU 1 – *Morganellaceae*), coloured by the presence of zOTU 11 (*Rickettsia*). Individuals with and without *Rickettsia* are represented by pink circles and black squares, respectively. Ellipses indicate 95% confidence intervals. Retrieved from **Chapter II**.

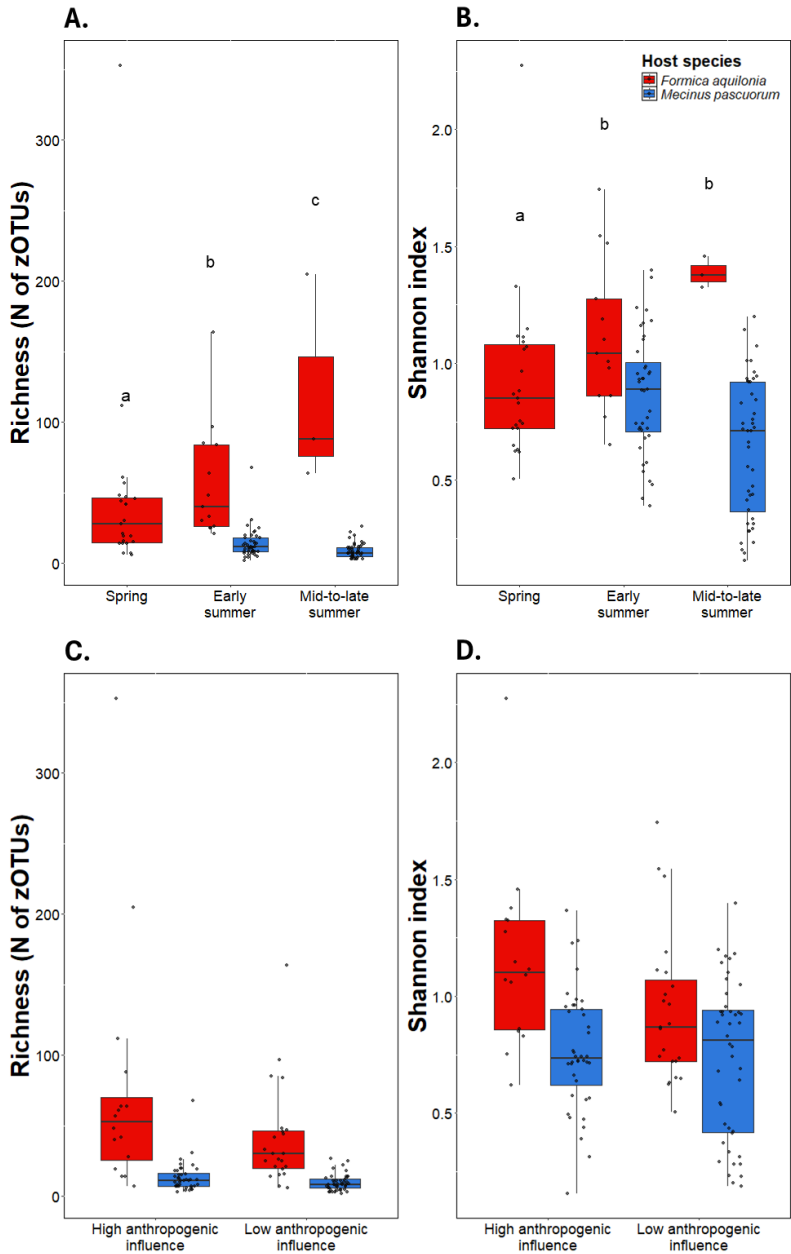


Figure 8. Boxplots showing bacterial richness (number of zOTUs) and Shannon diversity index for the whole microbiota of *Formica aquilonia* (red, N = 39) and *Mecinus pascuorum* (blue, N = 84), across seasons and landscape types. (A–B) Seasonal variation (spring, early summer, mid-to-late summer), and (C–D) landscape type (low vs. high anthropogenic influence), with richness

shown in panels A and C, and Shannon index in B and D. Boxes represent the median and inter-quartile range. Letters above the boxes indicate significant differences ($p < 0.05$) between groups for insect species in categorical variables with more than two levels. Figure retrieved from **Chapter II**.

(b) Temperature

In **Chapter III**, I examined how temperature experienced by the parental generation and across developmental stages of the offspring influences the F1 microbiota of *Melitaea cinxia* and *Hyposoter horticola*. In these species, the microbiota composition was not affected by temperature treatments. However, in both the butterfly and its parasitoid, the thermal conditions experienced by the maternal generation (F0) influenced the microbial diversity indexes. These findings suggest that environmental effects on microbiota may manifest transgenerationally. The mechanism underlying this transgenerational effect remains unknown. Offspring may inherit part of their microbiota from their mother through intra- or extracellular vertical transmission, or the temperature experienced by the mothers could influence early microbiota assembly, leading to lasting effects (Guilhot *et al.*, 2023). Similar transgenerational effects have been observed in the cockroach *Diploptera punctata*, where the temperature experienced by the parental generation had a stronger impact on offspring microbiota than the offspring's own thermal environment (Bridson, 2020). In the context of ongoing climate change, such effects are particularly important, as shifts in microbiota may only become apparent in subsequent generations.

In *H. horticola* specimens carrying *Wolbachia* (see the “Microbe-microbe interaction” section), the effect of parental temperature on offspring's richness and Shannon index was modulated by temperatures experienced across life stages, whereas *Wolbachia*-free specimens showed a weaker overall response to temperature treatments. For instance, the *Wolbachia*-infected individuals showed lower bacterial richness when their parents were reared at 28 °C, while the same temperature treatment significantly increased their Shannon index (Figure 9C-D). This pattern may be linked to the presence of *Wolbachia*, as richness and Shannon index also varied more among infected individuals, possibly due to the bacterium's sensitivity to temperature fluctuations (Hague, Caldwell & Cooper, 2020; Hague *et al.*, 2022). Changes in *Wolbachia* abundance likely influence the overall microbiota composition of the parasitoid, affecting the relative abundance of other bacterial taxa (Li *et al.*, 2022) (see the “Microbe-microbe interaction” section).

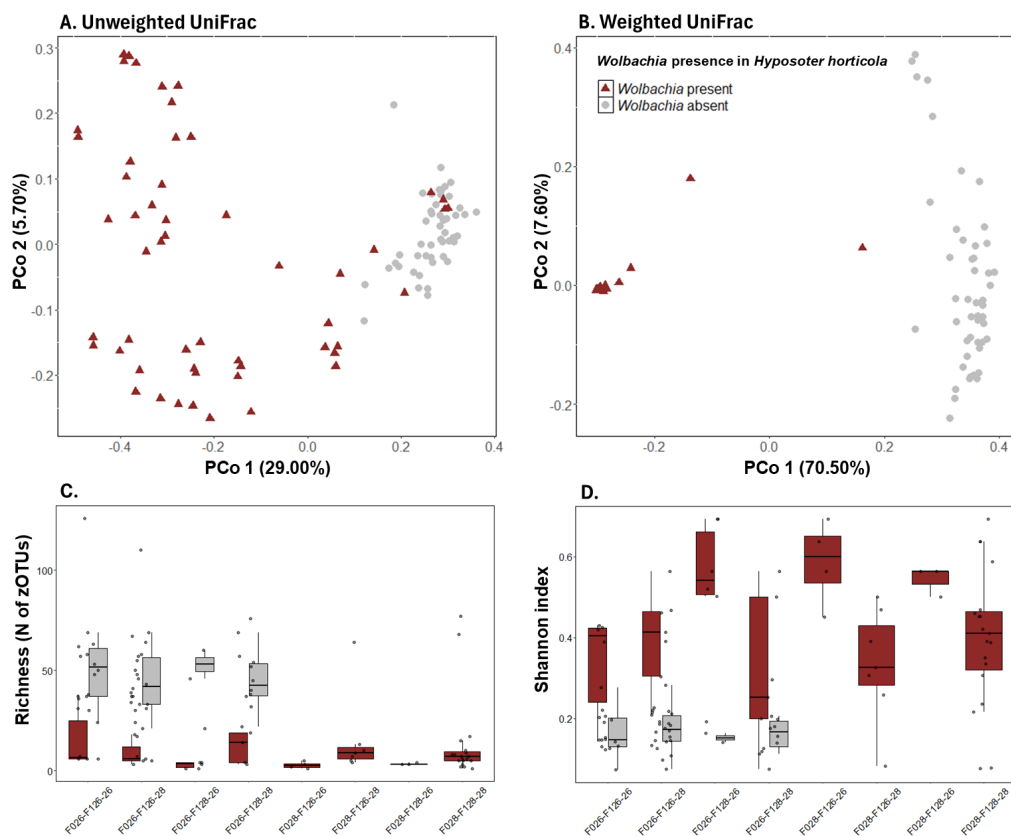
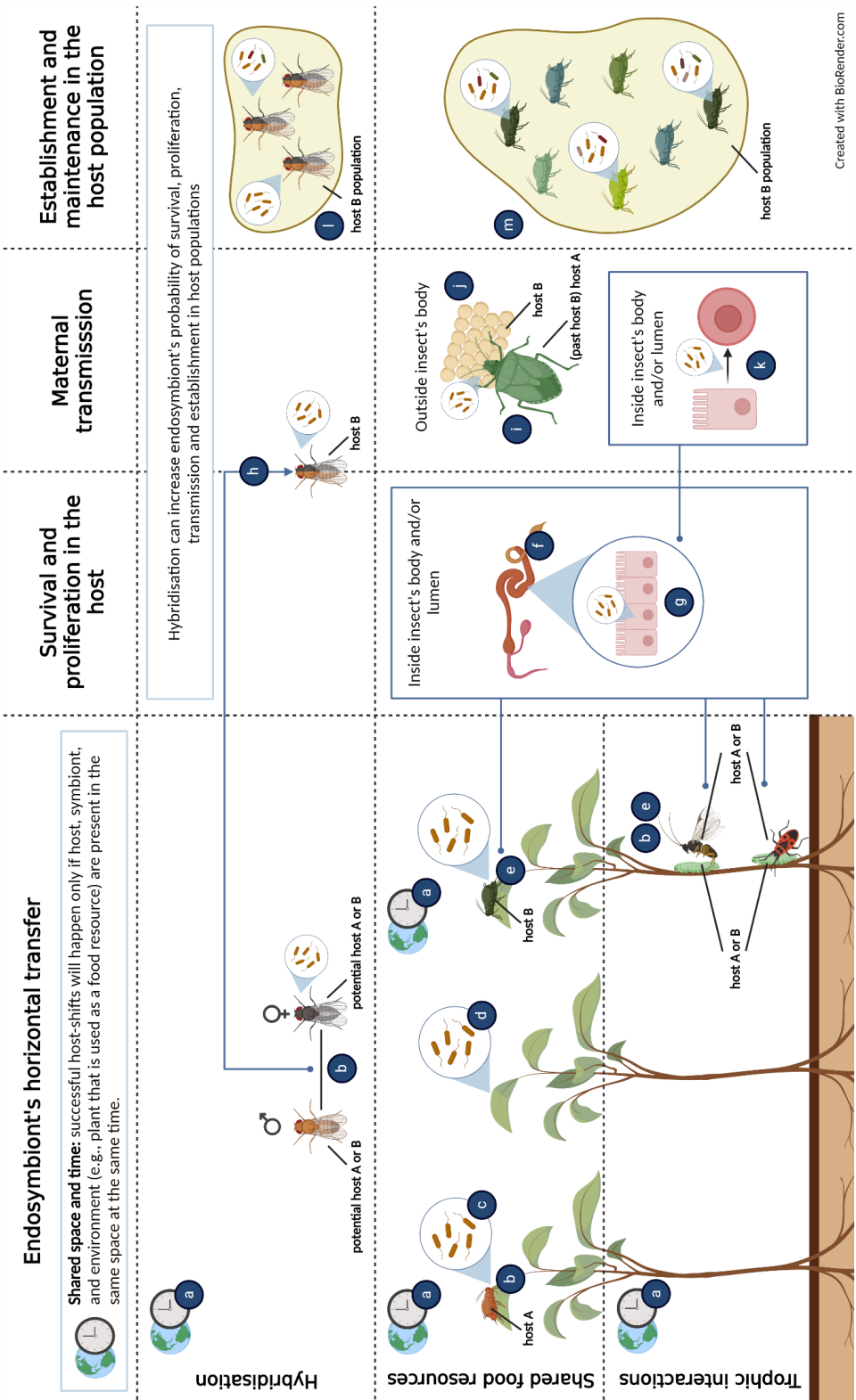


Figure 9. Microbiota composition, richness and Shannon index of F1 adult *Hyposoter horticola*. A-B. PCoA plots using (A) Unweighted and (B) Weighted UniFrac distances. Shapes represent individuals with or without *Wolbachia* (dark red triangle = *Wolbachia* present; gray = *Wolbachia* absent). C-D. Richness (N of zOTUs) and Shannon index, respectively, per temperature treatment for individuals with (dark red) and without *Wolbachia* (gray). Retrieved from **Chapter III**.

(4) Effects of environmental factors on host shifts

In **Chapter IV**, the analysis of dominant biological systems in symbiotic host shifts revealed distinct biases in the study of insect symbionts, their hosts, and the abiotic factors shaping their associations. Bacteria were the most frequently studied symbionts, often as single endosymbionts (e.g., *Wolbachia*, *Buchnera*) and sometimes as microbial communities. While insect-bacteria associations have been extensively examined (Gurung, Wertheim & Falcao Salles, 2019), research on fungal and viral symbioses remains limited. Unsurprisingly, Hemiptera, particularly aphids, and Diptera, especially *Drosophila*, were disproportionately represented, likely due to their suitability as laboratory models (Douglas, 1998; Sandström, Telang & Moran, 2000; Detcharoen *et al.*, 2020; Hague *et al.*, 2020, 2022; Radousky *et al.*, 2023). Among abiotic factors, temperature dominated, appearing in 74% of studies, followed by climate and precipitation. This emphasis reflects temperature's fundamental role in insect physiology and microbial metabolism (Gilbert & Raworth, 1996; Sunday *et al.*, 2014; Knapp & Huang, 2022). However, other rapidly changing environmental factors, such as humidity and pollution (Wagner, 2020), are comparatively less explored.

Within the four main steps required for a symbiotic host shift — (i) acquisition from the environment or a donor host, (ii) proliferation within the naïve host, (iii) persistence through vertical transmission, and (iv) spread within the host population (Sanaei *et al.*, 2021) — I identified key stages (“events”) that may be influenced by environmental conditions (Figure 10). Drawing from the literature, I examined how abiotic factors can influence each stage. While host shifts are often described as processes vulnerable to abiotic stress at multiple stages — for example, *Wolbachia* titer is known to decline at high temperatures, compromising transmission fidelity (Hague *et al.*, 2020, 2022) —, environmental changes can also create opportunities for novel symbiotic associations by, for instance, causing the movement and overlap of potential donor and recipient insect species (Gornish & Tylianakis, 2013; Carbonell *et al.*, 2017; Yang *et al.*, 2021; Fenn-Moltu *et al.*, 2022). Together, this underscores the context-dependent nature of abiotic influences on host shift dynamics.



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Figure 10. Conceptual framework of the four main steps required for the establishment of a naïve endosymbiosis. The A hosts are donors, and the B hosts are recipients of endosymbionts. Lower case letters indicate events within the main steps that can be affected by environmental conditions: (a) host shifts occur if hosts (donor A or recipient B), endosymbiont, and environment (e.g., shared resource) share the same space at the same time; (b) endosymbiont's titer and tropism in the donor host A; (c) immediate (short-term) survival of the endosymbiont outside host A's cells or body; (d) long-term (permanence for a period, season) survival of the endosymbiont outside host A's cells or body; (e) endosymbiont's titer and tropism in the recipient host B; (f) endosymbiont's ability to bear host's metabolic changes; (g) endosymbiont's ability to overcome host's immunity; (h) hybridisation as a shortcut for promoting survival and proliferation in the novel host; (i) mechanism that maintains extracellular vertical transmission in host A; (j) endosymbiont's survival to environmental exposure and acquisition by the offspring (host B); (k) endosymbiont's survival to the cell-to-cell transmission until reaching the germline; (l) hybridisation as a shortcut for promoting establishment in the novel host population; (m) fitness outcome of the interaction endosymbiont-host B promote endosymbiont's establishment in host B's population. Retrieved from **Chapter IV**.

(5) Art as a tool to communicate my PhD thesis

In **Chapter V**, I explored how the art activity *MicrobiART* can be used as a learning tool to foster discussions on host-microbe interactions. After creating the exhibition wall (Figure 11), participants were invited to reflect on the emerging patterns, identifying two key insights: (i) environments, along with their respective hosts and abiotic factors, play a crucial role in shaping interactions, and (ii) the duration of the interaction influences the results, with longer interactions leading to denser patterns (Figure 11). Participants noted that despite some common elements across environments, paintings differed significantly due to the interplay between abiotic factors and host backgrounds. They also acknowledged the impact of their own role in the activity by choosing players and moving the tray, suggesting that this could represent individual variation within populations.

MicrobiART effectively transforms abstract concepts into tangible experiences through accessible materials and adaptable methods. Participants were highly engaged, experimenting with different conditions and voluntarily creating multiple paintings. While formal learning assessments were not conducted, feedback suggested that the activity helped students grasp how interaction outcomes depend on the specific combination of host, microbe, and environment. Recent research in science communication emphasizes that visual representations in scientific practices should not only facilitate cognitive understanding but also actively engage participants in the process of visualisation (Evagorou, Erduran & Mäntylä, 2015; Davies & Horst, 2016). *MicrobiART* aligns with this perspective by requiring students to create their own representations of otherwise invisible species interactions. Additionally, through public engagement, it is possible to foster emotional connections with participants while also learning from their perspectives, creating a more interactive and reciprocal knowledge exchange (Davies & Horst, 2016; Zaelzer, 2020). By blending creativity, interaction, and dialogue, *MicrobiART* not only enhances understanding of host-microbe interactions but also exemplifies how participatory approaches can make science more engaging, inclusive, and impactful.



Figure 11. Paintings representing the outcomes of host-microbe interactions in environments A and B. (A) Environment with abiotic factors represented by warm-coloured paints. (B) Environment with abiotic factors represented by cool-coloured paints. Black arrows highlight example paintings where interactions lasted longer, resulting in denser patterns, while red arrows indicate paintings with shorter interactions, leading to more sparse patterns. Retrieved from **Chapter V** (Souza Beraldo, 2025).

V. Conclusions and future directions

Environmental conditions influence not only insect species and their communities but also the microbial partners they host. Changes in host-associated microbial communities can have significant effects on host fitness and offspring success. Exploring how microbial community composition and diversity vary in wild insects in response to both ecological interactions and environmental factors offers valuable insight into host–microbe dynamics, while also shedding light on the ecological and evolutionary dimensions of these interactions. In this thesis, I investigated the insect community associated with *Plantago lanceolata* in the Åland Islands (**Chapter I**), examining how ecological interactions and environmental variables influence microbiota composition and diversity across multiple insect species (**Chapters I–IV**). In addition, I considered how artistic approaches can support science communication by making host–microbe–environment relationships more accessible to the public (**Chapter V**).

This thesis produced four main outcomes. Firstly, in **Chapter I**, I sequenced both the *COI* and *16S rRNA* genes when characterising the microbiota of the insect individuals. Because *COI* amplification captures not only the host's genetic material but also that of potential prey, parasitoids, and symbionts within the target sample, this so-called *COI* "contamination" can offer evidence of previously undocumented trophic interactions. Using this approach, I **expanded the known network of insect species associated with *P. lanceolata*, while also adding a novel layer of diversity that includes interactions with symbionts.** This result highlights the value of integrating *COI* barcoding with microbiota studies in wild species. Moreover, the expansion of the *P. lanceolata* insect network (Figure 4) was made possible by building on existing literature, underscoring the cumulative nature of scientific knowledge. Future research should continue to explore the insect community and its overlooked interactions, such as those involving Pentatomidae bugs and *Blastochitridia* sp. or *Escherichia/Shigella* infections (**Chapter I**), as well as investigate the potential for symbiont transfer within the *P. lanceolata* insect community (**Chapter II**).

Secondly, across the insect species studied (**Chapters II and III**), **host identity consistently emerged as the primary determinant of microbiota composition**, a pattern that aligns with previous research (Malacrinò, 2022; Maritan *et al.*, 2024). The strong role of host species in structuring insect microbiota raises the question whether phylosymbiosis occurs across insect groups. Although, previous studies have found no evidence for this pattern at broad taxonomic scales within Insecta, Malacrinò and colleagues (2022) proposed that phylosymbiosis may be difficult to detect at such scales and could instead be more evident within specific subclades. Phylosymbiosis have indeed been observed in smaller taxonomic groups, such as *Formica* ants (Jackson *et al.*, 2023). Therefore, a valuable next step would be to investigate phylosymbiosis signal within other genera of the *P. lanceolata* insect community. Furthermore, given that each insect species harboured a distinct microbiota, future studies should aim to uncover the functional roles of these microbial communities, as well as the mechanisms by which they are transmitted and maintained within their hosts.

Thirdly, although all insect species were either sympatric in the Åland Islands or involved in close interactions (host–parasitoid relationships), **environmental conditions influenced microbial diversity unevenly across species, with distinct, species-specific responses** (**Chapters II, III, and IV**). The observed seasonal variation in the microbiota of *Formica aquilonia* and *Mecinus pascuorum*

deserves further investigation to determine whether these patterns are indeed driven by changes in diet or by differences between generations, respectively (**Chapter II**). In **Chapter III**, I found that the temperature experienced by the parental generation had a stronger effect on offspring microbiota diversity than the temperature experienced during the offspring's own development. Future research should explore the mechanisms underlying such transgenerational effects, specifically whether temperatures experienced by mothers affect the microbiota transmitted to their offspring, or whether parental conditions more broadly shape microbial community assembly in the next generation. To contribute to the understanding of microbial transmission within and between insect species, in **Chapter IV**, I demonstrated that although host shifts are often seen as vulnerable to abiotic stress, environmental change can also facilitate novel symbiotic associations. Gaining insight into what makes each step of the host shift process more or less sensitive to environmental conditions is essential for predicting insect–microbe responses to anthropogenic change. To reveal broader patterns in host shift dynamics, future research should move beyond model systems and integrate a wider range of environmental factors.

Fourthly, art-based approaches proved to be an effective communication tool in engaging the public in discussions about host-microbe interactions (**Chapter V**). The *MicrobiART* activity demonstrated that artistic representations of microbiota dynamics can stimulate public engagement and spark conversations about the effects of ecological and environmental factors on host-microbe relationships. This method offers a promising tool for science communication, facilitating interdisciplinary dialogue between scientific research and the broader public.

In conclusion, my thesis highlights the central role of host–microbe relationships in shaping insect microbiota, while also showing that microbial community diversity is influenced by both environmental context and interactions among microbes. To communicate the interplay between these factors, the art-based approach proved effective in making complex relationships more tangible. These underscores the value of integrating diverse forms of knowledge, such as science and art, to foster new ways of thinking and approaching science.

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