Ecological Genetics of Habitat-Forming Plants

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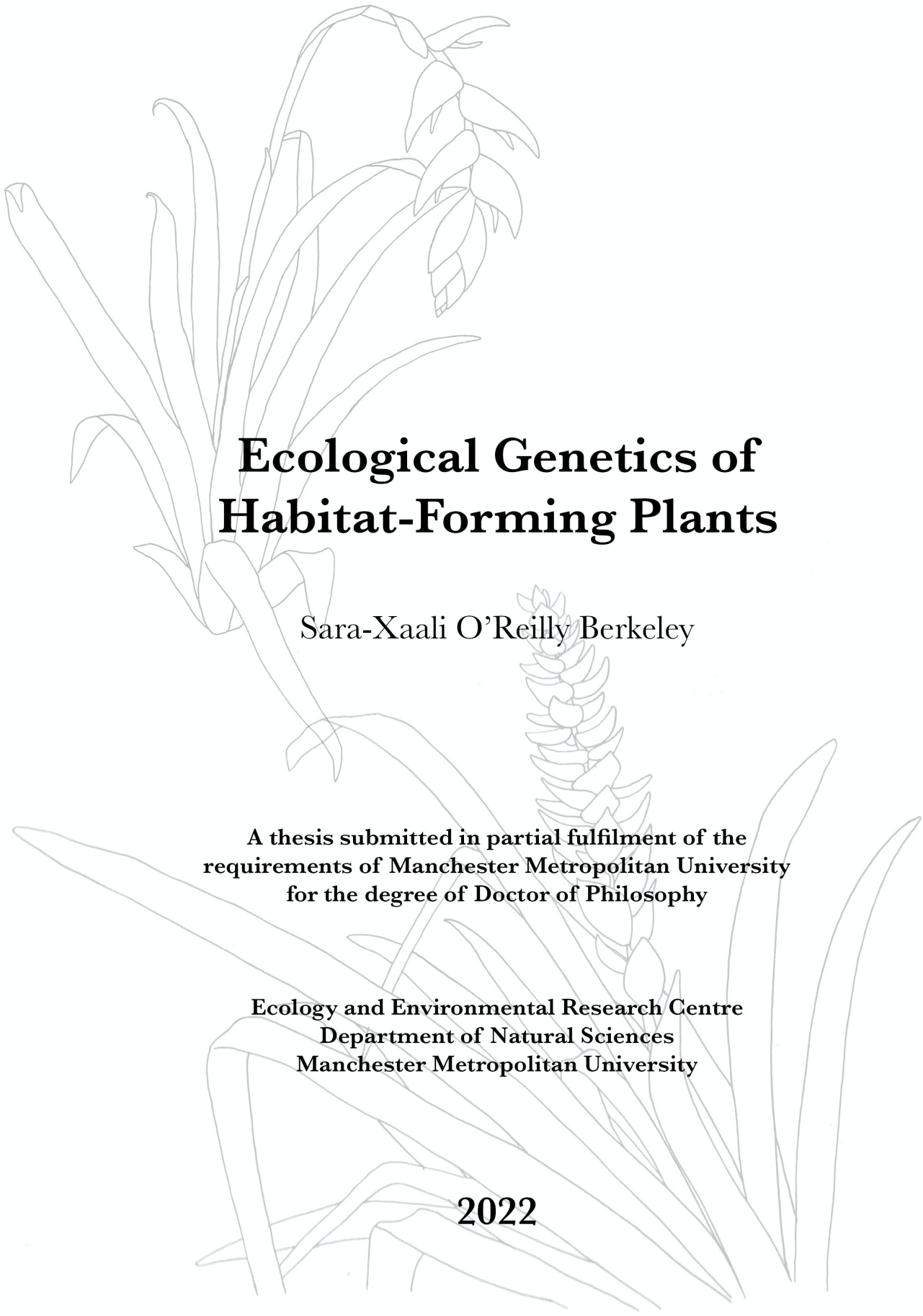
PhD 2022



Ecological Genetics of Habitat-Forming Plants

Sara-Xaali O'Reilly Berkeley

A thesis submitted in partial fulfilment of the requirements of Manchester Metropolitan University for the degree of Doctor of Philosophy

Ecology and Environmental Research Centre
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Manchester Metropolitan University

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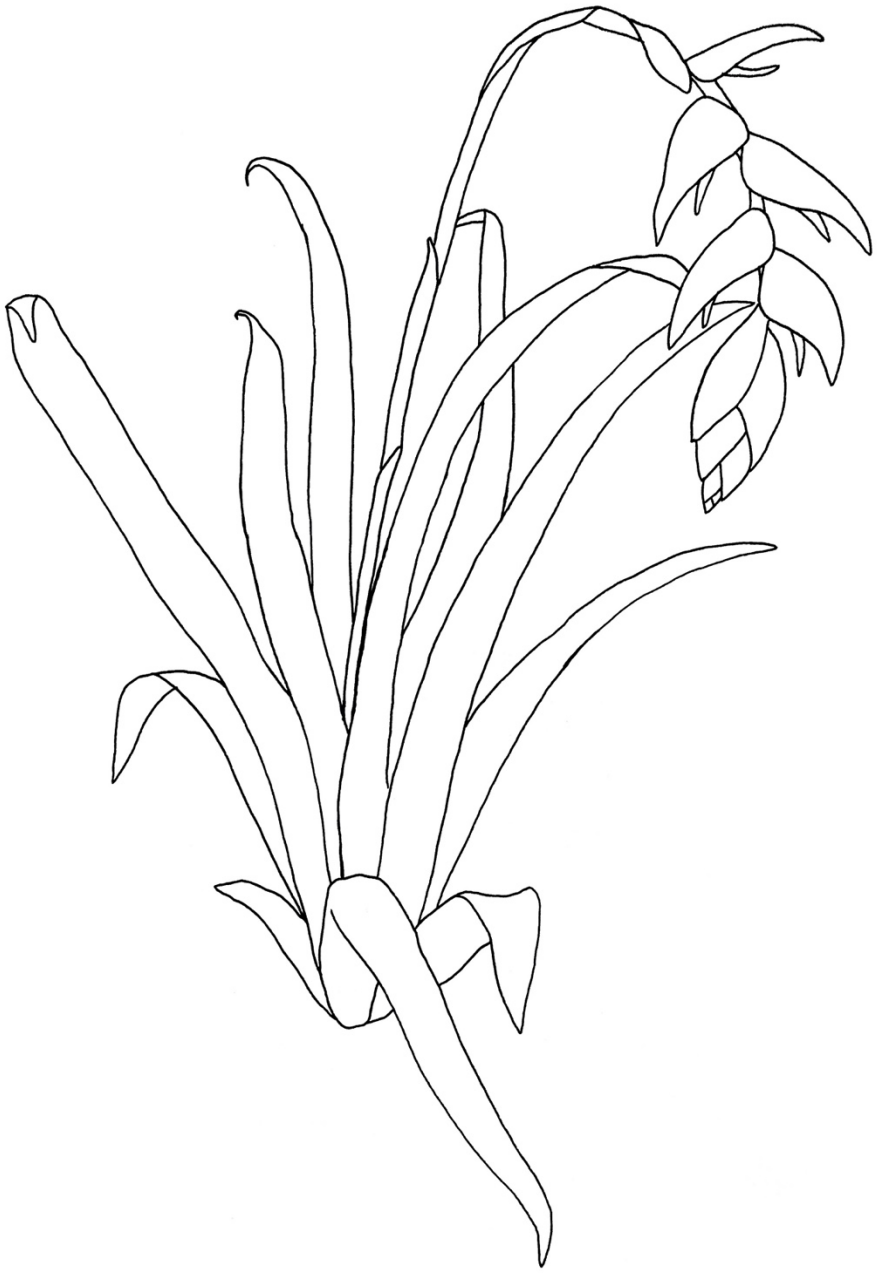
Contents

Declaration

I declare that I have produced all the contents of this PhD thesis unless otherwise acknowledged.



Tillandsia dyeriana

General Abstract

Genetic diversity is an important component of biodiversity. Studying genetic diversity within species has become a key aspect of conservation biology, but is often neglected when studying the interactions among species. Increasingly, community genetics research is revealing relationships between plant genetic diversity and the community composition of associated organisms. However, the majority of these studies have taken place in temperate systems. In this thesis, we explore relationships between plant genotype and wider species diversity in an area of tropical rainforest in the Ecuadorian Amazon, using epiphytic bromeliads and the false-bird-of-paradise plant *Heliconia stricta.* Using a combined observational and experimental approach, we aimed to establish whether plant genotype influences community composition in these highly diverse tropical systems and their importance relative to environmental variables. We studied natural bromeliad communities of *Aechmea hoppii* and *A. nidularioides in-situ,* first establishing what environmental and plant factors affected the composition of their invertebrate and prokaryotic communities (Chapters II and III), and then analysing whether bromeliad genetic distance correlated with community distance (Chapter IV). We established *in-situ* experimental plots of multiple *Heliconia stricta* genotypes and analysed the effect of plant genotype on herbivory (Chapter V). Finally, we designed an *ex-situ* experiment using *Neoregelia schultesiana* bromeliads in a greenhouse to compare effects of plant genotype in the absence of environmental variation (Chapter VI). In the wild, bromeliad size, forest type, and the height in the tree at which the bromeliad was found to explain small but significant proportions of the variation in invertebrate and prokaryotic communities (Chapter II), depending on what taxonomic level was considered (Chapter III). However, bromeliad genotype did not correlate with prokaryotic or invertebrate community composition (Chapter IV). Contrary to this, *Heliconia* genotype did affect herbivory and growth rate – some genotypes were consumed or grew consistently more than others (Chapter V). Finally, there were genotypic differences between bromeliads in a greenhouse setting (Chapter VI). Taken together, the results of this thesis suggest that, despite the diversity and complexity of megadiverse tropical systems, plant genotype may affect some interactions with other organisms, but this cannot be assumed to be true for all interactions. It may be that the

more organisms are involved and the less direct the interaction, the less likely it is that plant genotype will have an appreciable effect on wider community composition or ecosystem processes. We encourage the exploration of community genetics in a wider range of ecosystems, particularly in ecosystems and geographic regions where such studies have been absent or sparse.

Aechmea hoppii

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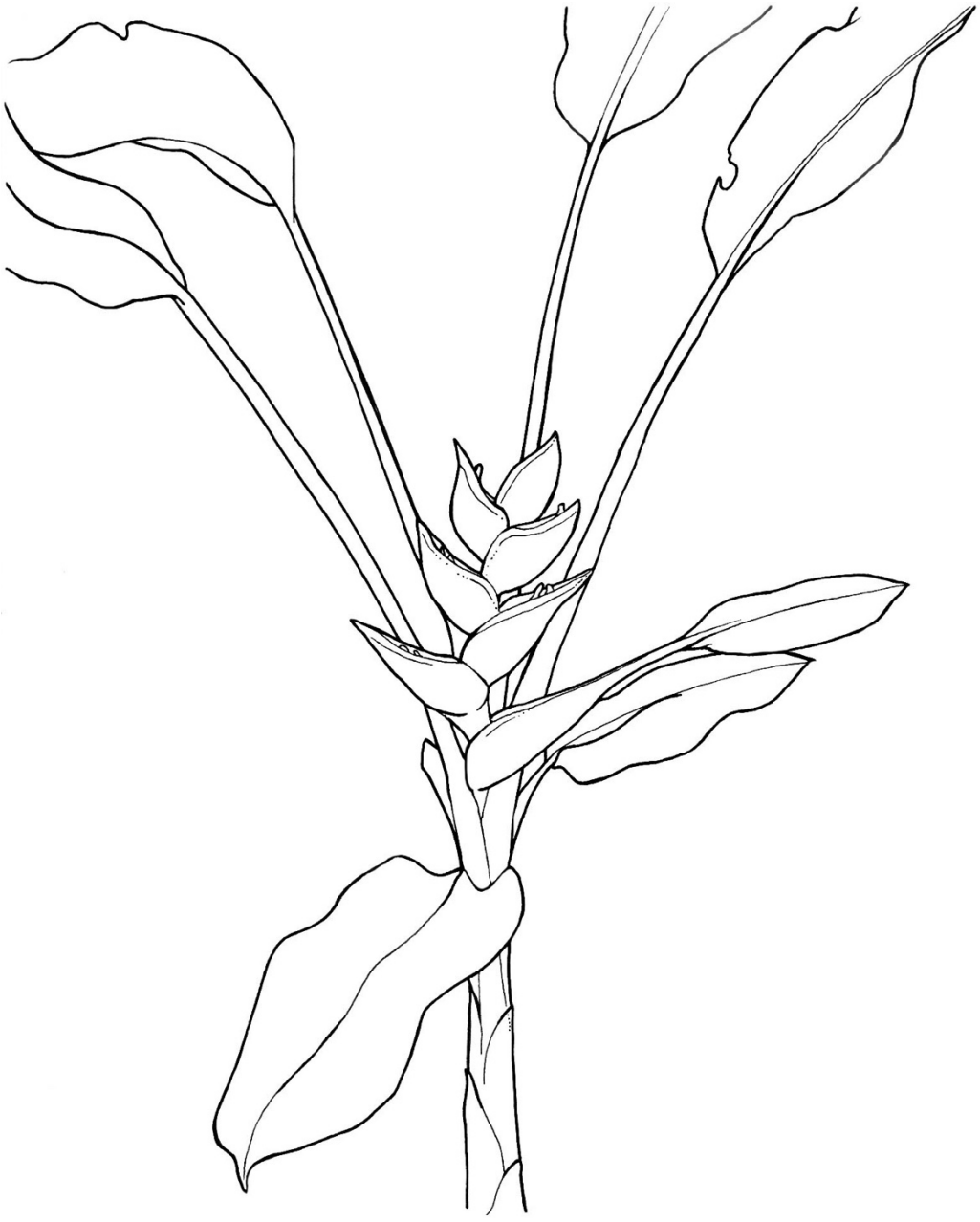
Dedication

To my family –

To my siblings, Nathan, Lily, Calloway, and Ruben, who are a constant source of inspiration.

And above all, to my parents, Orna O'Reilly and Jon Berkeley. You know how much you do.



Heliconia stricta

Acknowledgements

Innumerable people have helped me along this journey, but I could not have embarked upon it without the faith and support and opportunities offered to me by my principal supervisor, Prof Richard Preziosi. Thank you to my other supervisors – Dr Jennifer Rowntree, Dr Hannah Mossman, and Prof Liz Price for their advice, support, and feedback throughout.

I am particularly overwhelmed by the friendship and support of Alejandra Zamora Jerez and Sarah Griffiths, Alicia Bertolotti and Alyssa Smith. Without the friendship and guidance of Thomas Hughes, I would have been lost in the lab and the metabarcoding work in this thesis would not be what it is. Equally, Calum Bridson played an essential role in guiding me through the first steps of the bioinformatics and some of the statistical analysis. I wholeheartedly thank, in no particular order, Kern O'R., Jade N., Guuske T., Stefano C., Diana B., Darío C., Cristina G., "Orchid Olly", Miguel G., Emma R., Ciara S., Vanessa Y., Álvaro S. M., Joshua W, Anita H., Sarai N., Inés P., Meritxell M., and Jacob U. for everything along the way, for the fun and the laughs, the talks and the crying; this work not only benefitted indirectly from them by keeping me more or less sane, but directly benefitted from their knowledge and encouragement.

I am grateful for the numerous bits of field and identification work that various collaborators and students did and helped with in Ecuador, particularly Javier L. Patiño, Alex Pazmiño-Palomino, Hugh Tulloch, Sofía Trujillo Regalado, Andrea Hidalgo Cárdenas, Jagoba Malumbres-Olarte, and Abhiraj Chakraborty.

My friend Raphael "Iggy" Coleman thought doing a PhD didn't suit me – I wish he were still with us to prove him wrong! He encouraged me to pursue what I wanted to do nonetheless and believed in me. I hope Iggy knew what an inspiration he was to me in pursuing and fighting for the things I want and believe in.

– Sara-Xaali O'Reilly Berkeley, 2022

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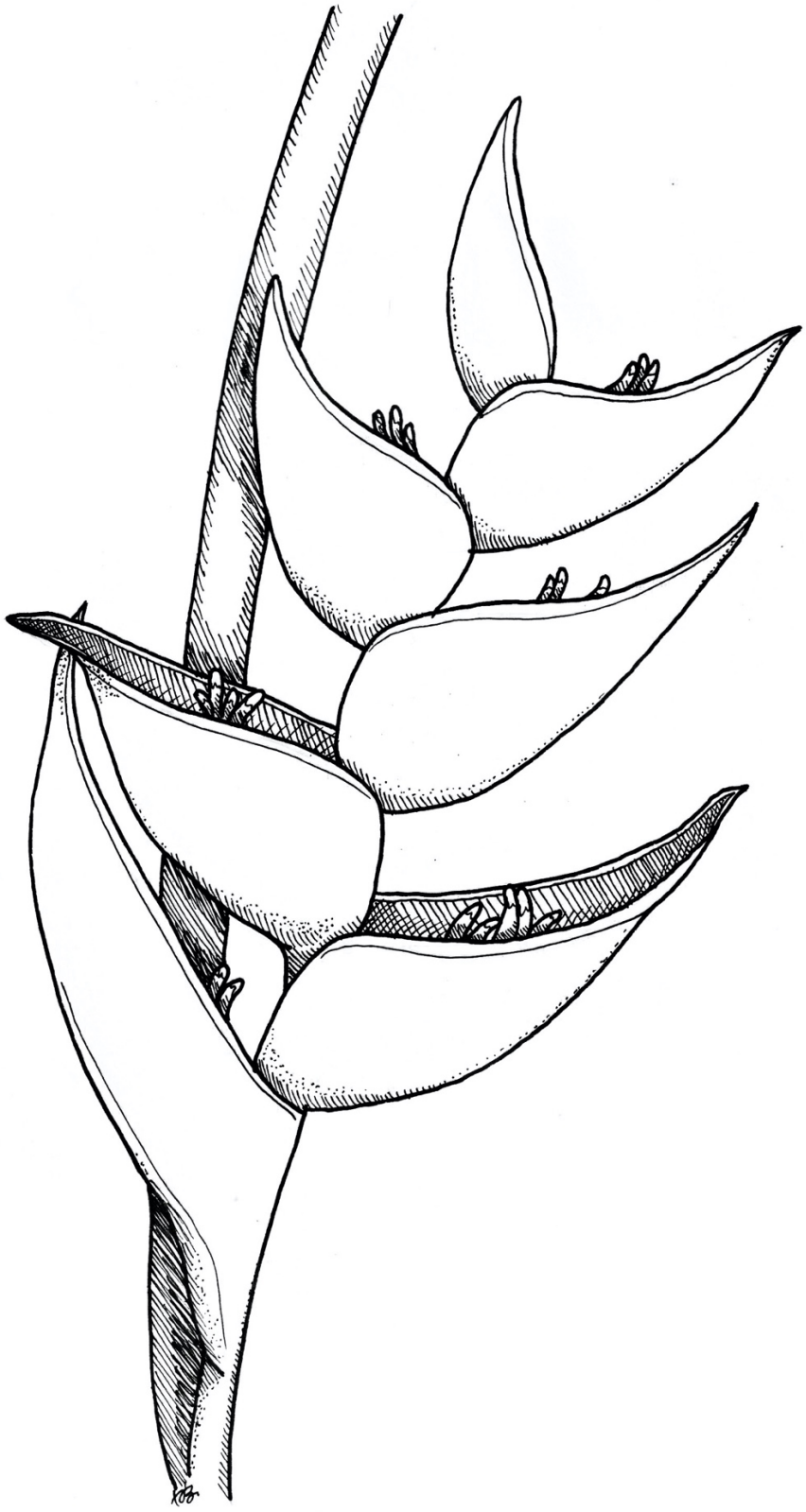
Chapter I

Introduction and thesis aims



1.1 Biodiversity

Biological diversity, as defined by the United Nation's Convention on Biological Diversity (United Nations 1992), refers to "the variability among living organisms from all sources (...) this includes diversity within species, between species and of ecosystems." This definition eliminates a common assumption that biodiversity refers to species diversity alone, by including intraspecific – and, therefore, genetic – variation. The taxonomic levels into which we classify organisms are by their very nature discreet categories, whereas biological variation is a continuum.

Many studies have demonstrated the importance of variation within taxa, particularly within species. For thousands of years, the phenotypic variation of animals and plants has provided a catalogue from which humans have been able to gradually enhance or lose traits in their stock and crops, and selective breeding continues to bear relevance even in the advent of molecular-assisted breeding and genetically modified organisms (Jaenicke-Després et al. 2003; Jonas and De Koning 2013; Wang et al. 2013; Janssen et al. 2017). Phenotypic variability within species is one of the conditions Charles Darwin recognised as necessary in order for natural selection to take place, along with heritability (Darwin 1859). We now know that inherited traits are genetic and epigenetic, and the rapid development and evolution of methods and tools to study this level of variation has allowed us to study biodiversity in an unprecedented way (Davey et al. 2011; Shokralla et al. 2012; McMahon et al. 2014; Haig et al. 2016).

In the past 30 years, the study of intraspecific diversity has become an important area of conservation research (Young et al. 1996; Manel and Holderegger 2013; Frankham et al. 2014). Population genetics has allowed us to assess the connectivity of wild populations of animals and plants (Reynolds et al. 2013; Truelove et al. 2017; Aavik and Helm 2018), whether they are threatened with inbreeding (Bensch et al. 2006; Ruiz-López et al. 2012), and what historical or current barriers deter them from mixing (Mcrae et al. 2005; Combe et al. 2016). In captivity, many *ex-situ* breeding programmes have moved beyond genealogies based solely on studbooks, in order to measure the genetic health of their populations or plan re-introductions (Laikre et al. 2010; Ochoa et al. 2016). There is now a trend towards assessing the resistance or resilience of wild populations or ecosystems, based on the genetic diversity of species in or across localities (Ehlers et al. 2008; Jahnke et al. 2015; Griffiths et al. 2020b).

Despite these advances, genetic variation is often overlooked when it comes to addressing relationships between different taxa or trophic levels within an ecosystem (Neuhauser et al. 2003; Des Roches et al. 2017). The structure of ecosystems is by definition dictated by the assemblies of organisms within it; it therefore follows, that the environment in which an organism finds itself is shaped by the combined phenotypes of every other organism in the community (Rowntree et al. 2011b). Additionally, interactions between organisms in a community tend to be lost before the populations or species involved go locally extinct, therefore understanding all aspects of community interactions – including the role of genetic diversity in natural systems – is urgent (Antonovics 2003; Arroyo et al. 2015; Des Roches et al. 2017).

1.2 The genetics of species interactions

Jim Collins and Janis Antonovics coined the term community genetics to refer to the study of the genetics of species interactions, both in an evolutionary and an ecological context (Antonovics 1992). The field of community genetics arose from the ever-growing recognition that the continuity of biological variation beyond the species unit implies that combinations of inherited traits will influence interactions between individuals and communities, in both ecological and evolutionary timescales (Wilson 1976; Collins 1986; Loehle and Pechmann 1988; Antonovics 1992).

To date, community genetics studies have revealed a genetic basis for multiple interactions within species, between species, and between species and their environment. These include direct interactions – such as genotype x genotype associations between competing individuals (Chase et al. 2000; Moya-Laraño 2011; Wolf et al. 2011), between predator and prey species (Ninkovic and Åhman 2009; Khudr et al. 2013), or between host and epiphytic species (Stireman et al. 2005; Ferrari et al. 2006; Johnson 2008; Zytynska and Preziosi 2011; Barbour et al. 2016) – and indirect relationships, such as genotype x genotype interactions between host plants and higher non-phytophagous trophic levels (Astles et al. 2005; Johnson and Agrawal 2005; Ninkovic et al. 2011; Barbour et al. 2015) or between above- and belowground organisms (Genung et al. 2012; Rowntree et al. 2014a; Singh et al. 2014). Much of this work has been carried out in agricultural systems. The abundance of work on barley and aphid systems – some of which is described in a following section (*1.5 How are community genetics interactions mediated*) – has already revealed how the genotypes of plant, herbivore, and parasitoids affect the outcome of the interaction between them (Johnson 2008; Zytynska et al. 2010; Rowntree et al. 2014; Singh et al. 2014). In an agricultural system where crops are dominated by monocultures of one or few genotypes,

considering the effects of genotypic interactions between plants, herbivores and their predators may provide alternative solutions to the use of damaging pesticides and the arise of pesticide-resistant pests (Neuhauser et al. 2003; Ninkovic and Åhman 2009).

In addition to informing agricultural practices, understanding the genetics of species interactions sheds light on important evolutionary questions, such as how ecological interactions are not just consequences of evolutionary change, but are also drivers of that change. For instance, the suppression of a selective pressure, such as herbivory, can cause a change in genotypic frequencies in plant populations; thus changing defence mechanisms in a community within an ecological timeframe (Agrawal et al. 2012). Conversely, the genotypic variation within plants can explain much of the variation within the associated invertebrate community (Johnson and Agrawal 2005).

The following sections contain examples of how genotypic diversity in foundation species can affect the biodiversity of associated communities, divided into three main questions which remain open in community genetics and ecology:

A) **Are community genetics effects ubiquitous? (1.3)** Whether these correlations between intraspecific diversity and ecosystem processes, community structure, or diversity are ubiquitous across different ecosystems. To genuinely assert this, exploratory field investigations can shed light on whether a connection exists in natural systems, but to prove causation underlying any correlation, manipulative experiments should be carried out.

B) **What is the relative importance of community genetics effects in relation to other environmental factors, ecosystem processes, and evolutionary forces? (1.4)** To what extent do correlations between intraspecific diversity and community assembly or ecosystem processes matter. Patterns can be appreciated from laboratory experiments and *in-situ* manipulations, but without *in-situ* data from natural systems, it is difficult to truly draw conclusions on the relative weight of genetic interactions.

C) **How are community genetics effects mediated: through what phenotypes are these genetic patterns manifesting themselves in order to have an ecological effect? (1.5)** The phenotype through which genetic variation within different species can influence other organisms or ecosystem processes. This point is more difficult to assess and can only be done experimentally. It is briefly addressed in this introduction, but is beyond the aims and scope of the chapters in this PhD.

1.3 Are community genetics effects ubiquitous across different ecosystem types?

Intraspecific genetic and genotypic diversity has been shown to affect direct and indirect ecological interactions and ecosystem processes in a relatively small selection of systems. The bulk of community genetics research has been carried out primarily in North American and European systems, particularly cottonwood stands (*e.g.* Wimp et al. 2005; Bailey et al. 2009; Barbour et al. 2015) and in closed *ex-situ* plant-insect herbivore experiments (*e.g.* Ferrari et al. 2006; Tétard-Jones et al. 2011; Genung et al. 2012; Agrawal et al. 2013). Very few community genetics studies have been carried out in complex tropical systems, although even in these, invertebrate community composition has been found to be affected by genetic or genotypic diversity of plants (Marquis 1990; Zytynska et al. 2011a, 2012a; Campos-Navarrete et al. 2015a). These studies have mainly been on terrestrial systems, with a few notable exceptions (Hughes and Stachowicz 2004, 2009; Reusch et al. 2005; Rudman et al. 2015a)**.** Most of these aquatic studies, however, have focused on the effect of eelgrass (*Zostera marina*) genotype on shoot density and meadow resistance to disturbance, rather than on relationships with other organisms in the community. There are even fewer studies examining systems with interplay between terrestrial and aquatic compone**nts** (LeRoy et al. 2006a; Zytynska et al. 2012a)**.** This therefore leaves a significant gap in our knowledge of systems with closely linked aquatic and terrestrial components, as well as a gap in tropical habitats.

In the systems that have been studied, the estimated contribution of interspecific diversity to biotic interactions varies greatly. In some part, this is likely to be due to different experimental designs, sampling techniques, and analyses; however, a large degree of this variation is likely to be due to intrinsic differences and varying degrees of complexity between the ecosystems themselves (Hendry 2019). There is also the issue of which elements of the community are studied and how much how strong the relationships between these elements and other components of the community are (Hendry 2019; Chase and Knight 2003). It is therefore impossible to extrapolate results from a highly simplified common garden experiment to a wild system, or from a European woodland to a Neotropical rainforest. A combined approach may elucidate more than studies of either experimental or natural systems on their own, but elements of the community to focus must still be chosen.

The focus of community genetics studies tends to be on interactions between organisms that are known or presumed to have strong relationships, or centred around species which are thought to exert a disproportionate influence on others – for example, keystone species, foundation species, specialised plant-herbivore interactions (Whitham et al. 2012; Crutsinger 2016). The reason for this may be justified in arguing that indeed if genotypic variation is likely to influence the rest of a community, then it will be in such organisms that disproportionately influence their surroundings (Crutsinger 2016). However, it is then important not to take this as an indication that the effect of genetic diversity upon other organisms will be equal across different sets of organisms. It may be worth continuing to explore systems in which there is unlikely to be a significant genetic basis for interactions, if only for proof of principle; for instance, in megadiverse systems where one would assume there is more redundancy in the roles and interactions between organisms.

In summary, before we can start making a fair comparison of community genetics effects in different ecosystems, different habitats need to be assessed more evenly. This includes increasing the variety of geographical locations in which *in-situ* studies are conducted and *ex-situ* studies are sourced, and expanding research to understudied environments such as freshwater, marine, and canopy systems, as well as expanding the types of relationships under investigation.

1.4 What is the relative ecological importance of community genetics?

It is unclear how important community genetics effects are in ecosystems when compared to other environmental or biotic factors (Hersch-Green et al. 2011b). Studying the genetics of species interactions in natural systems is particularly complex. Nonetheless, several efforts have been made to quantify these effects in semi-natural settings. Common garden experiments and field studies on North American cottonwood (*Populus spp.*) ecosystems have revealed several community genetics interactions *in-situ,* such as the similarity between invertebrate community composition on genetically similar cottonwood individuals (Wimp et al. 2004a, 2005; Bangert et al. 2005); the correlation of avian predation patterns and cottonwood relatedness (Bailey et al. 2006a); and how genetically-determined plant traits can predict certain ecosystem processes (Schweitzer et al. 2004). Ultimately, the Cottonwood Ecology Group in Northern Arizona University has provided ample evidence that, even in complex common garden experiments and natural systems, genotypic diversity within a species can influence biotic interactions and community composition, suggesting there may be a certain heritability to trophic interactions (Whitham et al. 2003; Bailey et al. 2006a, 2009b). Work on another North American plant, the evening primrose (*Oenatheria*

biennis), lends further support to the idea of whole communities evolving through genotypic interactions, by experimentally demonstrating how plant and invertebrate community composition and dynamics vary according to the selection of primrose genotypes in large common garden plots (Johnson and Agrawal 2005; Agrawal et al. 2012).

Combined, these studies make a strong case that a foundation organism's genetics has consequences beyond the individual and beyond the species in naturally complex systems. However, what only a handful of studies have attempted to decipher, is to what extent do intraspecific genetics affect community interactions and explain community variation in the wild (Johnson and Stinchcombe 2007; Rowntree et al. 2011b). There have been impressive claims from studies estimating the relative importance of intraspecific diversity in determining community composition or interactions between organisms, in some cases where up to 60% of the observed variation in communities or ecosystem processes was suggested to be explained by genetic diversity (Schweitzer et al. 2004; Shuster et al. 2006; Bailey et al. 2009b). However, the lack of environmental variables and trophic complexity in a controlled system enables identification of phenotypes through which community genetics may be mediated, but this same simplification of processes will change biological the strength of biological interactions (Hersch-Green et al. 2011b; Hendry 2019a). For instance, even in large-scale common garden experiments, the inclusion or exclusion of a trophic level or single invertebrate species in the system can alter the rest of the community (Dickson and Whitham 1996; Ninkovic et al. 2011), as can manipulating invertebrate population numbers (Chase et al. 2000; Chase and Knight 2003). This means that in most experimental cases, it is difficult to translate the proportion of variation explained by genetic diversity to a complex natural setting. Controlling for geographic variation in the genotypes used in manipulative experiments can also alter the impact that genotypic diversity has on communities (Tack et al. 2012). Because species exhibit local variation over their range, in many cases it is likely that genotypes and phenotypes of conspecifics collected from across an extensive space may differ more than co-occurring individuals do. Therefore, it is possible that the high percentage of variation explained by genotypic diversity would be exaggerated in such cases and unrepresentative of natural scenarios, especially where a mesocosm presents fewer variables than a natural system (which for experimental reasons, it must) (Hersch-Green et al. 2011b; Tack et al. 2012).

The only plausible way of understanding the strength of community genetics effects in relation to other factors in natural ecosystems, is to combine the information ascertained from laboratory and common garden experiments, with work *in-situ* (Hersch-Green et al. 2011b). Although they are in shorter supply, there have been field studies on the effect of

plant genotype on invertebrate community composition, and indeed the estimated community divergence credited to host genotypic diversity in these studies is much more conservative. In a Belizean rainforest, Zytynska et al. (2012) found that bromeliad (*Aechmea bracteata*) genotype explained 1% of the variation in the community of invertebrate larvae found within its phytotelmata. Although a small figure, in a tropical rainforest it may be biologically significant (Zytynska et al. 2012a), which highlights the importance of taking into account the complexity of the system in question.

As already discussed in the previous section (*1.3 Are community genetics effects ubiquitous across different ecosystem types*), the extent to which genetics plays a role in species interactions is likely to vary between different environments and depend on the focal community of the study. This could be due to the species richness and complexity of different systems, how stable and seasonal they are, or the directness and intimacy of the relationship studied (for instance, a specialist herbivore has a direct and intimate relationship with its host plant species, while a generalist predator will have an indirect and relationship with the host plant species of its prey). Therefore extending the reach and variety of field studies will help to build a picture of the relative ecological importance of community genetics, even if non-genetic factors cannot be tightly controlled as they are in *ex-situ* experiments.

1.5 How are community genetics interactions mediated?

There is mounting evidence to suggest interspecific genotype interactions affect community dynamics and composition in an ecosystem, yet the mechanisms through which community genetics effects occur have been investigated in relatively few experimental systems. For instance, several studies have revealed genetic patterns in plant-aphid systems, demonstrating that different aphid genotypes associate with specific host plant genotypes (Via 1991; Dickson & Whitham 1996; Johnson 2008), that parasitoid wasp genotype affects the behaviour of infected aphids (Khudr et al. 2013), and that genotypic interactions between plants and aphids can determine the fitness of parasitoid wasps (Zytynska et al. 2010)**.** However, the presence of a genetic basis to these associations does not tell us the mechanism or phenotype mediating particular genotypic interactions.

One of the difficulties in determining these mechanisms is that they will vary depending on the system. Plant-invertebrate interactions offer an ideal system in which to understand how community genetics can be manifested. The chemical environment of plants is highly genetically-determined and some studies have demonstrated how differences in volatile

chemicals released by different genotypes affects the distribution and fitness of associated invertebrates (Ninkovic and Åhman 2009; Ninkovic et al. 2011). For instance, aphid host choice is influenced by the composition of volatile emissions from specific barley genotypes and the interaction of volatile emissions from different genotypes (Pettersson et al. 2007; Ninkovic and Åhman 2009; Dahlin et al. 2014). In the same system, predator distribution unsurprisingly follows that of prey availability, but when aphids are excluded from the system, the distribution of their lady bird predators is still largely explained by the chemical environment of different barley genotypes (Ninkovic et al. 2011). In more complex common garden experiments, Iason et al. (2011) found that distinct Scots pine volatiles affect various types of herbivores in different ways, while Schweitzer et al. (2004) found that genes involved in tannin production were a better predictor of soil nitrogen than abiotic factors such as soil temperature and moisture. Thus, the specific chemical environment of a particular plant genotype will elicit different outcomes depending on the herbivore species and genotype (Ninkovic and Åhman 2009; Ninkovic et al. 2011; Iason et al. 2011) and can affect ecosystem processes (Schweitzer et al. 2004). If the chemical environment of plants affects species differently and within species has different effects on specific genotypes, it leaves us with a very complex picture of how community genetics might influence a system and resonate through trophic levels.

Although there are some clear advances towards uncovering plant phenotypes mediating genotypic interactions with invertebrates, it is unlikely that the plant chemical environment is the only phenotype through which community genetics effects are taking place. Common garden experiments with coastal willow (*Salix hookeriana*) which took into account trait variation and heritability among several different willow genotypes, demonstrated that plant genotype significantly influenced invertebrate community assembly and suggested that this was likely mediated through several plant characteristics (Barbour et al. 2015, 2016). Differences in cuticle surface genes have been found to completely change the bacterial community composition on thale cress (*Arabidopsis thaliana*) leaves (Reisberg et al. 2013; Bodenhausen et al. 2014). Therefore, cuticle surface in this system is a mechanism community genetics acts through, but it does not exclude the possibility that (a) other physical or chemical features of the plants affect the bacterial or higher trophic levels if they were present, or that (b) the presence of other organisms in the system might change the direction or degree to which cuticle genes affect bacterial diversity; in addition to any other abiotic and biotic factors involved in natural systems which may interact with one another (Johnson and Agrawal 2005; Zytynska et al. 2010; Campos-Navarrete et al. 2015a).

Even when the phenotypes through which community genetics effects take place can be determined, it doesn't necessarily reveal *how* that phenotype influences the rest of the community. For instance, in the case of plant-invertebrate systems, even when it is known that plant volatiles are a phenotype steering an interaction between genotypes, we do not necessarily know whether this is the case because invertebrates are attracted to or avoid specific phenotypes (active choice), or because they do not survive on specific phenotypes (differential survival). Zytynska and Preziosi (2011) demonstrated that aphids (*Sitobion avenae*) choose their barley host (*Hordeum vulgare*), but this is not the case for all genotypic interactions. For instance, in the case of epiphyllic bacteria on *A. thaliana,* it seems differential survival of bacterial strains due to differences in cuticle waxiness determines the ultimate community composition (Reisberg et al. 2013; Bodenhausen et al. 2014). Similarly, sterilised bromeliads kept in the same conditions develop very different bacterial communities according to bromeliad species, despite receiving the same source bacteria (O'Reilly-Berkeley 2014, *unpublished data*).

1.6 A combined approach

A growing body of interesting evolutionary ecology research is elucidating the roles of community genetics. However, many questions which still remain unanswered in this field, including the seemingly basic questions of how widespread these genetic effects on communities are, how they are mediated, and their relative importance in natural ecosystems. Megadiverse and tropical systems have remained largely unexplored in this area, as have aquatic systems. Although still largely unknown and likely diverse, the phenotypes mediating the interactions of genotypes can be picked apart using highly controlled systems, while these same controlled systems cannot reveal whether these patterns have a significant role in natural systems. Approaches which combine *ex-situ* and *in-situ* experiments and field studies elucidate different aspects of ecological genetics; each can be justified depending on the specific questions being asked of these systems, and all will be necessary moving forwards in the field of community genetics.

This thesis is a preliminary exploration into the effect of plant genotype on associated microbial and invertebrate communities in the megadiverse Amazon rainforest. It focuses on the communities of two main habitat-forming plant systems: epiphytic tank bromeliads (Bromeliaceae) and false-birds-of-paradise or *Heliconia* (Heliconiaceae). The field work of this these was carried out in San José de Payamino, an area on the edge of the Tropical Andes Biodiversity Hotspot and within the buffer zone of the Sumaco Napo-Galeras National Park, in the Sumaco UNESCO Biosphere Reserve (Figure 1.1).

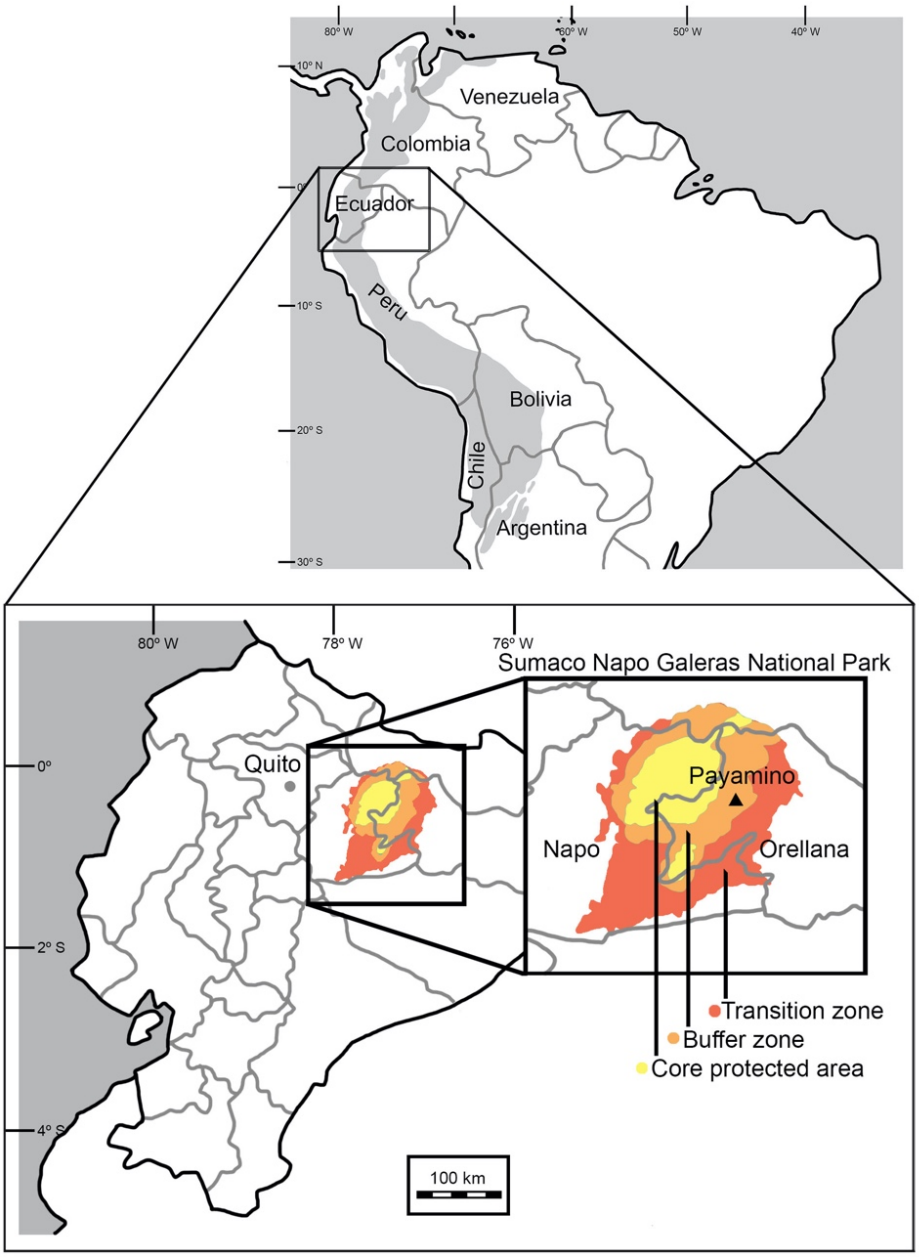


Figure 1.1 Map of South America and Ecuador. The Sumaco Napo-Galeras National park is coloured, with San José de Payamino marked with a triangle within the park buffer zone.

1.7 Study systems

Tank bromeliads and *Heliconia stricta* are monocotyledonous plants that have spaces and cavities formed by parts of the plant, called phytotelmata (*s. phytotelm*). In both cases, these phytotelmata are colonised by communities of species of a range of phyla. They commonly reproduce clonally, which makes them easy systems to obtain genetic replicates from. Epiphytic bromeliads were chosen as a manageable mini-ecosystem that inhabit the canopy, an environment mostly neglected in community genetics. *Heliconia* were chosen as a model

system with which to design an *in-situ* common garden experiment, due to how easily they can be split from a parent rhizome and grown into separate genetic replicates.

1.7.1 Bromeliads

Bromeliads comprise over 3200 species within the family Bromeliaceae (order Poales) (APG III 2009). All bar one African species are native to the Neotropics. Although they occur throughout the tropics and subtropics of the Americas in a variety of habitats, their greatest diversity lies in the rainforests and cloud forests. At least one third of species, particularly those in forests, are epiphytic, their position in the canopy granting them first claims on sunlight, rainwater, and discarded foliage (Zizka et al. 2019a). Amongst the myriad forms of vascular epiphytes in humid Neotropical forests, bromeliads stand out because of their characteristic morphology: strap-like leaves arranged in a rosette around a central axis, which may form an open cavity or "tank" (Figure 1.2). While not all Bromeliaceae share this morphological plan, the shape of many bromeliads do share this common blueprint. One of the major advantages this structure and leaf-arrangement provides the plant, is various spaces between the leaves and in the centre (in tank bromeliads) which collect rainwater and canopy debris. These spaces and cavities, or phytotelmata, also provide opportunities for a plethora of other organisms (Figure 1.3).

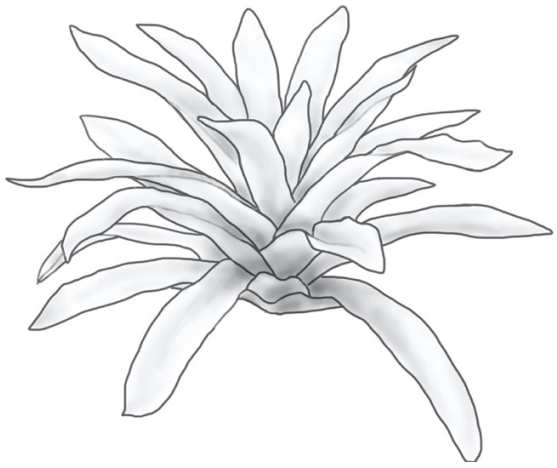


Figure 1.2. Morphology of a typical tank bromeliad.

The communities of organisms found in tank bromeliads are fully-functional microcosms. They contain everything from primary-producing microorganisms (Brouard et al. 2011), nitrogen-fixing bacteria (Leroy et al. 2017), zooplankton and detritovores (Farjalla et al. 2012), and predators (Srivastava et al. 2005; Hénaut et al. 2014). Canopy bromeliads in particular represent islands of resources surrounded by less-appealing habitat. Although the

bromeliad microcosm is not independent from external inputs nor isolated from passing organisms, they are complex and compact semi-contained systems which can be used as a model for studying ecosystem processes and dynamics (Srivastava et al. 2004).

This thesis uses tank bromeliads for most of its studies. The closely-related *Aechmea caudata* and *A. hoppii* are the focus for the *in-situ* study carried out in Chapters II and III, and *A. hoppii* is the focus of Chapter IV. The communities of bacteria and invertebrates living in individuals of these two species will be characterised in order to evaluate (1) what microhabitat and environmental factors influence community composition within these phytotelmata and (2) whether the genetic distance of individual bromeliads is one of the variables that influences community composition. The smaller and horticulturally-available *Neoregelia schultessiana* will be used to evaluate how bromeliad genotype influences their phyllosphere in an *ex-situ* experiment with a fixed number of replicated genotypes.

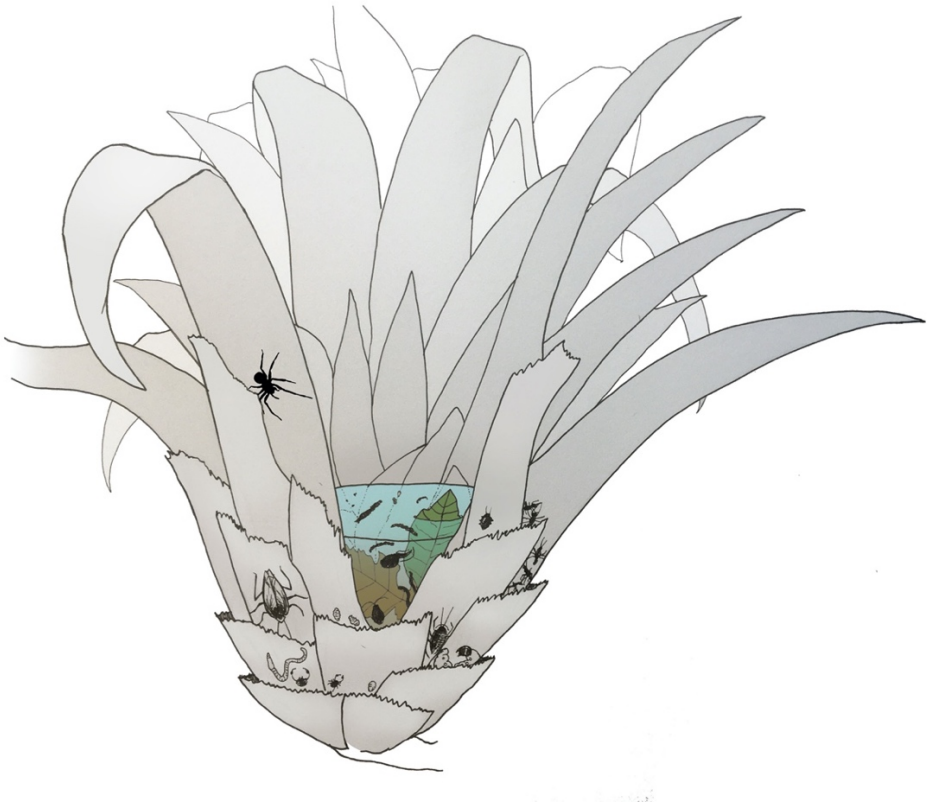


Figure 1.3. Dissected illustration of a tank bromeliad with several types of organisms living in its accumulated water and between its leaves.

1.7.2 Heliconia

Heliconia is the single genus within the monocotyledonous family Heliconacea (order Zingiberales). The 240-plus species are native to the neotropics and commonly called false-bird-of-paradise plants, due to their similarity to *Strelitzia* bird-of-paradise plants. Like bird-of-paradise plants, they are known for their showy inflorescences. However, *Heliconia*

species have a diverse suite of inflorescences, mostly compost of hard bracts arranged along a central axis. Many species present pendulant inflorescences, thus it is the species with upright inflorescences which are able to collect water and debris and accommodate communities of aquatic invertebrates. The plants themselves emerge as shoots from an underground rhizome, which can run for several meters in any direction, giving separate shoots of *Heliconia* the appearance of being separate individuals (Figure 1.4).

Figure 1.4. Illustration of a Heliconia, depicted with a simplified underground rhizome system.

Due to the temporary nature of flowering, *Heliconia* inflorescences can contain short-lived (months-long) communities principally of the aquatic larva of invertebrates. However, many other invertebrate larvae are associated with the plants and particularly their leaves, which suffer herbivory from various insects (Seifert 1982a; McCoy 1984). Several species of hispine beetles (Chrysomelidae, Coleoptera) in particular specialise in consuming unrolled

leaves and are thought to have co-evolved with *Heliconia,* despite the relatively low nutritional content of *Heliconia* leaves (Wilf et al. 1975)**.**

In this thesis, *Heliconia stricta* will be used in an *in-situ* experiment to investigate whether plant genotype affects the level of leaf herbivory they suffer compared to other genotypes of the same species. *Heliconia* were chosen for their fast-growing nature and the ease of obtaining genetic replicates *in-situ* by splitting their rhizomes (Figure 1.5), as well as the fact their inflorescences form phytotelmata and the susceptibility of their leaves to herbivory. Although there was an intention to sample phytotelm communities to represent a more comparable system to those of bromeliad phytotelmata, due to the Covid-19 pandemic it was not possible to return to the field site once the inflorescences had grown. Nonetheless, the effect of plant genotype on *Heliconia stricta* growth and herbivory will be analysed, which will contribute to the study of community genetics in megadiverse tropical systems.

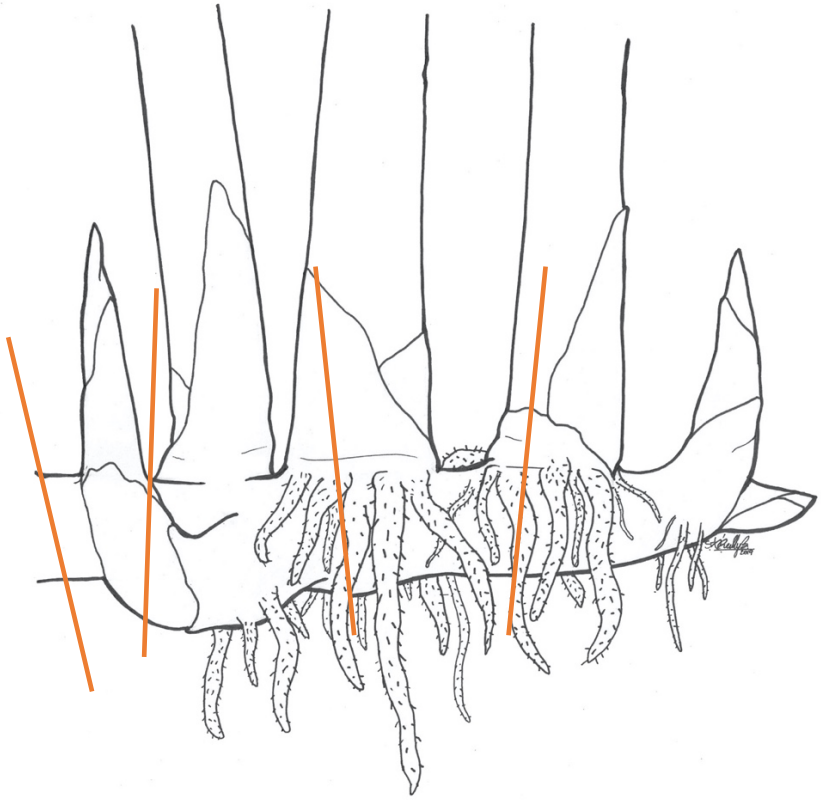


Figure 1.5. Illustration of the rhizome of a Heliconia with various shoots, with an indication (orange lines) of how this may be split to make genetically-identical replicates.

1.8 Thesis aims and objectives

This thesis addresses the ubiquity of community genetics effects by investigating whether they occur in two plant-based systems in a mega-diverse tropical habitat, the Amazon Rainforest. The relative contribution of intraspecific plant genetic diversity to the

composition of bacterial and invertebrate communities compared to environmental factors will be assessed *in-situ* and *ex-situ.* To this end, the environmental and biotic factors that influence the invertebrate and prokaryote community composition in bromeliads *in-situ* will be studied independently of bromeliad genotype, prior to analysing the influence of genetic distance relative to environmental variables. Additionally, an *in-situ* manipulative experiment will demonstrate whether plant genotype can determine the degree of herbivory damage in this mega-diverse habitat.

The specific aims of this thesis are therefore four-fold:

1. To determine the amount of variation in phytotelm community composition determined by environmental and plant factors.
2. To establish whether plant genotype affects the community composition of bacteria and invertebrates in the bromeliad phytotelm in the rainforest.
3. To test the effect of *Heliconia* genotype on plant growth and leaf herbivory in a manipulated *in-situ* experiment.
4. To test the effect of bromeliad genotype on bacterial community composition in an *ex-situ* greenhouse experiment.

This thesis contains five data chapters:

- **Chapter II – Height in the canopy, forest type, and microhabitat size affect the invertebrate communities of epiphytic bromeliads (*Aechmea spp.*)**
  Specific aims: To determine the environmental and physical plant characteristics that influence invertebrate community composition in bromeliad phytotelmata.
  This chapter looks at the environmental and plant variables that influence the composition of the invertebrate communities in bromeliads, with a particular focus on the effect of the forest type and height at which bromeliads are found on the most common orders of invertebrates, as well as the effect of bromeliad size and complexity on these orders. This is a field study on the two closely-related and morphologically-similar bromeliads *A. hoppii* and A. *nidularioides*.

- **Chapter III – The effect of environmental and plant characteristics on prokaryotic diversity in bromeliad phytotelmata (*Aechmea spp.*)**
  Specific aims: To determine the environmental and physical plant characteristics that influence microbial community composition in bromeliad phytotelmata.
  This chapter examines what factors influence the structure of the prokaryotic communities within the phytotelmata of *A. hoppii* and A. *nidularioides*.

Environmental and plant characteristics are examined in relation to their influence on taxonomic and functional diversity. This is a field study on the two closely-related and morphologically-similar bromeliads *A. hoppii* and A. *nidularioides*.

- **Chapter IV – No effect of plant genotype on phytotelm community composition in an epiphytic bromeliad (*Aechmea hoppii*)**
  Specific aims: To establish how much of the variation in invertebrate and prokaryotic communities is due to genetic distance between host bromeliads.
  This chapter investigates correlations between bromeliad genetic distance, prokaryotic community composition, and invertebrate community composition in *A. hoppii*. This contributes to answering whether community genetics effects are significant and ubiquitous across diverse systems. This is a field study the bromeliad *A. hoppii*.
- **Chapter V – Plant genotype influences growth rate and leaf herbivory in an Amazonian disturbance plant (*Heliconia stricta*)**
  Specific aims: To measure the growth of replicates of Heliconia genotypes and determine whether there is more variation in growth among or within genotypes. To measure leaf herbivory and establish whether some plant genotypes are consistently consumed more or less than others.
  This chapter asks whether *Heliconia stricta* genet identity determines the growth of plants and the degree of herbivory they suffer. The principal of this project is to look at to what extent herbivore activity is affected by the host plant genotype in an *in-situ* setting while manipulating genotypic diversity. This is an *in-situ* experimental study.
- **Chapter VI – *Ex-situ* study of the effect of plant genotype (*Neoregelia schultesiana*) on prokaryote communities**
  Specific aims: To establish whether bacterial community composition varies more between or within genotypes of bromeliads when external in-situ influences are removed.
  This chapter uses a greenhouse experiment to remove the complexity of the environmental variables in a natural setting. It asks whether, all else being equal, plant genotype explains a significant proportion of variation between bacterial communities in bromeliads. This is an *ex-situ* experimental study.

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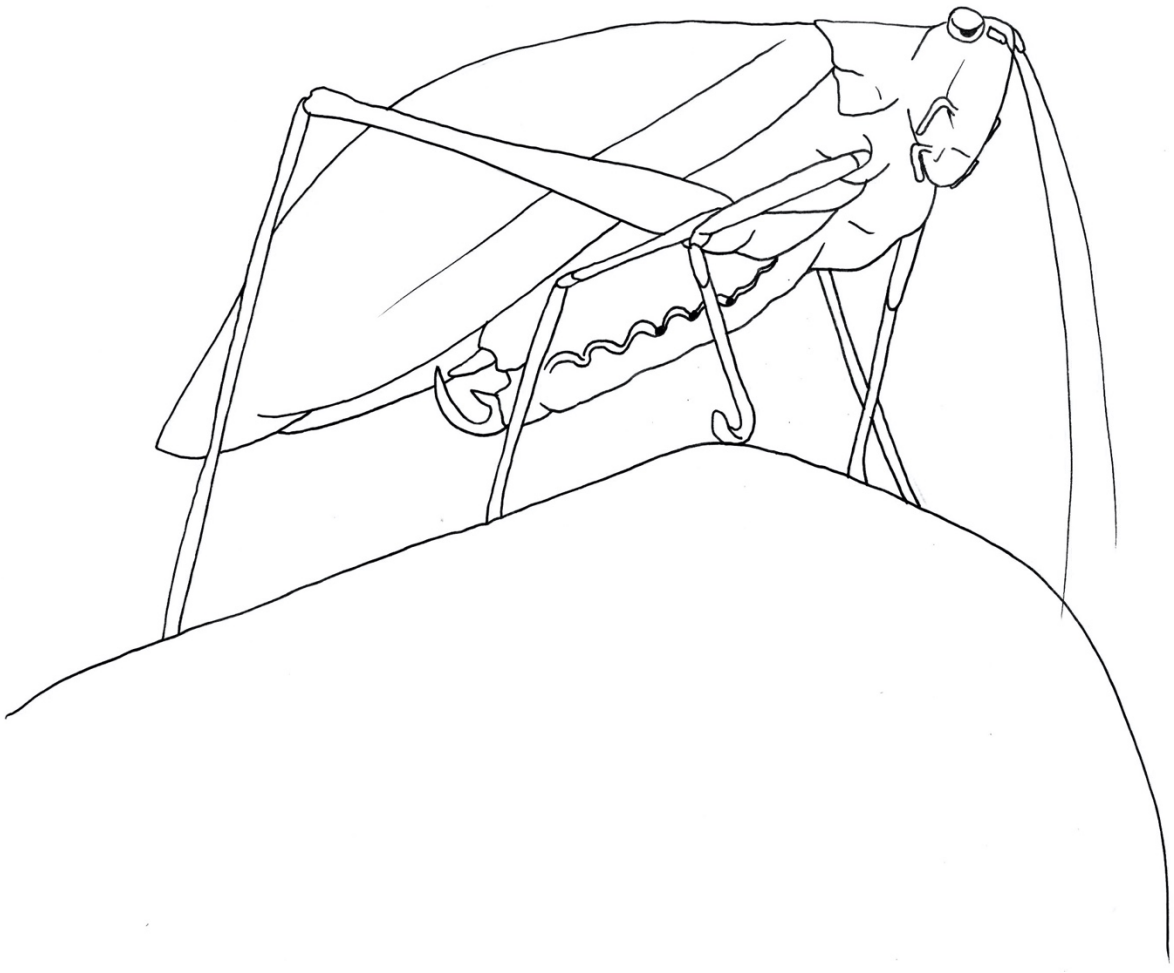
Chapter II

Height in the canopy, forest type, and microhabitat size affect the invertebrate communities of epiphytic bromeliads (*Aechmea spp.*)



2.1 Abstract

Tank bromeliads are found in rainforests across the Neotropics, and house diverse communities between their leaf axes. Various factors influence the species richness and composition of invertebrate communities in bromeliad phytotelmata, most notably, habitat size. However, there has been precious little work on invertebrate communities in the western Amazonia. We collected 63 epiphytic bromeliads (*Aechmea hoppii, A. nidularioides*) within the Tropical Andes Biodiversity Hotspot in Ecuador and explored what biological and physical variables explained the differences in composition among bromeliads. We extracted a total of 7524 individual macroinvertebrates and identified 300 morphospecies belonging to 27 Orders. We recorded information on bromeliad location within the forest (height in canopy, primary or secondary forest), plant size (phytotelmata volume, base circumference, length of longest leaf) and phytotelm pH and temperature, and counted the number of leaves as a measure of habitat complexity. Overall, forest type (primary/secondary), height in the canopy, and habitat size explained statistically significant portions of the variation in the whole invertebrate community. Bromeliad size was positively correlated with alpha diversity, whereas height in the canopy affected beta diversity. Forest type affected community composition but not abundance or richness within bromeliads. Taken together, our results echo those of other phytotelmata and canopy studies, and fill in a geographic gap in this body of work.

2.2 Introduction

Bromeliads constitute one of the most diverse families of flowering plants in the neotropics, with over 3500 species accounted for, nearly half of which are epiphytic and many possess a tank-like morphology (Zizka et al. 2019b). The elongated strap-like leaves of tank bromeliads are arranged in a rosette around a central gap, known as a tank or phytotelm (pl. *phytotelmata*). This provides a space in which rainwater and debris from the canopy accumulate, forming a protected oasis in the canopy, rich in nutrients courtesy of diverse microbial communities of detritovores, primary producers, and nutrient cyclers within them (Bermudes and Benzing 1991; Carrias et al. 2001, 2014; Brouard et al. 2011; Louca et al. 2016a; Brandt et al. 2017).

Bromeliads have been called "biodiversity amplifiers", due to the concentration and diversity of invertebrate fauna they harbour in their phytotelmata, compared to the surrounding habitat (Rocha et al. 2000; Hénaut et al. 2014). They house both aquatic and terrestrial invertebrates, serve as nurseries for amphibians (Almendáriz et al. 2000; Mccracken et al. 2007; Sabagh et al. 2017) and provide nutrition and hydration for birds and Andean bears (Piacentini and Varassin 2007; DeMay et al. 2014; Palacios-Mosquera 2018; Goldstein and Goldstein 2019).

Most members of the aquatic community within bromeliads tend to be stationary for at least the larval part of their life cycle, whereas the terrestrial community is usually composed of more mobile foragers or predators which visit bromeliads briefly to feed or breed, or temporary tenants which use the plant for nourishment and shelter, but can move on when they need to (Zillikens et al. 2005). Given the different niches and needs of these broadly distinguishable communities within bromeliads, studies typically analyse them separately, revealing that the aquatic community is more sensitive to variation among bromeliad microhabitats (Cotgreave et al. 1993; Lopez et al. 2009; Marino et al. 2013; Jocque and Field 2014a). Habitat size and water volume have been shown to be the bromeliad features that best correlate with species richness and abundance of aquatic communities within them (Srivastava 2006; Srivastava et al. 2008a; Jocque and Field 2014a; Méndez-Castro et al. 2018). Detrital content (Armbruster et al. 2002; González et al. 2014; Dézerald et al. 2017), bromeliad species (Jabiol et al. 2009; Marino et al. 2013), and even bromeliad genotype (Zytynska et al. 2012) have been shown to explain between 1-60% of the variation among bromeliad aquatic invertebrate communities, depending on the combination of factors examined, the portion of the community included, and the location of the study. However,

there are geographic differences in the factors that influence community composition and trophic interactions within bromeliads (LeCraw and Srivastava 2019).

Despite the rich literature on bromeliad invertebrate communities across the neotropics, many geographical areas and bromeliad species remain unexplored. Western Amazonia is particularly unmapped in terms of bromeliad invertebrate communities. This is significant because the northwestern corner of Amazon rainforest falls within the Tropical Andes biodiversity hotspot, by some measures the most biodiverse place on Earth (Myers et al. 2000). This area includes the northern section of the Andes mountain range, as well as its flanking regions as it descends into the Amazon basin. Additionally, between the period of 1990 to 2007, Ecuador lost the highest relative amount of forest cover in South America (Peres et al. 2010). To the authors' knowledge, the only comprehensive study of whole bromeliad-associated invertebrate communities in Ecuador was that of Armbruster *et al.* (2002), nearly two decades ago and not quite within the Tropical Andes hotspot.

Additionally, most bromeliad studies focus on plants on or near to the ground. The forest canopy is an important reservoir of floral and invertebrate diversity in tropical forests (Davis and Sutton 1998; Davidson et al. 2003; Stork and Grimbacher 2006; Cascante-Marín and Nivia-Ruíz 2013; Quaresma et al. 2017). In the Neotropics, forest canopies are laden with layers of epiphytes, including a high diversity of vascular epiphytes such as orchids, aroids, and bromeliads (Gentry and Dodson 1987; Freiberg and Freiberg 2000; Flores-Palacios and García-Franco 2006; Hietz et al. 2009; Quaresma et al. 2017; Hayward et al. 2018), of which bromeliads in particular are important reservoirs of arthropods. Studies which have considered the effect of distance from the ground on communities in bromeliads have found a negative relationship (Brouard et al. 2012; Kratina et al. 2017), or no relationship (Antonetti et al. 2021) between microfauna and the height of the bromeliad on the phorophyte. However, the range of heights occupied by bromeliads has a positive effect on spider diversity, with bromeliad species occupying a greater range of heights housing a greater abundance of spiders (Gonçalves-souza et al. 2011). The bromeliads in these studies were all found under 2.5 m from the ground, yet average canopy height in the Amazon is around 30 m (Helmer and Lefsky 2006) (Sullivan 2018, *unpublished data*), although emergent tree heights of up to 88 m have been reported (Gorgens et al. 2019).

Here, we aim to address the western Amazonian gap in the bromeliad-invertebrate literature, by examining potential factors that may explain the variation in macroinvertebrate communities within two congeneric and vegetatively indistinguishable epiphytic bromeliad species, *Aechmea hoppii* (Harms)(Smith 1953a) and *A. nidularioides* (Smith 1953b) in San

José de Payamino, Ecuador. This relatively understudied region of northwestern Amazonia falls on the Eastern edge of the Tropical Andes biodiversity hotspot, we thus anticipated we would find a larger overall number of morphospecies amongst the bromeliads than Armbruster *et al.* (2002) and a distinct set of variables explaining differences in community structure among bromeliads. We expected that environmental and plant characteristics in our more geophysically diverse environment would explain community patterns to a lesser degree than in the lower, flat regions of Amazonia. We discuss the complex set of patterns that emerge in the whole, aquatic, and terrestrial invertebrate communities in the bromeliads, focusing in particular on the effect of plant volume, height from the ground, plant structural complexity, and forest type on the most common orders of invertebrate found in these bromeliads.

2.3 Materials and Methods

2.3.1 Study site

Bromeliads were collected in San José de Payamino, Orellana, Ecuador, hereafter referred to as Payamino. Payamino is a 17000 ha expanse of Amazon rainforest owned and managed by an indigenous Kichwa community, on the eastern edge of the Tropical Andes Biodiversity Hotspot. It is in the buffer zone of the core Sumaco Napo Galeras National Park and within the Sumaco UNESCO Biosphere Reserve.

Elevation of the area ranges between 200 – 714 meters above sea level (m.a.s.l.). Mean tree height is 18.2 m (Sullivan 2018, *unpublished data*). The area is a patchwork of mature primary and old secondary forest, interspersed with small areas of basic family properties (*fincas*) consisting of small open wooden houses or platforms and cultivated land (*chacras*). Henceforth, we use the term *primary forest* to refer to mature areas of rainforest with minimal or no human disturbance in the collective memory of the local community. *Secondary forest* will refer to regenerated or anthropogenically disturbed areas of rainforest (Brown and Lugo 1990); this includes areas formerly used as farmland that are now fully forested, areas that look mature but may be scarred by frequently used trails, and patches of forest that are selectively weeded or logged.

The forest is humid tropical forest. Average rainfall is 3661 $mm.y^{-1}$. There are no defined seasons in the area, with a mean annual temperature of 24.0ºC, varying between 23.4ºC and 24.4ºC between the coldest and warmest quarters of the year. Evapotranspiration does not

surpass precipitation at any point throughout the year, so there is no dry season (Sullivan et al. 2020). There are seasonal patterns in fruiting trees (Stafford et al. 2016).

2.3.2 Field sampling

The two focal species of bromeliad were picked *in-situ* following exploratory surveys of the area prior to sampling. *Aechmea hoppii* and *A. nidularioides* were chosen owing to their abundance and apparent ubiquity throughout primary and secondary forest on both sides of the main river. The physical vegetative characteristics of these species make them virtually indistinguishable from each other, but distinguishable from the many other epiphytic bromeliads in the area, allowing identification even when no inflorescence is present.

Sampling took place in January-February and July-August 2018. Suitable bromeliads were selected from the ground by the same person throughout the study, based on the morphospecies and accessibility. Host tree species was not recorded. Bromeliads were reached using double-rope canopy access methods or ladders, depending on their position in the canopy, which ranged from 1.96 m to 20.20 m.

When a suitable bromeliad was identified, forest type (i.e. primary [n=28] or secondary [n=35]) and GPS coordinates were recorded. The vertical position of the bromeliad in the canopy was measured from the base of the bromeliad to the ground directly beneath it, using a standard 50 m tape measure. Central phytotelm pH was recorded using a handheld pH meter prior to removing the bromeliad from its host tree. The bromeliad was then sawn off the tree at the base and lowered to the ground in a large heavy-duty bin bag, to avoid losing the contents or inhabitants of the epiphyte.

Collected bromeliads were returned to the Timburi Cocha Research Station (18 M 0245706 9946597) for processing. The entire contents of the collection bag, including the plant and any debris or water that may have been loosened from it, were emptied into a large glass aquarium. This allowed plant characteristics to be measured without losing invertebrates. Total phytotelm capacity, base circumference, and the length of the longest leaf were measured, in addition to counting the total number of leaves on the bromeliad. The total capacity of the phytotelmata was measured by filling the central cavity and leaf axes with known volumes of water until they overflowed. Base circumference was measured around the section of the plant base or stem where the bottom leaves ended and the stem's diameter evened out.

2.3.3 Invertebrate identification

Following measurement, bromeliads were dissected leaf-by-leaf and scoured for macroinvertebrates visible to the naked eye (>2 mm in length). All leaf litter, soil, and water from the bromeliads was examined in equal detail. All collected invertebrate specimens were collected whole and preserved in 96º ethanol for later identification. There was occasion for some ants to escape, and so they cannot be accounted for, but this was generally only a few individuals when it occurred. Invertebrates were transferred to the entomology laboratory of the National Biodiversity Institute (Instituto Nacional de Biodiversidad del Ecuador, INABIO) in Quito, Ecuador, for processing.

Individual invertebrates were defined as either aquatic or terrestrial. Whether an invertebrate was considered aquatic or terrestrial was based on its life stage at the time of collection; e.g. aquatic beetle larvae are considered part of the aquatic community, even if the adult instars would not be had they continued to grow. All collected invertebrates were classified at least down to the level of order, and classified into morphospecies. Both general (Gavin 2000; Kočík et al. 2002; Triplehorn and Johnson 2005) and taxon-specific keys (Hebard 1924; Kury 2002; Silvestre et al. 2003; Merritt et al. 2008; Domínguez and Fernández 2009; Andersen 2010; Prat et al. 2010; Silva and Brandão 2010; Vidlicka 2014; Brito and Borges 2015; Grismado et al. 2015) were used to classify the samples and then split into morphospecies.

2.3.4 Data analysis

All statistical analyses were carried out in R (R Core Team 2020), unless otherwise specified.

Descriptives

The relative percentage of aquatic and terrestrial invertebrates in the total sample was calculated in terms of the number of individual invertebrates and morphospecies richness. Likewise, the abundance of different Orders across the samples was calculated as a percentage of the number of individuals within each class and each Order over the total number of invertebrates, as well as the percentage of the number of morphospecies per class or order over the total number of invertebrates.

Generalised linear models of alpha diversity

To investigate whether continuous plant and environmental variables had a linear relationship with the number of morphospecies or number of individuals in the bromeliads. generalised linear models (GLMMs) were used. We simplified the models by selecting a few relevant factors to analyse: logged bromeliad volume, height on host, forest type, as well as a measure of complexity, number of leaves. The models were fitted with a quasipoisson family distribution due to the nature of the count data.

This was performed for the whole invertebrate dataset, the aquatic and terrestrial subsets, as well as the most common order (by number of morphospecies) in the dataset; these were Aranae, Blattodea, Coleoptera, Diptera, Hymenoptera, Isopoda, Lepidoptera, and Oligochaeta. We treated Oligochaeta as an order as we did not possess the taxonomic expertise to classify these samples further; however we did not have morphospecies data for Oligochaeta either and so only the number of individuals were analysed for this group.

Multivariate analysis

To investigate how plant and environmental factors explained the variation amongst invertebrate communities, six separate permutational multivariate analyses were run on different subsets of the invertebrate data, depending on whether they were aquatic or terrestrial invertebrates. The subsets are defined in Table 2.1. There are no clearly marked seasons in this part of Ecuador, therefore because the community variance of the earlier sampling period (January/February) was found to be a subset within the later sampling period (July/August), it was not considered as a true factor in the permutational analyses, and the data were pooled.

The permutational multivariate analyses (PERMANOVA) were run using Bray-Curtis distance matrices with the *adonis2* function of the *vegan* package in R (Oksanen et al. 2020). Eight variables were analysed simultaneously for each of the subsets defined in Table 2.1: forest stage (primary / secondary), height of the bromeliad on tree, log bromeliad volume (phytotelmata capacity), longest leaf of the bromeliad, number of leaves, base circumference, and phytotelm pH and temperature. Although phytotelm capacity, longest leaf, and base circumference are all measures of bromeliad size, all were included as they were not highly correlated ($r < 0.4$). The number of leaves is considered a measure of structural complexity. The PERMANOVAs were then simplified by sequentially removing non-significant factors until they only contained significant factors or the minimum combination of forest + height + log(V). AIC scores were checked to ensure the simplified

models had lower scores than the original eight-factor models. However, simplified and eight-factor models did not differ in their overall results.

Sixty percent of invertebrate morphospecies present were only found once in the whole dataset (181 singletons found only once across all bromeliads), therefore we repeated the permutational analyses treating the data as binary (presence/absence), essentially removing abundance data. Removing singletons from the dataset produces the same results, therefore we retained them.

Table 2.1. Definition of subsets of data on which permutational multivariate analyses were performed, due to the distinct nature of each of the community subsets. Presence-absence data was preferred due to the rarity of most morphospecies in the whole dataset.

	All invertebrates	**Aquatic invertebrates**	**Terrestrial**
Presence-absense [N=63]	*Whether or not any and each of the 300 morphospecies were present in each bromeliad*	Aquatic morphospecies presence/ absence per bromeliad	Terrestrial morphospecies presence/ absence per bromeliad
Abundances [N=63]	Morphospecies and their abundance in each bromeliad	Aquatic morphospecies and their abundance in each bromeliad	Terrestrial morphospecies and their abundance in each bromeliad

Effect of height on community composition

To further investigate the effect of height on the relative abundance of different taxa, Fisher's Exact Test was performed to compare the relative abundance of orders between strata. Stacked bar charts were constructed using the abundances of each order and the number of morphospecies of each order. The vertical gradient was divided into four strata based on the sampled heights (up to 20.20 m above the ground): 0-4.9 m, 5-9.9 m, 10-14.9 m, and 15-20.20 m above the forest floor. Fisher's Exact Test was used due to the low count data in some orders, both in terms of morphospecies and individuals. The tests were carried out for all four strata at once, and between each pair of strata.

2.4 Results

2.4.1 Environmental and plant variation

Bromeliad size was highly variable. Phytotelmata capacity ranged from 80 mL to 1400 mL (mean 389.36 ± 245.86 SD); longest leaf, 19-238 cm (mean 141.48 ± 40.22 SD); base circumference, 9-25 cm (mean 14.34 ± 3.05 SD); number of leaves, 8-29 (mean 17.98 ± 4.20 SD). Central phytotelm pH was mostly acidic but with a great deal of variation, from strongly acidic pH=3.4 to few phytotelmata exhibiting neutral or mildly alkaline pH=8.2 (mean 5.83 ± 0.93 SD); 14.3% had a pH between 3.4-4.9, 61.9% between 4.9-6.4, and 23.8% between 6.4-8.2. Phytotelm temperature varied between 22.2 ºC and 30.0ºC (mean 25.71 ± 1.71 SD). Height at which bromeliads were collected ranged between 1.96 and 20.20 m above the ground (mean 7.77 ± 4.14 SD), with 97.33% of bromeliads found above 2.5 m from the ground. The distributions of these variables can be found in SI-1 (Supplementary Information). None of these variables were strongly correlated (Figures 2.1).

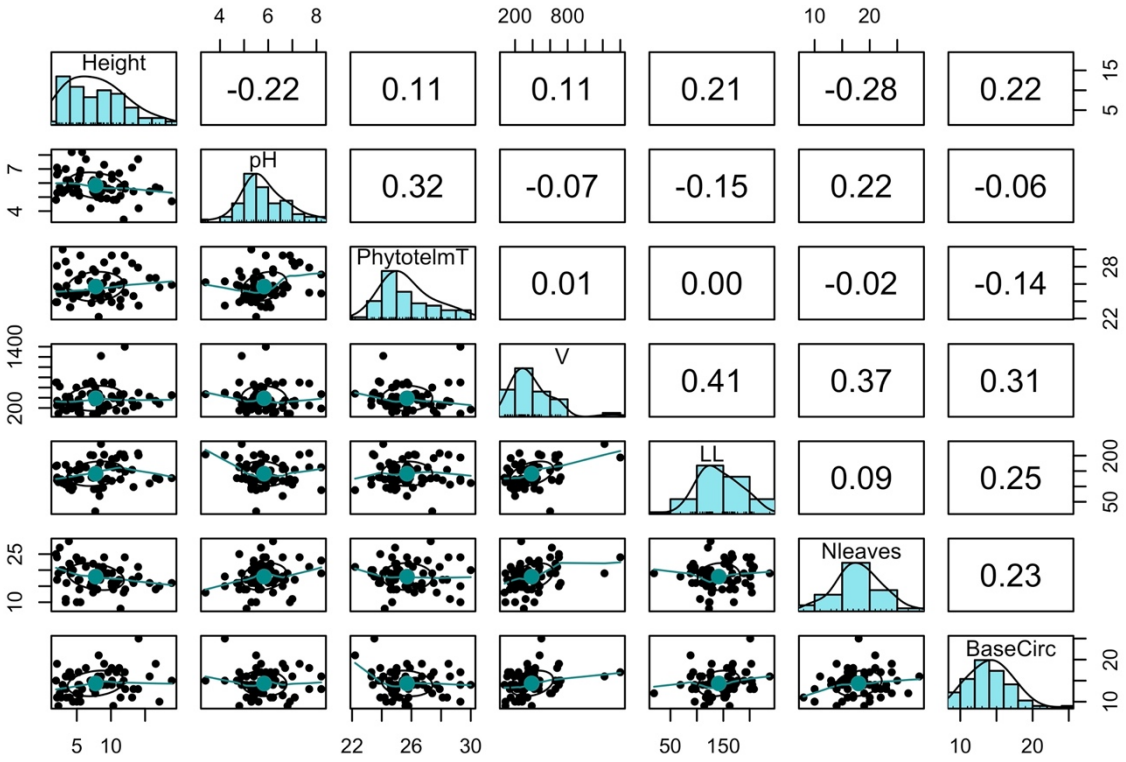


Figure 2.1 Correlation coefficients between measured variables.

2.4.2 Invertebrate descriptives

A total of 7524 individual macroinvertebrates (length > 1 mm) belonging to 300 different morphospecies were collected from the 63 bromeliads, the vast majority of which were arthropods. The terrestrial community consisted of 78.9% of all individuals and 76.0% of morphospecies, while 18.7% and 15.7% of individuals and morphospecies, respectively, were aquatic. The lifestyle of 8.3% of morphospecies were both terrestrial and aquatic, and that of 2.4% of individuals was indeterminable.

Insects comprised 89.2% of individuals; crustaceans, 5.0%; arachnids, 2.6%; other arthropod classes made up fewer than 1% each. The predominant non-arthropod classes in terms of numbers of individuals were Clitellata (phylum Annelida) and Rhabditophora (phylum Platyhelminthes), but these only accounted for 2.2% and 0.1% of individuals, respectively. Hymenoptera accounted for 66.0% of all individuals; in fact, of the 4944 hymenopterans collected, all individuals apart from two were ants (family Formicidae). Beetles (order Coleoptera) were the next most abundant group, comprising 16.3% of individuals, with the remaining 25 orders accounting for fewer than 17.7% of individuals.

In terms of morphospecies richness (rather than abundance), Insecta was still the most prominent class, comprising 202 of the 291 morphospecies of macroinvertebrates (69.4%). Arachnids were the next most speciose class with 65 morphospecies (22.3% of total), including 42 morphospecies of spiders (Aranae) and nine morphospecies of harvestmen (Opiliones). We counted five, three, and one morphospecies of Pseudoscorpionida, Scorpionida, and Schizomida respectively, but for lack of taxonomic expertise cannot guarantee that the 30, 39, and 10 individuals found of each of these orders do not comprise more or fewer morphospecies. Following arachnids in terms of number of morphospecies per class, were myriapods (Diplopoda, 3.1%; Chilopoda, 1.0%) and malacostracans (1.7%), although these classes did not contain the most speciose orders. The orders with the greatest number of morphospecies were Hymenoptera (26% of all morphospecies, all but one of which were ants); Coleoptera (19.2%); Aranae (14.4%); Diptera (9.6%), and Blattodea (6.2%). More detail on the invertebrates can be found in SI-2 (Supplementary Information).

2.4.3 Generalised linear models of alpha diversity

Logged phytotelm capacity was the only variable analysed which significantly correlated with species richness. This was true for the whole community as well as when aquatic and terrestrial communities were analysed separately (Table 2.2; Figure 2.2). The longest leaf of

bromeliads was also correlated to the number of morphospecies and number of individuals in the aquatic community. Other than that, the number of individual invertebrates within bromeliads did not correlate significantly with any of the analysed variables (Table 2.2; Figure 2.3).

*Table 2.2. Results of the GLMMs performed on different factions of the community. Models were simplified sequentially, but volume, height, and forest type were retained in all models as they were of interest to us. Significance (P<0.050) indicated by *.*

Community	Variable	Morphospecies richness		Abundance	
		Estimate* ± *SE	***P***	***Estimate* ± *SE***	***P***
Whole community	**Volume (log)**	0.315 ± 0.077	< 0.001*	0.300 ± 0.334	0.372
	Height	0.003 ± 0.011	0.757	-0.042 ± 0.049	0.395
	Forest type	-0.127 ± 0.094	0.179	0.161 ± 0.402	0.689
Aquatic community	**Volume (log)**	0.329 ± 0.110	0.004*	0.462 ± 0.245	0.064
	Height	-0.001 ± 0.014	0.924	-0.006 ± 0.033	0.853
	Forest type	0.019 ± 0.131	0.882	-0.062 ± 0.298	0.836
	Longest leaf	-0.003 ± 0.001	0.050*	-0.008 ± 0.003	0.021*
Terrestrial community	**Volume (log)**	0.346 ± 0.088	< 0.001*	0.316 ± 0.389	0.420
	Height	0.009 ± 0.013	0.463	-0.049 ± 0.058	0.408
	Forest type	-0.232 ± 0.108	0.036	0.156 ± 0.468	0.739

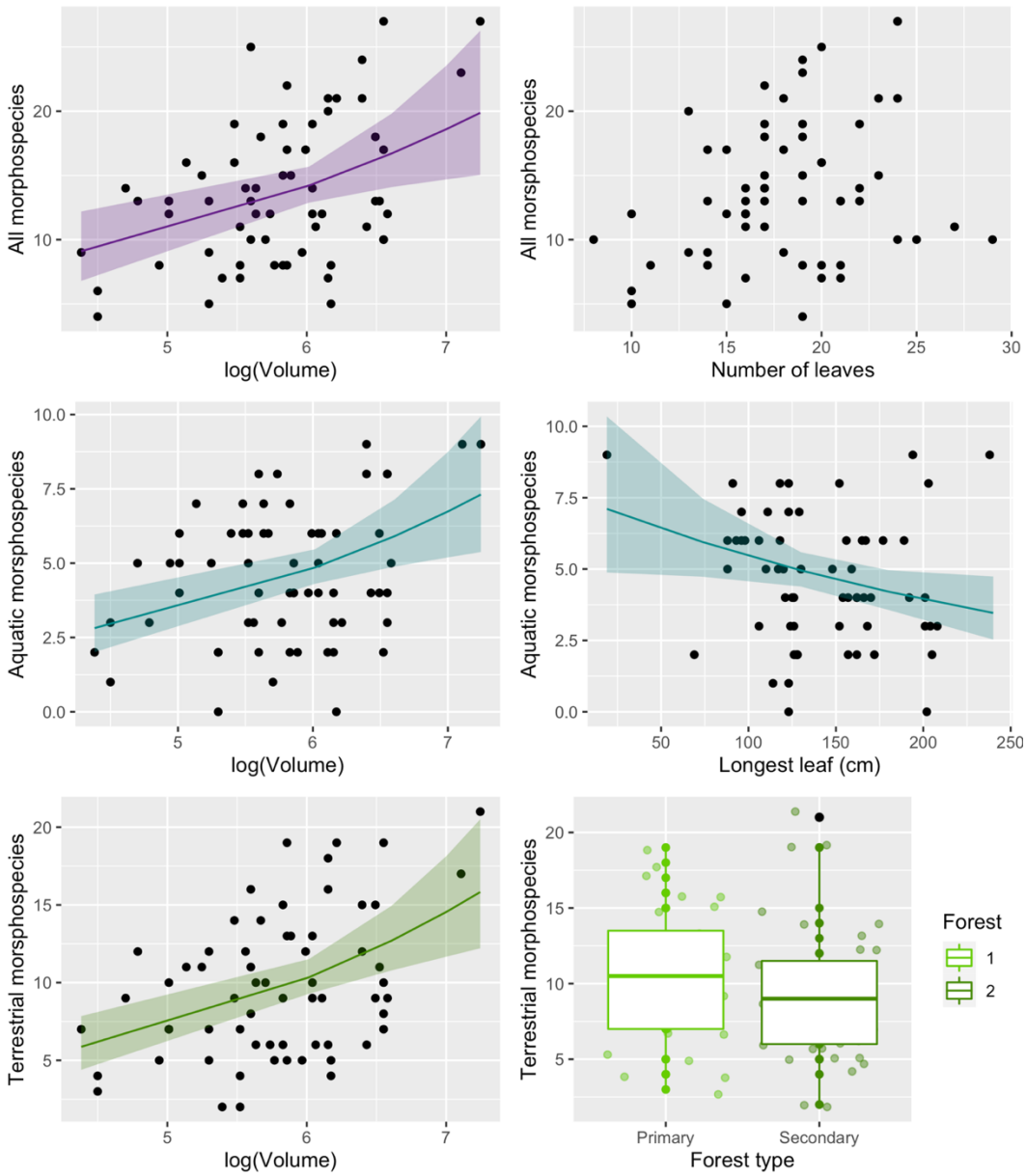


Figure 2.2. Trends in species richness according to different bromeliad traits.

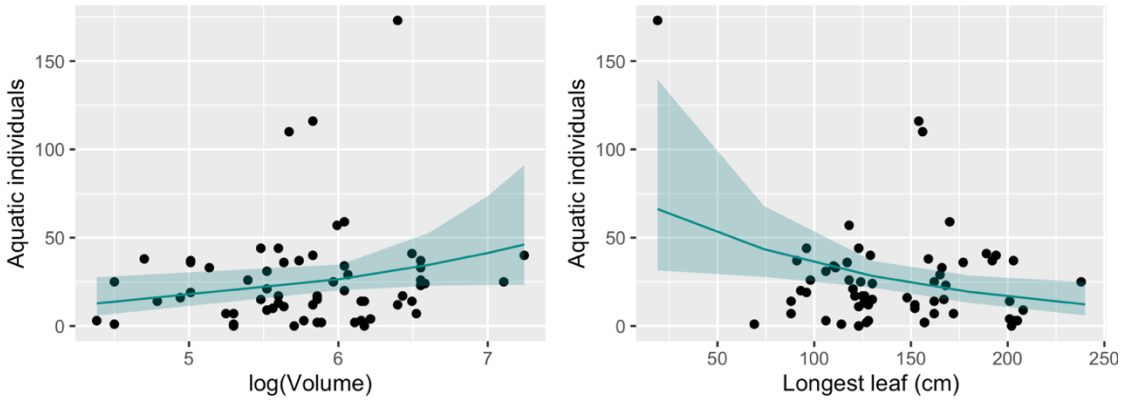


Figure 2.3. Trends in the number of individuals according to different bromeliad traits in the aquatic community, which was the only section of the community with significant trends in the number of individuals according to bromeliad traits.

*Table 2.3. Results of the GLMMs performed on the most common orders. Forest type did not have a significant effect on any of the groups, which is why it is not shown. None of these variables correlated significantly with the abundance of any order, with the exception of Blattodea abundance with number of leaves (P=0.044), so results of the abundance analysis are not shown. Significance (P<0.050) indicated by *.*

Order	Variable	Morphospecies richness	
		Estimate* ± *SE	***P***
Aranae	**Volume (log)**	-0.241 ± 0.269	0.381
	Height	0.034 ± 0.025	0.187
	Nº of leaves	0.004 ± 0.028	0.889
	Forest type	0.149 ± 0.234	0.530
Blattodea	**Volume (log)**	0.475 ± 0.143	0.002*
	Height	0.005 ± 0.019	0.806
	Nº of leaves	-0.000 ± 0.019	0.976
	Forest type	-0.207 ± 0.138	0.141
Coleoptera	**Volume (log)**	0.249 ± 0.135	0.071
	Height	0.035 ± 0.019	0.064
	Nº of leaves	0.039 ± 0.020	0.058
	Forest type	-0.089 ± 0.147	0.544
Diptera	**Volume (log)**	-0.006 ± 0.161	0.096
	Height	-0.033 ± 0.020	0.117
	Nº of leaves	-0.003 ± 0.025	0.888
	Forest type	0.348 ± 0.174	0.052
Hymenoptera	**Volume (log)**	0.274 ± 0.136	0.049*
	Height	0.023 ± 0.019	0.223
	Nº of leaves	0.004 ± 0.019	0.836
	Forest type	0.086 ± 0.149	0.566
Isopoda	**Volume (log)**	0.172 ± 0.110	0.124
	Height	-0.026 ± 0.018	0.154
	Nº of leaves	-0.029 ± 0.018	0.105
	Forest type	-0.108 ± 0.125	0.388
Lepidoptera	**Volume (log)**	0.051 ± 0.134	0.707
	Height	0.009 ± 0.019	0.634
	Nº of leaves	0.018 ± 0.025	0.470
	Forest type	0.015 ± 0.173	0.933

Bromeliad volume, height, number of leaves, and forest type had different effects on the richness and abundance of orders (Table 2.3). The abundance within orders wasn't affected

by any of the tested variables, with the exception of Blattodea, whose abundance was significantly and positively correlated with the number of leaves (0.084 ± 0.040, P = 0.043). The morphospecies richness of most orders was mostly unaffected by the tested variables, although the richness of Blattodea, Coleoptera, and Hymenoptera were positively correlated with bromeliad volume (Table 2.3).

2.4.4 Multivariate analysis

Overall, the eight environmental and physical factors explained 13.36% (individual abundance data included) or 15.83% (presence/absence data) of the variation seen in the whole invertebrate community composition among bromeliads. When abundance data was included, no single factor explained a significant portion of community variation. For presence/absence data, height of the bromeliad on the host and forest type explained statistically significant portions of variation in the invertebrate community composition, but still only accounted for 2.9% and 2.7% of this variation, respectively (Table 2.4). Phytotelm capacity was the plant trait measured that explained the most variation between overall invertebrate communities, explaning 2.3% (Table 2.4).

*Table 2.4. Results from PERMANOVAs of different factions of the community. We have only included the statistics for forest type, height, and volume because these were the variables which explained significant portions of the variation. Significance indicated by *.*

Community	**Variable**	**Presence-absence**				**Abundance**			
		F	**df**	**R^2**	**P**	**F**	**df**	**R^2**	**P**
Whole community	**Forest**	1.755	1, 59	0.027	0.004*	1.019	1, 54	0.016	0.416
	Height	1.923	1, 59	0.029	0.001*	1.106	1, 54	0.018	0.282
	Volume (log)	1.489	1, 59	0.023	0.033*	1.171	1, 54	0.019	0.229
Aquatic community	**Forest**	2.965	1, 56	0.047	0.006*	1.515	1, 56	0.025	0.120
	Height	3.012	1, 56	0.048	0.004*	2.013	1, 56	0.034	0.032*
	Volume (log)	0.698	1, 56	0.011	0.678	0.738	1, 56	0.010	0.829
Terrestrial Community	**Forest**	1.252	1, 59	0.019	0.148	1.009	1, 59	0.016	0.444
	Height	1.489	1, 59	0.023	0.033*	1.076	1, 59	0.017	0.302
	Volume (log)	1.719	1, 59	0.027	0.009*	1.463	1, 59	0.023	0.015*

When the aquatic community was considered separately from the terrestrial invertebrates, height and forest type were the only variables accounting for statistically significant portions of the differences between communities using the presence/absence data (Table 2.4), whereas when abundances were included, only height was statistically significant (Table 2.4). Overall 13.9% (presence-absence) or 18.4% (with abundances) of the variation between aquatic communities was explained by the measured variables when all eight factors were included in the model.

Forest type did not explain a significant portion of the variation between terrestrial communities. Height on host tree and phytotelm capacity explained small but significant portions of the variation among bromeliads (Table 2.4). The eight measured variables explained 14.8% of the variation between terrestrial communities. When abundance data was included, only phytotelm capacity was statistically significant (Table 2.4) and 14.2% of the variation was explained by the measured variables.

2.4.5 Community composition across strata and forest types

Fisher's Exact Test was used to compare the community composition in terms of abundance and morphospecies richness of invertebrates orders between strata and forest types. Although the number of morphospecies in each order did not vary significantly between vertical strata, the abundances of orders varied significantly across all strata ($P < 0.001$, across all strata and between pairwise analyses of strata). Both abundances ($P < 0.001$) and morphospecies richness ($P = 0.026$) of orders varied between forest types.

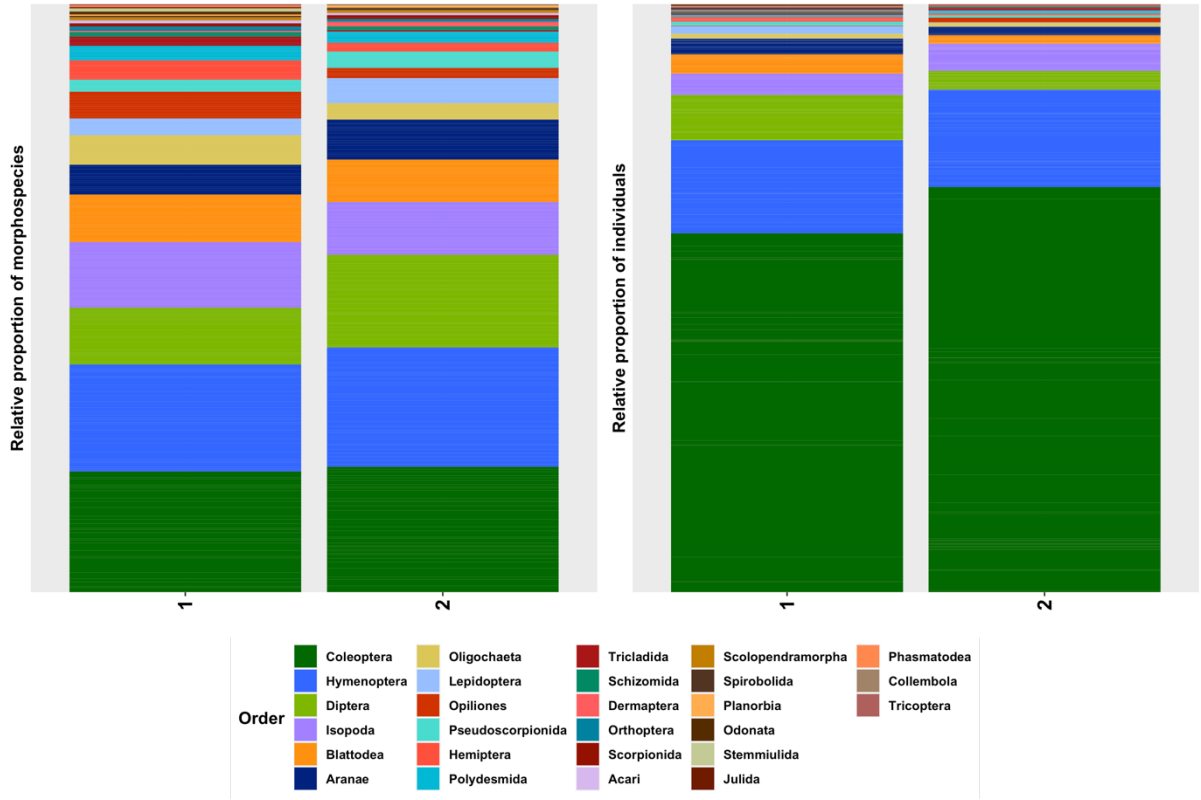


Figure 2.4. Composition of orders in primary and secondary forest. (A) shows the relative number of individuals within each order in primary (1) and secondary (2) forest. (B) shows the relative number of morphospecies within each order. Composition was significantly different between forest types

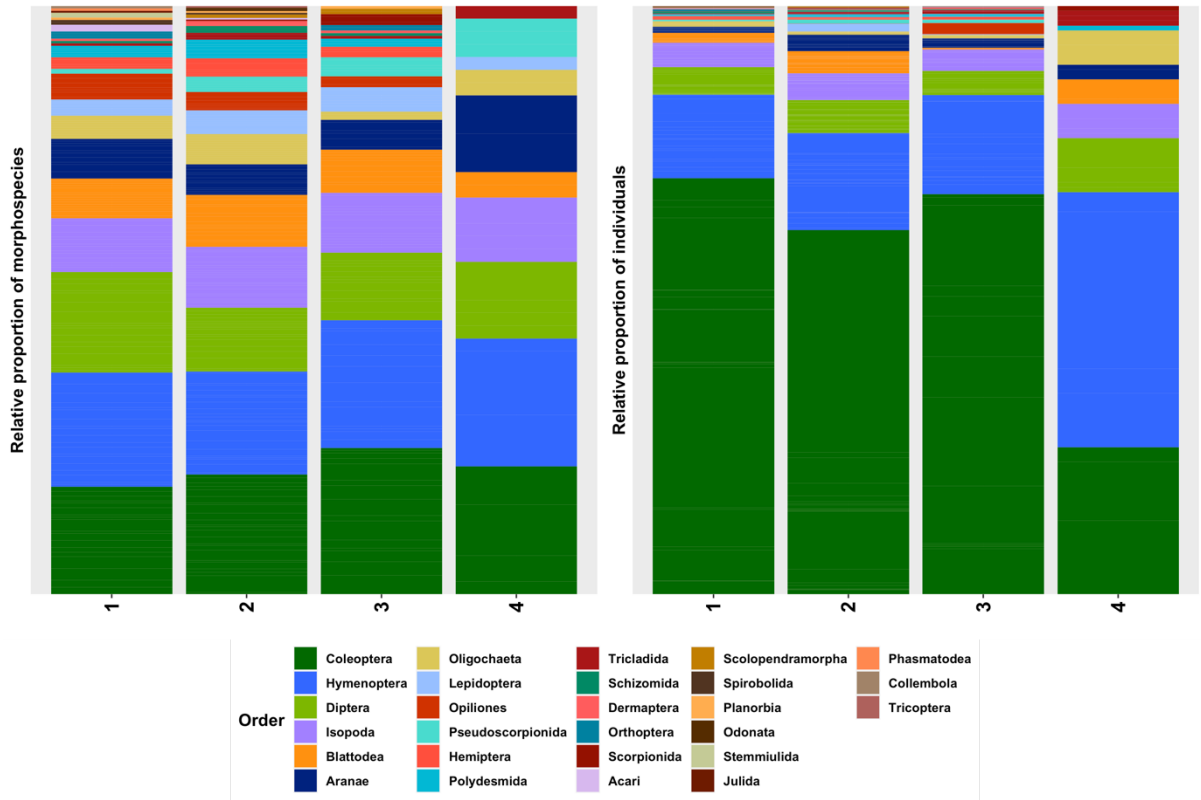


Figure 2.5. Composition of orders across vertical strata. (A) shows the relative number of individuals within each order across four vertical strata. (B) shows the relative number of morphospecies within each order.

2.5 Discussion

Different environmental and plant variables explained different proportions of invertebrate community composition in bromeliad phytotelmata, depending on the subset of the whole invertebrate community was examined. Whether presence-absence data or abundances data was included in the multivariate analyses influenced whether variables had a significant effect on the invertebrate community. This discrepancy between multivariate analyses using abundance and presence-absence data, could in part be explained by the high proportion (60.3%) of the invertebrates being identified as singletons in the data, not an unusual phenomenon in tropical forest datasets (Novotný and Basset 2000; Armbruster et al. 2002; Stork et al. 2016). Regardless, forest type, the height at which bromeliads were attached, plant volume, and the number of leaves on the bromeliad all had some effect on part of the invertebrate communities (aquatic or terrestrial), whether on the richness and abundance of communities. These significant effects on parts of the community lead to changes in community composition.

2.5.1 Effect of forest type

Although forest type was suggested as a significant cause of variation in community structure in the multivariate analyses, species richness and number of individuals, when analyzed separately, did not differ between primary and secondary forest. This last point seems initially encouraging, as it suggests that the fauna of bromeliads may recover adequately to levels comparable to before disturbance, in secondary forests. Certainly, bromeliads act as a buffer against numerous environmental conditions (Scheffers et al. 2014; Fernandez Barrancos et al. 2017) and might appear to suggest that human disturbance may be one of them. Although we did not find significant differences in species richness or abundance in our separate linear models, community composition was significantly different between primary and secondary forest bromeliads, both in terms of abundances and morphospecies within orders. Additionally, we recorded at least one species of invasive ants in the bromeliads we sampled: *Monomorium floricola* (Wetterer 2009), emphasizing that these assemblages are not immune to human disturbance. Other studies have found that local environmental conditions (Ngai et al. 2008) or differences in the occurrence and size of bromeliads themselves among forest types (Srivastava et al. 2005), drive differences between primary and secondary forest communities in bromeliads. Local environmental conditions such as canopy cover or shade have been also been found to affect invertebrate communities in bromeliads (Méndez-Castro and Rao 2014; Busse et al. 2018), and it can be expected that different stages of forest succession exhibit different canopy densities and

light conditions (Matsuo et al. 2021). However, without having recorded the traits of the forest types sampled, it is impossible to say what variables drive the differences between communities in primary and secondary forest here, and so it is hard to compare our study with those outlining the effect of environmental conditions on invertebrate communities in bromeliads.

2.5.2 Effect of height

There are a number of reasons why vertical stratification may occur in forests, such as the contrasting environmental variables (e.g. temperature, humidity) between ground, understorey, and canopy layers, the uneven availability of resources (such as leaf litter and fresh leaf tissue), and the behavioural traits and dispersal capabilities of different taxa (Basset et al. 2003). Despite the results of the multivariate analyses using presence-absence data, there was no effect of attachment height on the number of morphospecies within a bromeliad in our univariate analyses, in either the aquatic or terrestrial macroinvertebrate communities. Species richness and abundances did not vary significantly with increasing height, but the effect size was small. This mirrors other studies which found no effect of height on phytotelmata communities (Jocque and Field 2014a; Gossner and Petermann 2022), although contrasts with other studies of phytotelmata (Yanoviak 1999; Kratina et al. 2017) and canopy invertebrates in general (DeVries et al. 1997; Chapin and Smith 2019) which found a decrease in diversity as one ascends towards the top of the canopy.

Of the individual orders analysed, only beetle (Coleoptera) species richness was marginally ($P=0.064$) correlated with height on the host tree. Whereas as, as in this study, spider richness and abundance has been known not to vary vertically (Kitching et al. 1993; Yoshida et al. 2021), we expected both cockroaches and flies to increase with height as Dial et al. (2006) found in a tropical forest in Borneo; however, this was not the case here. This may be due to the fact that none of our bromeliads were found in the harsher upper canopy, as well as that bromeliads may offer an oases and buffer against diversity inclines throughout the canopy. When vertical strata were compared, the differences between pairs of strata and all four 5 m strata were compared, differences were driven by the abundances within orders, while the number of morphospecies per order was very similar across all measured strata. The differences caused by height indicated by the multivariate analyses therefore, are likely due to differences at lower taxonomic levels and species turnover, rather than differences in alpha diversity.

2.5.3 Effect of bromeliad size and complexity

Bromeliad capacity explained a significant portion of the variation between terrestrial communities. Richness was positively correlated with capacity, as has been shown by previous studies on bromeliad invertebrates (Jabiol et al. 2009; Jocque and Field 2014a; Méndez-Castro et al. 2018). However, this pattern was not uniform across the community and was driven by patterns within certain taxa. Habitat size is known to affect different invertebrate taxa to different extents, with predators generally requiring a larger area (Srivastava et al. 2008b, 2020; Romero et al. 2016). The only fully predatory group at the order level that was common enough to analyse separately from the rest of the community were spiders, which seemed unaffected by any of the measured variables. However, we did detect patterns in some of the most common orders: ant, cockroach, and beetle species richness increased with plant volume.

In addition to size, plant architecture and complexity has previously also been shown to affect bromeliad invertebrate communities (Armbruster et al. 2002; Gonçalves-Souza et al. 2011; Carrias et al. 2014), however, here we found no effect of the number of leaves on the community in terms of richness or composition. Instead the longest leaf of bromeliads was slightly but significantly correlated with the richness and abundance of the aquatic community. Longest leaf did not correlate significantly with any of the other size variables measured, and so the reason for its effect on the aquatic community here is unclear.

2.5.4 Conclusion

In this study, we studied the entire macroinvertebrate communities in two species of *Aechmea* bromeliads in a part of the Ecuadorian Amazon and Tropical Andes Biodiversity Hotspot, in the context of a range of environmental and plant measurements. The average number of morphospecies per bromeliad (300 morphospecies in 63 bromeliads) was higher than in the last comprehensive study of bromeliad invertebrate communities in Ecuador (354 morphospecies in 209 bromeliads) (Armbruster et al. 2002). We found that community structure differed in bromeliads in primary and secondary forest and that differences in communities found at different heights were driven by abundances within orders and community composition, rather than species richness. Not all taxa responded uniformly to an increase in plant size, but those that did increased in species richness with increasing plant volume. Our study fills in a geographic gap in the study of bromeliad fauna and to the limited literature on the communities of bromeliads higher in the canopy.

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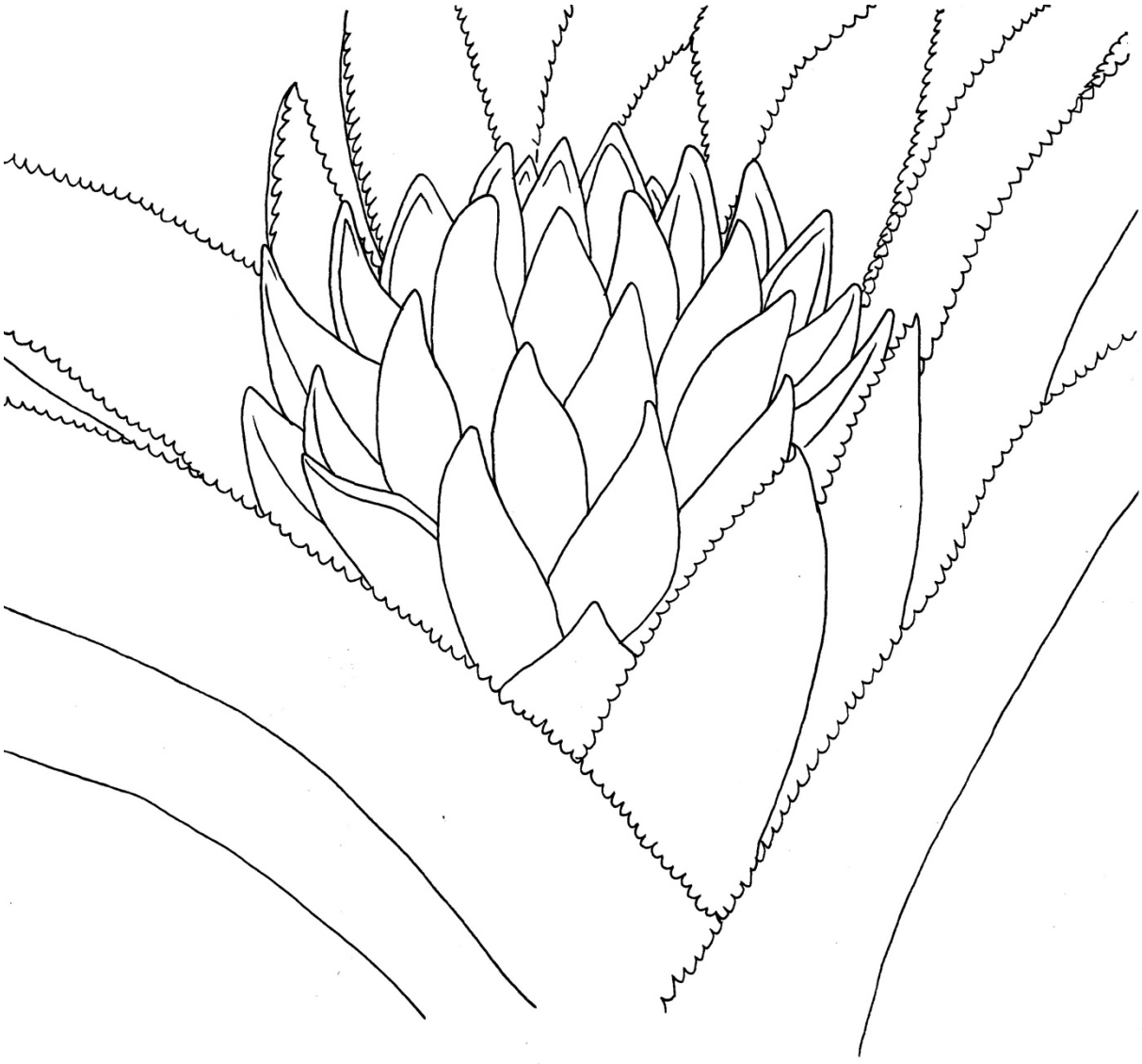
Chapter III

The effect of environmental and plant characteristics on prokaryotic diversity in bromeliad phytotelmata (*Aechmea spp.*)



3.1 Abstract

Tank bromeliads in the neotropics harbour complex communities of invertebrates and even provide food, water, and shelter to various vertebrate species. However, few studies have sequenced the microbial communities within bromeliad phytotelmata. We sequenced the 16S rRNA subunit extracted from swabs rubbed in the central phytotelm of epiphytic bromeliads *Aechmea hoppii* and *A. nidularioides*. We used generalised linear models to test what factors influence the alpha diversity of prokaryote communities in bromeliads, and permutational multivariate analyses of variance to analyse what factors influence the beta-diversity of these communities. Height on the tree and size of the bromeliad influenced the alpha-diversity within plants. Depending on which taxonomic level was analysed and whether abundance or presence-absence data was used, different factors explained significant proportions of variation in the prokaryotic communities. At the Amplicon Sequence Variant level, forest type, height from the ground, and pH of the phytotelm explained a small proportion of the variation among communities. We used FAPROTAX to assign taxa to functional groups and found that a complex picture of correlations between function and variables emerges. This is the largest metabarcoding study of bromeliad phytotelmata prokaryotes to date and contributes to the growing knowledge of these highly diverse systems.

3.2 Introduction

Knowledge of the microbial diversity in the tropical understory and canopy is limited (Nakamura et al. 2017), but there have been some notable studies exploring this diversity on suspended soils (Donald et al. 2020; Eskov et al. 2021)**,** leaf surfaces (Lambais et al. 2006; Kembel et al. 2014), and phytotelmata (Pittl et al. 2010; Gilbert et al. 2020)**.** Bromeliads form important phytotelmata in neotropical habitats, including in megadiverse forests such as Amazonia. These plants form phytotelmata by virtue of their spiral rosetted structure, which allows for rainwater to collect between their leaf axils. The phytotelmata are colonized by diverse communities of eukaryotes and prokaryotes, although work on the microorganisms inhabiting these spaces has been relatively limited compared to studies on their invertebrate communities. Eukaryotic microorganisms in bromeliads include rotifers, ciliates, tardigrades, yeasts, and crustaceans, amongst others (Gomes et al. 2015; Simão et al. 2017; Mercado-Salas et al. 2021)**.** Prokaryotic microorganisms (Bacteria and Archaea) in bromeliads have been shown to be incredibly diverse, with previous studies identifying between 23-51 different phyla in a few samples collected within close proximity of each other (Rodriguez-Nuñez et al. 2018; Herrera-García et al. 2022)**.** Although there are similarities in the microbial communities among individual bromeliads from the same species (Louca et al. 2017a), there is still a high degree of variation in community structure among individuals (Carmo et al. 2014; Louca et al. 2017a), and even between separate phytotelmata within the same bromeliad (Giongo et al. 2019), emphasizing a role for environmental and stochastic processes in structuring these communities (Farjalla et al. 2012).

Prokaryotes perform a wide range of functions in bromeliad phytotelmata, from breaking down plant and animal detritus (Goffredi et al. 2011b), to methane production and nitrogen cycling (Inselsbacher *et al.*, 2007; Goffredi, Kantor and Woodside, 2011; Kotowska and Werner, 2013), to primary production (Lehours et al. 2016). These functions benefit both the plants themselves**,** as well as their associated eukaryotic communities by providing important nutrients to these microcosms (Inselsbacher *et al.*, 2007; Gonçalves *et al.*, 2014; Leroy *et al.*, 2016)**.**

There have been various approaches to studying the prokaryotic communities in bromeliads. Non-DNA-based methods have included studying bacterial growth or chemical production (Haubrich et al. 2009; Martinson et al. 2010; Brandt et al. 2017), as well as flow cytometry and staining, which are useful for counting microorganisms and identifying them based on morphology without the need to culture them. Despite the benefits of these approaches they

have low taxonomic resolution and it is when used often unfeasible to obtain full coverage of complex communities. DNA-based approaches such as DGGE electrophoresis has allowed allowed the discrimination of taxonomic richness and abundance based on size and intensity of amplified DNA fragments (Farjalla 2012, Cereghino 2020), while DNA metabarcoding with high-throughput sequencing has enabled an analysis of whole microbial communities at greater taxonomic resolution, based on a barcode or barcodes of choice (Louca et al. 2017a; Rodriguez-Nuñez et al. 2018). However, few studies have used metabarcoding and next-generation sequencing to characterise bromeliad-associated communities, although they are now increasing in number, with at least six publications on prokaryote communities to date (Louca et al. 2017a; Louca et al. 2017; Rodriguez-Nuñez et al. 2018; Giongo et al. 2019; Simão et al. 2020; Herrera-García et al. 2022).

Several biogeochemical factors have been shown to influence prokaryotic communities in bromeliads. For instance, phytotelmata pH, ammonium ion concentration, water temperature, water colour and turbidity, and methane concentrations (Haubrich et al. 2009; Goffredi, Kantor, et al. 2011; Louca et al. 2017), as well as algal biomass and tank depth (Brouard et al. 2011; Goffredi et al. 2011a) have been shown to be related to prokaryotic taxonomic or functional diversity. Additionally, some external environmental factors may affect these prokaryotic communities, such as the presence or absence of surrounding vegetation, canopy cover, and detrital content (Louca et al. 2017; Herrera-García et al. 2022). However, generally very little variation in bacterial communities has been attributed to external environmental variables on the prokaryotic communities in bromeliads (Haubrich et al. 2009; Céréghino et al. 2020).

In this study, we looked at how habitat and physical plant characteristics influence the prokaryotic phytotelm communities of two epiphytic bromeliads in one of the most biodiverse regions on Earth, the Tropical Andes Biodiversity Hotspot. We collected data from two vegetatively cryptic species of bromeliads, which were later identified using inflorescences and molecular methods. We characterised the prokaryote communities by sequencing a section of the 16S rRNA subunit and add to the small but growing body of literature on bromeliad community metabarcoding. We analysed the effects of forest regeneration stage (primary or secondary), height on the host tree, pH and temperature of the phytotelm, size of the plant, complexity of the plant, as well as the bromeliad species and sampling season.

3.3 Materials and methods

3.3.1 Focal species

Two species of congeneric bromeliad were selected in this study, *Aechmea hoppii* (Harms)(Smith 1953a) and *A. nidularioides* (Smith 1953b). They were chosen based on their abundance in the field site and are physically indistinguishable when no inflorescence is present. The clustering of plants based on distances calculated with specially-designed microsatellite markers was used to separate the two species, as this clustering coincided with the separation of plants by their inflorescences. The molecular methods are described in detail in the *Materials and methods* of Chapter IV of this thesis.

3.3.2 Field sampling

This study was carried out in San José de Payamino, Orellana, Ecuador, from here on referred to as Payamino. The site is described in Chapter II of this thesis. Field sampling took place in January-February and July-August 2018. Although Payamino does not have marked seasons (Irvine 1987; Sullivan et al. 2020), sampling period was included as a factor in all analyses.

Bromeliads were collected between 1.96 and 20.20 m off the ground, using either a ladder or double-rope canopy access techniques. The height of the bromeliad was determined by dropping the end of a tape measure to the ground, whilst holding the top against the base of the plant. Whether the site a bromeliad was found in was primary or secondary forest was determined by the author and the expertise of the local guides.

Upon accessing a bromeliad, a sterile swab was rubbed for 10-20 seconds in the base of the central phytotelma and immediately placed in a sterile 2.0 cryovial. Samples were frozen in liquid nitrogen upon return to the base camp that day. Following swabbing, a handheld pH meter was used to record pH and temperature of the central phytotelm. The bromeliad was then removed from the tree and transported back to camp for further processing, where invertebrates were extracted for other studies (Chapters II and IV). Measurements of the plants themselves included measuring the base circumference and the longest leaf, and counting the number of leaves. Plant volume was measured as the total capacity of the bromeliad, by filling the phytotelmata with a known quantity of water until full.

3.3.3 DNA extraction

DNA was extracted from 63 swabs using the DNeasy® PowerSoil® DNA kit (Qiagen) following the manufacturer's protocol with the following modification to the beginning of the protocol: as a first step, swabs were snapped off into the bead tubes provided in the kit, the C1 solution was added, and samples were left to incubate at 65ºC for 2 hours while shaking gently. For each set of extractions, extraction controls were done using a clean swab and following the same steps as with sample extractions, as a negative extraction control. To obtain a positive control for later amplification, a swab was dipped in a ZymoBIOMICS™ Microbial community standard (D6300) and extracted following the same protocol as the sample extractions.

3.3.4 DNA amplification

A subset of samples were amplified with qPCR to determine the sample concentration and cycle number that most evenly amplified the samples. The 10 µl qPCR reaction consisted of 5 µl SYBR® Green Master Mix (Sigma-Aldrich), 2 µl of 1 M forward primer, 2 µl of 1 M reverse primer, and 1 µl of sample (4ng/µl DNA) of 1/10, 1/20, and 1/40 dilutions. All samples were then normalised to 4 ng/µl and diluted to 1/10. The ZymoBIOMICS™ Microbial community standard was left at a dilution of 1:200.

Samples were amplified in plates consisting of 12 blank wells (at least one per row and column), 4 PCR negative controls, 4 positive controls extracted from the ZymoBIOMICS™ Microbial community standard, 4 extraction controls, and 72 DNA extractions from samples. These 72 samples include least 3 replicates of each community sample. This resulted in 3 plates. Forward and reverse primers were tagged with phased barcodes. The PCR recipe was a 10 µl reaction consisting of 4.9 µl AmpliTaq Gold 360 (Applied Biosystems), 0.1 µl GC Enhancer (Applied Biosystems), 2 µl of 1 M forward primer, 2 µl of 1 M reverse primer, and 1 µl of sample (0.4ng/µl DNA). Amplification was achieved with the following programme: 10 minutes at 95ºC, 31 cycles of 30 seconds at 95ºC**,** 1 minute at 50ºC, and 90 seconds at 72ºC, followed by 30 minutes at 72ºC and held at 4ºC.

3.3.5 Library preparation and sequencing

Plates were pooled by taking 5 µl of each well from each amplified plate. Plate pools were quantified by Qubit® dsDNA HS (High Sensitivity) Kit (Life technologies) for broad-range double-stranded DNA. Each plate pool was normalised by dilution with molecular grade

water to the lowest concentration of the plate pool. Two pools were created from the three plates. This was possible at this stage due to every primer combination being unique, due to the phased tags.

Library preparation of the two pools was performed in conjunction with a third pool of plates for another project. Library preparation was carried out using TruSeq® DNA PCR-Free Library Prep kit (Illumina) and following the manufacturer's instructions. The TruSeq® DNA Single Indexes Set A (Illumina) adapters 005, 006, and 012 were used. Library size was ascertained by an Agilent 7500 Bioanalyzer chip with the High Sensitivity DNA Kit (Agilent Technologies), from which DNA-adapter ratios were established to estimate the concentration of the prepared libraries. Libraries were pooled to obtain 20 µl of 4 nM library. The pooled library was diluted and denatured following Illumina® Dilute and Denature protocol in order to dilute the library to 20 pM and then the final dilution. The pooled library that was sequenced was diluted to 8 pM and spiked with 5% 20 pM PhiX, due to the low-diversity of the libraries. 600 µl of library were loaded onto an MiSeq Reagent Kit v2 (Illumina) flow cell and sequenced on an Illumina® MiSeq for 2 x 301 cycles, at the Manchester Metropolitan University (Manchester, UK).

3.3.6 Prokaryotic data processing

Paired end reads were merged using the function *illuminapairedend* in Obitools v.1 (Boyer et al. 2016)**.** Reads with an alignment score of over 50 were kept and demultiplexed using the function *ngsfilter*, which sorts reads by their barcode tag combination to their corresponding samples and removes the tag and primer sequences. The resulting data was exported using *obisplit* and imported into QIIME2 (Bolyen et al. 2019)**.** The *Deblur* function (Amir et al. 2017) was used to denoise the data, remove chimeras, and trim sequences to 252 base pairs. At this point, sequences shorter than 252 were removed along with sequences which appeared fewer than twice throughout the dataset. Denoising resulted in 3,005,775 reads. Taxonomic assignment was performed with a Naive Bayes pre-trained SILVA 138 classifier (*silva-138-99-515-806-nb-classifier*) (Bokulich et al. 2018; Robeson et al. 2020), trained on the specific region of interest (515F/806R). A phylogenetic tree was constructed in order to calculate UniFrac community distances, using the *MAFFT* function in QIIME2 to align the sequences and the *FastTree* function to construct the tree, to which a midpoint was added using *phylogeny midpoint-root*. Files were saved as QIIME2 artifacts and imported into R as a *phyloseq* object using the *qiime2R* package (Bisanz 2018). The *qza_to_phyloseq* function imports and combines the outputs from the QIIME2 pipeline: the

ASV (amplicon sequence variant) or feature table, the phylogenetic tree, the taxonomic data, and the information about the samples in the dataset.

ASVs were considered contaminants if they were present in the PCR and extraction controls but not in the sequencing controls or "blanks" (empty wells in the PCR plates). These were therefore removed using the *prune_samples* and *prune_taxa* functions in *phyloseq* (McMurdie and Holmes 2013). We calculated tag jump as the average number of reads present in sequencing controls over the average number of reads present in samples. Although this equated to 3.44% of reads, we did not attempt to remove tag jump as more common ASVs are more likely to "jump" than less abundant ones (Taberlet et al. 2012), and so removing 3.44% of reads from samples would likely bias the results. Contamination during extraction was calculated as the average number of reads present in extraction controls over the average number of reads present in samples. This equated to 0.98% and no effort was made to remove reads based on this number.

Functional group assignment was done using FAPROTAX (Louca et al. 2016b) in Python 3.7. FAPROTAX is a Python script which assigns ASVs to functional groups based on the known functions of other members of a taxonomic group. It is therefore a rough method of functional grouping based on the functional roles of related taxa, rather than an accurate classification of an ASV's functional role, as most will be unknown. This process assigned 1319 ASVs to groups, leaving 3585 unclassified. Functional groups that could be collated into larger groups were merged (*e.g.* "methanogenesis using formate" and "methanogenesis by CO2 reduction" and similar were all included under the umbrella "methanogenesis").

3.3.7 Statistical analysis

All statistical analyses were performed in R 4.1.1 (R Core Team 2020). Sample replicates were merged using *merge_samples2* from *speedyseq* (McLaren 2020) and controls removed prior to analysis. Observed Richness, Shannon, and Inverse Simpson indexes were calculated for each bromeliad using raw count data (McMurdie and Holmes 2014). Generalized Linear Models (GLMM) with quasi-poisson distribution were used to test whether each alpha diversity metric was correlated with any of the measured variables: bromeliad species; forest type; sampling season; height of the bromeliad on the tree; the volume of the bromeliad; the number of leaves; the base circumference; the longest leaf; and the pH and temperature of the phytotelm. Attempts to simplify the models did not significantly improve them, therefore all variables were retained.

Diversity index ~ height + log(Volume) + pH + phytotelm temperature + longest leaf + nº of leaves + base circumference + forest type + bromeliad species + season, family = quasipoisson

For beta diversity analyses, data was transformed to relative abundances. Weighted UniFrac (Lozupone et al. 2007) and Unweighted UniFrac (Lozupone and Knight 2005) distances were calculated using the *phyloseq* package in R. UniFrac distances take into account phylogenetic relationships between ASVs, with Unweighted UniFrac distances considering only presence-absence counts while Weighted UniFrac uses abundance data as well. Permutational multivariate analyses of variance (PERMANOVA) with each distance metric were performed with the *adonis* function in the *vegan* package (Oksanen et al. 2013) with 999 permutations, to see if any of the following variables affected taxonomic composition within the bromeliads: bromeliad species, forest type, height on the tree, volume (logged), length of the longest leaf, base circumference, number of leaves, collection season, and phytotelm temperature and pH. Models were simplified by stepwise removal of the least significant variables until only significant explanatory variables remained. The PERMANOVAs were performed with ASV data, Family data, and Phylum data. Non-metric MultiDimensional Scaling (NMDS) was used to visualise effects of significant variables from the PERMANOVAs.

To see whether environmental and plant variables correlated with different functional groups, Pearson's correlations were used for the continuous variables (height, pH, phytotelma temperature, volume, longest leaf, number of leaves, base circumference), while Chi-Square tests were performed for the categorical variables (forest type, season, species). The results of the Pearson's correlations and residuals [(observed - expected) / square root (expected)] of the Chi-Square tests were visualised with *corrplot* (Wei and Simko 2021).

Stacked bar charts of taxomomic data were produced using the *barplot* function in *phyloseq*, showing composition in different bromeliads and in the two forest types. These were produced at the Phylum and Family level due to the amount of unidentified entities at the ASV level and the intractability of the number of ASVs. Stacked bar charts of functional groups were constructed in *ggplot2* (Wickham 2016).

3.4 Results

3.4.1 Plant characteristics

Sixty-three bromeliads (56 *A. hoppii* and 7 *A. nidularioides*) were collected. The range of plant measurements and environmental variables are described in the *Results* section of Chapter II of this thesis.

3.4.2 Prokaryotic alpha diversity

Fifty-six phyla, 171 classes, 452 orders, and 806 families were found across the dataset from 63 epiphytic bromeliads. A total of 4904 ASVs including sequences were found.

Using Observed Richness, alpha diversity was not correlated with any of the measured variables. However, using Shannon and Inverse Simpson indexes, diversity was correlated with height, volume, longest leaf, and season, depending on which index was used (Table 3.1). Alpha diversity by categorical variables is plotted in Figure 3.1, and by continuous variables in Figures 3.2 and 3.3.

*Table 3.1. Results of the GLMMs. For simplicity, only the indexes and variables with significant results are shown The GLMM of Observed Richness yielded no significant correlations. Significance indicated by *.*

	Shannon			**Inverse Simpson**		
Variable	**Estimate ± SE**	**t**	**P**	**Estimate ± SE**	**t**	**P**
Height	-0.009 ± 0.004	-2.041	0.046*	-0.055 ± 0.023	-2.368	0.021*
Volume (log)	-0.056 ± 0.032	-1.749	0.086	-0.413 ± 0.159	-2.587	0.012*
Longest leaf	0.001 ± 0.000	2.337	0.023*	0.005 ± 0.002	1.812	0.075
Season (Jan-Jul)	-0.132 ± 0.047	-2.845	0.006*	-0.506 ± 0.244	-2.066	0.044*

3.4.3 Prokaryotic beta diversity

PERMANOVAs of both Unweighted UniFrac and Weighted UniFrac yielded very similar results at the ASV level, with forest type, height, and pH explaining small but significant proportions of the variation (Table 3.2). However, at the Family and Phylum levels, different variables were significant depending on whether Weighted or Unweighted UniFrac was used to calculate distances, a common occurrence due to the fundamentally different ways in which they calculate distances (Lozupone et al. 2007).

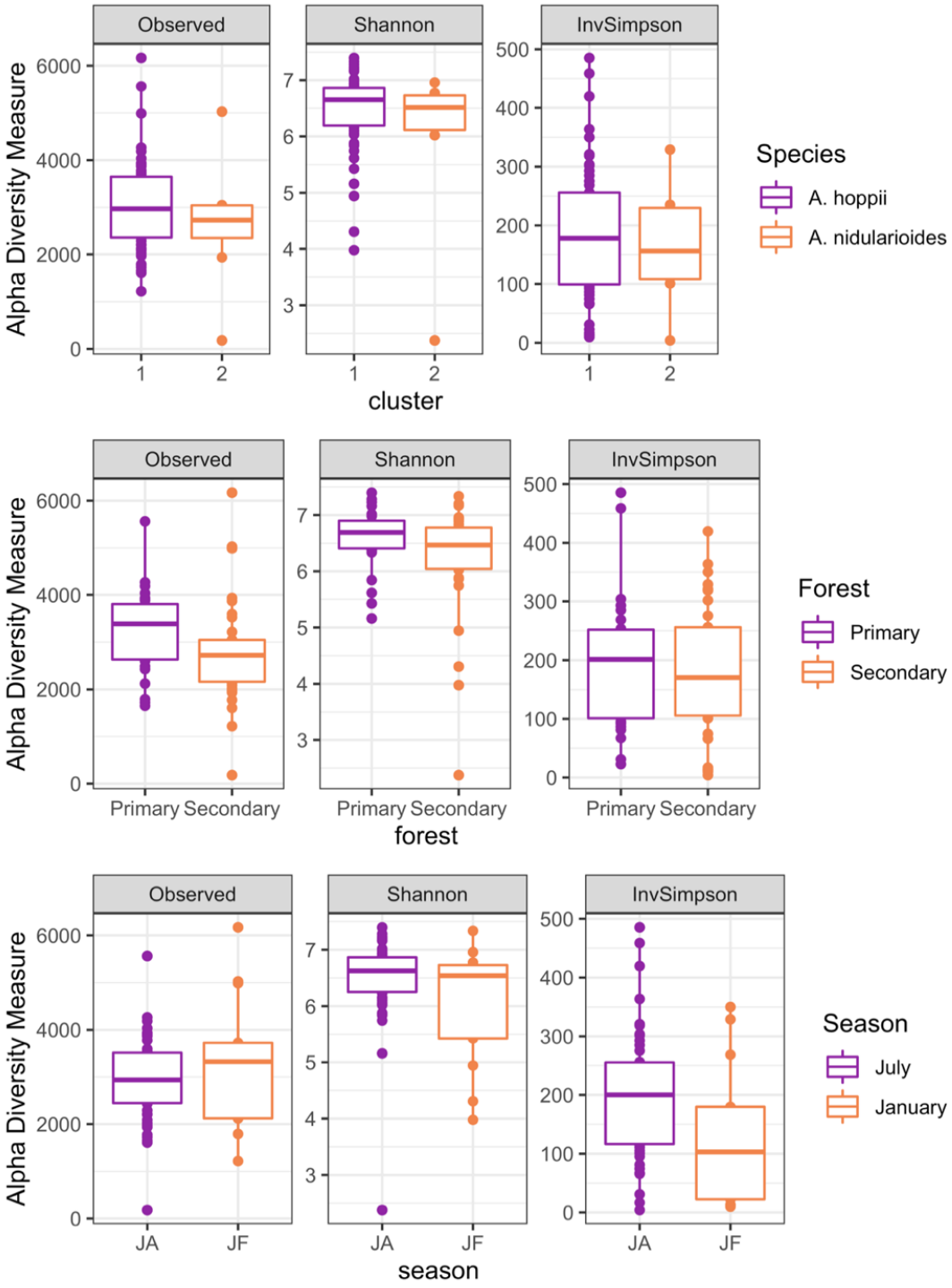


Figure 3.1. Alpha diversity as Observed Richness, Shannon index, and Inverse Simspon index, according to categorical variables: bromeliad species, forest type, and sampling season.

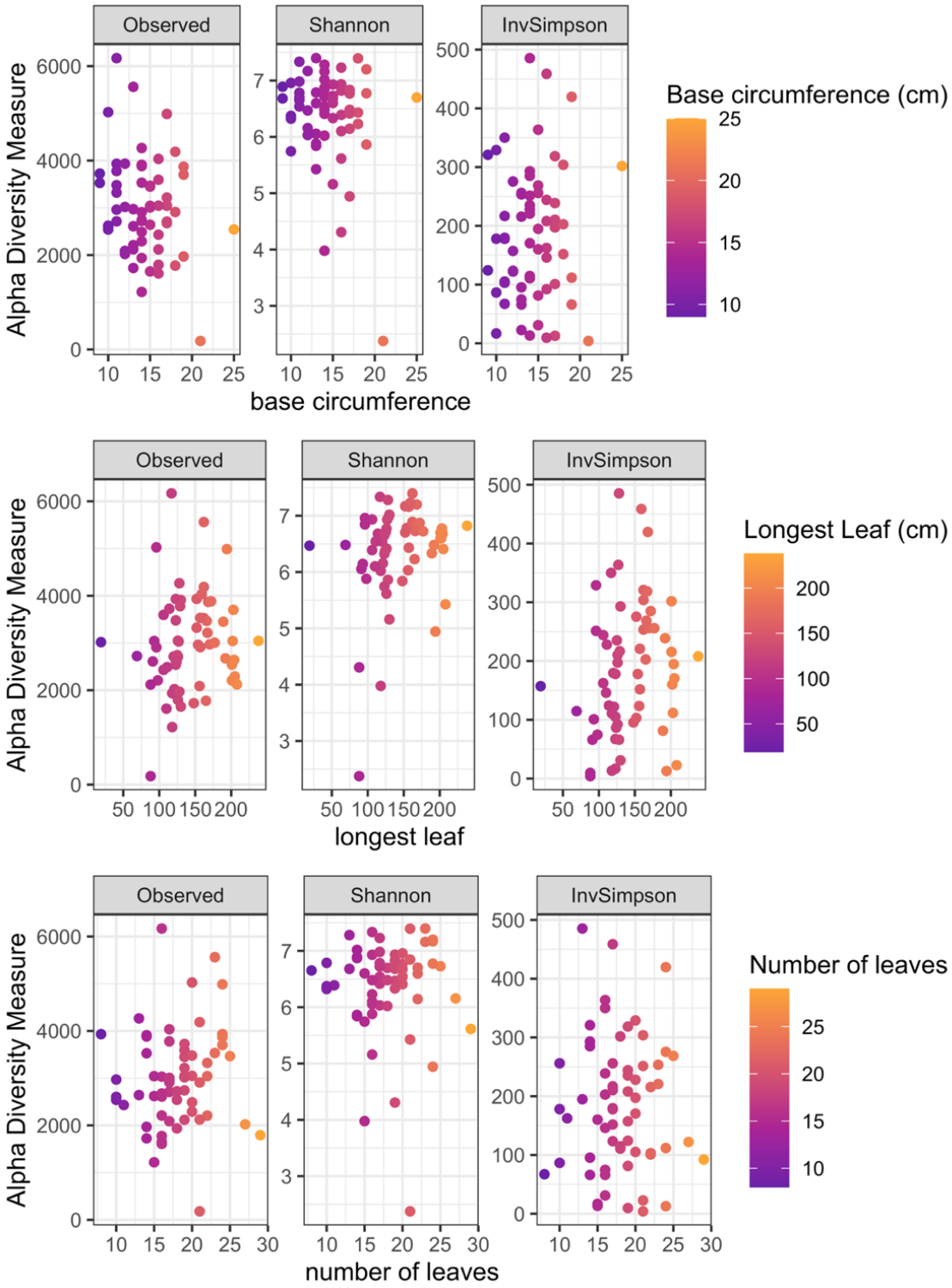


Figure 3.2. Alpha diversity as Observed Richness, Shannon index, and Inverse Simspon index, according to base circumference, longest leaf, and number of leaves.

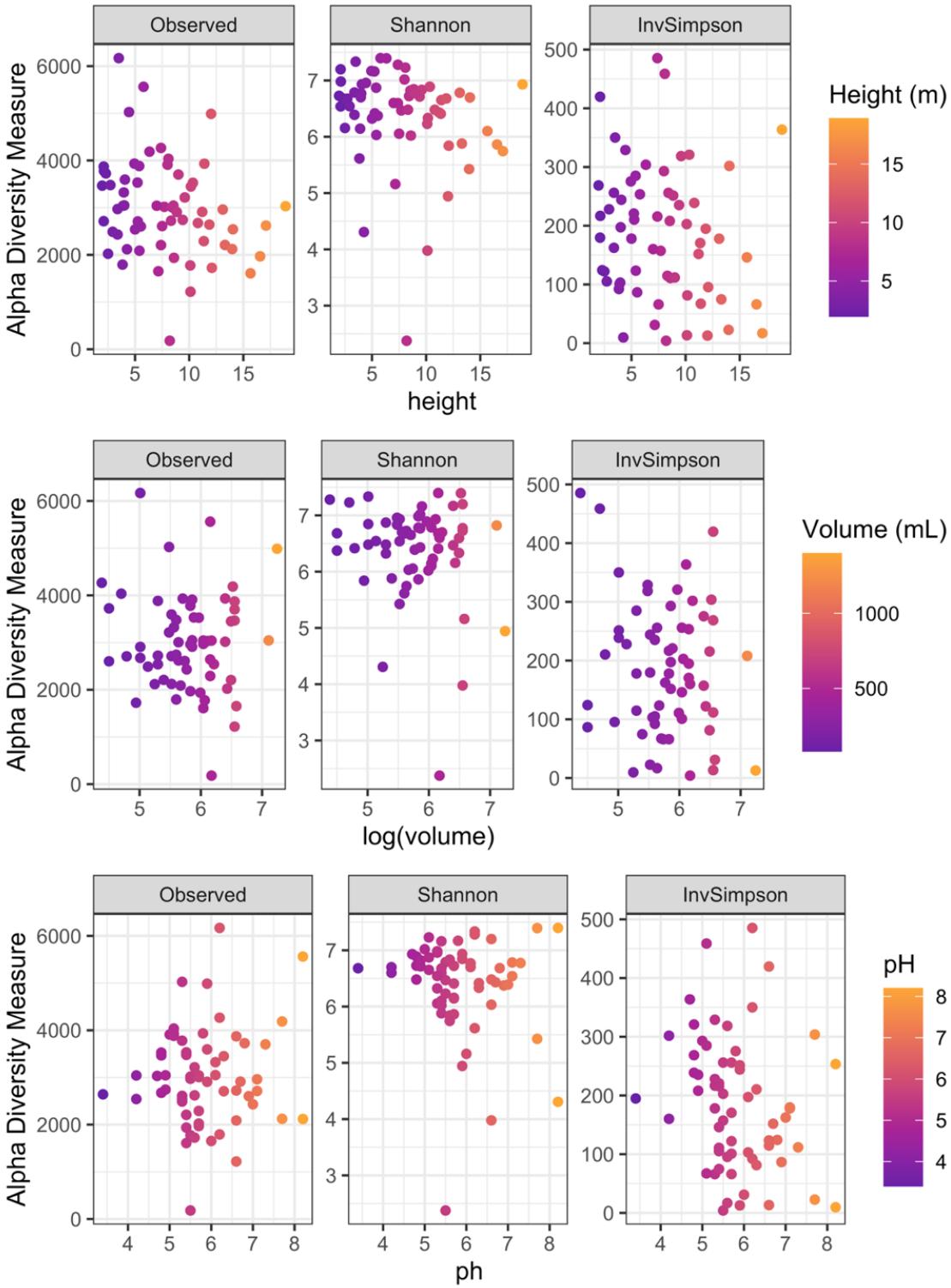


Figure 3.3. Alpha diversity as Observed Richness, Shannon index, and Inverse Simspon index, according to height on the tree, bromeliad volume (log), and phytotelm pH.

*Table 3.2. Results of the reduced PERMANOVAs of UniFrac distances at the ASV level. Significance indicated by *.*

		Weighted UniFrac				Unweighted UniFrac			
Taxon	**Variable**	**Df**	**F**	**R^2**	**P**	**Df**	**F**	**R^2**	**P**
ASV	Forest	1, 59	1.320	0.021	0.016*	1,59	1.168	0.018	0.015*
	Height	1, 59	1.371	0.022	0.017*	1, 59	1.122	0.019	0.005*
	pH	1,59	1.434	0.023	0.005*	1, 59	1.304	0.020	0.001*

*Table 3.3. Results of the reduced PERMANOVAs of UniFrac distances at the Family and Phylum levels. Significance indicated by *.*

	Weighted UniFrac				
Taxon	**Variable**	**Df**	**F**	**R^2**	**P**
Family	Forest	1,59	1.998	0.029	0.038
	pH	1,59	4.169	0.062	0.002
	Volume (log)	1,59	2.244	0.033	0.027
	Unweighted UniFrac				
	Variable	**Df**	**F**	**R^2**	**P**
	Height	1, 58	2.309	0.034	0.004*
	pH	1, 58	2.306	0.034	0.004*
	Longest left	1, 58	1.818	0.027	0.022*
	Base circumference	1, 58	1.930	0.029	0.017*
Phylum	**Weighted UniFrac**				
	Variable	**Df**	**F**	**R^2**	**P**
	pH	1, 60	4.720	0.069	0.007*
	Volume (log)	1, 60	3.871	0.057	0.010*
	Unweighted UniFrac				
	Variable	**Df**	**F**	**R^2**	**P**
	Height	1, 60	4.369	0.065	0.001*
	Phytotelm temperature	1, 60	2.812	0.042	0.022*

Due to the small proportion of variance explained by the analysed factors, most NMDS were not helpful to visualise these differences, therefore only NMDS of the effect of forest type on communities are shown (Figure 3.4). Bar charts at the ASV level were too diverse to include figure legends (Figure 3.5) and bar charts at the Family and Phylum level show the top 20 taxa at each level in order to visualise the data more easily and permit the inclusion of figure legends (Figure 3.6).

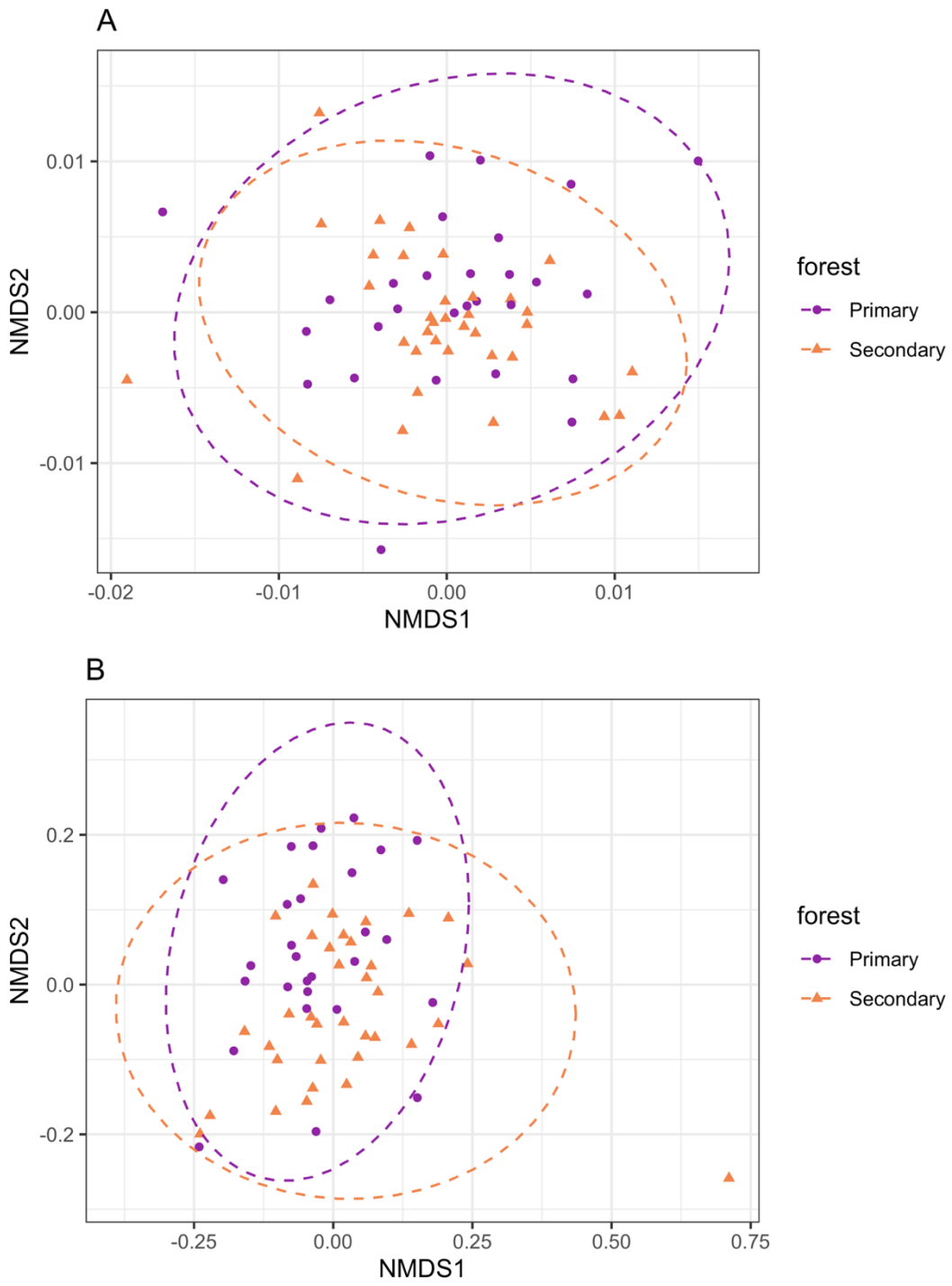


Figure 3.4. NMDS of forest type at the ASV level. Distances are either (A) Weighted UniFrac or (B) Unweighted UniFrac distances.

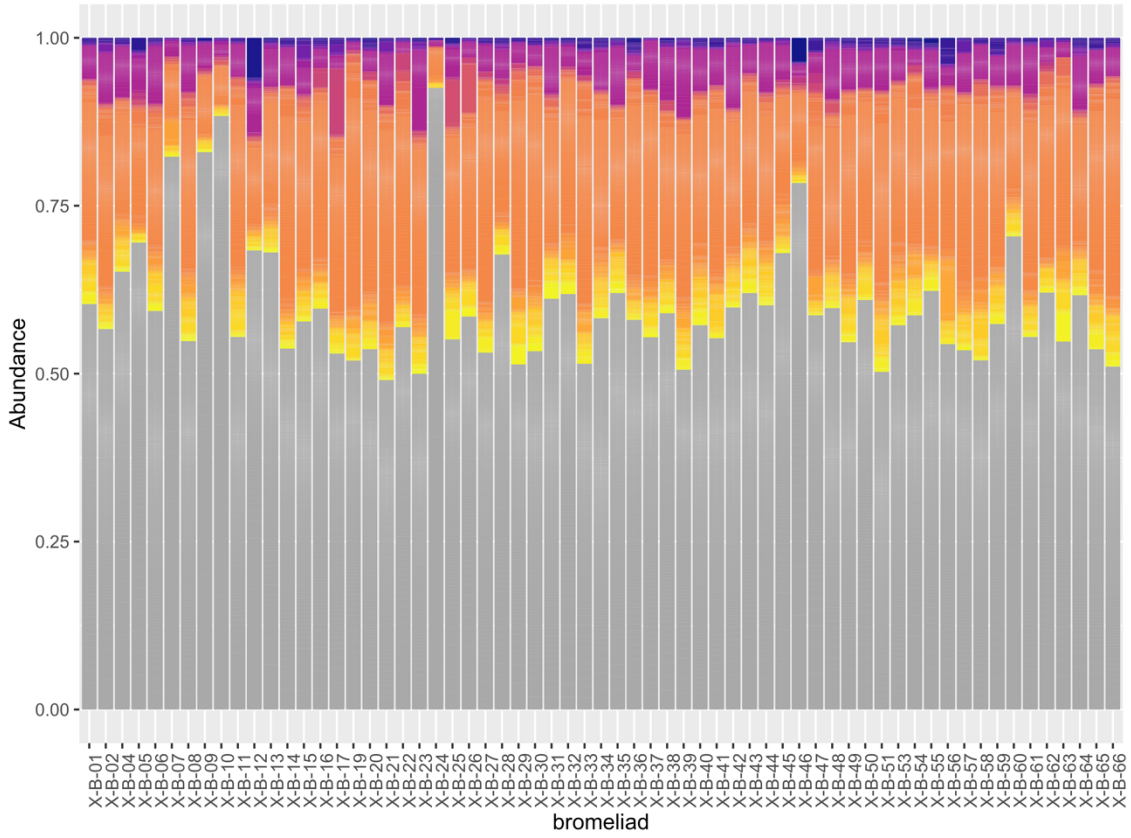


Figure 3.5. Relative abundance stacked bar chart at the ASV level. Each bar represents one bromeliad sample, composed of the pool of the technical replicates.

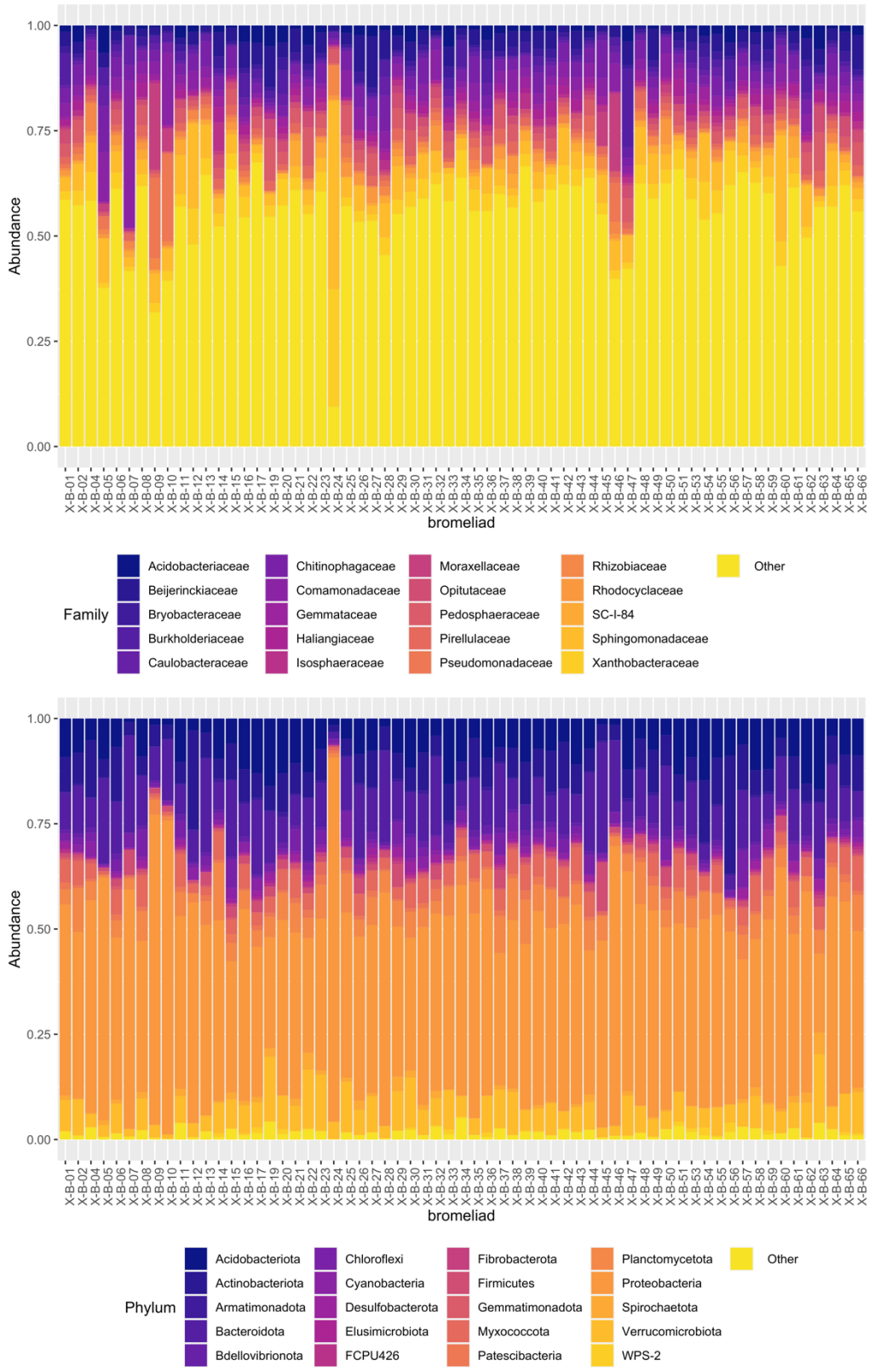


Figure 3.6. Relative abundance stacked bar chart at the Family (top) and Phylum (bottom) levels. Each bar represents one bromeliad sample, composed of the pool of the technical replicates.

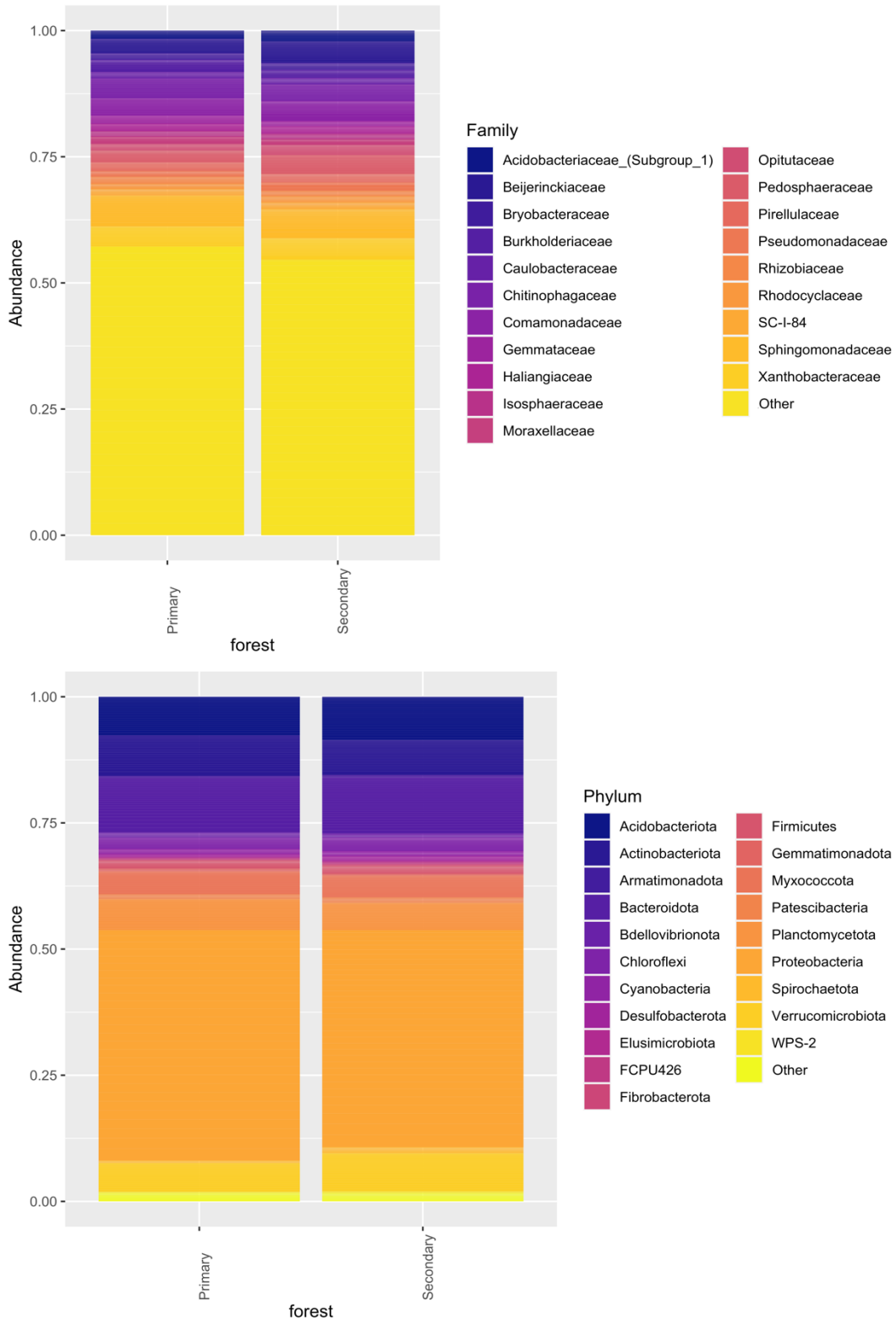


Figure 3.7. Relative abundance stacked bar chart at the Family (top) and Phylum (bottom) levels. Each bar represents primary or secondary forest, composed of the pools of bromeliads from primary and secondary forest.

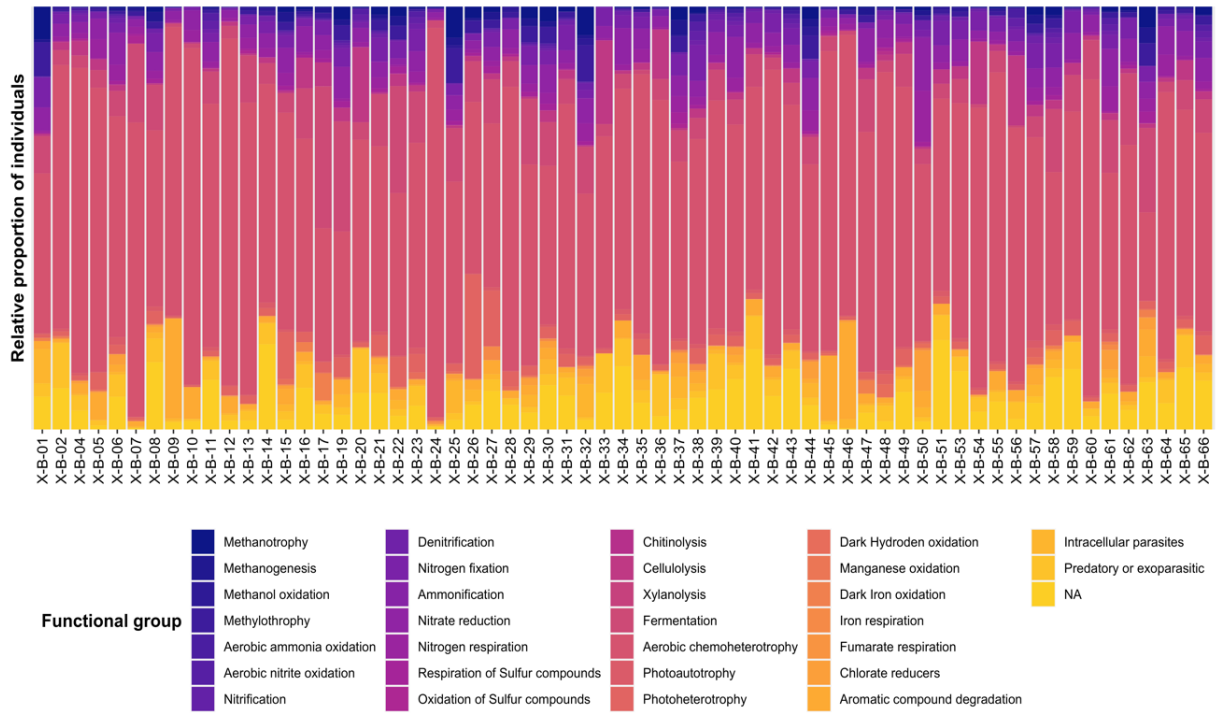


Figure 3.8. Barplot of functional group abundance in individual bromeliads.

3.4.4 Functional diversity

Using FAPROTAX, 1319 ASVs were successfully sorted into functional groups, leaving 3585 unclassified. Phytotelm pH had the highest number of associations with function, being significantly positively correlated with denitrification, nitrogen respiration, oxidation of Sulfur compounds, chitinolysis, xylanolysis, fermentation, aerobic chemoheterotrophy, and dark Hydrogen oxidation, but was negatively correlated with cellulolysis. Height on the tree was negatively correlated with denitrification, nitrogen fixation, and nitrogen respiration. Phytotelm temperature was correlated with aromatic compound degradation. Measures of plant size and complexity (volume, longest leaf, number of leaves, and base circumference) were variably negatively correlated with methanogenesis, aerobic ammonia oxidation, nitrification, cellulolysis, photoheterotrophy, Iron respiration, Chlorate reduction, and predatory or exoparasitic groups. Volume was additionally positive correlated with aerobic chemotrophy and aromatic compound degradation, while the number of leaves was positively correlated with xylanolysis (Figure 3.9).

It is not possible to obtain p-values for the significance of individual group differences between categorical variables, however some functional groups were more strongly associated with certain a forest type, species, or sampling season. For instance, fermentation was more strongly associated with secondary forest than primary forest (Figure 3.10).

Several group differences were found between sampling season, including nitrogen fixation, cellulolysis, aerobic chemoheterotrophy, photoheterotrophy, aromatic compound degradation, and predatory or exoparasitic groups (Figure 3.10). Between species of bromeliad, differences between the two mostly manifested themselves as stronger affiliations or dissociations between *A. nidularioides* and certain functions, such as methanotrophy, methanogenesis, methylotrophy, nitrogen fixation, cellulolysis, fermentation, aerobic chemoheterotrophy, aromatic compound degradation, hydrocarbon degradation, and predatory or exoparasitic groups (Figure 3.10).

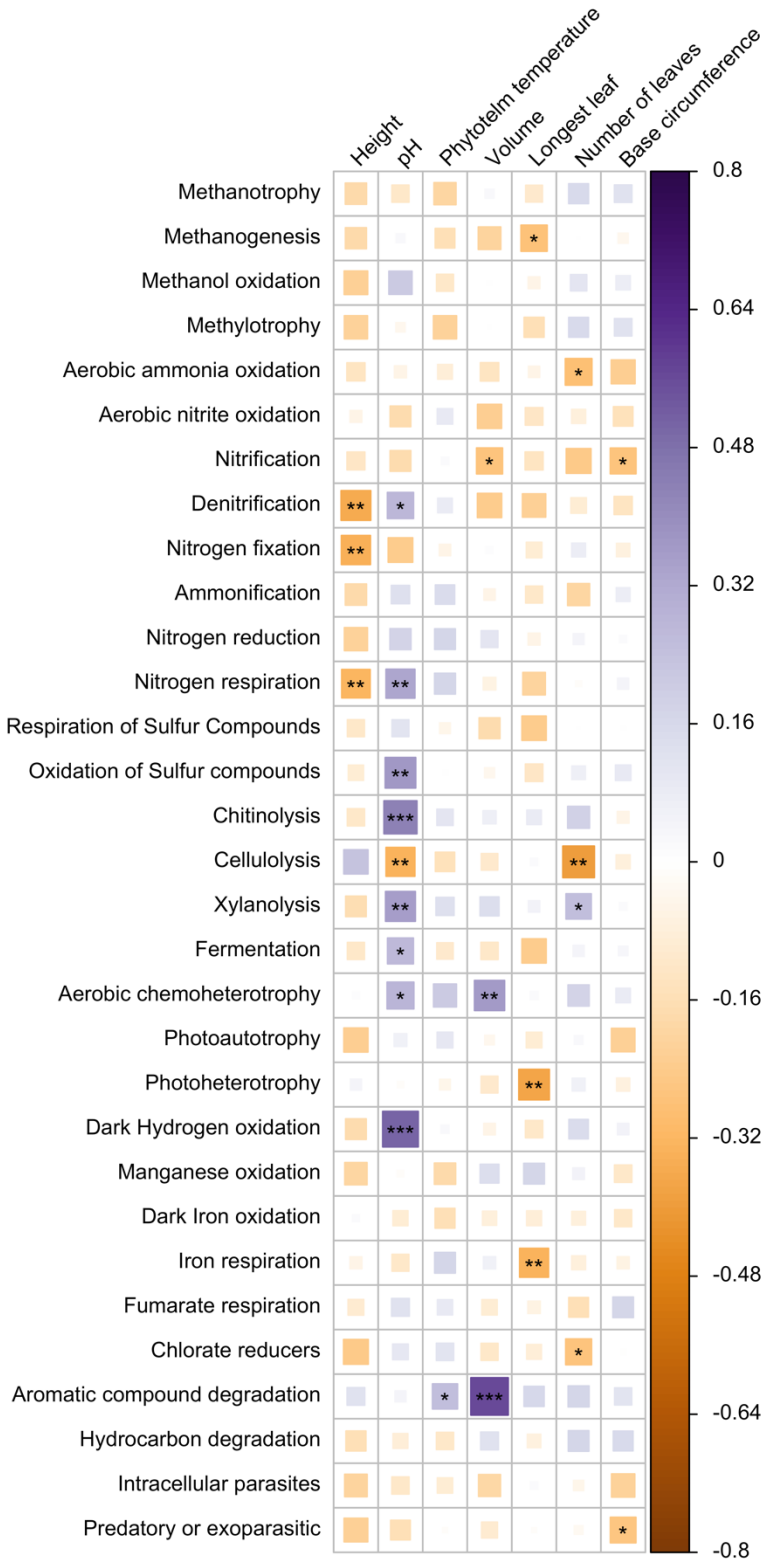


*Figure 3.9. Correlation plot of functional groups of microbial taxa in bromeliads according to different continuous variables. Legend shows strength and direction of correlation. Asterisks show degree of significance: * 0.05, ** 0.01, *** 0.001.*

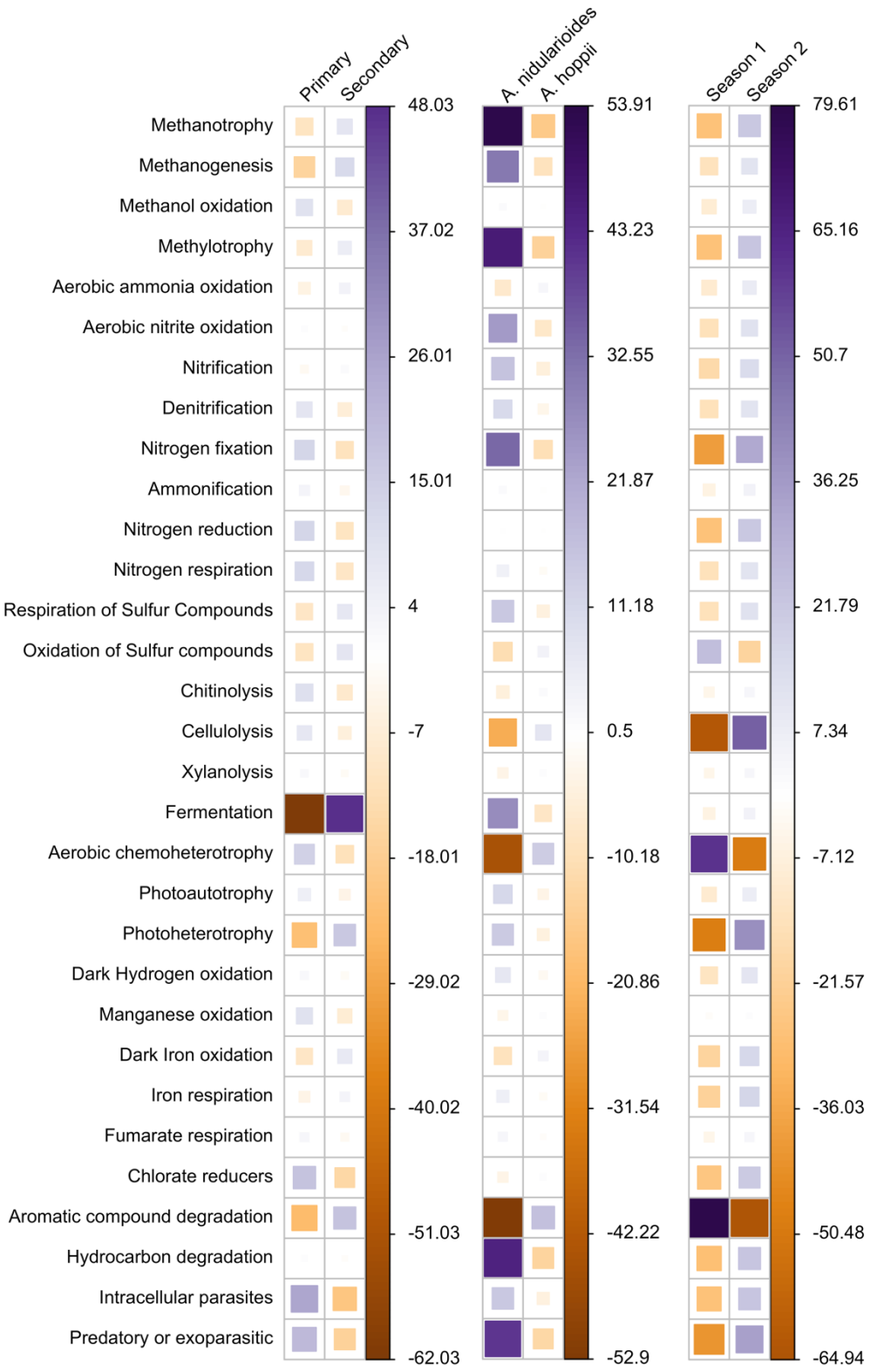


Figure 3.10. Correlation plot of functional groups of microbial taxa in bromeliads according to different categorical variables. Legend shows strength and direction of residuals of Chi-squared test between each variable.

3.5 Discussion

Bromeliads host diverse and complex prokaryotic communities which carry out a range of functions within the phytotelm microcosm (Inselsbacher *et al.*, 2007; Lehours, Perrie and Sabatier, 2016; Leroy *et al.*, 2016)**.** These communities are taxonomically distinct but functionally similar to each other amongst bromeliads (Brandt, Martinson and Conrad, 2017; Louca *et al.*, 2017a; Simão *et al.*, 2020). Although to our knowledge this is the largest bromeliad community metabarcoding study to date in terms of sample size (Louca *et al.*, 2017a; Louca *et al.*, 2017; Rodriguez-Nuñez, Rullan-Cardec and Rios-Velazquez, 2018; Giongo *et al.*, 2019; Simão *et al.*, 2020; Herrera-García *et al.*, 2022), like most of the other six such studies it focuses on bromeliads within a tropical forest and adds to our knowledge of these systems (Louca *et al.*, 2017a; Louca *et al.*, 2017; Rodriguez-Nuñez, Rullan-Cardec and Rios-Velazquez, 2018; Giongo *et al.*, 2019; Simão *et al.*, 2020).

3.5.1 Alpha diversity

We found 4904 ASVs belonging to 56 Phyla across the 63 bromeliads in this study. This represents a greater number of phyla overall than seen in the next-most diverse study of bromeliad phytotelm communities which found 51 Phyla in 16 bromeliads (Rodriguez-Nuñez et al. 2018). This may be reflective of our greater sampling size, as the average number of Phyla per bromeliad is lower than in the aforementioned study (Rodriguez-Nuñez et al. 2018). The high number of taxa in general in this study is likely due to the high level of general biodiversity where the study was carried out, on the Eastern edge of the Tropical Andes Biodiversity Hotspot, one of the biologically richest places on Earth. It also mirrors the large diversity of macroinvertebrates found in the same bromeliads (Chapter II of this thesis).

The degree of richness in taxa suggests our sampling technique was successful in capturing microbial diversity, which differs to that of most bromeliad metabarcoding studies because we used swabs, although there are exceptions (Greenspan et al. 2019). Given our results, it appears that swabbing the phytotelm is an effective means of sampling its biodiversity. Although in this case only the central phytotelm was swabbed for consistency, this could be expanded to other leaf axils as the resulting samples take up less space than traditional water samples, are easier to obtain than filtering water, and require no storage buffer (which can bias community preservation, Tatangelo et al. 2014), but they do still require deep freezing.

Observed Richness of ASVs did not appear to be influenced by any of the measured variables, whereas diversity summarised as Shannon and Inverse Simpson indexes was influenced to different extents by height on the tree, volume of the bromeliad, the length of the longest leaf, and season. For both indexes, height was negatively correlated with alpha diversity, though this correlation was not strong. A previous study found no effect of height on bacterial density in bromeliads (Brouard et al. 2012), but they only looked at bromeliads up to 2 m off the ground. Other studies of canopy microbiomes have found decreasing levels of alpha diversity as well as differences in community composition in the phyllosphere depending on position in the canopy, as seen within the bromeliad phytotelmata here (Izuno et al. 2016; Stone and Jackson 2019; Herrmann et al. 2021). Although bromeliads form canopy oases with different conditions to their surrounding environment, it appears that some patterns in microbial diversity follow general patterns of canopy phyllosphere microbial communities. In the case of other phyllosphere communities, richness may decrease with increasing height due to the more extreme conditions (higher exposure to UV, heat, wind, rain) of the canopy (Stone and Jackson 2019). However, none of the bromeliads sampled here were exposed to the extreme conditions of the upper canopy, so the lower prokaryotic diversity may in part be related to less rich surrounding communities. We did not record microclimatic differences at the heights sampled and so we cannot rule out that these could also affect prokaryotic phytotelm communities.

Similarly, although perhaps surprisingly, the Inverse Simpson index was slightly negatively correlated with volume. The opposite tends to be true for bromeliad invertebrate communities (Richardson 1999; Jocque and Field 2014b; Srivastava et al. 2020) and other microbial communities (Bell et al. 2005; Delgado-Baquerizo et al. 2018). The opposite was true for another proxy of bromeliad size, the length of the longest leaf, which was slightly but significantly correlated with the Shannon index. This could be related to some unmeasured variable affecting both the length of leaves and the prokaryotic community, such as irradiation or plant age. Alternatively, due to their roles in facilitating plant nutrition (Inselsbacher *et al.*, 2007; Leroy *et al.*, 2016), characteristics of the microbial community could influence the length of leaves, but this is impossible to untangle with our data.

Somewhat surprisingly, pH did not affect alpha diversity as it has been found to in previous studies (Goffredi et al. 2011b). However, as discussed below, pH did affect community composition.

3.5.2 Beta diversity

Previous work has found no or weak habitat associations with bacterial densities in bromeliads (Brouard et al. 2012; Farjalla et al. 2012; Leroy et al. 2017; Céréghino et al. 2020). These studies used non-molecular methods of quantifying bacteria. Epilithic bromeliads from vegetated or non-vegetated sites contained some families exclusive to each habitat type, however these were generally families which occurred in low abundances in each habitat (Herrera-García et al. 2022). Nonetheless, although there is consistently huge taxonomic variation between bromeliads (Louca *et al.,* 2017a; Herrera-García *et al.*, 2022), previous research has suggested that taxonomic composition is not completely random or due to neutral processes (Louca *et al.*, 2017a; Pascual-García and Bell, 2020). In this study, different combinations of factors influenced different taxonomic levels of community composition. Whether qualitative (Unweighted UniFrac) or quantitative (Weighted UniFrac) measures were used to calculate distances between communities also affected the results, which is unsurprising as Unweighted UniFrac is more sensitive to differences in taxa found at low abundances, than the abundance-reliant Weighted UniFrac distance (Lozupone et al. 2007). At the ASV level, the same factors had a significant effect on the prokaryotic community regardless of which distance metrics were used, suggesting abundances did not affect the influence of forest type, height on the tree, or pH. At the Family and Phylum levels, results differ, although there are common themes. While, for instance, the composition of Families and Phyla vary according to height and phytotelm temperature, these differences are obscured by the differential abundances across taxa. Instead, quantitative distances reveal differences in communities according to forest type (Family level), pH and bromeliad volume (Phylum and Family levels). Across all measured taxa, however, forest type, height on the tree, and pH affected beta diversity of the prokaryotic communities, albeit explaining very small proportion of variation amongst these communities.

Invertebrate assemblages in bromeliads have also been shown to differ between primary and secondary forest (Srivastava et al. 2005) or with proximity to human settlements (Docile et al. 2017) but in the former case this appeared to be driven by differences in the size and abundance of bromeliads in primary and secondary forest, which was not observed between primary and secondary here. None of the measured plant variables differed significantly between primary and secondary forest (data not shown), and so whatever is driving these differences remains unclear. Even canopy cover over the bromeliads did not differ between primary and secondary forest, although irradiation has not been shown to affect bacterial densities in other studies (Brouard et al. 2012; Leroy et al. 2017). In the case of Family

diversity, forest type only explained some of the variation when using Weighted UniFrac distances, suggesting that both forest types share phylogenetically similar taxa present in different abundances (Lozupone et al. 2007).

Similarly, height on the tree has been shown to affect invertebrate communities in bromeliads in some cases (Brouard et al. 2012; Kratina et al. 2017). Although to our knowledge there hasn't been an association drawn between bacterial densities and height of the bromeliad on the tree (Brouard et al. 2012), we detected an effect of height on communities at all three of the analysed taxonomic levels when using Unweighted UniFrac distances, suggesting previous non-molecular studies may have failed to detect differences in community composition. Certainly, studies of other phyllosphere systems in the canopy have shown that position in the canopy can be a stronger determinant of bacterial community composition than other facts such as rain disturbance or (sometimes) tree species identity (Izuno et al. 2016; Laforest-Lapointe et al. 2016; Stone and Jackson 2019; Herrmann et al. 2021).

Phytotelm temperature and pH have previously been shown to affect prokaryotic communities in bromeliads, as they did here (Haubrich et al. 2009; Goffredi et al. 2011b). The pH of the environment affects bacterial community composition in a range of different habitats, from soil (Zhalnina et al. 2015; Yun et al. 2016) to gut communities (O'May et al. 2005; Duncan et al. 2009), with different taxa tolerating different levels of acidity. The effect of temperature on the community seems less clear, but it has been suggested that higher temperatures may limit bacterial growth in bromeliads as they do on a daily basis in larger bodies of water (Farjalla et al. 2005; Apple et al. 2006). However, temperature was not correlated with alpha diversity but affected qualitative community composition (Unweighted UniFrac distances), suggesting temperature does not affect all taxa equally, similar to other aquatic systems (Sjöstedt et al. 2012).

Although it is not easy to find examples of the effect of habitat size on community composition (rather than richness) on prokaryotic communities, Madsen (2020) reported an effect of maximum rock pool size on the community composition of bacteria. In this case, similarly to here with our bromeliads, volume only explained a small proportion of the variation (2%) (Madsen 2020). In the case of the bromeliads, an effect of volume on microbial community composition was seen using Weighted UniFrac distances but not Unweighted, which may be related to the higher colonisation surface allowing for different abundances of taxa to inhabit the tank.

3.5.3 Functional diversity

There was also a large diversity of functional groups. Although functional group classification should be viewed with a caution due to the coarse manner in which FAPROTAX (Louca et al. 2016) translates taxonomic data into functional data (see section 3.3 Materials and methods), the number of groups into which ASVs were classified reflects the diverse roles and functions that microbes carry out in the bromeliad phytotelm, which include methanogenesis, nitrogen cycling, and degradation of plant and animal tissues such as xylem and chitin. Previous work on the functional diversity of bromeliad bacteria has shown that different functions correlate with various biogeochemical conditions within phytotelmata (Louca *et al.*, 2017). Here we correlated more external variables with functional groups and found some links between the abundance of ASVs performing certain functions and habitat and plant characteristics. As in previous work elsewhere, pH was correlated with the abundances of some functional groups, although some of these correlations differed to previous research (Louca *et al.*, 2017). For instance, we observed a significant positive relationship between chitinolysis and pH, which Louca et al. (2017) did not find in terrestrial bromeliad phytotelmata; however, research in other systems has found pH to be a predictor of microbial chitinase activity (Ramirez-Coutiño et al. 2010; Kielak et al. 2013). Among the most surprising relationships, was a negative correlation between nitrogen activity and height of the plant on the tree, which could perhaps be caused if higher bromeliads were dryer habitats, which we did not measure (Bell et al. 2008). There weren't many differences in function between primary and secondary forest, other than in the rate of fermentation, which was higher in secondary forest. There were also seasonal and species differences between some functional groups, although these may be due to differing chemical conditions within the bromeliads, which we did not measure (Louca *et al.*, 2017) Additionally, sample sizes of the different sampling season and bromeliad species were very different (13 versus 50 and 7 versus 56, respectively), therefore we would caution against drawing definitive conclusions from these particular comparisons.

Without metagenomics data or more measurements of the conditions inside the bromeliads, it is hard to draw strong conclusions from this data. However, it provides a number of hypotheses to further test and serves as a basis upon which future research can explore these epiphytic microbial systems. For instance, the effects of bromeliad size and complexity on cellulolysis and aromatic compound degradation could be due to differences in the collection of material. One would expect bromeliads of larger volume to accumulate more leaf litter, while those with more leaves may not accumulate as much leaf litter as fewer-leafed, more open bromeliads. This hypothesis would have to be tested by quantifying leaf

litter accumulation at varying volumes and in bromeliads with different numbers of leaves. However, although Louca et al. (2017) also found a positive correlation between bromeliad volume and aromatic compound degradation, they found a negative correlation with detrital content. The decrease in various nitrogen-related processes with height could be due to a number of reasons, some physical or some geochemical. Nitrogen fixation, respiration, and denitrification negatively correlated with height, but previous research hasn't linked these processes significantly to the geochemical conditions within the tank (Louca *et al.*, 2017), As mentioned before, it could be that greater dryness as one ascends the canopy or generally harsher conditions could contribute to less activity by these functional groups (Burke et al. 1997; Murakami et al. 2022). The assumption of greater dryness and harsher conditions would have to be tested in this case, especially as none of these bromeliads were collected from the upper parts of the canopy where conditions are known to be more extreme. Nonetheless, relative humidity has been shown to be negatively correlated with canopy height even below the top layer of the outer canopy (Murakami et al. 2022).

3.5.4 Conclusion

We present a broad and preliminary picture of the prokaryotic diversity inside epiphytic bromeliads in one of the most biodiverse regions on Earth. Various plant and habitat characteristics were correlated with alpha, beta, and functional diversity, and so we consider it is important to include such measurements in future work on the microbial communities in phytotelmata.

3.6 Acknowledgements

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Chapter IV

No effect of plant genotype on phytotelm community composition in an epiphytic bromeliad (*Aechmea hoppii*)

4.1 Abstract

Community genetics studies the interactions among the genotypes of organisms and with the environment. A large number of studies have demonstrated patterns of preferences of organisms for particular genotypes or combinations of genotypes, and elucidated correlations between host genotypes and the composition of their associated communities. However, most studies of community genetics have been carried out in temperate terrestrial systems. Here, we investigated whether epiphytic bromeliad genotype correlated with community composition within its phytotelma, in Western Amazonia. Tank bromeliads are neotropical plants which host diverse communities of microbes and invertebrates. All macroinvertebrates were collected from the bromeliads and classified into morphospecies, and the prokaryotic community was sequenced from eDNA from the phytotelm. We designed novel microsatellite markers in order to estimate genetic distance between *Aechmea hoppii* bromeliads. We found no effect of genotype on the invertebrate or prokaryotic communities. However, aquatic invertebrate community distances correlated with prokaryotic community distances, suggesting that these two community components influence each other's composition. Our results are contrary to many studies of host genotype effects on associated communities, and may suggest that in megadiverse tropical habitats such as Amazon rainforest, genotype effects may be drowned out by the sheer diversity of organisms in these systems.

4.2 Introduction

The natural environment is made up of the interactions between the phenotypes of different organisms and the abiotic conditions surrounding them. It therefore follows, that every habitat is the result of the interactions among the expressed genotypes of individuals and those expressed genotypes with the abiotic environment (Rowntree et al. 2011b).

Under the moniker of community genetics, patterns to this effect have been detected in a variety of systems, particularly between plants and their associated communities. For example, weevils show a preference for particular apple cultivars (Mody et al. 2015) and in barley fields with mixed genotypes, arthropod predators have been found to be attracted to particular mixtures of barley varieties, due to the resulting combination of volatile compounds they emit (Ninkovic et al. 2011, 2019). Broader correlations have also been found in more complex systems, with a particular amount of work having been carried out in North American cottonwood systems, in which the composition of arthropod communities and lichen cover varies among cottonwood genotypes and hybrids (Wimp et al. 2005; Bangert et al. 2006; Lamit et al. 2018). Similar patterns have been demonstrated in the epiphyte communities on aspen trees and fungal communities on ash trees (Davies et al. 2014; Griffiths et al. 2020a). As well as community composition, plant host genotype has been shown to mediate plant responses to fungal endophytes as well as the population growth rates of aphid communities (Rowntree et al. 2011a; Hughes et al. 2020). Additionally, several reviews listing many more examples of such relationships are available (Neuhauser et al. 2003; Whitham et al. 2003; Hersch-Green et al. 2011a; Rowntree et al. 2011b).

Despite the wealth of examples of relationships between plant genotype and community composition, most of these are restricted to temperate terrestrial environments. Exceptions to this include a few studies in temperate aquatic systems such as streams and seagrass meadows (Hughes and Stachowicz 2004; LeRoy et al. 2006b; Wainwright et al. 2018), as well as tropical studies in mahogany in Mexico and epiphytes in Belize (Campos-Navarrete et al. 2015; Zytynska et al. 2011; Zytynska et al. 2012). These studies have shown correlations between host genetics with some components of the associated communities, but not with others. For instance, epiphyte assemblages on breadnut in Belize are associated with host tree genotype (Zytynska et al. 2011), while mycorrhizal communities were not determined by seagrass genotype in the Indian Ocean (Wainwright et al. 2018). In mahogany, the effect of genetic diversity within a plot influenced different factions of the invertebrate community differently, with predator but not herbivore species richness

positively correlated with host genetic diversity (Campos-Navarrete et al. 2015b). In a rainforest study in China, adaptive genetic diversity (but not neutral genetic diversity) in different tree species had different effects on species diversity in the community (Li et al. 2022). Therefore, it is unclear how widespread these community genetics effects are, and they at least appear to depend on the system or components of a system studied.

In cases where environmental variables have been measured alongside genotype effects, environmental variables, geographic separation, and plant trait plasticity have stronger effects on communities than genotype (Tack et al. 2010; Barbour et al. 2019a; Gosney et al. 2021). Therefore, it might be expected that in systems with greater environmental variation caused by higher levels of biodiversity, community genetics effects may be weaker. However, this is difficult to study in complex megadiverse systems such as many of those in the tropics.

In order to investigate this in such an environment, here we study correlations between plant genetic distance and community distance in a diverse but relatively simplified system: epiphytic bromeliads in a tropical forest. Zytynska et al. (2012) demonstrated a correlation between aquatic invertebrate community composition and bromeliad genetic distance in the bromeliad *Aechmea bracteata* in Belize, showing that more closely-related individual *Aechmea* housed slightly but significantly more similar communities of invertebrates. This was similar to the degree of variation explained by geographic distance between the bromeliads (1%).

An important component of bromeliad phytotelm communities is their microbial community (Leroy et al. 2016b). Bacteria and Archaea community structure is influenced by the conditions within the phytotelm, while at the same time these organisms contribute to conditions within this microhabitat (Leroy et al. 2016; Louca et al. 2017; Carrias et al. 2020). It is not known whether bromeliad prokaryotic communities correlate with genotype as the arthropod communities have been shown to, but in both bromeliads and other tropical plants there is evidence of species-specific communities of surface bacteria (Lambais et al. 2006; Louca et al. 2017a).

Here, we examined the microbial and macroinvertebrate communities of epiphytic bromeliads in the Ecuadorian Amazon, in relation to the genetic distance between plants. We explored whether prokaryotic and invertebrate communities within the bromeliad phytotelm correlated with the genetic distance between plants. We employed eDNA sampling, metabarcoding, whole invertebrate identification, and developed bespoke

microsatellite markers to study the communities of the epiphytic bromeliad *Aechmea hoppii* in an area on the edge of the Tropical Andes Biodiversity Hotspot. The focus of this work was to study correlations between bromeliad genotypes and their associated communities, while the environmental variables influencing the invertebrate and microbial communities have been described previously in Chapters II and III of this thesis.

4.3 Materials and methods

4.3.1 Field sampling

This study was carried out in San José de Payamino, Orellana, Ecuador, from here on referred to as Payamino. The field site is described in the *Materials and methods* section of Chapter II of this thesis. Sampling took place in January-February and July-August 2018.

Bromeliads *A. hoppii* and *A. nidularioides* were spotted from the ground and accessed either with a ladder or using double-rope canopy access techniques. Collection heights ranged from 1.96 m to 20.20 m. GPS coordinates were taken on the ground below the selected bromeliad and the stage of the forest (primary [n=28] or secondary [n=35]) was recorded.

Prior to any other disturbance to the plant, a sterile swab was inserted into the central phytotelm of the bromeliad and rubbed for 10-20 seconds and inserted into a sterile 2.0 cryovial. Following this, a handheld pH meter was inserted into the central phytotelm to record pH and temperature at time of sampling. The bromeliad was then sawed off the host tree at the base, inserted into a large heavy-duty refuse bag, and lowered to the ground with a rope.

Collected bromeliads were taken back to the Timburi Cocha Research Station for processing. At the research station, the contents of the bag containing the bromeliad was emptied into a large glass aquarium to ensure no invertebrates escaped. Plants measurements were then taken for Chapters II and III of this thesis.
Bromeliads were dissected leaf-by-leaf to extract any macroinvertebrates over approximately 1-2 mm in size. The water, soil, and leaf litter remaining in the collection bag was also emptied into the aquarium and searched for invertebrates. All specimens were preserved whole in 96º ethanol until identification. All invertebrate specimens were transferred to the entomology department of the National Biodiversity Institute (Instituto Nacional de Biodiversidad del Ecuador, INABIO) in Quito, Ecuador, for processing and identification.

Whole invertebrate samples were identified to morphospecies and identified to at least order, using both general (Gavin 2000; Kočík et al. 2002; Triplehorn and Johnson 2005) and taxon-specific keys (Hebard 1924; Kury 2002; Silvestre et al. 2003; Merritt et al. 2008; Domínguez and Fernández 2009; Andersen 2010; Prat et al. 2010; Silva and Brandão 2010; Vidlicka 2014; Brito and Borges 2015; Grismado et al. 2015).

4.3.2 Prokaryote community metabarcoding

Details of DNA extraction, library preparation, sequencing, and raw data processing are described in Chapter III of this thesis, along with an analysis of the environmental and plant variables which affect the prokaryotic communities within these bromeliads.

4.3.3 Plant DNA extraction

Approximately 20 mg of plant tissue was macerated in 2.0 ml tubes with 4-8 metal ball bearings in a Retch 440 mixermill for 40 seconds at 30,0 x g. Samples were centrifuged briefly and 750 ml of pre-warmed OPS Diagnostics CTAB Extraction Buffer was added to the tubes, before being returned to the mixermill for a further 40 seconds maceration. Samples were then incubated at 65ºC for 60 minutes. From then, the extraction protocol by Inglis et al. (2018) was followed with the following modifications: at step 11 of the DNA extraction, DNA was precipitated using 1/10 volume of 0.2 M sodium chloride and 1.5x volume of ethanol absolute, and samples were then left at -20ºC overnight. The next day, samples were centrifuged for 40 (rather than 10) minutes. Thereafter, we proceeded as Inglis et al. (2018). DNA was quantified by Nanodrop™ 2000 (Thermo Fisher).

4.3.4 Microsatellite development and plant genotyping

Bromeliads were genotyped using microsatellite markers developed specifically for *A. hoppii* and *A. nidularioides*, following Fox et al. (2019) and Griffiths et al. (2016). In order to separate the two species, microsatellite markers were developed which cross-amplified in *A. hoppii* and *A. nidularioides*. Marker development followed the Multi-individual Microsatellite identification (MiMi) method described in Fox et al. (2019). DNA of eight individuals of flowering bromeliads (four *A. hoppii* and four *A. nidularioides*) was extracted and library preparation was performed using the Nextera® XT Library Prep Kit (Illumina) following the manufacturer's instructions.

Initial microsatellite identification for each bromeliad was carried within the Galaxy Centaurus server of the University of Manchester's Core Bioinformatics Facility, following Griffiths *et al.* (2016). Trimmomatic (Bolger et al. 2014) within Pal_filter (Griffiths et al. 2016) was used for quality control and trimming reads, with the following settings: sliding window size = 4bp, quality = 20, leading = 3, trailing = 3, minlen = 50. Reads were paired using PANDAseq (Masella et al. 2012) and potentially amplifiable microsatellite loci were identified using Pal_finder (Castoe et al. 2012). Primers for potentially amplifiable loci were designed in Pal_finder using Primer3 (Untergasser et al. 2012), with the following parameters: primer_opt_size = 21, primer_min_size = 20, primer_max_size=30, primer_min_GC = 40.0, primer_max_GC = 60.0, primer_min_TM = 59.0, primer_max_TM = 68.0, primer_opt_TM = 60.0. These parameters were set so that downstream, primers could be amplified using the Type-It® Microsatellite Kit (Qiagen).

Identified primer regions for each of the eight individuals were then compared using MiMi (Fox et al. 2019). MiMi was run separately for each bromeliad species, using the same terms. MiMi was set to find primers of polymorphic loci found in at least 50% of individuals.

The MiMi pipeline identified 91 and 65 potential microsatellite markers fitting the specified parameters in *A. hoppii* and *A. nidularioides,* respectively, and filtered 12 and 13, respectively, as being of high quality. All 25 of these markers amplified in both species of bromeliad. PCR conditions for testing markers were as follows: 5 minutes at 95ºC, 35 cycles of 30 seconds at 95ºC, 90 seconds at 60ºC, 30 seconds at 72ºC, followed by 30 minutes at 60ºC. A universal "tail" sequence was added to the 5' end of the forward primer of successful primer pairs, in order to cost-effectively label the primers with fluorophores according to Culley *et al.* (2013) and Blacket *et al.* (2012). The labelled forward primer was then included in the reaction as a third primer (Culley et al. 2013).The fluorophores added to the tail primers were either 6 FAM, HEX, or ROX, added to the tails "Tail C" (CAGGACCAGGCTACCGTG) (Blacket et al. 2012), "Tail B" (GCCTTGCCAGCCCGC) (Blacket et al. 2012)**,** or "M13modB" (CACTGCTTAGAGCGATGC) (Culley et al. 2013), respectively. Markers were tested with fluorophores and primers which appeared to amplify fewer than 5/8 of samples (absence of bands on an agarose gel) were excluded from further testing.

Twelve markers were selected for Fragment Length Analysis, three of which were thereafter excluded due to producing several alleles per locus in one case and little variation in the

others; a further marker was excluded due to linkage disequilibrium. Eight markers were used in the final genotyping.

Table 4.1. Microsatellite primer sequences, allele repeat motifs and size ranges, with tail-dye combinations. BC = "Tail C" (Blacket et al., 2012), BB = "Tail B" (Blacket et al., 2012), M13B = "M13modB" (Culley et al., 2013).

Primer	Forward sequence	Reverse sequence	Motif	Size range	Tail -dye
AH1	TCAATACCGTCACCA ATAGCC	TTCCCCTCTATTAGC CATTCC	AG	311-365	BC - 6FAM
AH5	CAAATTGTGAATGCG AGAAGG	CTCAATCGGACATT CCAAAGG	AC	350-365	BC - 6FAM
AH9	GAAAGAAATTGACAA CCGTGC	CTTTGTTGTCAAAG GTGATTGC	AT	349-453	BB - HEX
AN5	TCTTTCTCACTTTTCTC CCCG	TAGATGGCACTGAT GATGACG	TC	220-337	M13B - ROX
AN7	GCGATTCCAACTATTT TGACG	CAGTTTCCACTCTG CGAGC	TC	399-420	M13B - ROX
AN10	TATCGGACTCCTCTCT CCTGC	AAGAAGCGCACACT AAAACCC	TC	175-184	M13B - ROX
AN12	TCCTCCACTATGCCTC TACCG	GGCATAGAAGAAA AGTAGCATAAAGGG	TCG	147-191	M13B - ROX
AN13	CCTCAACGATTGCCTT TGC	CTCGGCGTTACCAT TAGATCG	TTC	212-237	M13B - ROX

Amplification of eight microsatellite regions was performed in singleplexes for each of the eight markers in 5 µl reactions, using the Type-It® Microsatellite Kit (Qiagen) under the following conditions: 5 minutes at 95ºC, 35 cycles of 30 seconds at 95ºC, 90 seconds at 60ºC, 30 seconds at 72ºC, followed by 30 minutes at 60ºC. Following amplification, 2 µl of water was added to the PCR amplicons. Plates were sent to the DNA Sequencing & Services Facility of the University of Dundee (Dundee, UK), where 0.5 µl of amplicon was prepared with 0.5 µl LIZ500 size standard (Thermo-Fisher Scientific) and 9 µl HiDi Formamide (Thermo Fisher Scientific). Amplicons were sized using a 3730 DNA analyser (Applied Biosystems). Alleles were then scored using *Fragman* (Covarrubias-Pazaran et al. 2016) and bins were established and checked using *MsatAllele* v1.05 (Alberto 2009) in R v4.1.1 (R Core Team 2020).

4.3.5 Plant genetic analysis

All genetic analyses were performed in R v4.1.1 (R Core Team 2020). Markers were checked for linkage disequilibrium using the *GENEPOP* package v4.6 (Raymond and Rousset 1995; Rousset 2008). Due to linkage disequilibrium between markers AH2 and AN5, AH2 was removed from further analysis. The remaining eight microsatellites were used to calculate observed and expected heterozygosity, allelic richness, and check for Heidy-Weinberg disequilibrium using *GENEPOP* v4.6.

Clusters of genetically similar samples were identified using the *find.clusters* function of the *adegenet* package v2.1.5 (Jombart 2008). This function uses a Bayesian Information Criterion (BIC) to compare different clustering solutions for an algorithm which analyses the variance between potential clustering groups. The number of two clusters was selected to use for further analysis, based on the BIC not lowering substantially beyond this value (Jombart et al. 2010). Discriminant Analysis of Principal Components (DAPC) was used to assess the relationship between the two clusters (Jombart et al. 2010). Rogers' genetic distances were calculated between bromeliads using eight of the microsatellite markers in *adegenet* (Rogers 1972; Jombart 2008). Non-metric multidimensional scaling (NMDS) was constructed using the *vegan* v2.5.7 package (Oksanen et al. 2020), based on the Rogers' genetics distances. Two clear clusters were visible in both the DAPC and NMDS plots (Figure 4.1). One cluster included all the known *A. nidularioides* individuals based on inflorescence presence. The seven individuals in this cluster were removed in order to focus solely on *A. hoppii* for further analysis. Another 14 samples were removed due to amplification in fewer than 7/8 of the microsatellite loci. This left 42 individuals of *A. hoppii* for further analysis.

4.3.6 Statistical analysis

All statistical analyses were performed in R v4.1.1. Invertebrate community distances matrices using Bray-Curtis dissimilarities were calculated using the *vegdist* function in *vegan*. This was performed separately for the whole invertebrate community and the terrestrial and aquatic subsections. Unweighted UniFrac and Weighted UniFrac distances were calculated for the prokaryotic communities using *phyloseq* (McMurdie and Holmes 2013)**,** both take into account phylogeny but Weighted UniFrac uses abundance data while unweighted UniFrac only uses presence-absence data.

A geographic distance matrix was constructed using UTM coordinates for the bromeliad collection locations with the *dist* function in base R. Partial Mantel tests of community, genetic, and geographic distances were then performed to assess whether bromeliad genetic distance correlated with invertebrate community similarity. The partial Mantel tests with geographic and bromeliad genetic distances were performed for the whole, terrestrial, and aquatic invertebrate communities, and for the prokaryotic communities.

To test for correlations between invertebrate and microbial communities with bromeliad genetic distance, partial Mantel tests were performed on invertebrate community distances, microbial community distances, and bromeliad genetic distances. This was carried out for the whole, terrestrial, and aquatic invertebrate communities, using Roger's genetic distance for bromeliads, Bray-Curtis distances for invertebrates, and UniFrac distances for microbes.

To test for association between just the invertebrate and prokaryotic communities, Mantel tests of invertebrate community distances and microbial community distances were performed using the whole, terrestrial, and aquatic invertebrate community datasets. Additionally, distances for invertebrate and prokaryotic communities were calculated at the Order level and analysed in the same manner.

Pearson correlations between Observed, Shannon, and Inverse Simpson indexes of prokaryotic and invertebrate (whole, aquatic, terrestrial) diversity were calculated using the *cor* function.

4.4 Results

4.4.1 Plant genetic analysis

Two microsatellite markers, AH2 and AN5, were in linkage disequilibrium, so AH2 was excluded from further analyses. The following results are for the eight remaining markers. Two clear clusters differentiated NMDS of Rogers genetic distances (Figure 4.1). Further analysis only include those belonging to the largest cluster, identified based on the inflorescences collected from individuals of this cluster to be *A. hoppii.*

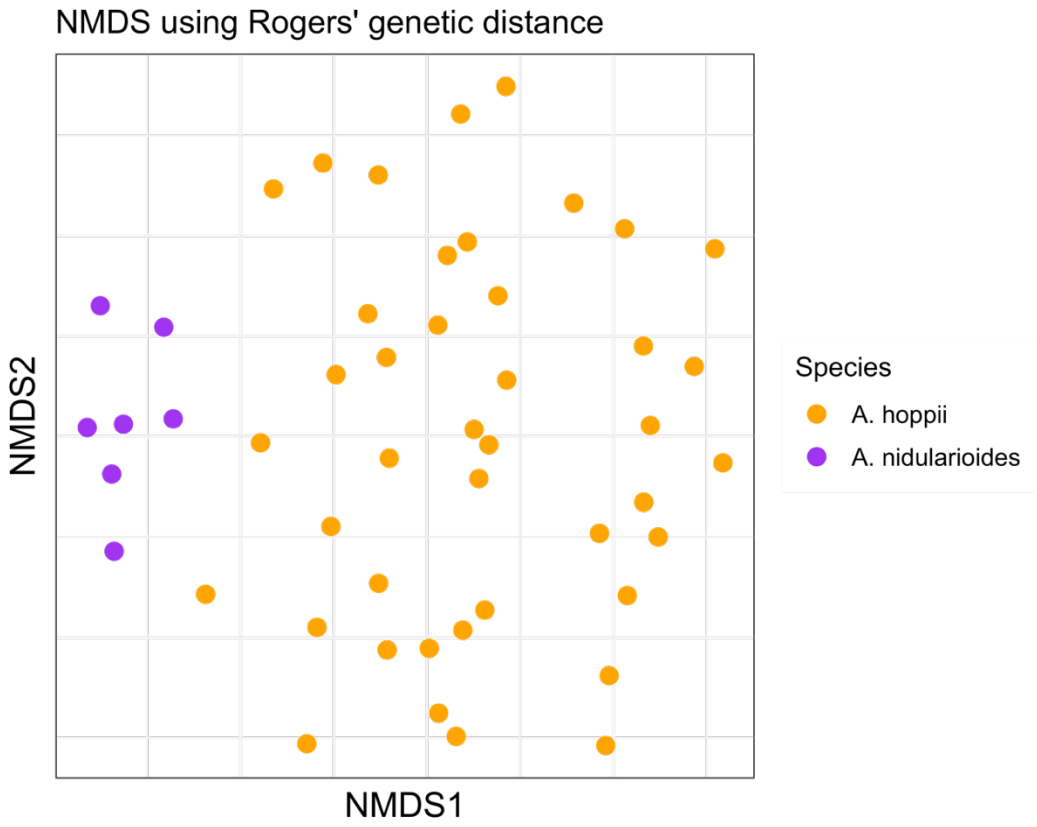

Figure 4.1. NMDS Using Rogers' genetic distance. Two clear clusters were regarded to represent the two species as inflorescences of each species pertained to one cluster or the other.

The number of alleles and observed heterozygosity per locus are presented in Table 4.2. The overall observed heterozygosity was much lower (0.479) than the expected heterozygosity (0.732). Only one marker was in Hardy-Weinberg equilibrium when P-values were adjusted for multiple tests (AN10, P = 0.554). Overall allelic richness was 10.423 ± 2.670.

Table 4.2. Statistics for individual microsatellite markers used.

Marker	Alleles per locus	Observed heterozygosity	Expected heterozygosity
AH1	14	0.68	0.89
AH5	4	0.57	0.60
AH9	7	0.34	0.59
AN5	26	0.44	0.93
AN7	5	0.29	0.64
AN10	4	0.56	0.61
AN12	15	0.67	0.84
AN13	9	0.29	0.76

4.4.2 Statistical analysis

Geographic distance did not correlate with any aspect of the bromeliads or their associated communities. That is, there was no relationship between geographic distance and genetic distance among bromeliads, nor did geographic distance have any relationship with distances between the whole, aquatic, terrestrial, or prokaryotic communities ($P > 0.05$). The only exception was a marginally significant correlation ($R = 0.1017$, $P = 0.049$) between invertebrate distances at the order level and geographic distance.

Bromeliad genetic distance did not correlate with community distances. This was true for the whole, aquatic, and terrestrial invertebrate communities, as well as for the prokaryotic communities ($P > 0.05$).

For the most part, elements of the invertebrate and prokaryotic communities were not correlated. However, there was a significant relationship between prokaryotic abundances at the ASV level (Weighted UniFrac distances) and the terrestrial component of the invertebrate community (Table 4.3). Weighted UniFrac distances at the ASV level were also correlated with aquatic community distances at the order level ($R = 0.1958$, $P = 0.027$), but not morphospecies level (Table 4.3). There was a significant relationship between Unweighted UniFrac distances at the ASV level of the prokaryotic community with both morphospecies and order distances in the aquatic invertebrate community (invertebrate order level, $R = 0.263$, $P = 0.001$; for results using distances based on invertebrate morphospecies see Table 4.3).

When distances were calculated at the level of prokaryote orders, there was a correlation between the overall community of invertebrates. However, this appears to be driven by correlations between presence-absences of prokaryotes with the aquatic invertebrate community, as there was no relationship between prokaryotic orders with terrestrial morphospecies and the effect on the overall community disappeared when using Weighted rather than Unweighted UniFrac distances (Table 4.3).

There were no strong correlations between invertebrate and prokaryote alpha diversity indexes, all being between -0.24 and 0.34.

*Table 4.3. Results of the Mantel tests between prokaryotic and invertebrate community distances. Invertebrate community distances are calculated with morphospecies, using either abundance or presence-absence (PA) data. Significance indicated by *.*

Invertebrate abundance / PA	Invertebrate community	Prokaryote taxonomic level	UniFrac distance	R	P-value
Invertebrate abundance data	**Whole**	**ASV**	Weighted	0.093	0.116
			Unweighted	0.077	0.076
		Order	Weighted	0.063	0.153
			Unweighted	0.128	0.021*
	Aquatic	**ASV**	Weighted	0.093	0.100
			Unweighted	0.178	0.002*
		Order	Weighted	0.093	0.057*
			Unweighted	0.249	0.001*
	Terrestrial	**ASV**	Weighted	0.151	0.012*
			Unweighted	0.038	0.204
		Order	Weighted	-0.028	0.706
			Unweighted	0.042	0.209
Invertebrate presence-absence data	**Whole**	**ASV**	Weighted	0.045	0.296
			Unweighted	0.063	0.149
		Order	Weighted	0.023	0.371
			Unweighted	0.120	0.014*
	Aquatic	**ASV**	Weighted	0.000	0.497
			Unweighted	0.217	0.001*
		Order	Weighted	0.215	0.002*
			Unweighted	0.310	0.001*
	Terrestrial	**ASV**	Weighted	0.153	0.049*
			Unweighted	0.014	0.395
		Order	Weighted	-0.134	0.969
			Unweighted	0.019	0.389

4.5 Discussion

Observed heterozygosity was lower than expected heterozygosity in our sample of 42 *A. hoppii* bromeliads, based on eight polymorphic loci. This implies relatively low genetic variability in our sample. This is fairly common amongst bromeliads, although the discrepancy between observed and expected heterozygosity in our sample is greater than in most other studies (Zanella et al. 2012); however, the sample size is also low.

Bromeliad genotype did not have an appreciable effect on the community composition of associated organisms, invertebrate or prokaryotic. This is surprising, as most other studies examining such patterns have repeatedly demonstrated that genotype-community associations do exist (Bailey et al. 2005; Whitham et al. 2006; Rowntree et al. 2014b; Gosney et al. 2017), including other studies in the tropics (Zytynska et al., 2011; Campos-Navarrete et al., 2015; Li et al., 2022) and another study in a different bromeliad species in Belize (Zytynska et al. 2012b). The absence of an association in our study could suggest that the effect of plant genotype on their associated communities is not as strong in megadiverse tropical systems as it is in temperate ones. After all, the effect found in other tropical systems, although significant, was small (1-2% of variation explained) and depended on the level of the community examined (Zytynska et al. 2011a, 2012b; Campos-Navarrete et al. 2015b). The present study found more macroinvertebrates in fewer bromeliads. It may be that in areas of extremely high diversity, the effects of host genetic diversity become less relevant in structuring the community compared to other biological or environmental factors. This seems especially plausible given the results of Chapter VI of this thesis, in which bromeliad genotype in a constrained environment did correlate with differences in the bacterial community structure and richness.

However, it is important to bear in mind methodological differences in these studies. For instance, Zytynska et al. (2012b) used amplified fragment length polymorphisms (AFLPs) and we used microsatellite markers. AFLP panels amplify many more polymorphisms than our reduced panel of eight microsatellites. Similarly, Bailey *et al.* (2005) studied genotypes identified by 35 species-specific alleles using restriction fragment length polymorphism (RFLPs). It is possible that our genetic markers did not reveal sufficient genetic diversity to elucidate any correlation between community and genetic distance. Certainly, the use of a different type of genetic markers, could explain the contrasting results of this study compared to previous work (Bailey et al. 2005; Zytynska et al. 2012b). However, although the observed heterozygosity was relatively low, the allelic richness was high compared to some bromeliad studies (Barbará et al. 2009; Goetze et al. 2015, 2018; Sheu et al. 2017;

Soares et al. 2018), suggesting it is unlikely that was the issue. Nonetheless, it is worth considering the difficulty of comparing results between community genetics studies with different methodologies, including but not limited to molecular techniques.

The lack of correlation between bromeliad genotype and phytotelm communities may be seen in a positive light – it implies high genetic diversity among bromeliads is not necessary to promote high biological diversity in its associated communities, due to a disassociation between genotype and community structure. Of course, this does not negate the importance of genetic diversity more generally, given its importance for adaptive potential, but it does have consequences for restoration efforts. For instance, when restoring bromeliads in an effort to promote invertebrate diversity, clones of few genotypes would be equally as effective in promoting complex phytotelm communities as individual genotypes (which would take much longer to source and grow from seed individually than to harvest clones from a few mother plants).

In terms of correlations between subsets of the phytotelm communities, prokaryotic and whole invertebrate community composition at the ASV level were not correlated either, although prokaryotic community composition at the order level using abundance data did correlate with invertebrate composition at the species level. This pattern seemed to be driven by the aquatic invertebrate community, which correlated with prokaryotic community composition at both the ASV and order levels. The terrestrial invertebrate community only correlated in terms of abundance of invertebrate species with prokaryotic ASV composition.

The general correlation between prokaryotic communities with aquatic invertebrate communities rather than terrestrial communities seems to reflect a closer association between microorganisms and aquatic invertebrates. There could be several reasons for this. To begin with, the prokaryote samples were taken from the leaf surface near the bottom of the central phytotelm, which is where most aquatic organisms in bromeliads dwell due to the central phytotelm holding the greatest proportion of water (*pers. obs.*). Additionally, microorganisms modify the environmental conditions (e.g. pH, nutrient availability, etc.) within which they live, therefore affecting the habitat shared by higher organisms. This will likely have a stronger effect on the largely resident aquatic communities in direct contact with the phytotelm water, than on the visiting or transient terrestrial invertebrates with greater dispersal capabilities (Jocque and Field 2014b). Unfortunately, we did not measure geochemical conditions of the phytotelm water, apart from phytotelm pH and temperature, and so cannot speculate what conditions may mediate such a correlation between prokaryotic and aquatic invertebrate diversity. However, we do know from Chapters II and

III of this thesis that both types of organisms are affected by some of the same habitat variables, such as forest type and height from the ground.

Our data contained only macroinvertebrates, thus the correlation between prokaryote and aquatic invertebrate communities was unlikely due to direct trophic associations. However, an important intermediate portion of the population was not investigated here. This includes algae and zooplankton, studying which by molecular methods would have required several additional sets of markers (Santoferrara 2019), and by manual methods would have required more *in-situ* processing which was not possible in the conditions in which this study took place (Leakey et al. 1994; Carrias et al. 2001). Both algae and zooplankton carry out important roles in the bromeliad microcosm (Carrias et al. 2001; Brouard et al. 2011)**,** and so we recognise that the exclusion of these groups is not ideal. While algae contribute to the primary production within the bromeliad phytotelm, many planktonic animals and protozoa feed on bacteria or aid in the breakdown of organic matter by feeding on the matter left behind by larger shredders (Carrias et al. 2001; Farjalla et al. 2016; Durán-Ramirez et al. 2019)**.** We expect these communities of eukaryotic microorganisms may also be significantly correlated with that of prokaryotic microorganisms and the rest of the aquatic community, which would be interesting to investigate in future.

Previous research has suggested that bacterial communities in bromeliads are more influenced by stochastic processes while invertebrates show niche-based patterns of structuring, namely habitat-filtering (Farjalla et al. 2012). It is likely true to some extent that the dispersal-limited prokaryotic community is more susceptible to stochastic processes, which would partly explain the huge variation in taxonomic composition between individual bromeliads (Louca *et al.,* 2017; Simão *et al.*, 2020). However, at least within some functional groups, there appear to be non-neutral processes involved in structuring microbial communities in bromeliads, implying the phytotelm environment plays some role in structuring these communities as well (Louca *et al.*, 2017a). This is further supported by the data in Chapter III of this thesis (where various habitat and plant features affected prokaryotic community composition) and the correlations between prokaryote and aquatic invertebrate community composition seen here.

However, prokaryote and invertebrate alpha diversity (measured by observed species richness, Shannon, or Inverse Simpson indexes) were not correlated, not even between the prokaryotic and aquatic invertebrate communities. This could be a reflection of different drivers of alpha diversity in these communities: while bromeliad volume was the main drivers of invertebrate species richness (Chapter II of this thesis), height from the ground

and volume were both negatively correlated with prokaryote diversity (Chapter III of this thesis).

4.5.1 Conclusions

Bromeliad genotype did not affect the composition of associated communities in this study, contrary to many studies of plant-associated communities in other habitats (Wimp et al. 2005; Rowntree et al. 2011b; Davies et al. 2014; Hughes et al. 2020). We speculate that this may be due to the higher level of biodiversity in our study habitat. This study was conducted in one of the most biological rich regions on Earth (Myers et al. 2000)**.** Therefore, it could be that any effect of plant genotype on community structuring may be outweighed by the extremely high level of diversity in this system. Although previous research has shown that the effect of habitat-filtration in bromeliads increases with organism size (Farjalla et al. 2012)**,** we found a significant relationship between prokaryote community composition and aquatic invertebrate composition, but not with terrestrial community composition. This could be due to the first two components of the community sharing largely stationary lifestyles within the aquatic phytotelm habitat, while many terrestrial organisms in bromeliads are generally more mobile and transient. We encourage community genetics research in other tropical systems, particularly in regions where there have been none, to elucidate patterns of community structure further.

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Chapter V

Plant genotype influences growth rate and leaf herbivory in an Amazonian disturbance plant (*Heliconia stricta*)

5.1 Abstract

Plant phenotype is influenced by both the individual's genotype and its interaction with the environment and other organisms. Although this has important implications in crop systems and on pest control, in natural megadiverse diverse tropical habitats there has been relatively little exploration of the importance of plant genotype on plant growth and its biotic interactions. We set-up three adjacent plots in rainforest at the foothills of the Andes (Orellana, Ecuador) where we planted clones of 35 individual *Heliconia stricta* rhizomes collected from up to 4 km away. The 35 rhizomes were split into at least three individual pieces each, resulting in three genetically identical replicates. At least one representative of each genotype was planted in each plot. Shoot height was measured every few of months. Nine months after planting, all leaves of the plants were photographed for leaf herbivory analysis. When the herbivory analysis took place, neither shoot height nor total leaf area varied significantly between genotypes; however, the total percentage of leaf area consumed per plant was significantly different between genotypes. Whether or not there was a significant difference in shoot height between genotypes depended on the timepoint at which the plants were measured. However, the overall growth rate over a period of 1.5 years was significantly different between genotypes. These results suggest that even in megadiverse systems and despite the plasticity of plant responses, plant genotype can exert a strong role on growth rate and biotic interactions such as herbivory.

5.2 Introduction

Plants are the main primary producers in terrestrial habitats and as such interact with most organisms in ecosystems, either directly or indirectly. Insect herbivory on plants has spurred adaptations in both plants and their invertebrate diners, from wide-ranging chemical responses and unsavoury exudates in the former, to specialised proteases and tough mandibles in the latter (Burkepile and Parker 2017). Bottom-up and top-down processes have been shown to mediate different aspects of these interactions. For instance, herbivory can have top-down consequences for the outcomes of interactions between plants and the rest of their herbivore community (Bailey and Whitham 2003, 2006; Ode et al. 2016), while plant productivity has bottom-up consequences for ecological interactions (Moore et al. 2003; Báez et al. 2006).

Plant responses to herbivory include direct and indirect chemical and physical characteristics. Physical defences can include leaf waxiness, trichomes, latex, and thorns, which directly deter herbivores (Tian et al. 2012); while chemical defences include toxins, as well as volatile compounds which either deter herbivores or attract their predators (Zhu-Salzman et al. 2008; Ninkovic and Åhman 2009; Gantner and Najda 2013)**.** Although many of these defences are plastic and can be induced in response to an attack on the plant, some are also genetically-determined and vary among genotypes of the same species (Schweitzer et al. 2004; Bailey et al. 2006a; Ninkovic et al. 2011). For instance, *Arabidopsis thaliana* mutants with different levels of cuticle leaf waxiness host different bacterial communities on the leaf surface (Reisberg et al. 2013); both constitutive and facultative trichome density in Arabidopsis are genetically-determined (Bloomer et al. 2014); herbivore-induced plant volatiles vary between genotypes of single species, such as barley (Ninkovic et al. 2011); and active changes in the gene expression of hormone signalling pathways of plants control the variation in plant volatile emissions (Maffei et al. 2007; Mathur et al. 2013). These genetically-determined physical and chemical characteristics suggest that plant genetic diversity plays a role in the attraction and deterrence of arthropods.

Plant genetic diversity has also been found to influence the structure of associated communities and ecosystem processes (Fritz 1995; LeRoy et al. 2006a; Keith et al. 2010; Rowntree et al. 2011b). In experimental cottonwood stands with known crosses of hybrids, intraspecific genetic diversity amongst trees determined the structure of the associated arthropod community, and in wild stands, almost 60% of arthropod variation was explained by plant genetic diversity (Wimp et al. 2004b). A common garden experiment using coastal willow found that invertebrate community composition was indirectly associated with plant

genotype via traits related to resistance to herbivory (Barbour et al. 2016). Aspen clone has been found to influence the composition of their epiphyte communities (Davies et al. 2014) and ash genotype influences fungal community composition on leaves (Griffiths et al. 2020a). These studies provide evidence of plant genotype influencing the composition of their associated communities of arthropods. Plant genetic diversity may therefore have implications beyond the plants' own adaptability in the face of change and underline biotic interactions.

Although herbivores have the most direct interaction with plant hosts and the effect of plant genotype seems to be greater on them than other members of the arthropod community, the effect of plant genotype on arthropod community extends beyond a direct effect on their herbivores (Johnson and Agrawal 2005). Because of differences in aphid abundances, the communities of ant mutualists also differ between cottonwoods and hybrids, which in turn alters the composition and abundance of the rest of the arthropod community on the trees, including a negative effect on other invertebrate herbivores (Wimp and Whitham 2001). Different cottonwood genotypes have been shown to have specific responses to herbivory, and the interaction between genotype and herbivory has consequences for decomposition and thus nutrient fluxes (Schweitzer et al. 2005; LeRoy et al. 2007). The combination of plant volatiles emitted by mixed stands of barley genotypes is more attractive to ladybirds which prey on aphids attacking the barley, than those emitted by stands of a single barley genotype; this not only shows that plant genetic diversity can influence the structure of the associated invertebrate community, but that it has indirect as well as direct consequences on the amount of herbivory plants suffer (Ninkovic et al. 2011). Herbivory itself can have an effect on genotypic composition of plant populations, due to heritable differential resistance to it among genotypes (Agrawal et al. 2012).

Although there are more examples demonstrating a link between plant genotypic diversity and their associated invertebrate communities (Crutsinger et al. 2006; Bailey et al. 2009a; Barbour et al. 2015; Barker et al. 2018; Gosney et al. 2021), few experiments have been conducted in tropical systems *in-situ.* In tropical rainforests, both arthropods and plants are more diverse than at higher latitudes and so the likelihood of encountering the same species twice, is lower. Therefore, it is difficult to extrapolate the results of studies of relatively simpler temperate systems, to megadiverse tropical habitats. However, Zytynska *et al.* (2011) found that even in a megadiverse tropical system, tree genotype affected the composition of epiphyte communities and of bark-dwelling and leaf litter arthropods. In turn, epiphytic bromeliad genetic distance correlated with differences in the community of aquatic arthropods within the plants (Zytynska et al. 2012a).

Here, we were interested in the effect of genetic diversity on the level of herbivory experienced by tropical plants. We used a common garden experiment to test whether the genotype of *Heliconia stricta* explained the level of herbivory suffered by the plant *in-situ* in the rainforest, as well as whether plant growth rate was different between genotypes. Commonly-known as false-birds-of-paradise plants, *Heliconia* are fast-growing plants mostly native to the Neotropics and members of the ginger order, Zingiberales. As well as being horticultural favourites in the Americas and introduced around the world, they are common sights in a range of megadiverse tropical habitats, such as Amazon rainforest in South America (Berry and Kress 1991). Many provide homes to a range of aquatic invertebrate larva in their striking inflorescences, as well as providing shelter for animals like spiders which dwell on the underside of their large leaves. Various invertebrate herbivores consume their seeds, leaves and flowers, including leaf-cutter ants (Formicidae) and many species of rolled-leaf specialists like hispine beetles (Chrysomelidae) (Auerbach and Strong 1981; Seifert 1982b). There is palaeontological evidence of hispine beetles, lepidopteran larvae (Pyralidae and Choreutidae), and weevils (Curculionidae) consuming Zingiberales leaves since the time of the dinosaurs (Wilf et al. 1975; García-Robledo and Staines 2008). However, despite much work having been done on invertebrate-*Heliconia* relationships, whether *Heliconia* genotype has any effect on the outcome of these relationships has remained unstudied.

We cleared three plots in the rainforest and planted clones of 35 *Heliconia stricta* genotypes, measured growth and photographed leaves for herbivory analysis. We show that original genet or genotype has a significant effect on plant growth rate as well as leaf herbivory, with the replicates of some genotypes being consumed significantly more or less than others.

5.3 Materials and methods

5.3.1 Field site and permits

San José de Payamino (hereafter, Payamino) is a 17000 ha patchwork of primary and secondary Amazon rainforest, on the eastern edge of the Tropical Andes Biodiversity Hotspot, within the Sumaco UNESCO Biosphere Reserve, and within the buffer zone of the Sumaco Napo-Galeras National Park core protected area. Further details of this area are described in Chapter II.

This study took place in secondary rainforest near the Timburi Cocha Research (18 M 0245706 9946597). However, plants were collected across Payamino, within a 4 km distance (as the crow flies) of the research station.

5.3.2 Plot location

Three plots were set-up in secondary rainforest directly behind the research station. The understorey of approximately 5 x 50 m transects was cut down in order to make way for planting; however, surrounding vegetation around the plots was left intact and the soil was not cleared. Three parallel plots were cleared in this way, each approximately 40 m from one another and perpendicular to a man-made trail through the forest.

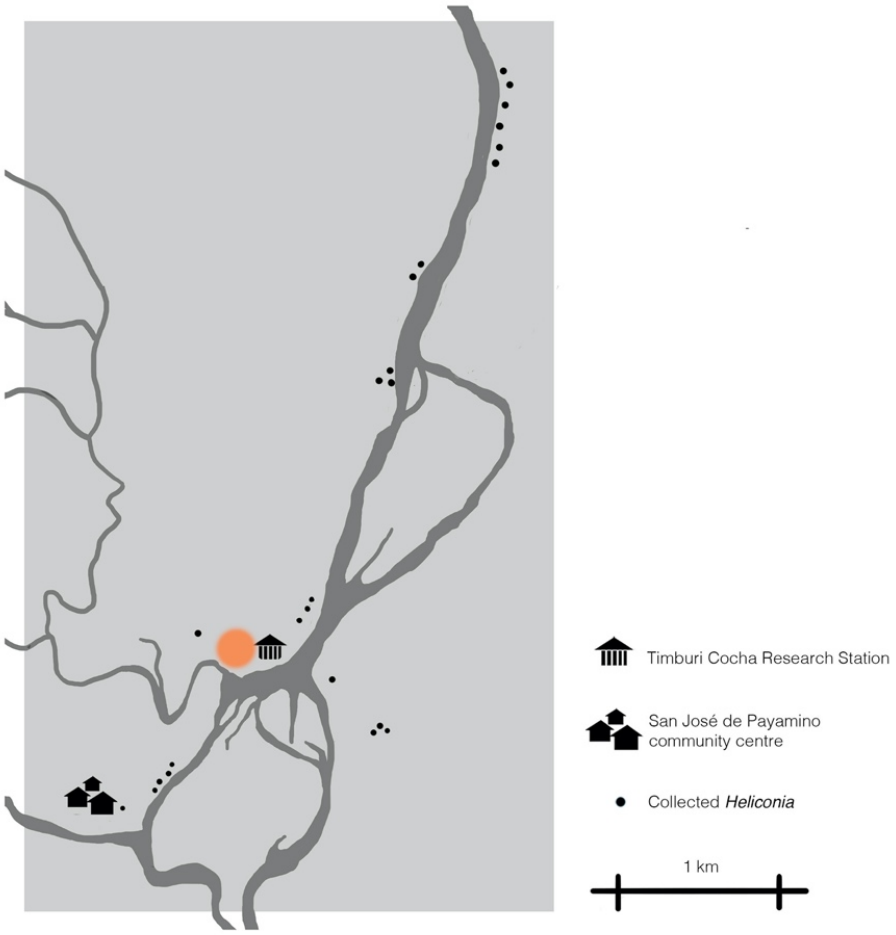

Figure 5.1. Heliconia rhizomes were collected throughout Payamino, mostly near the riverbanks (dark grey) of the Payamino River. The plots were located behind the Timburi Cocha Research Station, in the area of the orange circle.

5.3.3 Plant collection

Thirty-seven rhizomes of *H. stricta* were dug-up and collected around the forest and riverbanks of Payamino (Figure 5.1), where plants were easily detectable and transportable by canoe, in July 2018. Additionally, some of the plants from a pilot study were included if they had a large enough rhizome with at least three plants or shoots already growing from it; these rhizomes were dug up in the same week as when the rest of the collections were made. Rhizomes were collected and labelled before returning to the research station. Upon return to the research station, rhizomes were split into three or more (Figure 5.2), leaving a vertical shoot emerging from each rhizome. The vertical shoots were cut down to approximately 30 cm in height, following the advice of a local farmer (Oscar Aguinda, *pers. comm.*). Sections of rhizome were labelled according to which mother plant they came from and wrapped in newspaper, before being left against the ground under banana leaves for a few days.

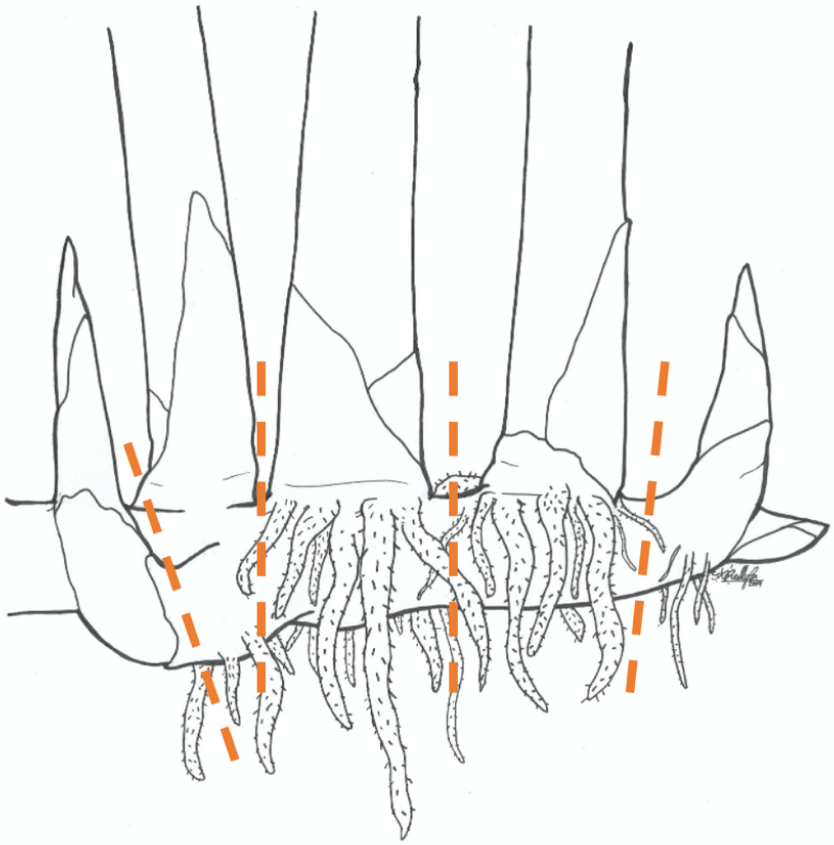

Figure 5.2. Line drawing of Heliconia rhizome. Dashed orange lines represent where rhizome was split in order to create replicates of a single genotype.

5.3.4 Plot design

Rhizome positioning within the plots was determined in excel prior to planting, to ensure each plot had at least one clone from each original plant, as well as a randomised (*RAND()* function) and unbiased planting pattern.

Planting only began once all rhizomes had been collected, sorted, and left for at least five days. This took place in July 2018. After this time, some sections of rhizome no longer looked viable (for instance, if they had rotted or turned brown) and were therefore discarded. This left 35 original plants that yielded 3-6 rhizomes sections each (118 pieces of rhizome in total), but resulted in each plot containing a different number of rhizomes (Plot 1: N = 43; Plot 2: N = 39; Plot 3: N = 36).

Each plot contained two rows of planted rhizomes. Each rhizome was separated from the next by two metres; the rows were staggered so as to ensure equidistance among plants (Figure 5.3).

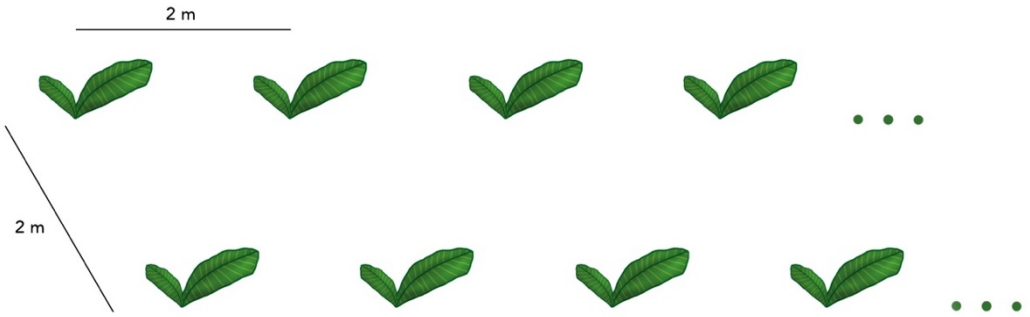

Figure 5.3. Arrangement of plant H. stricta within a plot. Two rows of rhizomes were planted per plot, staggered so as to achieve approximately 2 m between each plant.

5.3.5 Measurements

Survival rates were monitored per plot from the second month (September 2018) after planting, until the study ended in February 2020, with plants counted in September 2018, January 2019, April 2019, June 2019, July 2019, August 2019, September 2019, November 2019, January 2020 and February 2020.

Growth was measured from the 7th month after planting, with measurements taking place in February 2019, April 2019, June 2019, July 2019, August 2019, September 2019, November 2019, January 2020, February 2020. The number of shoots per plant were counted, and the height of the tallest and second tallest shoots were measured from the emergence of the shoot from the soil, until the tip of the tallest leaf, using a tape measure. Height measurements were taken from the point at which the shoot emerged from the ground until the tip of the tallest leaf, illustrated in Figure 5.4.

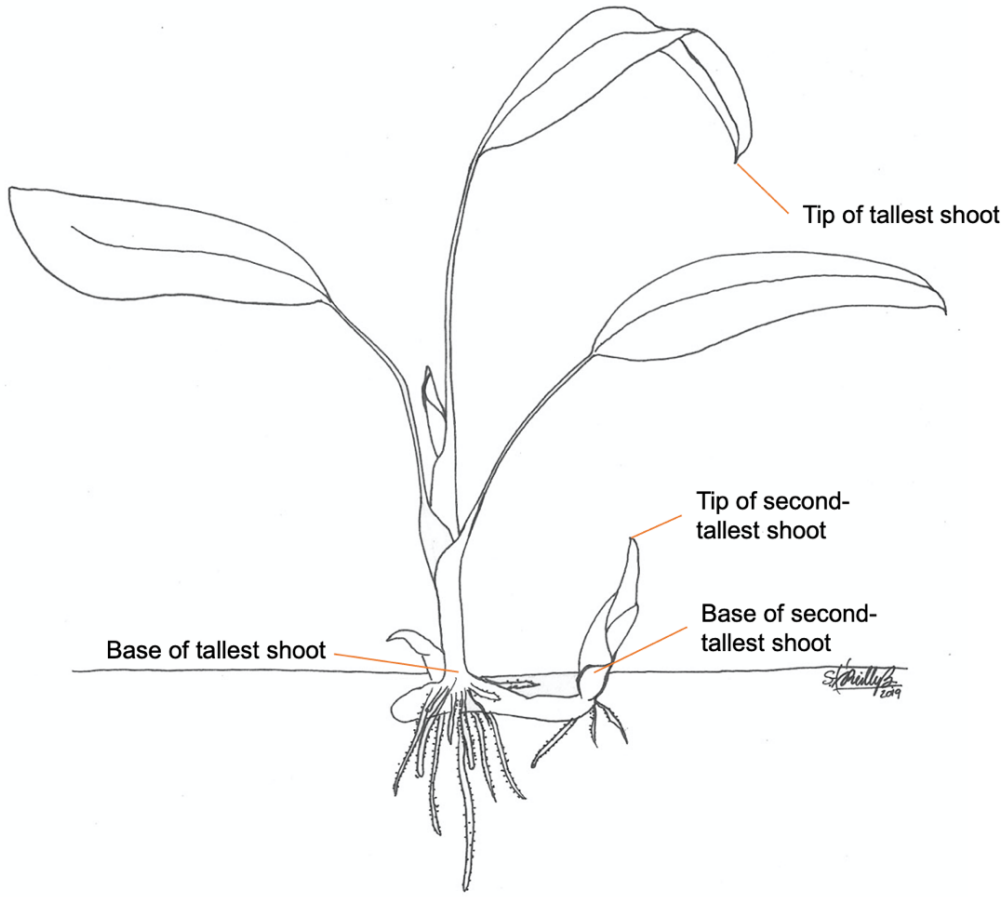

Figure 5.4. Diagram of H. stricta, showing above- and below-ground components. This plant is composed of two shoots connected by a single rhizome.

5.3.6 Leaf photos and analyses

In April 2019, every leaf of every plant was photographed individually against a white background. The leaves were not removed from the plant as the growth measurements were ongoing. After this time point, the leaves of most plants became too large and intractable to photograph.

The photos were edited in Adobe Photoshop (CC 2017) to extract the leaves from the shadows of their background. Drops of water and wet over-exposed parts of leaves were covered so as not to be confused with eaten sections of leaf. Leaf photos were edited individually so as to ensure there was no automated confusion between over-exposed elements and eaten sections, or between shadows and leaf edges.

Leaves were then fitted to a 4-point square scale, either of 30 cm^2, 60 cm^2, or 90 cm^2, depending on the size of the leaf. The consumed and remaining area of the leaf was analysed using the LeafByte (1.3.0) app (Getman-Pickering et al. 2020).

5.3.7 Statistical analysis

All statistical analyses were performed in R (4.1.1) (R Core Team 2020) using RStudio (R Core Team (R Foundation for Statistical Computing) 2021). The total original leaf area was analysed with a linear mixed-effects model using the *lme4* package (Bates et al. 2015), with total original leaf area and clone as fixed effects and plot as a random effect, to test whether leaf area varied between clones taking the potential effect of plot into account. Genotypes which only had one representative replicate remaining were removed from the dataset. Herbivory was measured as the total leaf area consumed (in absolute area, cm^2), and as a proportion of the leaf area consumed relative to the original leaf area of the plant ([leaf area consumed] / [total original leaf area] * 100). Separate linear mixed-effects models were fitted to total leaf area and to the percentage of leaf area consumed, with plot as a random effect. Significance values were assigned to the fixed effects of the models using the *Anova* function in the package *car* (Fox and Weisberg 2019) returning F statistics.

To compare growth between clones, the height of the tallest shoot was fitted to a linear mixed-effects model in *lme4* (Bates et al. 2015), with height and clone as fixed effects and plot as a random effect; a separate analysis was performed for every month the plants were measured in. The same analysis was undertaken for the height of the second tallest shoot of each plant. To model whether there was a difference in growth rate between clones over time, a linear mixed-effects model was used, taking into account the interaction between month and clone, with plot as a random effect. As above, significance values were assigned using the *Anova* function in *car* (Fox and Weisberg 2019) using F statistics. This was done using the tallest shoot and the second-tallest shoot of each plant.

A line plot was constructed using the packages *tidyverse* (Wickham et al. 2019) and *ggplot2* (Wickham 2016), where each line is drawn between the average height of the tallest shoots of genotypes at each time point.

A correlation analysis of height of the tallest shoot in April 2019 (when herbivory was measured) and percentage of consumed leaf area was performed to assess whether there could be a relationship between the herbivory and growth, in base R. The percentage of consumed leaf area was plotted against plant height and fitted with a regression line with *ggplot2*. The correlation analysis was repeated using only data from plants which were less than 20% consumed and plants which were more than 20% consumed.

5.4 Results

5.4.1 Survival rates

At the time the leaf photos were taken for herbivory analysis in April 2019, the proportion of plants alive relative to rhizomes planted were 88.37%, 87.18%, and 91.67% and 107 plants had at least one shoot with leaves. By the end of the study in February 2020, 91 plants remained across the three plots; the survival rate of the three plots relative to the individuals planted were 69.77% (N=30), 84.62% (N=33), and 77.78% (N=28).

5.4.2 Herbivory analysis

Neither the total original leaf area ($F_{31,68} = 0.980$, $P = 0.509$) nor the absolute area consumed by herbivores ($F_{31,68} = 1.171$, $P = 0.287$) varied significantly among clones (Figure 5.5). However the percentage of leaf area consumed by herbivores was significantly different among clones ($F_{31,68}=1.807$, $P = 0.021$) (Figure 5.6).

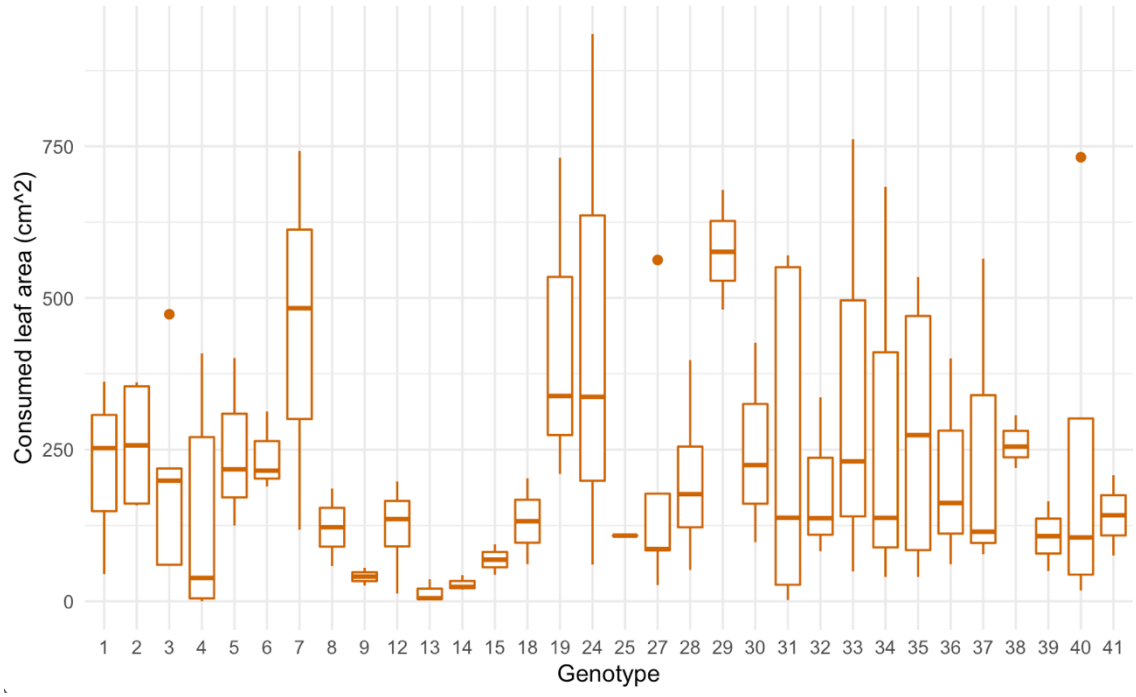

Figure 5.5. Absolute consumed leaf area (cm^2) of the tallest shoot of each genotype. Number of replicates per genotype vary, but genotypes represented by a single replicate were removed.

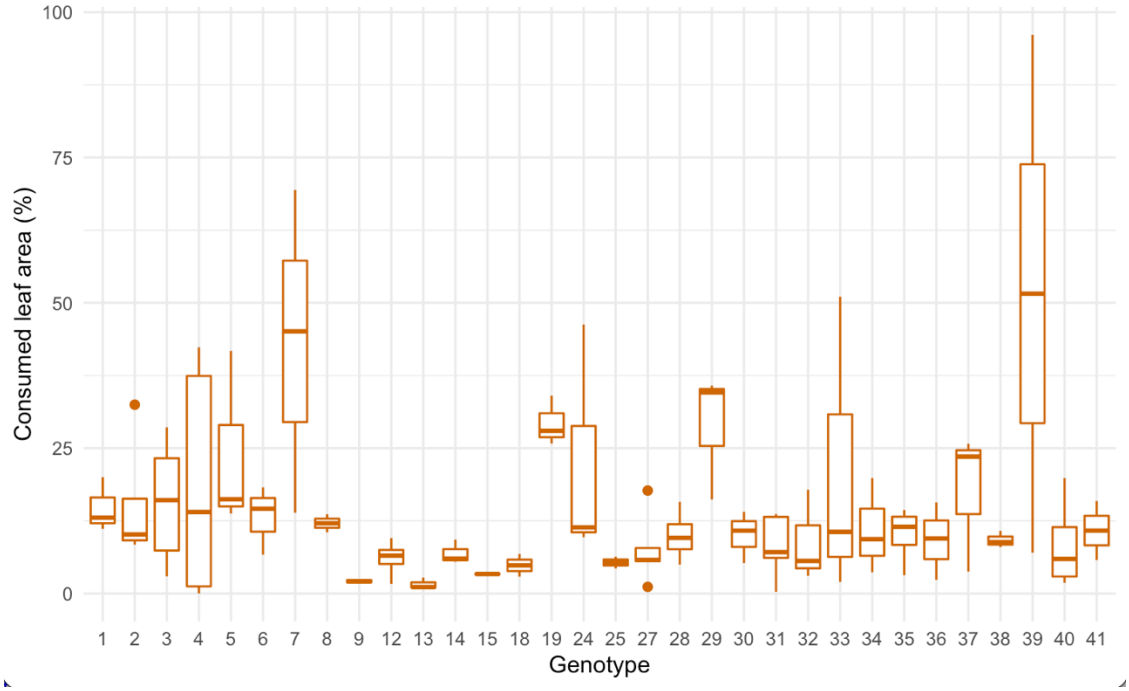

Figure 5.6. Percentage consumed leaf area (%) of the tallest shoot of each genotype. Number of replicates per genotype vary, but genotypes represented by a single replicate were removed.

5.4.3 Growth

Whether the height of the tallest and second-tallest shoots of different genotypes were significantly different from each other or not, varied across the months during which the plants were measured (Figures 5.7-5.8). However, the growth rate over time as measured as

the interaction between month and genotype, was significantly different between genotypes, both for the tallest shoot ($F_{34,840} = 2.31$, $P < 0.001$) (Figure 5.7) and the second-tallest shoot ($F_{34,515} = 1.78$, $P = 0.005$) (Figure 5.8).

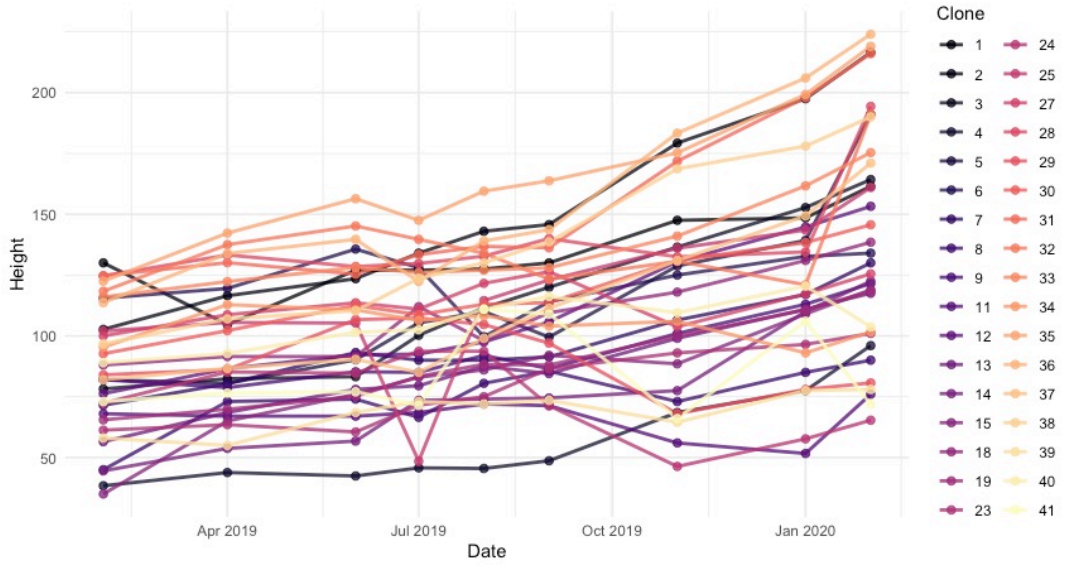

Figure 5.7. Growth rate of the tallest shoot. Each line represents a separate genotype. Each point is the average height of clones of each genotype at the time point measured.

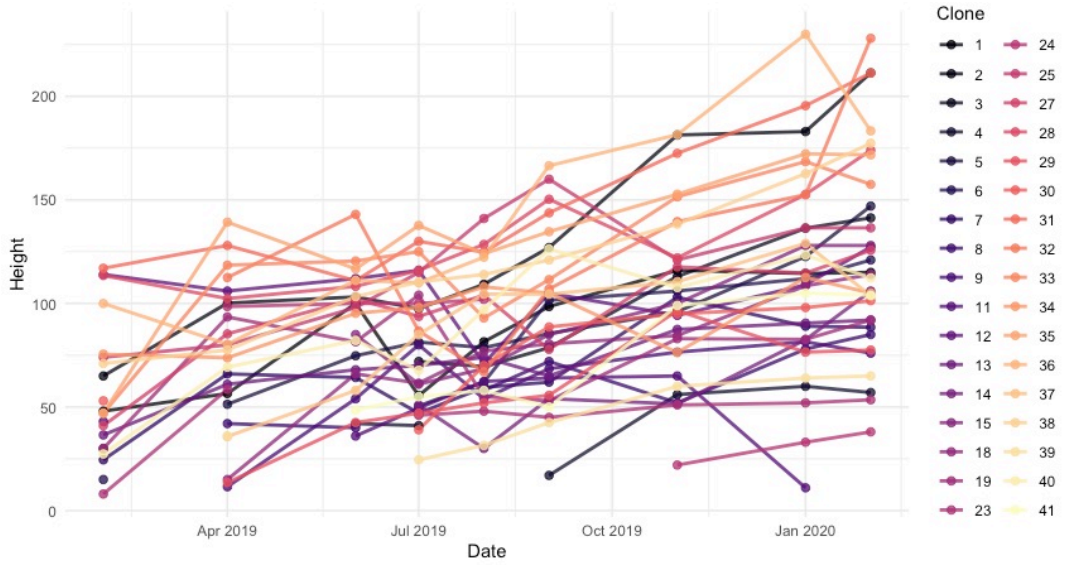

Figure 5.8. Growth rate of the second tallest shoot. Each line represents a separate genotype. Each point is the average height of clones of each genotype at the time point measured.

The height of the tallest shoot in April 2019 (when herbivory was measured) and the percentage of consumed leaf area appeared to be weakly negative correlated overall (Figure 5.9), although the P value was only marginally significant ($t = -1.9784$, $df = 103$, $P = 0.050$) and there was no relationship in plants that were consumed less than 20% ($t = 0.47378$, $df = 83$, $P = 0.6369$). The negative relationship disappeared when plants were split into under 20% consumed ($t = 0.47378$, $df = 83$, p-value = 0.6369) and over 20% consumed ($t = -0.074716$, $df = 18$, $P = 0.9413$).

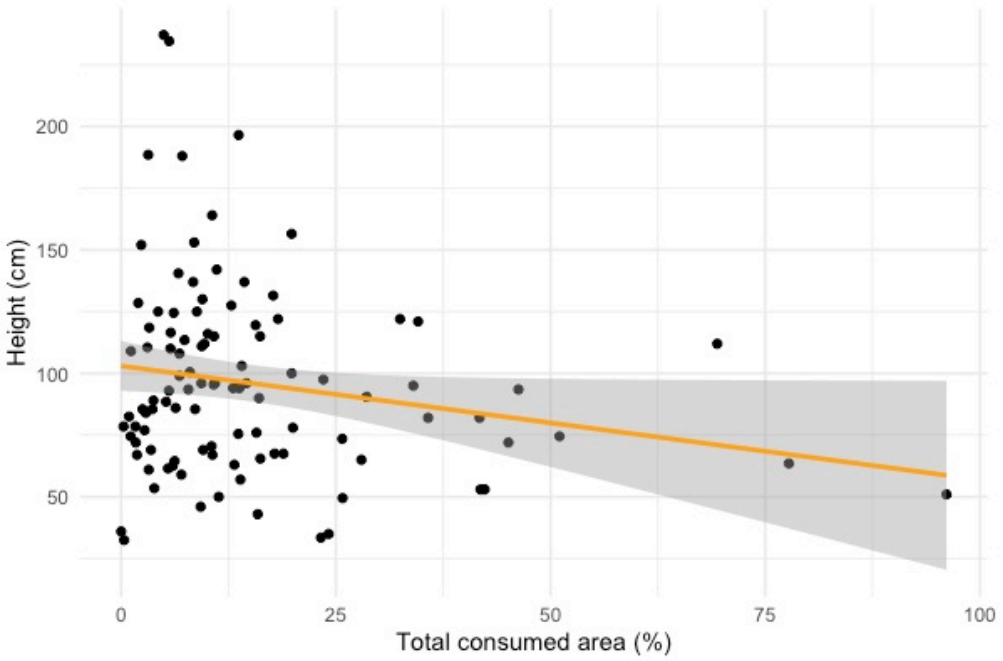

Figure 5.9. The percentage of total consumed leaf area plotted against the height of the plants at the time herbivory was measured. Each point represents the tallest shoot of a clone. The plot is fitted with a regression line.

5.5 Discussion

5.5.1 Herbivory

Here we demonstrate that the level of herbivory suffered by different genotypes differed significantly. However, the effect was not uniform. In the case of some clones, there was a lot of variation in consumption – for instance, genotype H39, which lost 7.02% of leaf area in Plot 1, 96.10% in Plot 3. There are various examples where the genotype was consumed to a higher extent in one plot and not another. However, specific genotypes were consistently consumed more or less than others. For instance, genotype H5 was consumed between 16.20%, 41.73%, and 13.76% across the three plots, whereas H12 and H32 suffered minimal loss to herbivory (H12: 6.22%, 9.54%, 6.79%, and 1.64%; H32: 5.59%, 3.05%, and 17.85%).

The most surface lost to herbivory on an individual leaf was 100% and the most leaf surface lost over an entire plant was 96.01% of the original estimated leaf area. However, 96.19% of all plants lost less than 50% of their total leaf area and 80.95% lost less than 20%. Low herbivory rates are often due to (a) an absence of herbivores, which is not the case in a tropical rainforest; (b) inaccessibility of plants to herbivores, which is unlikely in the case of

our forest plots; or (c) high levels of protection. However, the leaves of *H. stricta* are soft and bear no macroscopic physical defences. The phytochemistry of other *Heliconia* species such as *H. imbricata* and *H. latispatha* has been analysed elsewhere and revealed an absence of common chemical deterrents to herbivory (Williams and Harborne 1977; Auerbach and Strong 1981; Gage and Strong 1981), although some components like tannins and alkaloids have been detected in *H. angustifolia* (Strong and Wang 1977; Merh and Sabnis 1986). Silica is another known deterrent of herbivores (Reynolds et al. 2016), but has not been studied in relation to herbivory in *Heliconia,* although increases in silicon fertilisation do translate into an increase in foliar silica (Albuquerque et al. 2013) and reduced fungal infection (Fortunato 2009). Although the reason for low levels of herbivory in the apparent absence of classical chemical defences is unclear (Strong 1984), it has been suggested that *Heliconia* leaves may contain low levels of available nitrogen which could explain the slow larval development of *Heliconia* herbivores such as hispine beetles (Strong and Wang 1977; Auerbach and Strong 1981). An experiment analysing herbivory on different genotypes across plots with varying nitrogen and silicon treatments would be important to reveal whether these factors affect herbivory in *Heliconia*.

Another form of defence against herbivory to consider is volatile chemical compounds. García-Robledo and Horvitz (2009) used an olfactometer experiment to test whether four species of hispine beetle were able to distinguish between the smell of their host plants and whether they showed a preference when presented with two different plants, based on scent. The beetles were able to detect the smell of plants and three out of the four species of hispine beetle tested showed a preference for their host plant. Although the plants tested by García-Robledo and Horvitz (2009) were not *Heliconia*, they did include various Zingiberales, the order *Heliconia* belong to. It may be that *Heliconia* rely on volatile chemical signals as a mechanism of defence against herbivores or attraction of herbivore enemies, more than chemical defences within their leaves (Strong 1984). Although we did not measure any traits that may mediate this effect, there is a significant amount of literature indicating that genetically-determined traits such as volatile chemistry influence the outcome of herbivores and their predators (Ninkovic and Åhman 2009; Ninkovic et al. 2011), as well as evidence that polyphenolic compounds such as condensed tannins in leaves can have ecosystem effects beyond just the herbivores that consume the plants (Schweitzer et al. 2004; LeRoy et al. 2006a). Certainly, the flowers of *H. aemygdiana* emit volatile sesquiterpenes (Knudsen et al. 2004) and the erect inflorescence bracts of *Heliconia* are characteristically fowl-smelling (*pers. obs.*). Chemical analysis of *Heliconia* leaves would be interesting to establish what volatile chemicals are emitted by the vegetative parts

of the plants, whether they vary between genotypes, and how invertebrate herbivores or herbivore enemies respond to them.

The size of host plants can affect the communities of arthropods associated with them (Schlinkert et al. 2015; Barker et al. 2018; Barbour et al. 2019b). Additionally, there is often a trade-off between plant growth rate and defences against herbivores (Züst and Agrawal 2017). It would therefore be expected that plants with a faster growth rate, would suffer greater herbivory than slower growing plants. In our study, the height of the tallest shoot was weakly negatively correlated to the percentage of consumed leaf area. In cases of plants that lost a high proportion of leaf area due to herbivory, this may simply be due to the consumption of leaves at the top of the shoot shortening the height of plants. Because of how *Heliconia* grow, we measured the height from emergence from the soil to the tip of the tallest leaf, therefore it is possible for herbivore activity to shorten the height of the plant overall. However, amongst plants that did suffer more than 20% of leaf loss, the negative relationship between height and herbivory disappeared, with plants suffering over 20% of leaf loss being of an intermediate size rather than particularly tall. Equally, in plants that suffered less than 20%, there was no relationship between height and less than 20% of plants suffered more than 20% of leaf loss. Because we only analysed herbivory as a punctual measurement of lost leaf area, we could not determine the long-term relationship between growth rate and level of herbivory. Although measuring leaf consumption at one time point is likely to underestimate the true level of herbivory suffered by the plants (García-Robledo 2005), the goal of this study was to ascertain whether genotypic differences existed in the extent to which plants were damaged relative to each other, and so we deem that our methods were appropriate for this purpose. Nonetheless, it would be interesting to measure herbivory over time and compare it to plant traits, in order to understand the phenotypes through which the genotypic differences we observed occur.

5.5.2 Plant growth

The height of the tallest shoot was significantly different at different time points among genotypes, but at some time points, there was no difference between genotypes. We therefore measured growth rate over time as the interaction between month in which measurements were taken, and the heights of clones for each genotype. The overall growth rate was significantly different between genotypes over the period of the study. In another common garden experiment, Bruna and de Andrade, (2011) found there was significant variation within genotypes of *Heliconia acuminata* in terms of growth rate and above:below soil biomass ratios in different habitats (forest edge and understorey). We controlled for

possible effects of environmental differences by having the plots in the same area of forest and separated by only 40 metres from each other. Since edge effects have been demonstrated to influence Heliconia growth, our plots were longitudinal and only contained two plants across their width. Rowntree, McVennon and Preziosi (2011) used barley to look at the effect of nutrient level and genotype affect plant growth, as well as the population of aphids colonising the plants. Nutrient level, genotype, and the interaction of nutrient level and genotype all affected barley growth rate in an experimental setting. The aphid populations on the barley were also influenced by the interaction between nutrient treatment and barley genotype. Nutrient treatment affected different genotypes in different ways, with genotypes exhibiting different growth rates depending on the nutrient treatment. Although the Heliconia in our study were not subject to different conditions or nutrient levels, a similar pattern is reflected: although there was significant variation between genotypes in both growth rate and herbivory damage, some genotypes responded in specific ways – either they grew significantly faster or slower than other genotypes.

5.5.3 Community genetics effect in a common garden set-up

Tack, Johnson and Roslin (2012) warn against extrapolating the effect of genotype on communities in manipulated experiments, when genotypes have been collected across large spatial scales and thus may be locally-adapted to environmental conditions. The original rhizomes in this study were collected from different parts of Payamino, mostly from near clearing edges or above riverbanks, where they are conspicuous. However, they were not collected over a large geographic scale, the two furthest rhizomes collected from approximately 4 km from each other as the crow flies. Furthermore, Johnson and Agrawal (2005) found that although genotype x environment interactions explained variation in arthropod communities, environmental differences were caused by differences between habitats rather than microhabitats. However, plant genotype has been found to interact and mediate community responses to environmental conditions in other systems (Rowntree et al. 2011a; Barbour et al. 2019b). The three plots in our study were within close proximity to each other and within the same area of unbroken forest, and so shared the same habitat and environmental conditions. Nonetheless, future work could expand common gardens into other geographic areas using the same set of genotypes, to test whether the response of genotypes in terms of growth rate and herbivory remains significant despite environmental variation.

5.5.4 Conclusions

Genotype had a significant effect on leaf loss due to herbivory and on plant growth. Various plant traits are associated with influencing arthropod communities in other systems, including plant size which we measured here and did not seem to determine herbivory. However, we did not collect or identify the herbivores consuming the plant, and so are not able to assess whether there are differences in community composition of herbivores between genotypes or according to plant size, as has been seen in other systems (Barker et al. 2018; Barbour et al. 2019b). Nonetheless, we demonstrate that plant genotype can play a role in biotic interactions such as herbivory in a megadiverse tropical ecosystem.

We used various genotypes of a fast-growing plant, collected across a distance to ensure genetic differences without sampling different habitats. Our method of estimating herbivory across plants only constitutes a snapshot in the life of the plant and its associated community; however, it was sufficient to detect differences between plant genotypes. If clones were genotyped using molecular markers, it would be possible to analyse the degree of herbivory in correlation with the genetic distance between genotypes.

Although preliminary, we consider our study a step towards understanding the effect of plant genotype on associated communities in a megadiverse tropical ecosystem and recommend further exploration of the *Heliconia*-herbivore system in the context of plant traits and genotype. Future studies could assess the chemical profile of the leaves of different genotypes in order to determine the phenotypic characteristics mediating the genotypic pattern seen here.

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5.7 References

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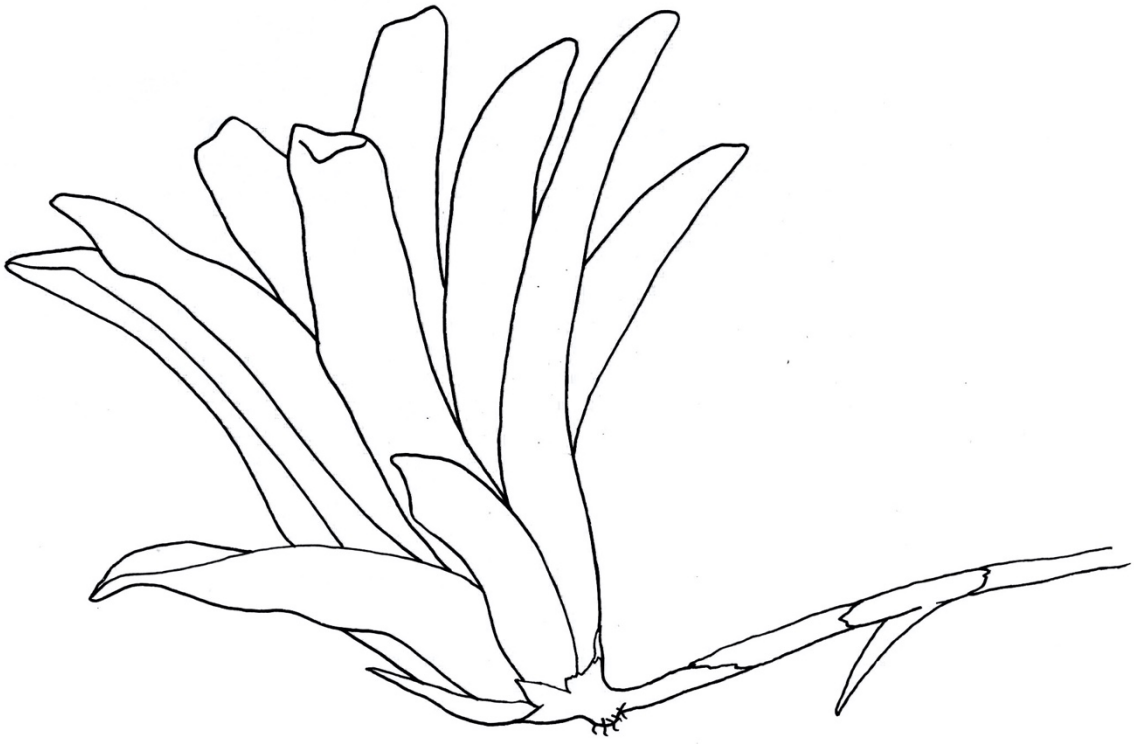
Chapter VI

Ex-situ study of the effect of plant genotype (*Neoregelia schultesiana*) on prokaryote communities



6.1 Abstract

Phyllosphere communities are those which live on the surface of plant leaves. The prokaryotes in these communities contribute to many aspects of plant health, from drought protection to plant growth. The composition of these communities is influenced by a range of biotic and abiotic factors, including plant genotype. Following a study on the prokaryotic communities in bromeliad phytotelmata – an extension of the phyllosphere – in the rainforest, where we found no link between plant genetic distance and community distance, we designed a greenhouse experiment to test whether the same patterns occurred in a less diverse environment in the absence of environmental variation.

We randomly arranged replicates of three varieties of the same species of bromeliad in a greenhouse , sampled their phytotelm communities, and sequenced the 16S rRNA region. Prokaryotic communities were significantly different among genotypes, although previous work revealed no link between bromeliad genetic distance and prokaryotic community composition in the rainforest. Using a spectrum of different genotypes in the greenhouse to imitate the *in-situ* system may have diluted genotype effects. However, we suggest that the higher diversity of the natural *in-situ* system may eliminate any potential significant effect of bromeliad genotype on the phytotelm community, whereas the effect of genotype is amplified in the simpler greenhouse system.

6.2 Introduction

The increasing attention on the interactions between plants and microbes has largely centred around the rhizosphere, the soil microbial communities associated with the roots of plants. Considerably less attention has been dedicated to what interactions lead to the structure of the phyllosphere communities (communities on plant surfaces) or a special extension of the phyllosphere, phytotelm communities (Vorholt, 2012)**.** Phytotelmata are cavities in plants which can collect and hold water. Phyllosphere and phytotelm bacteria contribute to their host plants and associated fauna in the form of nutrition through nitrogen mineralisation (Gonçalves *et al.*, 2014)**,** protection from pathogens (Innerebner, Knief and Vorholt, 2011; Vannier, Agler and Hacquard, 2019; Massoni *et al.*, 2020), alteration of leaf surface lubrication and drought protection (Bunster, Fokkema and Schippers, 1989; Sivakumar *et al.*, 2020)**,** and even promotion of plant growth (Lindow *et al.*, 2003). Despite receiving less attention than their related but distinct soil communities (Wagner *et al.*, 2016; Cregger *et al.*, 2018), the surface bacteria of plants are not without exploration: studies have elucidated various abiotic and biotic factors influence both the phyllosphere and phytotelm communities. For instance, seasonal variation, humidity, and UV levels have all been demonstrated to affect community composition in the phyllosphere (Jacobs and Sundin, 2001; Rastogi, Coaker and Leveau, 2013; Antonelli *et al.*, 2018)**.** Biotic factors such as the rhizosphere (Grady *et al.*, 2019)**,** the plant immune system (Pfeilmeier *et al.*, 2021)**,** detrital content (Louca *et al.*, 2017), and leaf anatomical and cuticle properties (Baldotto and Olivares, 2008; Bodenhausen *et al.*, 2014; Ritpitakphong *et al.*, 2016) also affect the composition of the phyllosphere and phytotelm communities.

In addition to the various abiotic and plant features which influence community structure, phyllosphere and phytotelm communities are more similar between individuals of the same species than they are to those other plant species, even when separated geographically (Lambais *et al.*, 2006; Knief *et al.*, 2010; Redford *et al.*, 2010; Kim *et al.*, 2012; Louca *et al.*, 2017). Species-level interactions – not just environmental variables – therefore, explain some of the community composition of the phyllosphere in both temperate and tropical plants (Lambais *et al.*, 2006; Redford *et al.*, 2010; Kim *et al.*, 2012; Louca *et al.*, 2017). In fact, there is plenty of evidence to suggest genetically closer individuals of the same species of plant share more similar communities than they do with non-related genotypes (Knief *et al.*, 2010; Redford *et al.*, 2010)**.** However, the effect of plant genotype on phyllosphere communities is not as strong as environmental factors such as geographic location or habitat (Wagner *et al.*, 2016; Cregger *et al.*, 2018; Singh *et al.*, 2018) and can depend on factors such as plant growth stage (Li *et al.*, 2021). Additionally, the effect of plant genotype on

their associated communities can vary according to the plant organ; for instance, plant genetics had a stronger effect on the phyllosphere communities of *Brassica stricta* than on its rhizosphere communities (Wagner *et al.*, 2016) and stronger on *Vitis vinifera* carposphere communities than on its phyllosphere assemblages (Singh *et al.*, 2018).

Bromeliads are neotropical plants that offer an excellent model to study bacteria in plant phytotelmata. The leaves of tank bromeliads are arranged in a rosette around a central phytotelm which harbors diverse and highly variable communities of microorganisms (Louca *et al.*, 2017a). In the wild, bromeliads provide habitats and resources for a diverse range of invertebrates and even vertebrates (Ladino *et al.*, 2019), and have been dubbed "biodiversity amplifiers" for this reason (Gonçalves-Souza *et al.*, 2010). Bacteria and other microorganisms in these microcosms have been shown to contribute to both the plants and the animal communities associated them through processes such as decomposition and mineralisation (Gonçalves *et al.*, 2014; Leroy *et al.*, 2016, 2017).

A range of factors influence the phytotelm bacteria of bromeliads, including geochemical conditions within the phytotelmata, bromeliad species, nutrient ratios, and surrounding vegetation (Louca *et al.*, 2017a; Louca *et al.*, 2017; Benavides-Gordillo *et al.*, 2019; Herrera-García *et al.*, 2022). Louca *et al.* (2017) showed that two sympatric species of bromeliad in Brazil hosted significantly different communities of microorganisms, while an *ex-situ* study showed that cultured microbial communities diverged between species over the course of a month following surface-sterilisation (O'Reilly Berkeley 2014, *unpublished data*). In terms of genotypic differences, bromeliad genotype did not correlate with prokaryote community distances in a megadiverse site in the Ecuadorian Amazon (Chapter IV of this thesis). However, nor did bromeliad genotype correlate with invertebrate community distances in the same system (Chapter IV of this thesis) while it did in a study in a theoretically less diverse tropical habitat in Belize (Zytynska *et al.*, 2012). It may be that the sheer diversity or environmental variation in the Ecuadorian rainforest drowns out any small effect genotype may exert on community structure, but in order to test this it is necessary to manipulate a simpler system.

The aim of this study was to determine whether bromeliad genotype influences differences in the community composition of phytotelm prokaryotes. Site and geographic distance play a role in the composition of the phyllosphere (Knief *et al.*, 2010; Redford *et al.*, 2010). To eliminate these effects, we investigated whether phytotelm bacterial communities diverged according to bromeliad species in a greenhouse. We randomly arranged bromeliads of the same species grown under the same conditions in a greenhouse and provided them with the

same soil and water source, which allowed us to assume that any differences in community composition on bromeliads was due to the selective survival of different bacteria (Grady *et al.*, 2019)**.**

6.3 Materials and methods

6.3.1 Botanical set-up

Eight individuals of 3 ‘strains‘ of *Neoregelia schultessiana* were obtained from one supplier. Genotype or strain will be used hereafter to refer to a variety of *N. schultessiana*. The supplier was a terrarium and horticultural, details of bromeliad varieties are listed in Table 6.1.

Table 6.1. Bromeliad ID, supplier, and variety information.

ID	Supplier	Website	Supplier variety name	Individuals
NS-E-(1-8)	Ben's jungle	bens-jungle.com	*Neoregelia schultesiana* ‘Purple Red’	8
NS-F-(1-8)	Ben's jungle	bens-jungle.com	*Neoregelia schultesiana* ‘Fireball’	8
NS-G-(1-8)	Ben's jungle	bens-jungle.com	*Neoregelia* ‘Fireball Rose’ Klein	8

Upon delivery, bromeliads were re-potted into standard 9 x 9 x 9 cm pots with the following compost mixture: 1/8 sand to 7/8 Sinclair All Purpose Gardening Medium (peat and sand compost mix, pH 6.0. Nitrogen N 192). Potted bromeliads were arranged in the greenhouse in rows following randomisation with the RAND() function in Excel. The temperature in the greenhouse ranged from 18-35ºC during the day, and 10-18ºC during the night.

6.3.2 Sampling

Sampling was conducted using sterile swabs. Two swabs per bromeliad were inserted into the base of the central phytotelm, one at a time, and rubbed on the surface of the leaf for 10 seconds. The swab was then immediately snapped into a clean 2 ml cryogenic tube and kept on ice while the remaining bromeliads were sampled, and the samples were transferred to a -80ºC freezer until DNA extraction took place (within 3-7 days after sampling).

Following sampling with the swabs, phytotelm pH and temperature was measured using a handheld pH meter (Hanna Halo pH wireless electrode).

At the end of the experiment, an area of approximately 10 x 1.5 cm of plant tissue was cut from one or two leaves from each bromeliad and dried in fine silica gel until DNA extraction.

6.3.3 Bacteria DNA extraction

DNA was extracted from 48 swabs (24 bromeliads x 2 swabs) using the Qiagen DNeasy® PowerSoil® DNA kit following the manufacturer's protocol with the modifications described in Chapter III of this thesis. For a positive control, a swab was dipped in a ZymoBIOMICS™ Microbial community standard (D6300) and extracted following the same protocol as the sample extractions.

6.3.4 Bacteria DNA amplification

Extractions were normalised to 4 ng/µl. The V4 region of the 16S rRNA gene was amplified using the forward primer 515F (Parada, Needham and Fuhrman, 2016) and the reverse primer 806R (Apprill *et al.*, 2015). Prior to final plating and amplification, a selection of samples at serial dilutions were amplified by qPCR with 5µl SYBR® Green Master Mix (Sigma-Aldrich), 2 µl of 1 M forward primer, 2 µl of 1 M reverse primer, and 1 µl of sample. This was done in order to establish the optimum number of cycles and concentration of DNA at which most communities amplified, without amplification plateauing. In accordance with the qPCR results, the normalised samples were then diluted 1/20 and the ZymoBIOMICS™ Microbial community standard, 1/40.

The final PCR programme was the following: 10 minutes at 95ºC, followed by 29 cycles of 30 seconds at 95ºC, 1 minute at 50ºC, and 90 seconds at 72ºC, followed by 30 minutes at 72ºC and held at 4ºC.The reaction was 10 µl: 4.9 µl AmpliTaq Gold 360 (Applied Biosystems), 0.1 µl GC Enhancer (Applied Biosystems), 2 µl of 1 M forward primer, 2 µl of 1 M reverse primer, and 1 µl of sample (4ng/µl DNA).

Phased tags were added to each primer in order to create a matrix of unique primer combinations as well as increase diversity, for sequencing purposes. PCR plate design consisted of 12 blank wells (at least one per row and column), 3 PCR negative controls, 5 positive controls extracted from the ZymoBIOMICS™ Microbial community standard, 4

extraction controls, and 72 DNA extractions from samples. Plates were designed with 288 bromeliad samples from another project, this totalled 14.5 plates. Three technical replicates of each sample were randomly arranged across matrices of 14.5 plates.

6.3.5 Bacteria library preparation and sequencing

Plates were pooled by taking 5 µl of each well from each amplified plate. Plate pools were quantified by Qubit® dsDNA HS (High Sensitivity) Kit (Life technologies) for broad-range double-stranded DNA. Each of the 7 pools was matched to the pool containing the closest concentration of DNA to its own, creating 2 pairs and 1 trio of pools. Each plate pool was normalised by dilution with molecular grade water to the lowest concentration in the matched pool pairs. 100 µl of each of the paired pools were then combined to create a pool of 2 or 3 plates, resulting in 3 pools. This was possible at this stage due to every primer combination in the matrix of 7 being unique, thanks to the phased tags. This process and the following steps was repeated with the second matrix of 7.5 plates.

Library preparation of the two sets of 3 pools was performed separately using Illumina® TruSeq® DNA PCR-Free Library Prep kit and following the manufacturer's instructions. The Illumina® TruSeq® DNA Single Indexes Set A adapters 005, 006, and 012 were used. Library size was ascertained by an Agilent 7500 Bioanalyzer chip with the High Sensitivity DNA Kit (Agilent Technologies), from which DNA-adapter ratios were established to estimate the concentration of the prepared libraries.

Library pooling, diluting, denaturing and sequencing were performed exactly as in Chapter III of this thesis, except it was performed for each of the two sets of three library pools.

6.3.6 Sequencing data processing

Paired end reads were merged using the function *illuminapairedend* in Obitools v.1 (Boyer *et al.*, 2016)**.** Reads with an alignment score of over 40 were kept and demultiplexed using the function *ngsfilter* in Obitools v.1. The demultiplexed reads were exported using *obisplit* and imported into QIIME2 (Bolyen *et al.*, 2019). The *Deblur* function (Amir *et al.*, 2017) was used to denoise the data, remove chimeras, and trim sequences to 252 base pairs. Sequences which appeared fewer than twice were removed. Denoising resulted in 21,475,066 reads. A pre-trained SILVA classifier (*Silva 138 99% OTUs from 515F/806R region of sequences*) (Bokulich *et al.*, 2018; Robeson *et al.*, 2020) was used for taxonomic assignment. The *MAFFT* function in QIIME2 was used to align the sequences in order to

construct a phylogenetic tree using the *FastTree* function. QIIME2 artifacts were imported into R as a *phyloseq* object using the *qiime2R* package (Bisanz, 2018). The resulting phyloseq object consisted of the ASV table, the phylogenetic tree, the taxonomic data, and the sample metadata.

We considered contaminant ASVs to be those which were found in PCR and extraction controls but not in sequencing controls, and were removed from the dataset using *prune_samples* and *prune_taxa* functions in *phyloseq* (McMurdie and Holmes, 2013).

We calculated tag jump as the average number of reads present in sequencing controls over the average number of reads present in samples. This only equated to 0.22% of reads, which we did not remove from the samples. Cross-sample contamination was calculated as the average number of reads present in extraction controls over the average number of reads present in samples, which was 0.21%; no further action was taken in regards to cleaning the data.

Functional group analysis was performed using FAPROTAX (Louca, Parfrey and Doebeli, 2016) as described in Chapter III of this thesis.

6.3.7 Statistical analysis

Statistical analyses were performed in R v4.1.1 (R Core Team, 2020). Biological and technical replicates were merged using *merge_samples2* from *speedyseq* (McLaren, 2020) and controls removed prior to analysis. Observed richness, Shannon, and Inverse Simpson indexes were calculated for each genotype (E, F, and G). ANOVAs were used to compare the alpha-diversity according to each diversity index among treatments and post-hoc Tukey tests performed for each.

Weighted UniFrac and Unweighted UniFrac distances were calculated using the *phyloseq* package in R. An initial Permutational multivariate analyses of variance (PERMANOVA) with each distance measure at the ASV level were carried out with the *adonis2* function in the *vegan* package (Oksanen *et al.*, 2020). PERMANOVAs were performed with genotype and pH as the explanatory variables. The PERMANOVAS were performed with ASV data, Family data, Order data, Class data, and Phylum data. Additionally, pairwise PERMANOVAs between genotypes were performed. NMDS were produced illustrating differences between communities according to genotype, using Weighted and Unweighted UniFrac distances.

Stacked bar charts were produced using the *barplot* function in *phyloseq*. These were produced at the Family and Phylum levels.

Functional groups were correlated with phytotelm pH and visualised using *corrplot* (Wei and Simko, 2021). We did not consider it appropriate to perform a correlation analysis based on different genotypes as there were so few replicates (N = 8) of each.

6.4 Results

In terms of alpha diversity, only Observed Richness differed significantly between genotype E and the other two. G and F were not significantly different to each other. However there was no difference between genotypes when Shannon or Inverse Simpson indexes were compared.

Table 6.2. Results of ANOVA and Tukey tests of species richness according to genotype..

	Observed richness			**Shannon**			**Inverse Simpson**		
Treatment	**Df**	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**
All	2, 21	5.126	0.015*	2, 21	1.583	0.229	2, 21	0.535	0.593
E - F			0.023*			0.240			0.586
E - G			0.035*			0.373			0.745
F - G			0.982			0.953			0.963

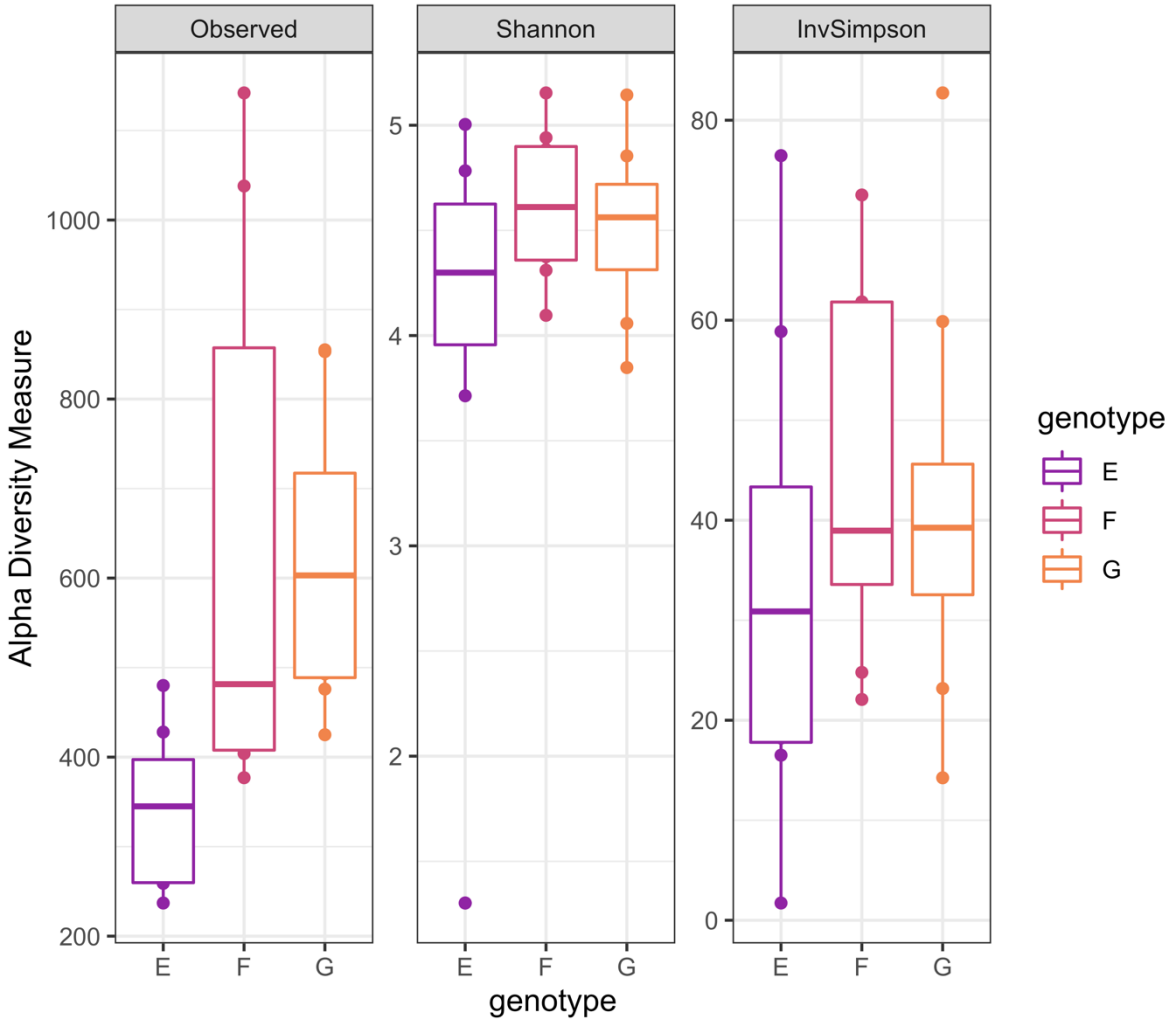


Figure 6.1. Observed Richness, Shannon, and Inverse Simpson indexes for each genotype.

Community composition differed between genotypes, depending on the taxonomic level examined and the distance metric used (Table 6.3). When abundance data was included in the PERMANOVA (by using Weighted UniFrac as the distance metric), communities were only significantly different at the ASV level. However, using Unweighted UniFrac, communities were significantly different between genotypes at all taxonomic levels examined. This implies the main differences in community composition across genotypes are likely due to the presence or absence of taxa rather than differences in abundances between the taxa present.

Phytotelm pH varied between 5.48 and 7.27. However, pH did not affect prokaryotic community composition.

Community composition at the ASV level was different between each genotype, using both distance metrics (Tables 6.4 and 6.5). However, the differences seen at other taxonomic levels using Unweighted Unifrac distances were explained mostly by differences between genotype E and the two others, while F and G did not differ significantly from each other (Table 6.5).

Table 6.3. Results of PERMANOVAs of the effect of genotype on the composition of the prokaryotic community at different taxonomic levels. Although pH was included in the model, values are not reported as none approached statistical significance.

	Weighted UniFrac				Unweighted UniFrac			
	Df	F	R2	P	Df	F	R2	P
ASV	2, 20	2.323	0.179	0.001*	2, 20	1.750	0.143	0.001*
Family	2, 20	1.449	0.119	0.082	2, 20	3.288	0.239	0.001*
Order	2, 20	1.376	0..114	0.126	2, 20	4.075	0.278	0.001*
Class	2, 20	0.964	0.083	0.476	2, 20	5.343	0.331	0.001*
Phylum	2, 20	1.412	0.112	0.209	2, 20	4.781	0.307	0.002*

Table 6.4. Posthoc PERMANOVAs of the effect of genotype on the composition of the prokaryotic community at different taxonomic levels, using Weighted UniFrac distances..

Weighted UniFrac												
	E-F				E-G				F-G			
	Df	F	R2	P adj.	Df	F	R2	P adj.	Df	F	R2	P adj.
ASV	1,14	2.944	0.174	0.003*	1,14	2.230	0.137	0.003*	1,14	1.785	0.113	0.012*
Family	1,14	1.236	0.081	0.642	1,14	1.336	0.087	0.537	1,14	1.213	0.279	0.837
Order	1,14	1.237	0.081	0.639	1,14	1.265	0.082	0.723	1,14	1.111	0.735	1.000
Class	1,14	0.403	0.0279	1.000	1,14	1.110	0.735	1.000	1,14	1.127	0.074	1.000
Phylum	1,14	0.422	0.0292	1.000	1,14	1.671	0.107	0.489	1,14	1.497	0.097	0.570

Table 6.5. Posthoc PERMANOVAs of the effect of genotype on the composition of the prokaryotic community at different taxonomic levels, using Unweighted UniFrac distances.

Unweighted UniFrac												
	E-F				E-G				F-G			
	Df	F	R2	P adj.	Df	F	R2	P	Df	F	R2	P adj.
ASV	1,14	1.958	0.123	0.006*	1,14	2.018	0.126	0.003*	1,14	1.245	0.081	0.069
Family	1,14	3.829	0.214	0.003*	1,14	4.418	0.239	0.003*	1,14	1.605	0.103	0.120
Order	1,14	5.104	0.267	0.003*	1,14	5.597	0.268	0.003*	1,14	1.175	0.077	0.735
Class	1,14	6.463	0.315	0.003*	1,14	6.931	0.331	0.003*	1,14	1.475	0.095	0.444
Phylum	1,14	6.670	0.322	0.006	1,14	5.249	0.272	0.015	1,14	1.939	0.122	0.366

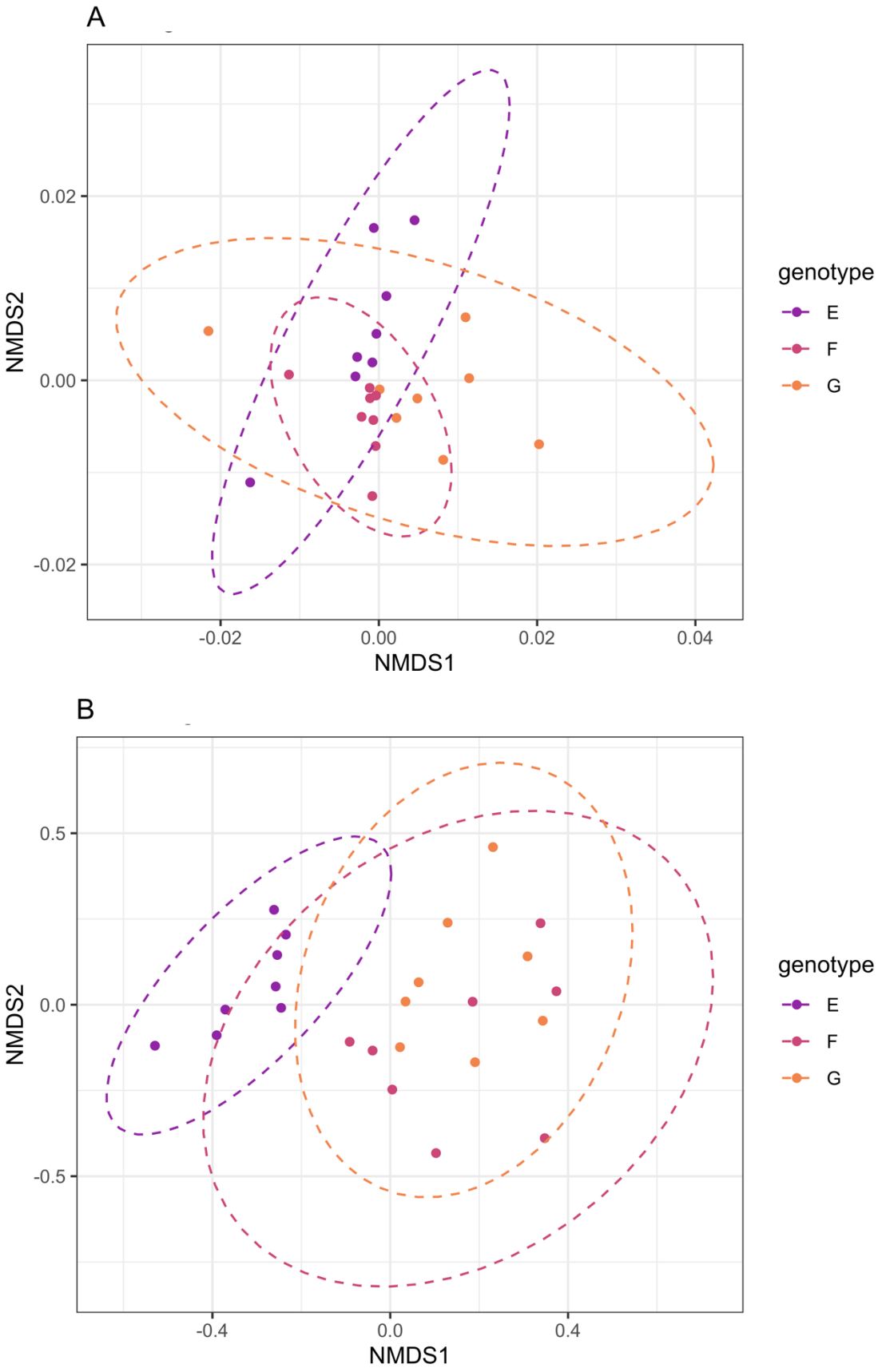


Figure 6.2. NMDS of UniFrac community distances by bromeliad genotype (A) Weighted UniFrac distances; (B) Unweighted UniFrac distances.

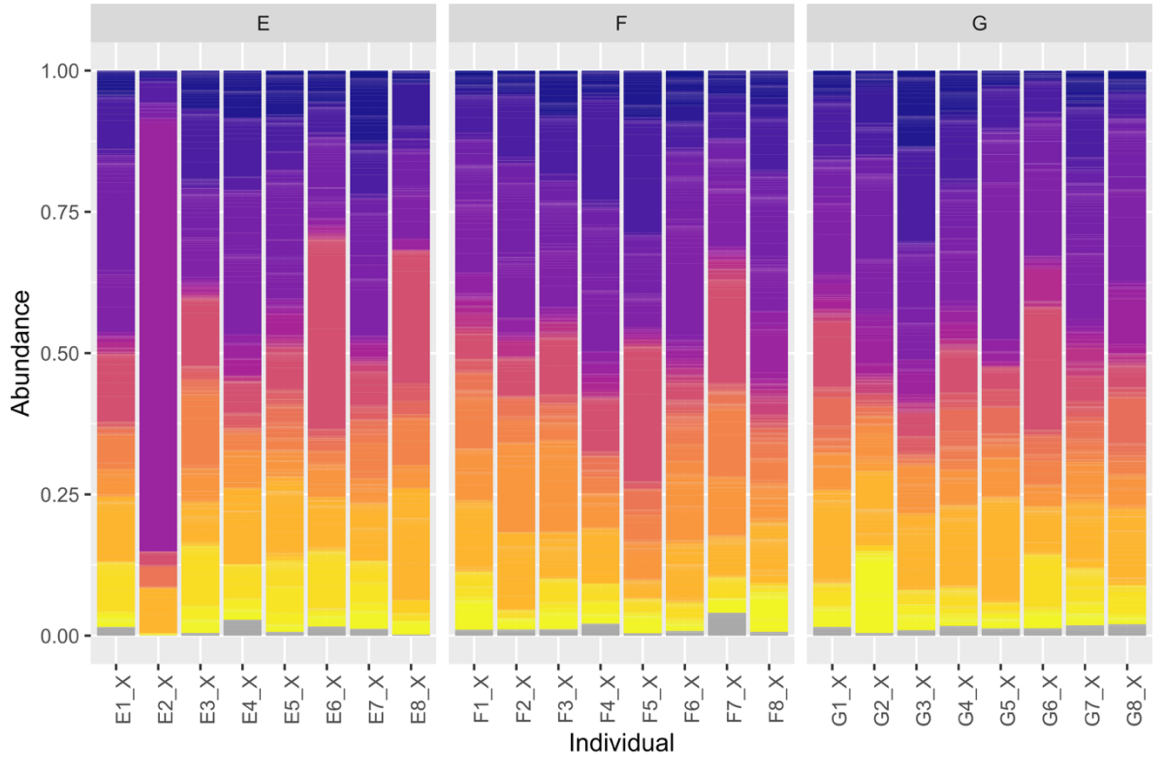


Figure 6.3. Stacked bar chart of transformed data at the Family level, split by individuals of each genotype. The legend is not included due to the large number of families.. Variation between individuals was clearly high, though some differences between genotypes can still be appreciated in Figure 6.4.

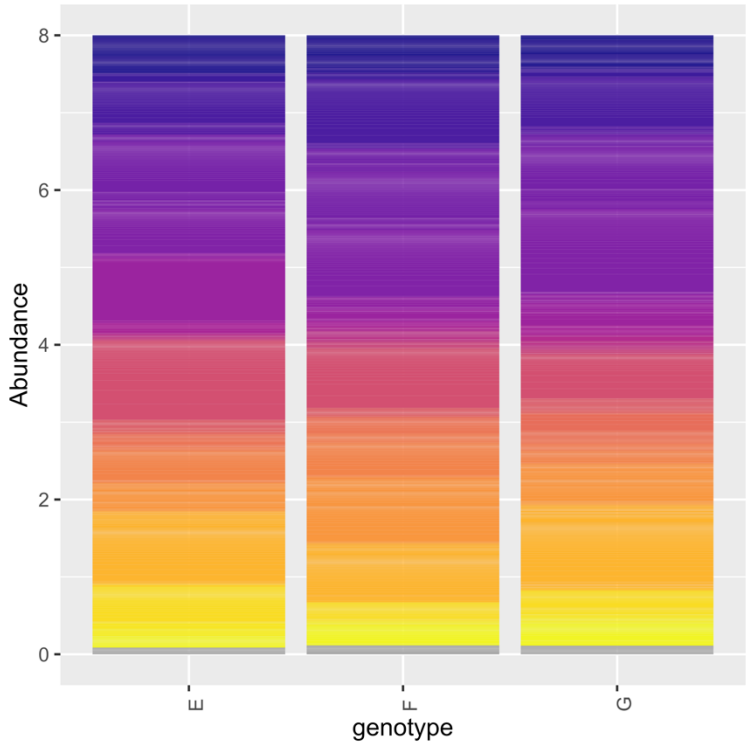


Figure 6.4. Stacked bar chart of transformed data at the Family level, by genotype. The legend is not included due to the large number of families.

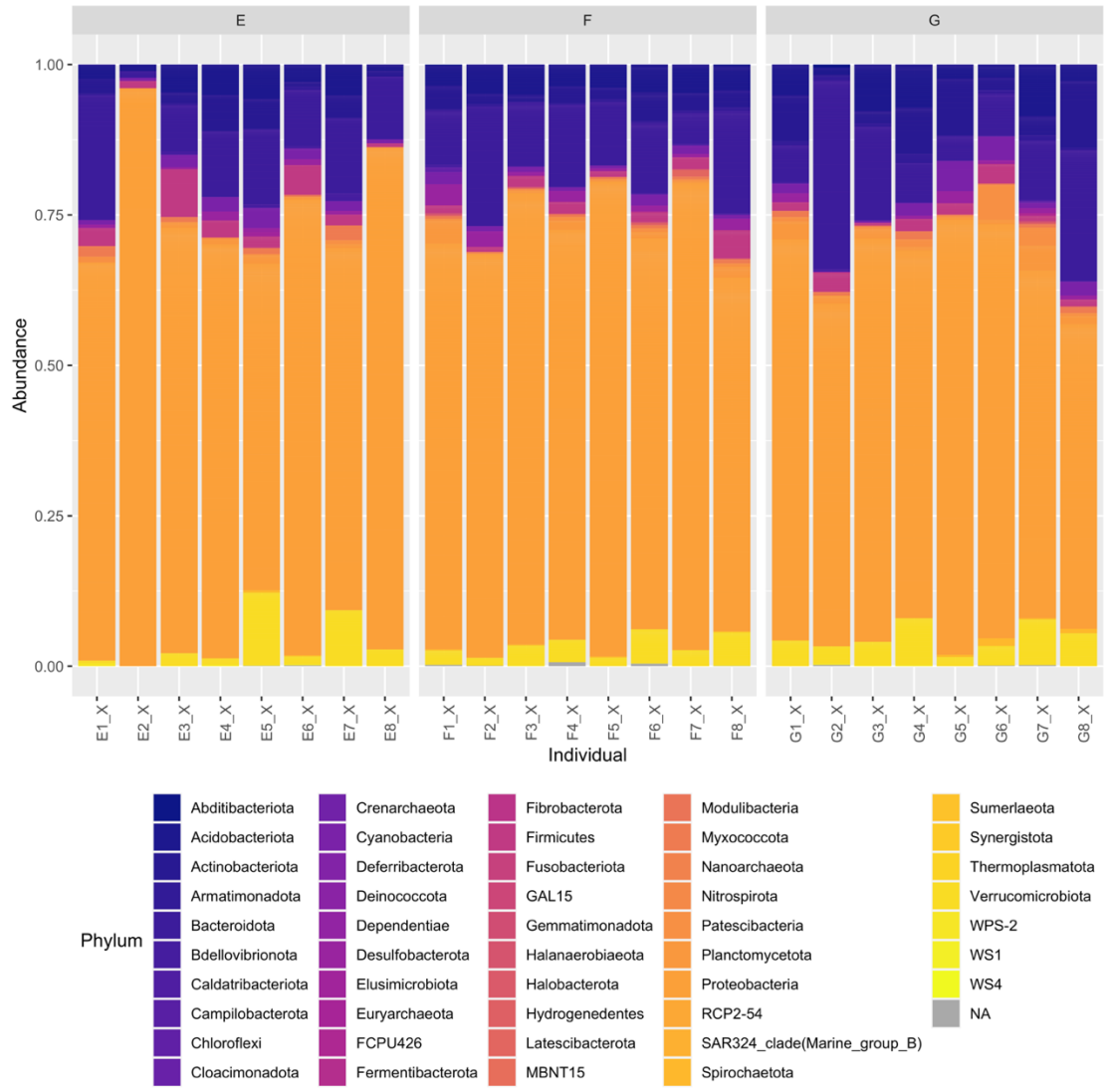


Figure 6.5. Stacked bar chart of transformed data at the Phylum level, split by individuals of each genotype. Variation between individuals was clearly high, though some differences between genotypes.

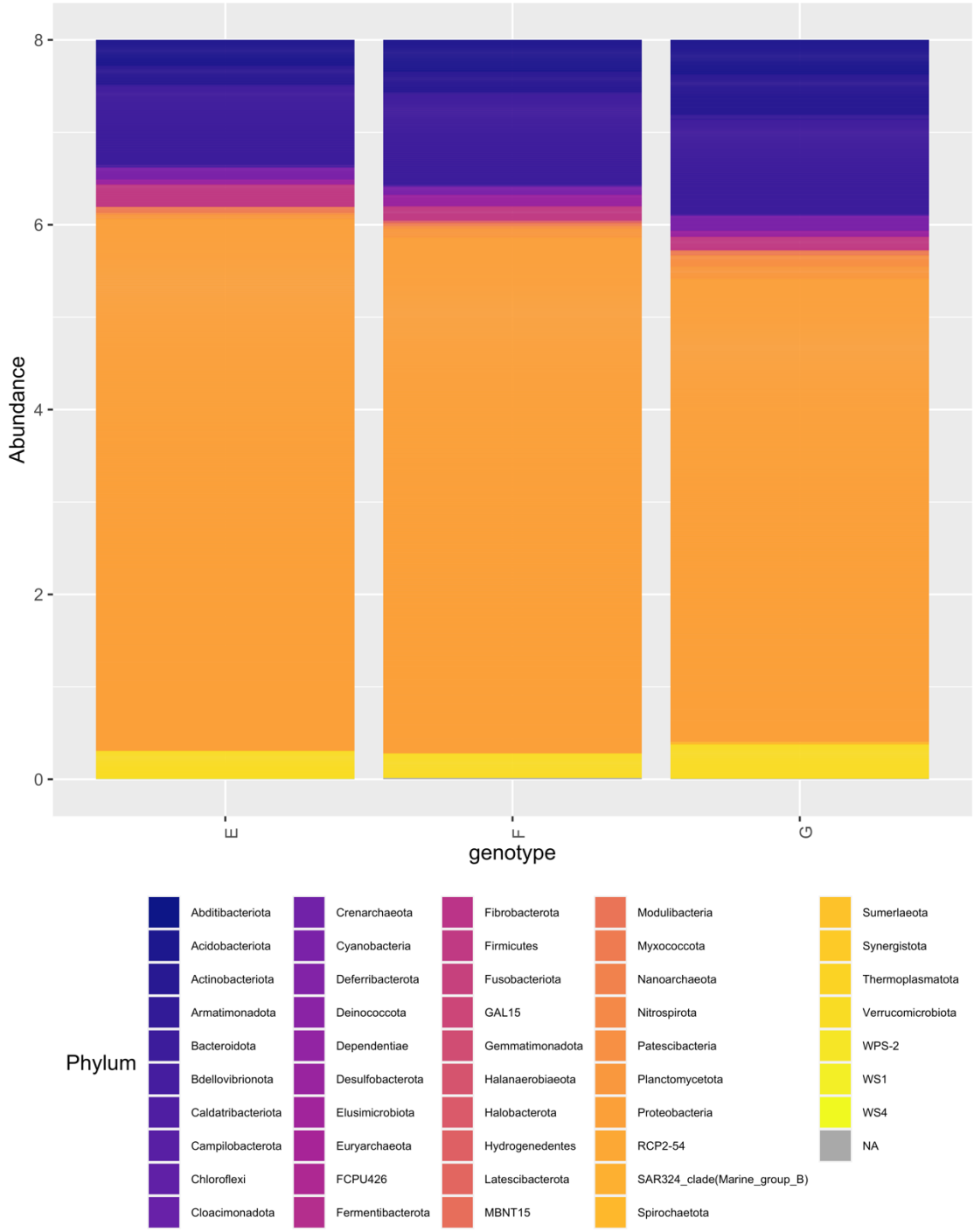


Figure 6.6. Stacked bar chart of the transformed data at each treatment stage at the Phylum level, by genotype..

Functional diversity analysis revealed that few functional groups were closely associated to variation in pH. Only methanol oxidation and methylotrophy were significantly associated with pH and this correlation was still relatively weak (0.44) (Figure 6.7). However, functional composition varied less between genotypes than taxonomic groups did (Figure 6.8).

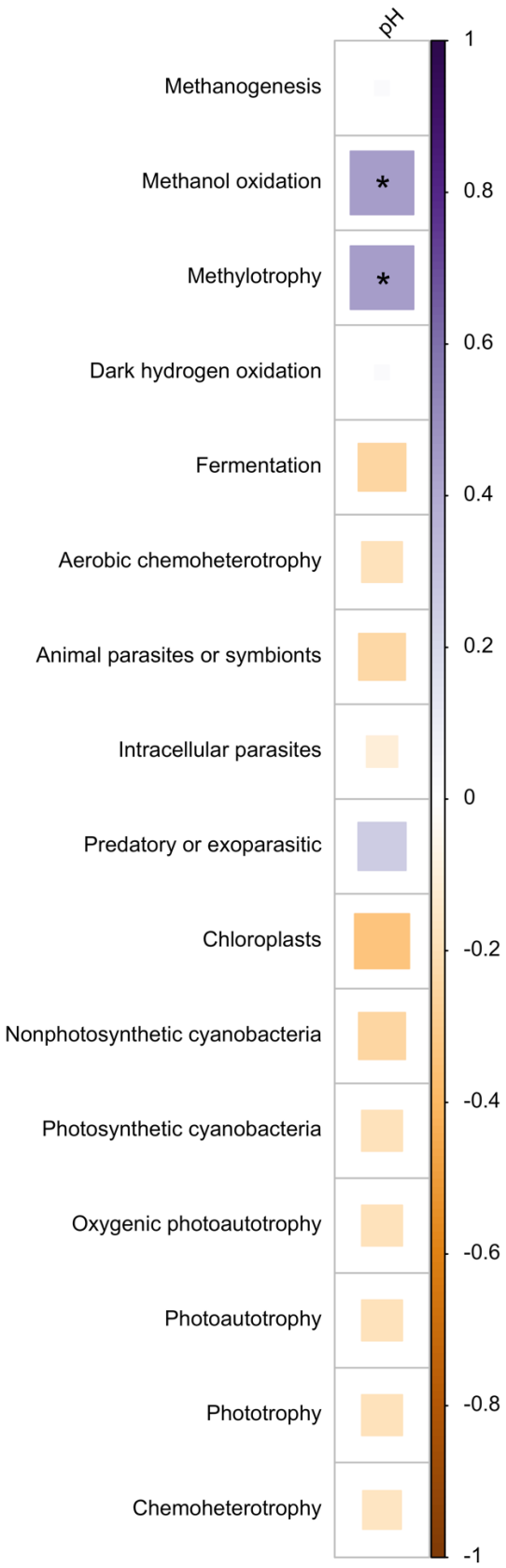


*Figure 6.7. Correlation plot of functional group association to pH. Legend shows strength and direction of correlation. Asterisks show degree of significance, * 0.05.*

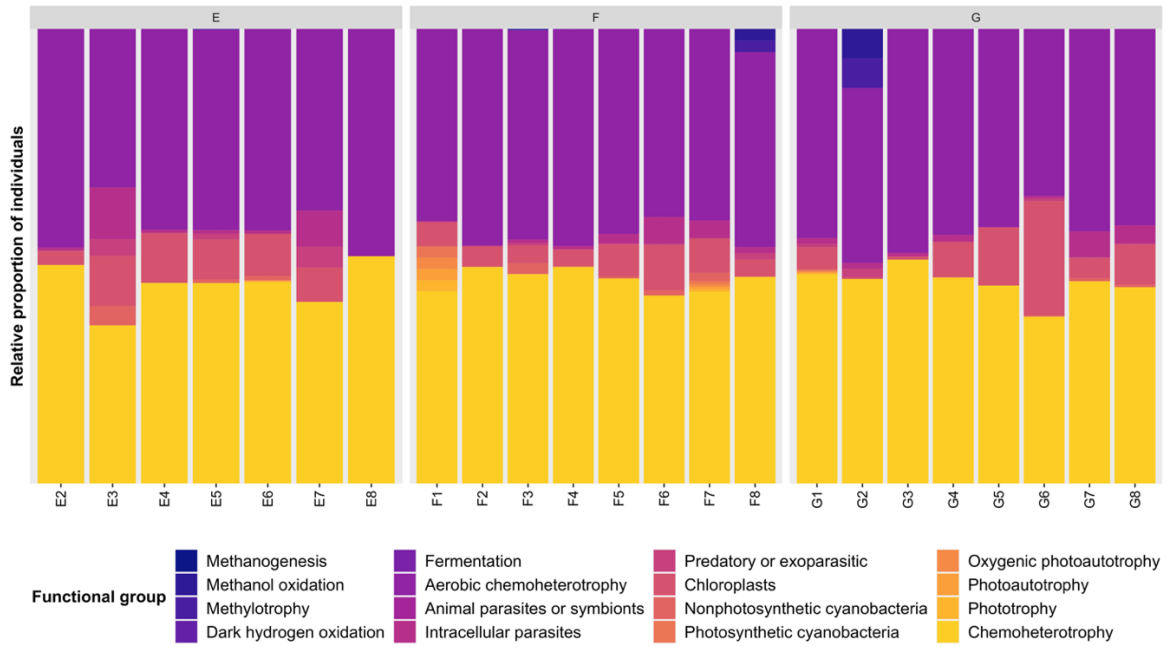


Figure 6.8. Relative proportion of functional groups across the three bromeliad genotypes.

6.5 Discussion

Bromeliad genotype affected the richness and composition of phyllosphere prokaryotic communities within the phytotelm. This mostly took shape in the form of one genotype being significantly different to the other two. This is in line with other experimental studies of phyllosphere bacteria, where for instance genetic differences between Arabidopsis correlate with changes in community structure (Reisberg *et al.*, 2013; Bodenhausen *et al.*, 2014). In natural systems, correlations between plant genetic distance have been observed (Redford *et al.*, 2010). However, the findings of this chapter differ from those of Chapter III, the latter of which revealed that genetic distance did not correlate with prokaryotic community distances in the rainforest.

The different results between these two studies could be explained by a number of factors. The aim of the experiment was to test whether bromeliad genotype influenced prokaryotic community all else being equal, removing environmental variation surrounding the bromeliads, by comparing communities among similarly sized clones of different genotypes in a common garden setting. However, in doing so, we used three varieties which do not necessarily usually occur together. Tack et al. (2012) warn about the amplification of genotype effects when plants are sourced from across wide geographic and experimented upon as if they occurred together naturally. While this should be considered, it is important to remember that these are horticultural varieties which may not have an exact

representation in nature. Nonetheless, it is possible that there was more genetic distance between the three *N. schultessiana* varieties we used in this experiment than between the *A. hoppii* sampled in the rainforest; unfortunately, we did not genotype the *N. schultessiana* so can only speculate. Regardless, rather than negate an effect of genotype, using genotypic differences not seen in nature would merely make any effect size calculated from the greenhouse study ecologically irrelevant.

In contrast to the observational study in Chapter III, all bromeliads in the greenhouse were subject to the same conditions four weeks prior to sampling and where they were cultivated (*pers. comm.* supplier Ben's Jungle). However, it seems plausible that given a lack of environmental variation, effects of genotype are amplified. Several studies in natural systems have found plant genotype effects on the wider community, from barley and yellow rattle to cottonwood forests (Bailey *et al.*, 2005; Bangert *et al.*, 2006; Ninkovic *et al.*, 2011; Rowntree, Cameron and Preziosi, 2011). However, others have not or have found that very little variation was explained by genetic distance (Zytynska *et al.*, 2011, 2012; Wainwright *et al.*, 2018). It may be that the greater the complexity of the system, the more diluted any existing effect of genotype becomes. This could be the case for a highly diverse tropical system versus a horticultural collection in a greenhouse. Certainly, there were more taxa in the rainforest samples than in the greenhouse (4909 ASVs within 56 phyla in the rainforest and 3083 ASVs in 48 phyla in the greenhouse).

We did not measure the full suite of geochemical factors known to influence bromeliad prokaryotic communities (Louca, Jacques, Aliny P.F. Pires, Juliana S Leal, *et al.*, 2017) as this was not the objective of the study, but we did measure pH. Although pH affected community composition at certain taxonomic levels in the rainforest study and is known to influence and be influenced by microbial communities, the present study did not detect an effect of pH on the taxonomic composition of communities. The range of pH variation was much greater amongst the rainforest bromeliads than in the greenhouse, however there was still a great deal of variation in the latter. Equally, pH did not correlate particularly strongly with any functional group although it did correlate significantly with methanol oxidation and methylotrophy, in contrast to the rainforest results. Functional diversity in general was much poorer than in the rainforest study (Chapter III of this thesis), which is to be expected in this constrained artificial environment (Williams and Marco, 2014; Dong *et al.*, 2019), although there was almost a complete overlap between groups in the rainforest and the greenhouse bromeliads. Functional group composition also appeared to vary less between individuals and genotypes than did taxonomic diversity, in line with previous studies (Louca *et al.*, 2017a; Chapter III of this thesis). The artificial nature of this experiment likely

constrained the functional diversity between bromeliads; nonetheless, functional structure appears to be more constrained than taxonomic diversity due to high functional redundancy in these systems (Louca et al., 2017a).

Overall, we found a diverse community of prokaryotes associated with the bromeliad phytotelm even in a greenhouse setting, which allowed us to assess the effect of genotype on the community. However, we cannot assume that this effect will extend to other parts of the phyllosphere, as previous studies on other plants have shown communities on different plant parts to have varying degrees of correlation with genotype (Wagner *et al.*, 2016; Singh *et al.*, 2018). It would be interesting, for instance, to look at the communities on the more exposed and dryer parts of the leaves, and compare these to the base of the leaves which we sampled within the phytotelm.

Our results indicate that in the absence of environmental variation, bromeliad genotype can determine the composition and richness of prokaryotic communities on the base of the leaves in the phytotelm. However, taken together with the results of Chapter III of this thesis, we suggest that this effect is small and ecologically not significant when other sources of variation are present and communities are more diverse.

6.6 Acknowledgements

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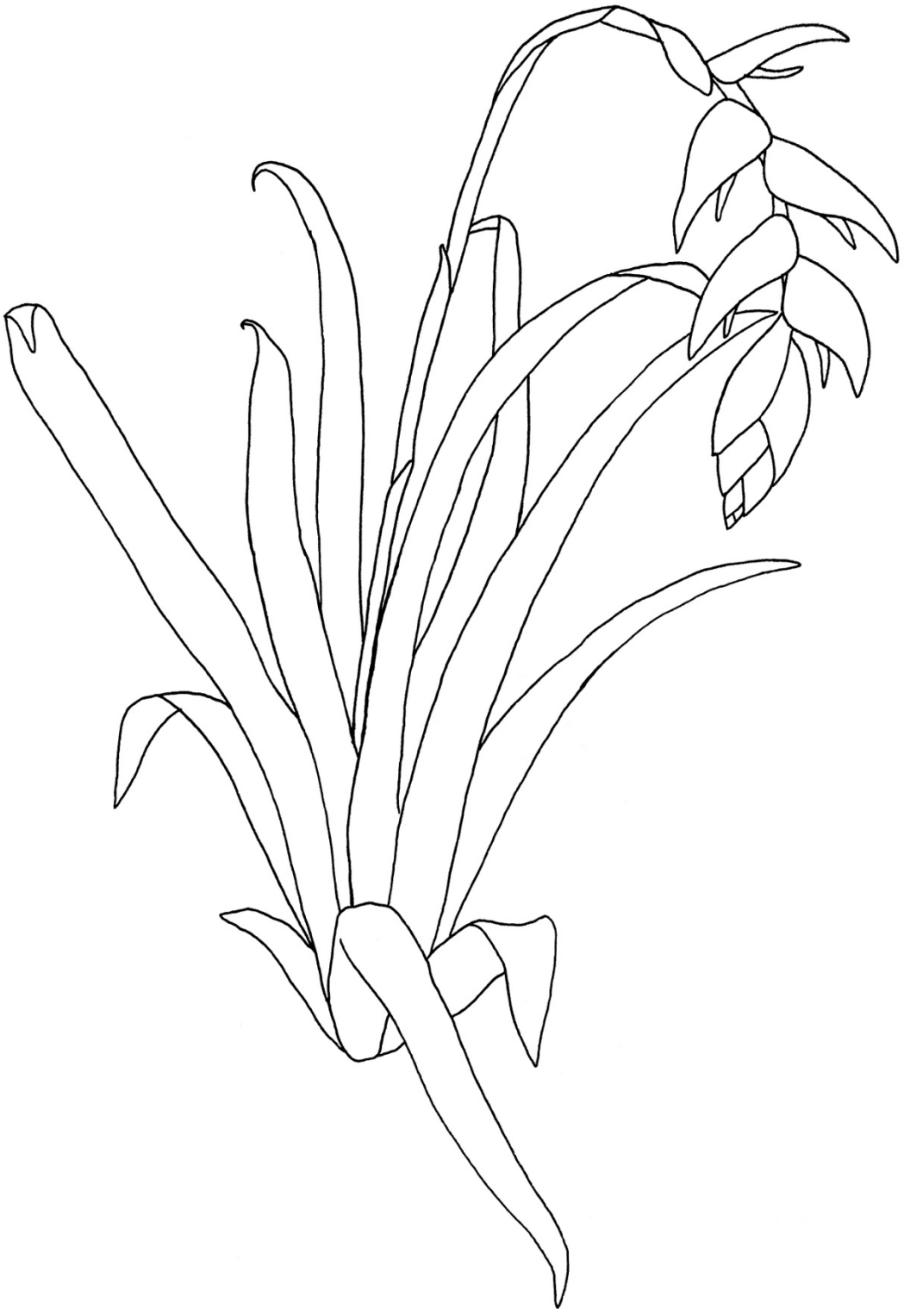
Chapter VII

General discussion



The effects of plant genetic diversity on their associated communities have been demonstrated in several systems repeatedly over the past 30 years since the term "community genetics" was coined (Antonovics 1992). From observational studies in standing forests to experimental laboratory set-ups using several trophic levels, plant genotype has been found to explain between 1% and 60% of surrounding community variation (Wimp et al. 2004b; Zytynska et al. 2012b; Bangert et al. 2013). These associations have usually been studied between plants and their arthropod associates, but also between plants and epiphyllic bacteria, other plants, and even vertebrate activity (Bailey et al. 2006b; Bodenhausen et al. 2014; Li et al. 2021, 2022). Plant genotype has also been studied in the context of its effect on wider ecosystem processes, such as leaf litter degradation and nutrient availability (LeRoy et al. 2006b; Rudman et al. 2015b; Semchenko et al. 2021). Multitrophic studies have even shown the effect of plant genotype on the rest of the community can depend upon the presence of other organisms, such as rhizobacteria (Tétard-Jones et al. 2007; Zytynska et al. 2010). Understanding the importance of genetic and genotypic diversity is important in the context of our changing world, both in terms of conservation and crop production (Mundt 2002; Neuhauser et al. 2003; Whitham et al. 2003). It also allows us to explore eco-evolutionary dynamics and ecosystem adaptability (Neuhauser et al. 2003). However, as discussed in Chapter I of this thesis, many questions still remain in the field of community genetics.

Three big questions can be summarised as the commonness of genotype effects on the community, their relative importance in relation to environmental interactions, and the mechanism through which such associations take place (Hersch-Green et al. 2011b; Rowntree et al. 2011b). Briefly, the vast majority of community genetics studies have taken place in temperate terrestrial systems, with exceptions (e.g. Marquis, 1990; Rudman *et al.*, 2015; Wainwright *et al.*, 2018). Many studies do not quantify the proportion of variation explained by genotype in conjunction with other habitat features, although again there are exceptions to this (e.g. Johnson and Agrawal, 2005; Bangert *et al.*, 2013; Barbour *et al.*, 2019; Gosney *et al.*, 2021). In most of the systems studied, the phenotypes through which genotype effects are taking place have not been identified, due to the theoretical and logistical complexity of investigating this; however, notable inlays in this area have been made in recent years (e.g. Ninkovic *et al.*, 2011; Barbour *et al.*, 2015; Silfver *et al.*, 2015).

In this thesis, we addressed two of these questions, by investigating genotype-community interactions in two tropical systems, bromeliads and *Heliconia.* By studying two tropical systems in a megadiverse habitat, we intended to contribute to addressing whether community genetics effects are ubiquitous. By simultaneously studying the environmental

and plant characteristics that affect bromeliad-associated communities, we intended to quantify the variation explained by these factors relative to any possible effect of genotype. We studied genotype-community interactions in an *in-situ* natural systems, an *in-stu* experimental system, and an *ex-situ* experimental system.

7.1 Community genetics of bromeliads and *Heliconia*

Contrary to expectations, we did not find any relationship between bromeliad genotype and the associated community in the wild, neither the prokaryotic nor the invertebrate contingents (Chapter IV). Our expectations were based on not only the wealth of evidence of plant genotype correlating with the composition of associated communities, but especially previous research on bromeliads (Zytynska et al. 2012b). Zytynska *et al.* (2012) found that the genotype of the bromeliad *Aechmea bracteata* explained 1% of the variation between aquatic invertebrate communities. Because microbes modify their surrounding environment, we considered that a correlation between plant genotype and prokaryotes could partially mediate a plant genotype and invertebrate association. Although statistically significant, the amount of community variation explained by plant genotype in the previous work in Mesoamerica, was low: 1% of the variation in the invertebrate community in bromeliads (Zytynska et al. 2012b), 2% in the epiphytic community on breadnut trees, 2% in the leaf litter community under breadnut trees, and 1% in the invertebrate trunk community on breadnut trees (Zytynska et al. 2011a). Additionally, our study area was at the edge of the Tropical Andes Biodiversity Hotspot and within the buffer zone of the Sumaco Napo-Galeras National Park, one of the most biodiverse regions on Earth. Vascular plant species diversity is estimated to be higher in the Tropical Andes than the Mesoamerican Biodiversity Hotspot (Myers et al. 2000), and so it is conceivable that the invertebrate species diversity may mirror this, as insect and plant species diversity often correlate (Siemann et al. 1998; Kemp and Ellis 2017; Shinohara and Yoshida 2021). It is therefore possible, that any effects that plant genetics could otherwise have on their associated communities are masked in an observational study in such a highly biodiverse area.

In our *in-situ* experiment with *Heliconia*, we did find an effect of plant genotype on both growth rate and leaf herbivory (Chapter V). We were unable to genotype the *Heliconia* using molecular methods, and so could not correlate genetic distance with herbivory. However, because we used clones obtained from a variety of widely-spaced parent rhizomes, we can be fairly certain that replicates were genetically identical (bar potential spontaneous mutations) and that many of the individuals were genetically distinct from each

other. Although in some genotypes there was a great deal of variation in the level of herbivory suffered by individual plants, others were consistently eaten more or less. The same was true for growth rate. Although this result appears to contrast with that of the *in-situ* bromeliad study, it is worth considering that measuring herbivory damage is a much coarser way of quantifying interspecific interactions than directly collecting invertebrates. All herbivory damage on *Heliconia* has been analysed in the same way regardless of the perpetrator, whereas by collecting and identifying specimens in bromeliads to morphospecies we obtained a much more detailed dataset. Leaf herbivory is also a very different type of interaction with the host plant compared to inhabiting it. Herbivores are generally more specific to particular food plants than other arthropod guilds, although cascading effects of plant genotype on higher trophic levels have been demonstrated elsewhere (Ninkovic et al. 2011; Rudman et al. 2015b; Moreira et al. 2016). Whereas certain genotypes of *Heliconia* may be more susceptible to herbivory due to a weaker phytochemical profile, the impact of phytochemical differences between different genotypes of bromeliad are less likely to impact their inhabitants, which generally do not feed on the leaves.

In the *ex-situ* experiment, genotype explained up to 26% of the variation between prokaryotic communities in the phytotelm, depending on the taxonomic level and pair of genotypes considered. This enormous statistic could be due to genotype effects being inflated in the absence of other sources of variation, as well as using varieties which may correspond to more distantly-related genotypes than one would encounter in close proximity *in-situ.*

If the absence of an association between plant genetic distance and community distance in the rainforest were indeed due to the extreme complexity of such a megadiverse system, we would expect to detect an association between plant genotype and the prokaryotic community in our *ex-situ* experiment – a much simpler system, with controlled environmental conditions. The presence of such an association in the greenhouse therefore supports this idea.

To summarise whether community genetics effects are ubiquitous, the straight forward answer must be no, and we are not the first to suggest this (Crutsinger et al. 2008; Whitham et al. 2012; Wainwright et al. 2018). However, it is not a simple case of plant genotype affecting community composition in some environments and not in others. In this thesis we studied two different systems in the same area of rainforest and found plant genotype to affect invertebrate activity in one but not the other. As discussed, there could be many reasons for this difference, but ultimately it suggests that the effect of plant genotype on the

associated community depends on the type of interaction being examined (Stiling and Rossi 1995; Crutsinger et al. 2008) and the level of complexity of the system, with weaker effects of plant genotype in more biodiverse environments.

7.2 Contribution of community genetics effects relative to other environmental variables

In a hypothetical scenario in which the *ex-situ* experiment worked and exposed a correlation between bromeliad genetic distance and prokaryotic community distance, it may have strengthened our suggestion that the lack of correlation *in-situ* was due to extremely high diversity. Whatever the reason, it appears that in *A. hoppii* bromeliads in this region of the Ecuadorian Amazon, the plant genotype has no significant influence on the community within the phytotelm. This leads us onto the next general question in community genetics that we intended to address: what is the relative contribution of community genetics effects compared with other environmental factors?

While did not find any effect of genetic distance, we found that the environmental and plant variables that we measured explained up to 18.4% of variation in the invertebrate community composition (Chapter II) and up to 12.6% in the prokaryotic community composition in wild bromeliads in the Ecuadorian Amazon (Chapter III). This value varied depending on which element of the invertebrate and prokaryotic community was measured, and on whether presence-absence or abundance data was examined. However, no single characteristic that we measured accounted for much of that variation on its own. The factor that explained the greatest amount of variation in the invertebrate community was height of the bromeliad from the ground and in the prokaryotic community it was pH, yet still these variables only accounted for 4.8% (of the aquatic community) and 6.9% (using Weighted UniFrac distances at the Phylum level) of the distances between communities, respectively. Whereas previous studies have found plant size and complexity to explain significant proportions of variation in the invertebrates communities between bromeliads (Srivastava 2006; Gonçalves-Souza et al. 2011; Jocque and Field 2014b), prokaryotic communities have been shown to exhibit fewer habitat-driven associations than invertebrates (Farjalla et al. 2012). Nonetheless, prokaryotic functional diversity within bromeliads does appear to be associated to biogeochemical traits within the phytotelm (Louca et al. 2017e) as well as wider habitat characteristics (Herrera-García et al. 2022) (Chapter III).

Similar factors explained statistically significant portions of variation between invertebrate and prokaryotic communities in bromeliads, although the alpha diversity of each of these communities correlated with different variables. Common factors influencing beta diversity in both invertebrate and prokaryotic communities in bromeliads included forest type, height on the tree, and bromeliad volume, whereas overall invertebrate alpha diversity only correlated with volume and prokaryotic alpha diversity correlated with height, volume, longest leaf, and sampling season (Chapters II and III). Additionally, prokaryotic and aquatic invertebrate community distances in bromeliads were correlated, implying that bromeliads containing more similar microbial communities contained more similar aquatic invertebrate communities (Chapter IV). As discussed in Chapter IV, this may be due the closer proximity in which these communities live, especially as the prokaryotic sequences were obtained from samples taken at the base of the central phytotelm of each bromeliad.

The relative contribution of environmental factors and plant genetics to the wider community varies depending on the interaction examined rather than the particular habitat type. However, this does not mean conserving genetic diversity should be neglected in systems where it is not found to exert an influence on the rest of the community, as there are other advantages to maintaining genetic diversity, such as adaptability to future environmental change.

7.3 Future work

An effect of plant genotype on associated communities is neither ubiquitous nor straightforward. Even when plant genetic diversity is linked to variation in the wider community, it is not necessarily biologically important compared to other environmental factors, although very small amounts of heritable genetic variation can lead to larger changes over time, so perhaps it is worth not dismissing low levels of variation. It is therefore necessary to explore the genetics of species interactions in a wider variety of systems to understand where genetic and genotypic diversity is relevant to management or conservation. In particular, there are huge geographic gaps in community genetics studies, the most striking being Africa, with very little representation in Asia, Oceania, or South America either. Only when community genetics effects have been identified, can research into mechanisms mediating these relationships take place in any particular system.
In terms of the systems studied in this thesis, it would be interesting to genotype the bromeliads of the *ex-situ* experiment (Chapter VI), in order to test whether the genotypes used were indeed more dissimilar than rainforest bromeliads were amongst each other. Given the relationship we found between the prokaryotic community and the aquatic

invertebrate community, it may be of interest to further study the links between different organisms within the bromeliad phytotelm. There are studies which have explored these multitrophic communities and included bacteria (Brouard et al. 2012; Farjalla et al. 2012; Carrias et al. 2020). However, generally the way in which bacterial communities have been characterised has been much less sophisticated than with the use of metabarcoding, resulting in much coarser profiles of the prokaryotic community. A multi-marker metabarcoding approach could reveal a more detailed picture of taxonomic diversity within bromeliads, although this can quickly become complicated as the classification of some groups of organisms (for instance, algae) can be difficult to resolve based on a single barcoding region (Hall et al. 2010).

Although taxonomic correlations are interesting, functional diversity may be more informative if our goal is to understand community dynamics, especially in megadiverse tropical ecosystems. We used FAPROTAX (Louca et al. 2016b) to roughly classify prokaryotic taxonomic groups into functional groups and did not have the expertise to classify the invertebrate specimens similarly into trophic guilds. However, a metagenomics or even transcriptomics approach to the prokaryotic community could more accurately characterise the functional diversity in these communities. This combined with classification of the invertebrate samples into functional groups could elucidate more ecologically meaningful interactions within the phytotelm community. Additionally, they could be studied in the context of the various habitat (Chapter III) and biogeochemical (Louca et al. 2017e) characteristics that influence different functional groups to different extents. Manipulations would be necessary to determine how much of the geochemical environmental within bromeliad phytotelmata is due to prokaryotic activity, wider environmental conditions, plant architectural traits, or even plant genetics.

The height at which the bromeliad was found to affect the composition of both invertebrate and prokaryotic communities (Chapters II and III). This largely neglected aspect in bromeliad studies (most take place on or near the ground) could prove interesting to explore in relation to microenvironmental variation throughout the canopy gradient. Additionally, it could be compared to the variation of the surrounding communities on trees at the same height as the bromeliads, to see if height has a similar effect on phytotelm and non-phytotelm communities in the same tree.

The *Heliconia* experiment revealed an effect of plant genotype on herbivory and growth rate. The next logical step would be to confirm whether this pattern is still true when microenvironmental conditions vary (i.e. over naturally distributed *Heliconia*) and to

investigate what could be driving this effect. In particular, it would be interesting to compare the phytochemistry among the different genotypes. Separately, once sufficient inflorescences have grown, it could be tested whether differences in the phytotelm communities exist between genotypes. Although some invertebrates consume *Heliconia* flowers (Seifert and Seifert 1976), most invertebrates and bacteria within these phytotelmata would have a less direct relationship with the plant than its foliovores. These are also much less diverse communities than those found in most rainforest bromeliads and so easier to study.

7.4 Closing words

This thesis explored two basic community genetics questions in two different systems in a megadiverse tropical rainforest, expanding the range over which community genetics studies have been carried out. We found community genetics effects in relatively-controlled experiments but not in the completely wild system, suggesting the type of interaction and level of complexity in the focal system determines the extent to which plant genotype may influence associated communities. Additionally, we explored the factors influencing invertebrate and prokaryotic communities in epiphytic bromeliads, extending the canopy height at which these communities are usually studied. Both bromeliads and *Heliconia* are exciting systems to work with and provide ideal microcosms within which to ask a wide range of ecological questions. Although some of the patterns we uncovered may arouse more questions than answers, we hope we provide a basis upon which further work can build.

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Supplementary Information

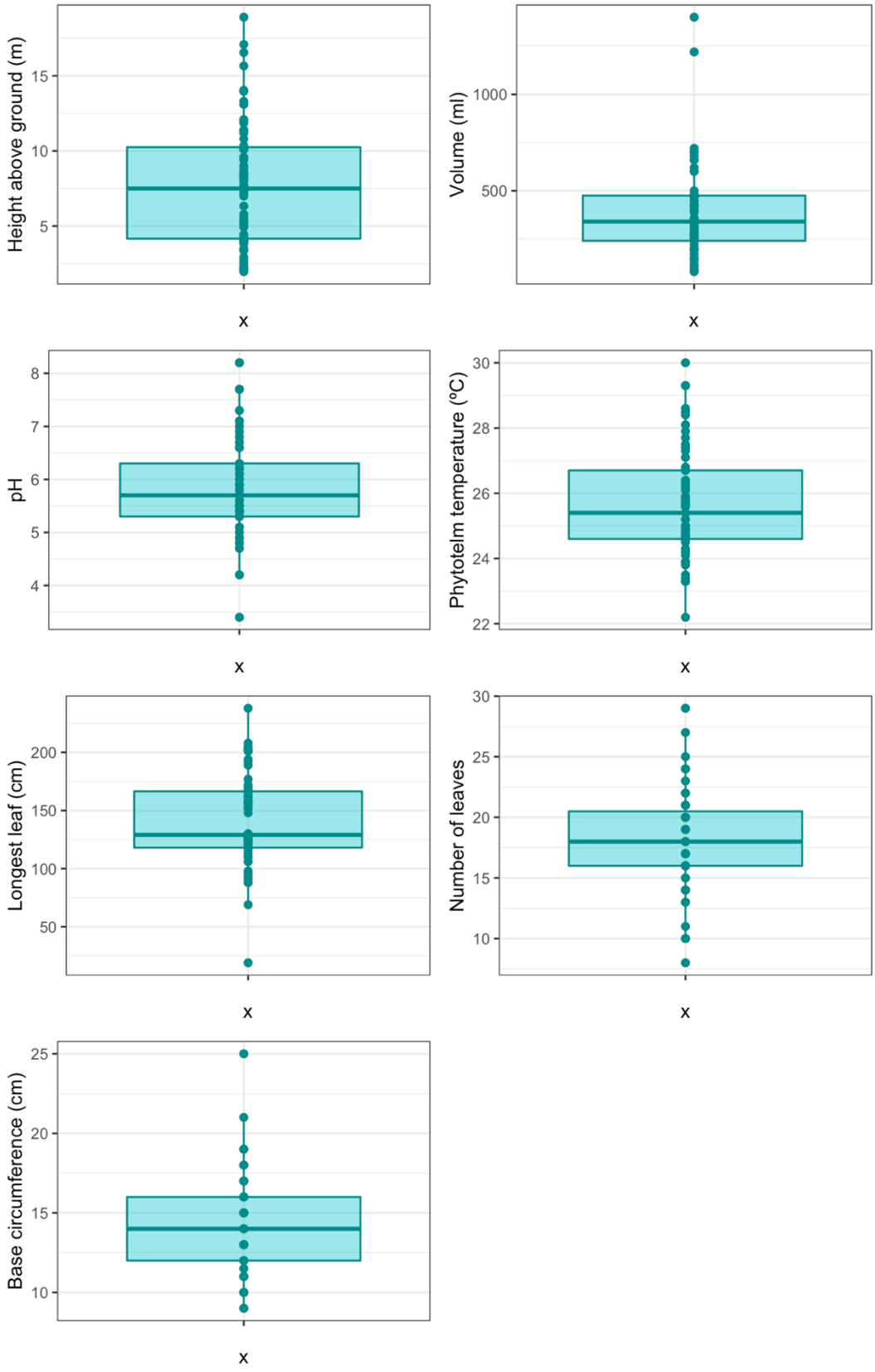


Figure SI-1. Distributions of the measured continuous variables.

Table SI-1. Invertebrates found in all 63 bromeliads in Payamino, used in the analyses in Chapter II.

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-01	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	4
X-B-02	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-04	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	14
X-B-06	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	2
X-B-13	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	13
X-B-15	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	4
X-B-24	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	2
X-B-25	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	4
X-B-27	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	16
X-B-29	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-30	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-31	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	2
X-B-34	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	5
X-B-35	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	3
X-B-38	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	15
X-B-39	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	13
X-B-40	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-41	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	5
X-B-42	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-43	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	3
X-B-45	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	2
X-B-47	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-48	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	6
X-B-49	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	3
X-B-50	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-51	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	2
X-B-53	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	1
X-B-55	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	5
X-B-57	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	3
X-B-60	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	4
X-B-64	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	4
X-B-65	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	9
X-B-66	Annelida	Clitellata	Oligochaeta		Olig. sp1		Aqu	14
X-B-13	Arthropoda	Arachnida	Acari		Acari1			1
X-B-64	Arthropoda	Arachnida	Acari		Acari2			1
X-B-64	Arthropoda	Arachnida	Acari		Acari3			1
X-B-43	Arthropoda	Arachnida	Acari		Acari4			6
X-B-19	Arthropoda	Arachnida	Araneae	Barychelidae	Araneae4		Trr	2
X-B-09	Arthropoda	Arachnida	Araneae	Caponiidae	Araneae8		Trr	1
X-B-28	Arthropoda	Arachnida	Araneae	Caponiidae	Araneae8		Trr	1
X-B-53	Arthropoda	Arachnida	Araneae	Cyrtaucheniidae	Araneae26		Trr	1
X-B-49	Arthropoda	Arachnida	Araneae	Dipluridae	Araneae35		Trr	1
X-B-41	Arthropoda	Arachnida	Araneae		Araneae2		Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-20	Arthropoda	Arachnida	Araneae		Araneae18		Trr	1
X-B-08	Arthropoda	Arachnida	Araneae		Araneae20		Trr	1
X-B-20	Arthropoda	Arachnida	Araneae		Araneae22		Trr	1
X-B-41	Arthropoda	Arachnida	Araneae		Araneae25		Trr	1
X-B-21	Arthropoda	Arachnida	Araneae		Araneae27		Trr	1
X-B-41	Arthropoda	Arachnida	Araneae		Araneae28		Trr	1
X-B-33	Arthropoda	Arachnida	Araneae		Araneae29		Trr	1
X-B-53	Arthropoda	Arachnida	Araneae		Araneae30		Trr	1
X-B-59	Arthropoda	Arachnida	Araneae		Araneae31		Trr	1
X-B-49	Arthropoda	Arachnida	Araneae		Araneae32		Trr	1
X-B-48	Arthropoda	Arachnida	Araneae		Araneae3		Trr	1
X-B-61	Arthropoda	Arachnida	Araneae		Araneae33		Trr	1
X-B-11	Arthropoda	Arachnida	Araneae		Araneae34		Trr	1
X-B-63	Arthropoda	Arachnida	Araneae		Araneae36		Trr	1
X-B-42	Arthropoda	Arachnida	Araneae		Araneae37		Trr	1
X-B-33	Arthropoda	Arachnida	Araneae		Araneae38		Trr	1
X-B-43	Arthropoda	Arachnida	Araneae		Araneae39		Trr	1
X-B-48	Arthropoda	Arachnida	Araneae		Araneae39		Trr	1
X-B-62	Arthropoda	Arachnida	Araneae		Araneae39		Trr	1
X-B-63	Arthropoda	Arachnida	Araneae		Araneae39		Trr	1
X-B-19	Arthropoda	Arachnida	Araneae		Araneae5		Trr	1
X-B-43	Arthropoda	Arachnida	Araneae		Araneae9		Trr	5
X-B-19	Arthropoda	Arachnida	Araneae		Araneae6		Trr	1
X-B-11	Arthropoda	Arachnida	Araneae		Araneae9		Trr	3
X-B-21	Arthropoda	Arachnida	Araneae		Araneae9		Trr	1
X-B-25	Arthropoda	Arachnida	Araneae		Araneae9		Trr	1
X-B-28	Arthropoda	Arachnida	Araneae		Araneae10		Trr	1
X-B-23	Arthropoda	Arachnida	Araneae		Araneae13		Trr	1
X-B-65	Arthropoda	Arachnida	Araneae		Araneae13		Trr	1
X-B-08	Arthropoda	Arachnida	Araneae		Araneae15		Trr	1
X-B-35	Arthropoda	Arachnida	Araneae		Araneae16		Trr	1
X-B-28	Arthropoda	Arachnida	Araneae	Salticidae	Araneae7		Trr	1
X-B-14	Arthropoda	Arachnida	Araneae	Salticidae	Araneae11		Trr	1
X-B-11	Arthropoda	Arachnida	Araneae	Salticidae	Araneae17		Trr	1
X-B-27	Arthropoda	Arachnida	Araneae	Salticidae	Araneae17		Trr	1
X-B-45	Arthropoda	Arachnida	Araneae	Salticidae	Araneae24		Trr	1
X-B-65	Arthropoda	Arachnida	Araneae	Salticidae	Araneae24		Trr	1
X-B-66	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae12		Trr	1
X-B-22	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae14		Trr	1
X-B-64	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae14		Trr	1
X-B-08	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae19		Trr	1
X-B-25	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae19		Trr	1
X-B-20	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae21		Trr	1
X-B-54	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae21		Trr	1
X-B-38	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae23		Trr	1
X-B-49	Arthropoda	Arachnida	Araneae	Sparassidae	Araneae40		Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-08	Arthropoda	Arachnida	Opiliones	Cosmetidae	Opiliones3	Mature	Trr	1
X-B-25	Arthropoda	Arachnida	Opiliones	Cosmetidae	Opiliones3	Mature	Trr	1
X-B-53	Arthropoda	Arachnida	Opiliones	Cosmetidae	Opiliones3	Mature	Trr	1
X-B-05	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones2	Mature	Trr	1
X-B-07	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones4	Immature	Trr	1
X-B-41	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones4	Mature	Trr	2
X-B-44	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones4	Mature	Trr	2
X-B-09	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	2
X-B-10	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	4
X-B-22	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	1
X-B-42	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	1
X-B-45	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	1
X-B-46	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	1
X-B-48	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	1
X-B-48	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	1
X-B-50	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	3
X-B-51	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones5	Mature	Trr	2
X-B-43	Arthropoda	Arachnida	Opiliones	Cranaidae	Opiliones9	Mature	Trr	1
X-B-10	Arthropoda	Arachnida	Opiliones		Opiliones1	Immature	Trr	2
X-B-51	Arthropoda	Arachnida	Opiliones		Opiliones1	Mature	Trr	1
X-B-43	Arthropoda	Arachnida	Opiliones	Manaosbiidae	Opiliones7	Mature	Trr	2
X-B-44	Arthropoda	Arachnida	Opiliones	Manaosbiidae	Opiliones7	Immature	Trr	1
X-B-07	Arthropoda	Arachnida	Opiliones	Manaosbiidae	Opiliones8	Immature	Trr	1
X-B-50	Arthropoda	Arachnida	Opiliones	Manaosbiidae	Opiliones8	Mature	Trr	1
X-B-53	Arthropoda	Arachnida	Opiliones	Manaosbiidae	Opiliones8	Immature	Trr	1
X-B-53	Arthropoda	Arachnida	Opiliones	Stygnidae	Opiliones6	Mature	Trr	1
X-B-09	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 1	Adult	Trr	5
X-B-25	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 1	Adult	Trr	2
X-B-31	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 1	Adult	Trr	1
X-B-65	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 1	Adult	Trr	2
X-B-29	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 4	Adult	Trr	1
X-B-33	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 4	Adult	Trr	2
X-B-34	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 4	Adult	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-53	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 4	Adult	Trr	1
X-B-57	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 4	Adult	Trr	1
X-B-60	Arthropoda	Arachnida	Pseudoscorpionida	Chernetidae	Pseudoscorpionida 4	Adult	Trr	1
X-B-28	Arthropoda	Arachnida	Pseudoscorpionida	Chthoniidae	Pseudoscorpionida 2	Adult	Trr	1
X-B-66	Arthropoda	Arachnida	Pseudoscorpionida	Chthoniidae	Pseudoscorpionida 2	Adult	Trr	1
X-B-49	Arthropoda	Arachnida	Pseudoscorpionida	Chthoniidae	Pseudoscorpionida 3	Adult	Trr	1
X-B-09	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	1
X-B-13	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	1
X-B-19	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	1
X-B-30	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	2
X-B-40	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	3
X-B-49	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	1
X-B-54	Arthropoda	Arachnida	Pseudoscorpionida		Pseudoscorpionida 5	Adult	Trr	1
X-B-23	Arthropoda	Arachnida	Schizomida	Hubbardiidae	Schizomida1	Adult	Trr	1
X-B-33	Arthropoda	Arachnida	Schizomida	Hubbardiidae	Schizomida1	Adult	Trr	5
X-B-34	Arthropoda	Arachnida	Schizomida	Hubbardiidae	Schizomida1	Adult	Trr	1
X-B-40	Arthropoda	Arachnida	Schizomida	Hubbardiidae	Schizomida1	Adult	Trr	1
X-B-43	Arthropoda	Arachnida	Schizomida	Hubbardiidae	Schizomida1	Adult	Trr	1
X-B-65	Arthropoda	Arachnida	Schizomida	Hubbardiidae	Schizomida1	Adult	Trr	1
X-B-13	Arthropoda	Arachnida	Scorpionida		Scorpionida1		Trr	1
X-B-33	Arthropoda	Arachnida	Scorpionida		Scorpionida1		Trr	36
X-B-36	Arthropoda	Arachnida	Scorpionida		Scorpionida2		Trr	1
X-B-10	Arthropoda	Arachnida	Scorpionida		Scorpionida3		Trr	1
X-B-09	Arthropoda	Chilopoda	Scolopendromorpha		Scolop2		Trr	1
X-B-34	Arthropoda	Chilopoda	Scolopendromorpha		Scolop2		Trr	37
X-B-49	Arthropoda	Chilopoda	Scolopendromorpha		Scolop2		Trr	1
X-B-30	Arthropoda	Chilopoda	Scolopendromorpha		Scolop3		Trr	1
X-B-42	Arthropoda	Chilopoda	Scolopendromorpha	Scolopocryptopidae	Scolop1		Trr	1
X-B-43	Arthropoda	Diplopoda	Julida		Julida1		Trr	1
X-B-48	Arthropoda	Diplopoda	Polydesmida		Polydesmida5		Trr	1
X-B-05	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-16	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-22	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-30	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-36	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-39	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-42	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-43	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	2
X-B-55	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-61	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-62	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-64	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	3
X-B-66	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida1		Trr	1
X-B-39	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida2		Trr	1
X-B-55	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida3		Trr	1
X-B-28	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida4		Trr	1
X-B-30	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida4		Trr	1
X-B-42	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmida4		Trr	1
X-B-17	Arthropoda	Diplopoda	Spirobolida		Spirobolida1		Trr	1
X-B-31	Arthropoda	Diplopoda	Spirobolida		Spirobolida2		Trr	1
X-B-38	Arthropoda	Diplopoda	Spirobolida		Spirobolida2		Trr	1
X-B-01	Arthropoda	Diplopoda	Stemmiulida		Stemmiulida1		Trr	1
X-B-11	Arthropoda	Diplopoda	Stemmiulida		Stemmiulida1		Trr	2
X-B-64	Arthropoda	Entognatha	Collembola	Entomobryidae		Larva	Aqu	1
X-B-05	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea3	1Mature/ 4Immature	Trr	5
X-B-37	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea5	1Mature/ 2Immature	Trr	3
X-B-38	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea5	Mature	Trr	1
X-B-55	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea5	Immature	Trr	1
X-B-07	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea6	Immature	Trr	1
X-B-08	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea6	Immature	Trr	1
X-B-14	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea6	Immature	Trr	1
X-B-45	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea6	Mature	Trr	1
X-B-45	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea9	Mature	Trr	1
X-B-64	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea10	Mature	Trr	1
X-B-16	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Immature	Trr	1
X-B-19	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Immature	Trr	2
X-B-42	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Immature	Trr	13
X-B-53	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Mature	Trr	1
X-B-63	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Immature	Trr	1
X-B-65	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Immature	Trr	1
X-B-66	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea11	Immature	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-57	Arthropoda	Insecta	Blattodea	Blattellidae	Blattodea12	5Immature/1Mature	Trr	6
X-B-09	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea4	Mature	Trr	1
X-B-11	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea7	Mature	Trr	1
X-B-09	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-13	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	6
X-B-17	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	2
X-B-22	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-27	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	5
X-B-28	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	10
X-B-30	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-36	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-42	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-44	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-48	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	2
X-B-56	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-63	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	3Immature/1Mature	Trr	4
X-B-65	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea8	Immature	Trr	1
X-B-43	Arthropoda	Insecta	Blattodea	Blattidae	Blattodea13	Immature	Trr	2
X-B-13	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	11
X-B-20	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	1
X-B-31	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	1
X-B-44	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	1
X-B-48	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	3
X-B-53	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	1
X-B-55	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	1
X-B-62	Arthropoda	Insecta	Blattodea		Blattodea2		Trr	3
X-B-55	Arthropoda	Insecta	Blattodea		Blattodea14	Immature	Trr	1
X-B-35	Arthropoda	Insecta	Blattodea		Blattodea15	Immature	Trr	5
X-B-54	Arthropoda	Insecta	Blattodea		Blattodea15	Immature	Trr	1
X-B-58	Arthropoda	Insecta	Blattodea		Blattodea15	Immature	Trr	2
X-B-28	Arthropoda	Insecta	Blattodea		Blattodea16	Adult	Trr	1
X-B-22	Arthropoda	Insecta	Blattodea		Blattodea17	Immature	Trr	2
X-B-07	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-09	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-13	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-22	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	3
X-B-25	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-27	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-30	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-36	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	3
X-B-37	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-41	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-47	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-48	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	2
X-B-49	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	3
X-B-50	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	1
X-B-55	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	2
X-B-63	Arthropoda	Insecta	Blattodea		Blattodea19		Trr	2
X-B-15	Arthropoda	Insecta	Blattodea	Termitidae	Blattodea18	Mature	Trr	1
X-B-13	Arthropoda	Insecta	Coleoptera	Dytiscidae		Larva	Aqu	1
X-B-55	Arthropoda	Insecta	Coleoptera	Dytiscidae		Larva	Aqu	2
X-B-09	Arthropoda	Insecta	Coleoptera	Elmidae		Larva	Aqu	1
X-B-12	Arthropoda	Insecta	Coleoptera	Elmidae		Larva	Aqu	3
X-B-13	Arthropoda	Insecta	Coleoptera	Elmidae		Larva	Aqu	2
X-B-53	Arthropoda	Insecta	Coleoptera	Heterocerida e		Larva	Aqu	1
X-B-29	Arthropoda	Insecta	Coleoptera	Hydrophilidae		Adult	Aqu	1
X-B-43	Arthropoda	Insecta	Coleoptera	Hydrophilidae		Adult	Aqu	9
X-B-05	Arthropoda	Insecta	Coleoptera	Hydrophilidae		Larva +Adult	Aqu	2
X-B-12	Arthropoda	Insecta	Coleoptera	Hydrophilidae		Larva +Adult	Aqu	2
X-B-25	Arthropoda	Insecta	Coleoptera	Hydrophilidae		Larva +Adult	Aqu	4
X-B-30	Arthropoda	Insecta	Coleoptera	Hydrophilidae		Larva	Aqu	1
X-B-62	Arthropoda	Insecta	Coleoptera	Hydroscaphid ae		Larva	Aqu	1
X-B-28	Arthropoda	Insecta	Coleoptera	Psephenidae		Larva	Aqu	1
X-B-06	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	1
X-B-09	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	3
X-B-13	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	1
X-B-14	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	1
X-B-42	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	1
X-B-48	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	2
X-B-53	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	3
X-B-60	Arthropoda	Insecta	Coleoptera	Ptilodactylida e		Larva	Aqu	1
X-B-07	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-08	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	4
X-B-10	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-30	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	11

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-46	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-47	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-10	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-25	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	5
X-B-29	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-37	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-35	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-01	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	26
X-B-04	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	9
X-B-05	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	11
X-B-06	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	21
X-B-07	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	4
X-B-08	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	30
X-B-09	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	13
X-B-10	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	9
X-B-13	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	9
X-B-14	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	5
X-B-15	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	15
X-B-19	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	11
X-B-20	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-22	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	19
X-B-25	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-28	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-29	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	28
X-B-35	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	3
X-B-36	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-37	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	33
X-B-45	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	14
X-B-46	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	17
X-B-47	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	10
X-B-49	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	107
X-B-55	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	11
X-B-23	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-26	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	3
X-B-27	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	76
X-B-07	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-09	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	7
X-B-11	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	4
X-B-12	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	4
X-B-13	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	7
X-B-14	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	7
X-B-15	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	9
X-B-17	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	27
X-B-21	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	4
X-B-23	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-24	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-26	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	17
X-B-27	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	43
X-B-31	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	18
X-B-32	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	7
X-B-33	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-35	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	8
X-B-38	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	13
X-B-40	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	26
X-B-43	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	13
X-B-44	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	32
X-B-50	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-57	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	29
X-B-58	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	21
X-B-61	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	54
X-B-62	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	13
X-B-63	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	88
X-B-26	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	1
X-B-27	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	14
X-B-32	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	19
X-B-34	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-35	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-45	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	4
X-B-55	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-60	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	2
X-B-63	Arthropoda	Insecta	Coleoptera	Scirtidae		Larva	Aqu	12
X-B-09	Arthropoda	Insecta	Coleoptera	Cantharidae	Coleoptera3	Larva	Trr	4
X-B-10	Arthropoda	Insecta	Coleoptera	Cantharidae	Coleoptera3	Larva	Trr	1
X-B-33	Arthropoda	Insecta	Coleoptera	Cantharidae	Coleoptera3	Larva	Trr	1
X-B-09	Arthropoda	Insecta	Coleoptera	Carabidae	Coleoptera11	Adult	Trr	1
X-B-27	Arthropoda	Insecta	Coleoptera	Carabidae	Coleoptera15	Adult	Trr	1
X-B-59	Arthropoda	Insecta	Coleoptera	Carabidae	Coleoptera40	Adult	Trr	2
X-B-13	Arthropoda	Insecta	Coleoptera	Carabidae	Coleoptera41	Adult	Trr	1
X-B-42	Arthropoda	Insecta	Coleoptera	Ceratocanthidae	Coleoptera26	Larva	Trr	1
X-B-10	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Coleoptera9	Adult	Trr	1
X-B-28	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Coleoptera9	Adult	Trr	3
X-B-42	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Coleoptera9	Adult	Trr	1
X-B-01	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera1	Adult	Aqu-Trr	2
X-B-39	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera1	Adult	Aqu-Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-13	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera12	Adult	Aqu-Trr	1
X-B-30	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera6	Adult	Aqu-Trr	1
X-B-53	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera7	Adult	Aqu-Trr	4
X-B-28	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera8	Adult	Aqu-Trr	1
X-B-64	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera18	Adult	Aqu-Trr	1
X-B-28	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera19	Adult	Aqu-Trr	1
X-B-45	Arthropoda	Insecta	Coleoptera	Curculionidae	Coleoptera25	Larva	Aqu-Trr	1
X-B-09	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera21	Larva	Trr	3
X-B-23	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera21	Larva	Trr	1
X-B-25	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera21	Larva	Trr	2
X-B-33	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera21	Larva	Trr	1
X-B-06	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera23	Larva	Trr	1
X-B-15	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera23	Larva	Trr	2
X-B-17	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera23	Larva	Trr	2
X-B-12	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	2
X-B-13	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	1
X-B-23	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	1
X-B-33	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	1
X-B-39	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	1
X-B-55	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	1
X-B-66	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera24	Larva	Trr	4
X-B-22	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	4
X-B-25	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	2
X-B-27	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-28	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	4
X-B-29	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-33	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-39	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-40	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-45	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-46	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	2
X-B-66	Arthropoda	Insecta	Coleoptera	Elateridae	Coleoptera20	Larva	Trr	1
X-B-48	Arthropoda	Insecta	Coleoptera	Lampyridae	Coleoptera34	Adult	Trr	1
X-B-16	Arthropoda	Insecta	Coleoptera	Lampyridae	Coleoptera35	Larva	Aqu-Trr	1
X-B-57	Arthropoda	Insecta	Coleoptera	Lampyridae	Coleoptera36	Larva	Aqu-Trr	1
X-B-44	Arthropoda	Insecta	Coleoptera	Melolonthidae	Coleoptera22	Adult	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-05	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera2	Adult	Trr	2
X-B-46	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera2	Adult	Trr	1
X-B-06	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera4	Larva	Trr	1
X-B-27	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera5	Larva	Trr	1
X-B-36	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera33	Larva	Trr	1
X-B-45	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera33	Larva	Trr	1
X-B-53	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera33	Larva	Trr	1
X-B-60	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera33	Larva	Trr	1
X-B-64	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Coleoptera33	Larva	Trr	1
X-B-36	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera31	Adult	Trr	6
X-B-54	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera31	Adult	Trr	1
X-B-36	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera29	Larva	Trr	2
X-B-01	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	1
X-B-11	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	3
X-B-13	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	1
X-B-20	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	4
X-B-41	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	1
X-B-45	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	1
X-B-48	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	14
X-B-49	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	2
X-B-51	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	2
X-B-61	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	4
X-B-64	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera13	Adult	Trr	1
X-B-39	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera28	Adult	Trr	1
X-B-43	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera39	Adult	Trr	1
X-B-48	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera14	Adult	Trr	3
X-B-06	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera17	Adult	Trr	1
X-B-13	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera27	Adult	Trr	1
X-B-63	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera27	Adult	Trr	1
X-B-28	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera30	Adult	Trr	1
X-B-48	Arthropoda	Insecta	Coleoptera	Staphylinidae	Coleoptera30	Adult	Trr	1
X-B-36	Arthropoda	Insecta	Coleoptera	Tenebrionidae	Coleoptera37	Adult	Trr	1
X-B-07	Arthropoda	Insecta	Coleoptera	Tenebrionidae	Coleoptera38	Adult	Trr	2
X-B-33	Arthropoda	Insecta	Coleoptera	Tenebrionidae	Coleoptera38	Adult	Trr	1
X-B-59	Arthropoda	Insecta	Coleoptera	Tenebrionidae	Coleoptera38	Adult	Trr	2
X-B-32	Arthropoda	Insecta	Dermaptera	Forficulidae	Dermaptera1	Adult	Trr	1
X-B-06	Arthropoda	Insecta	Dermaptera		Dermaptera3	Adult	Trr	1
X-B-13	Arthropoda	Insecta	Dermaptera	Pygidicranidae	Dermaptera2	Adult	Trr	1
X-B-17	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-22	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-37	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-50	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-16	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-44	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-62	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	2
X-B-64	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-44	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-10	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	3
X-B-17	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	2
X-B-26	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-27	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	11
X-B-32	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-37	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-38	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-40	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	9
X-B-43	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	2
X-B-57	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-58	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-60	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-66	Arthropoda	Insecta	Diptera	Ceratopogonidae		Larva	Aqu	1
X-B-65	Arthropoda	Insecta	Diptera	Chironomidae		Larva	Aqu	1
X-B-08	Arthropoda	Insecta	Diptera	Chironomidae		Larva	Aqu	2

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-17	Arthropoda	Insecta	Diptera	Chironomidae		Larva	Aqu	1
X-B-63	Arthropoda	Insecta	Diptera	Chironomidae		Larva	Aqu	1
X-B-36	Arthropoda	Insecta	Diptera	Chironomidae		Larva	Aqu	1
X-B-32	Arthropoda	Insecta	Diptera	Culicidae		Larva	Aqu	1
X-B-25	Arthropoda	Insecta	Diptera	Culicidae		Larva	Aqu	4
X-B-22	Arthropoda	Insecta	Diptera	Culicidae		Larva	Aqu	30
X-B-24	Arthropoda	Insecta	Diptera	Culicidae		Larva	Aqu	5
X-B-08	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-13	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	4
X-B-14	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-15	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-17	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-22	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-27	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-40	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-51	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-61	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	1
X-B-64	Arthropoda	Insecta	Diptera	Dolichopodidae		Larva	Aqu	2
X-B-38	Arthropoda	Insecta	Diptera		Diptera1	Adult		1
X-B-08	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	3
X-B-09	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	1
X-B-10	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	2
X-B-11	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	1
X-B-11	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	1
X-B-21	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	1
X-B-27	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	6
X-B-31	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	1
X-B-43	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	3
X-B-09	Arthropoda	Insecta	Diptera	Psychodidae		Larva	Aqu	1
X-B-08	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1
X-B-35	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	2
X-B-15	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-17	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1
X-B-21	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1
X-B-24	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	2
X-B-44	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1
X-B-57	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	2
X-B-60	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1
X-B-62	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	1
X-B-43	Arthropoda	Insecta	Diptera	Stratiomyidae		Larva	Aqu	6
X-B-07	Arthropoda	Insecta	Diptera	Syrphidae		Larva	Aqu	1
X-B-01	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-04	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	1
X-B-06	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	11
X-B-08	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	1
X-B-09	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	12
X-B-10	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-11	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	2
X-B-14	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	7
X-B-17	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-21	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	11
X-B-22	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	6
X-B-24	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	2
X-B-26	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-29	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	2
X-B-30	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	1
X-B-38	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	2
X-B-41	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	2
X-B-43	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	6

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-44	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	4
X-B-45	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	20
X-B-46	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-47	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-49	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	4
X-B-55	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	1
X-B-61	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	3
X-B-63	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	6
X-B-66	Arthropoda	Insecta	Diptera	Tipulidae	Diptera2	Larva	Aqu-Trr	2
X-B-61	Arthropoda	Insecta	Diptera	Tipulidae	Diptera3	Larva	Aqu-Trr	1
X-B-38	Arthropoda	Insecta	Diptera	Tipulidae	Diptera4	Larva	Aqu-Trr	1
X-B-45	Arthropoda	Insecta	Diptera	Tipulidae	Diptera4	Larva	Aqu-Trr	1
X-B-10	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	5
X-B-15	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-19	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-20	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-26	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-27	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	5
X-B-30	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-32	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-33	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-38	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-40	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-48	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	5
X-B-53	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	10
X-B-55	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-58	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	2
X-B-60	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-64	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	2
X-B-65	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	3
X-B-58	Arthropoda	Insecta	Diptera	Tipulidae		Larva	Aqu	1
X-B-09	Arthropoda	Insecta	Hemiptera		Hemiptera2	Nymph	Trr	1
X-B-11	Arthropoda	Insecta	Hemiptera		Hemiptera2	Mature	Trr	2
X-B-40	Arthropoda	Insecta	Hemiptera		Hemiptera4	Mature	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-27	Arthropoda	Insecta	Hemiptera		Hemiptera5		Trr	4
X-B-34	Arthropoda	Insecta	Hemiptera		Hemiptera5		Trr	1
X-B-40	Arthropoda	Insecta	Hemiptera		Hemiptera5		Trr	1
X-B-61	Arthropoda	Insecta	Hemiptera		Hemiptera5		Trr	1
X-B-38	Arthropoda	Insecta	Hemiptera		Hemiptera8		Trr	1
X-B-55	Arthropoda	Insecta	Hemiptera		Hemiptera9		Trr	1
X-B-13	Arthropoda	Insecta	Hemiptera		Hemiptera10		Trr	1
X-B-09	Arthropoda	Insecta	Hemiptera	Lygaeidae	Hemiptera1	Nymph	Trr	7
X-B-40	Arthropoda	Insecta	Hemiptera	Lygaeidae	Hemiptera1	Nymph	Trr	4
X-B-40	Arthropoda	Insecta	Hemiptera	Lygaeidae	Hemiptera3	Nymph	Trr	2
X-B-54	Arthropoda	Insecta	Hemiptera	Macroveliida e	Hemiptera7		Aqu-Trr	1
X-B-55	Arthropoda	Insecta	Hemiptera	Macroveliida e	Hemiptera7		Aqu-Trr	1
X-B-07	Arthropoda	Insecta	Hemiptera	Veliidae	Hemiptera6		Aqu-Trr	1
X-B-22	Arthropoda	Insecta	Hemiptera	Veliidae	Hemiptera6		Aqu-Trr	1
X-B-32	Arthropoda	Insecta	Hemiptera	Veliidae	Hemiptera6		Aqu-Trr	2
X-B-38	Arthropoda	Insecta	Hemiptera	Veliidae	Hemiptera6		Aqu-Trr	1
X-B-55	Arthropoda	Insecta	Hemiptera	Veliidae	Hemiptera6		Aqu-Trr	3
X-B-07	Arthropoda	Insecta	Hymenoptera	Braconidae	Hymenoptera1		Trr	1
X-B-44	Arthropoda	Insecta	Hymenoptera	Formicidae	Prionopelta_sp1	Mature	Trr	2
X-B-61	Arthropoda	Insecta	Hymenoptera	Formicidae	Prionopelta_sp1	Mature	Trr	9
X-B-62	Arthropoda	Insecta	Hymenoptera	Formicidae	Prionopelta_sp1	Mature	Trr	1
X-B-64	Arthropoda	Insecta	Hymenoptera	Formicidae	Prionopelta_sp1	Mature	Trr	2
X-B-36	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp1	Mature	Trr	1
X-B-40	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp1	Mature	Trr	1
X-B-45	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp1	Mature	Trr	18
X-B-05	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp2	Immature	Trr	38
X-B-14	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp2	Mature	Trr	12
X-B-46	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp3	Mature	Trr	1
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp4	Mature	Trr	1
X-B-51	Arthropoda	Insecta	Hymenoptera	Formicidae	Azteca_sp4	Mature	Trr	1
X-B-34	Arthropoda	Insecta	Hymenoptera	Formicidae	Brachymyrmex_sp 1	Mature	Trr	2
X-B-39	Arthropoda	Insecta	Hymenoptera	Formicidae	Brachymyrmex_sp 1	Mature	Trr	1
X-B-55	Arthropoda	Insecta	Hymenoptera	Formicidae	Brachymyrmex_sp 1	Mature	Trr	13
X-B-64	Arthropoda	Insecta	Hymenoptera	Formicidae	Brachymyrmex_sp 2	Mature	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-13	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_cf_mucronatus	Mature	Trr	1
X-B-09	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp1	Mature	Trr	4
X-B-29	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp1	Mature	Trr	1
X-B-46	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp1	Mature	Trr	5
X-B-20	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp2	Mature	Trr	11
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp2	Immature	Trr	84
X-B-29	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp3	Mature	Trr	1
X-B-29	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp4	Mature	Trr	1
X-B-13	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp5	Mature	Trr	4
X-B-46	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp5	Mature	Trr	14
X-B-48	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp6	Mature	Trr	2
X-B-49	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus_sp6	Mature	Trr	6
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Gigantiops_destructor	Mature	Trr	21
X-B-05	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Immature	Trr	86
X-B-12	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	5
X-B-13	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	9
X-B-37	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	31
X-B-49	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	2
X-B-51	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	7
X-B-61	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	32
X-B-62	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Mature	Trr	1
X-B-66	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp1	Immature	Trr	1
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp2	Mature	Trr	13
X-B-57	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp2	Mature	Trr	1
X-B-58	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp2	Mature	Trr	7
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp3	Immature	Trr	68
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp3	Immature	Trr	93
X-B-30	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp4	Mature	Trr	11
X-B-41	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp4	Mature	Trr	1
X-B-43	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp5	Immature	Trr	15
X-B-63	Arthropoda	Insecta	Hymenoptera	Formicidae	Nylanderia_sp5	Immature	Trr	121
X-B-05	Arthropoda	Insecta	Hymenoptera	Formicidae	Monomorium_floricola	Mature	Trr	1
X-B-13	Arthropoda	Insecta	Hymenoptera	Formicidae	Monomorium_floricola	Mature	Trr	1
X-B-63	Arthropoda	Insecta	Hymenoptera	Formicidae	Acromyrmex_sp1	Immature	Trr	999
X-B-44	Arthropoda	Insecta	Hymenoptera	Formicidae	Acromyrmex_sp2	Mature	Trr	3
X-B-45	Arthropoda	Insecta	Hymenoptera	Formicidae	Apterostigma_sp1	Mature	Trr	13
X-B-58	Arthropoda	Insecta	Hymenoptera	Formicidae	Apterostigma_sp2	Mature	Trr	2
X-B-35	Arthropoda	Insecta	Hymenoptera	Formicidae	Cephalotes_sp1	Mature	Trr	1
X-B-34	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp1	Mature	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-30	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 2	Mature	Trr	55
X-B-44	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 2	Mature	Trr	22
X-B-06	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	13
X-B-13	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	6
X-B-14	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	1
X-B-43	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	32
X-B-48	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	3
X-B-50	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	1
X-B-55	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 3	Mature	Trr	1
X-B-11	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 4	Mature	Trr	569
X-B-49	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 4	Mature	Trr	260
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 5	Mature	Trr	9
X-B-63	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 6	Mature	Trr	8
X-B-25	Arthropoda	Insecta	Hymenoptera	Formicidae	Crematogaster_sp 7	Mature	Trr	1
X-B-04	Arthropoda	Insecta	Hymenoptera	Formicidae	Myrmicinae_sp1	Mature	Trr	1
X-B-08	Arthropoda	Insecta	Hymenoptera	Formicidae	Nesomyrmex_sp1	Mature	Trr	1
X-B-08	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	3
X-B-10	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	6
X-B-20	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-22	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	2
X-B-25	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Immature	Trr	13
X-B-30	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-37	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	2
X-B-43	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	2
X-B-44	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-48	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-49	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-61	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1
X-B-62	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	14
X-B-63	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp1	Mature	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-09	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp2	Immature	Trr	145
X-B-32	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp3	Mature	Trr	295
X-B-16	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp4	Mature	Trr	5
X-B-20	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp4	Mature	Trr	1
X-B-21	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp5	Mature	Trr	5
X-B-23	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp5	Mature	Trr	1
X-B-60	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp5	Mature	Trr	3
X-B-53	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp6	Mature	Trr	31
X-B-34	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp7	Mature	Trr	18
X-B-35	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp7	Mature	Trr	1
X-B-56	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp7	Mature	Trr	2
X-B-61	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp7	Mature	Trr	2
X-B-63	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp8	Mature	Trr	15
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp9	Mature	Trr	5
X-B-64	Arthropoda	Insecta	Hymenoptera	Formicidae	Pheidole_sp9	Mature	Trr	82
X-B-14	Arthropoda	Insecta	Hymenoptera	Formicidae	Solenopsis_sp1	Mature	Trr	2
X-B-34	Arthropoda	Insecta	Hymenoptera	Formicidae	Solenopsis_sp1	Mature	Trr	1
X-B-64	Arthropoda	Insecta	Hymenoptera	Formicidae	Solenopsis_sp1	Mature	Trr	4
X-B-48	Arthropoda	Insecta	Hymenoptera	Formicidae	Solenopsis_sp2	Mature	Trr	1
X-B-08	Arthropoda	Insecta	Hymenoptera	Formicidae	Solenopsis_sp3	Mature	Trr	2
X-B-37	Arthropoda	Insecta	Hymenoptera	Formicidae	Solenopsis_sp3	Mature	Trr	32
X-B-07	Arthropoda	Insecta	Hymenoptera	Formicidae	Strumigenys_sp1	Mature	Trr	17
X-B-10	Arthropoda	Insecta	Hymenoptera	Formicidae	Strumigenys_sp1	Mature	Trr	1
X-B-41	Arthropoda	Insecta	Hymenoptera	Formicidae	Strumigenys_sp2	Mature	Trr	3
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Strumigenys_sp3	Mature	Trr	1
X-B-27	Arthropoda	Insecta	Hymenoptera	Formicidae	Tetramorium_sp1	Mature	Trr	1
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Tetramorium_sp1	Mature	Trr	1
X-B-49	Arthropoda	Insecta	Hymenoptera	Formicidae	Tetramorium_sp1	Mature	Trr	1
X-B-38	Arthropoda	Insecta	Hymenoptera	Formicidae	Wasmannia_sp1	Mature	Trr	1
X-B-42	Arthropoda	Insecta	Hymenoptera	Formicidae	Anochetus_sp1	Mature	Trr	3
X-B-07	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Immature	Trr	134
X-B-09	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Mature	Trr	3
X-B-30	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Mature	Trr	1
X-B-34	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Mature	Trr	3
X-B-36	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Mature	Trr	131
X-B-55	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Mature	Trr	51
X-B-56	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp1	Mature	Trr	291

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-01	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 2	Immature	Trr	53
X-B-54	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 2	Mature	Trr	27
X-B-57	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 2	Immature	Trr	129
X-B-64	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 2	Mature	Trr	1
X-B-37	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 3	Mature	Trr	1
X-B-39	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 3	Mature	Trr	1
X-B-27	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 4	Mature	Trr	11
X-B-31	Arthropoda	Insecta	Hymenoptera	Formicidae	Gnamptogenys_sp 5	Mature	Trr	4
X-B-36	Arthropoda	Insecta	Hymenoptera	Formicidae	Hypoponera_sp1	Mature	Trr	1
X-B-45	Arthropoda	Insecta	Hymenoptera	Formicidae	Hypoponera_sp1	Mature	Trr	1
X-B-63	Arthropoda	Insecta	Hymenoptera	Formicidae	Hypoponera_sp1	Mature	Trr	3
X-B-51	Arthropoda	Insecta	Hymenoptera	Formicidae	Hypoponera_sp2	Mature	Trr	1
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Hypoponera_sp3	Mature	Trr	2
X-B-28	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp1	Mature	Trr	17
X-B-29	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp1	Mature	Trr	1
X-B-42	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp2	Mature	Trr	1
X-B-06	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp3	Mature	Trr	1
X-B-27	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp4	Mature	Trr	1
X-B-36	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp4	Mature	Trr	1
X-B-57	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp4	Mature	Trr	1
X-B-22	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponera_sp5	Mature	Trr	1
X-B-35	Arthropoda	Insecta	Hymenoptera	Formicidae	Neoponeras_sp6	Mature	Trr	1
X-B-25	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_hastatus	Mature	Trr	1
X-B-27	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_hastatus	Mature	Trr	1
X-B-29	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_hastatus	Mature	Trr	8
X-B-31	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_hastatus	Mature	Trr	208
X-B-34	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_hastatus	Immature	Trr	274
X-B-59	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_hastatus	Mature	Trr	28
X-B-02	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 1	Mature	Trr	1
X-B-22	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 1	Mature	Trr	1

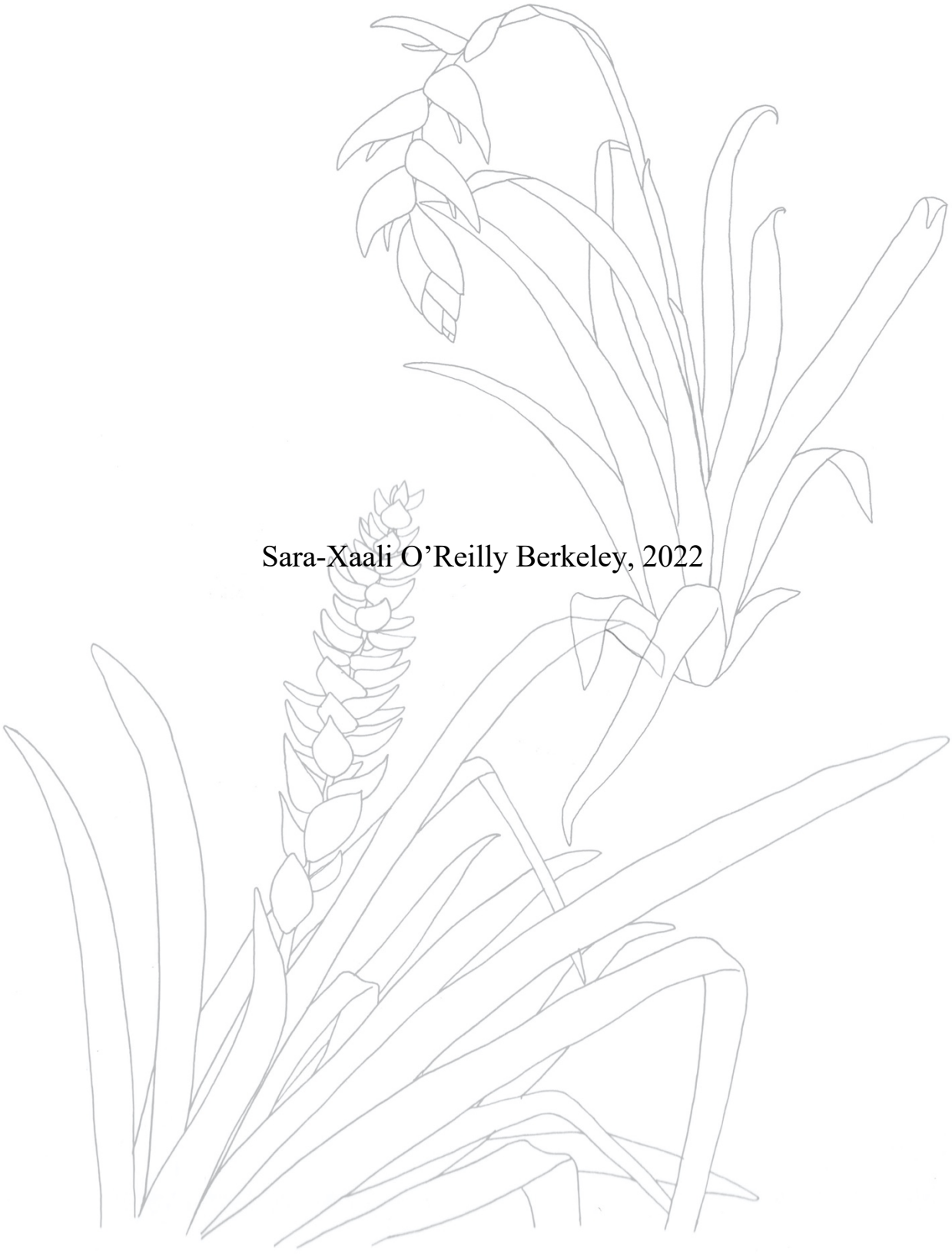
BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-10	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 2	Mature	Trr	1
X-B-20	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 3	Mature	Trr	2
X-B-21	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 3	Mature	Trr	1
X-B-24	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 3	Mature	Trr	17
X-B-32	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 3	Mature	Trr	3
X-B-33	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 3	Mature	Trr	2
X-B-45	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 3	Mature	Trr	3
X-B-36	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 4	Mature	Trr	1
X-B-62	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 4	Mature	Trr	12
X-B-58	Arthropoda	Insecta	Hymenoptera	Formicidae	Odontomachus_sp 5	Mature	Trr	2
X-B-30	Arthropoda	Insecta	Hymenoptera	Formicidae	Pachycondyla_sp1	Mature	Trr	2
X-B-36	Arthropoda	Insecta	Hymenoptera	Formicidae	Pseudomyrmex_sp 1	Mature	Trr	1
X-B-31	Arthropoda	Insecta	Hymenoptera		Hymenoptera2		Trr	1
X-B-65	Arthropoda	Insecta	Lepidoptera	Acrolophidae	Lepidoptera12	Larva	Trr	1
X-B-50	Arthropoda	Insecta	Lepidoptera	Arctiidae	Lepidoptera2	Larva	Trr	1
X-B-49	Arthropoda	Insecta	Lepidoptera	Arctiidae	Lepidoptera3	Larva	Trr	1
X-B-27	Arthropoda	Insecta	Lepidoptera	Choreutidae	Lepidoptera9	Larva	Trr	1
X-B-47	Arthropoda	Insecta	Lepidoptera	Choreutidae	Lepidoptera10	Larva	Trr	1
X-B-59	Arthropoda	Insecta	Lepidoptera	Choreutidae	Lepidoptera11	Larva	Trr	1
X-B-02	Arthropoda	Insecta	Lepidoptera	Hesperridae	Lepidoptera1	Larva	Trr	1
X-B-04	Arthropoda	Insecta	Lepidoptera	Limacodidae	Lepidoptera4	Larva	Trr	1
X-B-10	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera5	Larva	Aqu-Trr	1
X-B-33	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera5	Larva	Aqu-Trr	2
X-B-06	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-09	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-16	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	2
X-B-17	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-36	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-43	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-46	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-47	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-57	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-63	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-66	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera6	Larva	Aqu-Trr	1
X-B-09	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera7	Larva	Aqu-Trr	1
X-B-25	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera7	Larva	Aqu-Trr	1
X-B-63	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera7	Larva	Aqu-Trr	2
X-B-24	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera8	Larva	Aqu-Trr	1
X-B-39	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera8	Larva	Aqu-Trr	1
X-B-60	Arthropoda	Insecta	Lepidoptera	Noctuidae	Lepidoptera8	Larva	Aqu-Trr	1
X-B-65	Arthropoda	Insecta	Lepidoptera	Nymphalidae	Lepidoptera13	Larva	Trr	1
X-B-27	Arthropoda	Insecta	Lepidoptera	Pyralidae		Larva	Aqu	1
X-B-64	Arthropoda	Insecta	Lepidoptera	Pyralidae		Larva	Aqu	1
X-B-44	Arthropoda	Insecta	Lepidoptera	Pyralidae		Larva	Aqu	1
X-B-46	Arthropoda	Insecta	Odonata	Coenagrionidae		Larva	Aqu	2
X-B-55	Arthropoda	Insecta	Odonata	Coenagrionidae		Larva	Aqu	3
X-B-25	Arthropoda	Insecta	Orthoptera	Gryllidae	Orthoptera5		Trr	2
X-B-43	Arthropoda	Insecta	Orthoptera	Tetrigidae	Orthoptera4		**Trr**	1
X-B-33	Arthropoda	Insecta	Orthoptera	Tettigoniidae	Orthoptera2		Trr	1
X-B-12	Arthropoda	Insecta	Orthoptera	Tettigoniidae	Orthoptera3		Trr	1
X-B-01	Arthropoda	Insecta	Orthoptera	Tettigoniidae	Orthoptera1	Mature	Trr	1
X-B-11	Arthropoda	Insecta	Phasmatodea		Phasmatodea1	Adult	Trr	1
X-B-48	Arthropoda	Insecta	Trichoptera	Glossosomatidae		Larva	Aqu	1
X-B-02	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-04	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-05	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-06	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-07	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	4
X-B-08	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	7

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-09	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	6
X-B-10	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-12	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-13	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-14	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-16	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-17	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-19	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-23	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-24	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-25	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-26	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-27	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	13
X-B-29	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-30	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	4
X-B-32	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-34	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-35	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-36	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	16
X-B-37	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	6
X-B-38	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	40
X-B-39	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	5
X-B-41	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-42	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-43	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	3
X-B-45	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	6
X-B-46	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-47	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-48	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-49	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult(12)/ Immature(4)	Trr	16
X-B-51	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	4
X-B-53	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-54	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	2
X-B-55	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	7
X-B-56	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	6
X-B-57	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult(2)/I mmature(6)	Trr	8
X-B-58	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	1
X-B-60	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	4
X-B-64	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	14
X-B-66	Arthropoda	Malacostraca	Isopoda	Armadillidae	Isopoda3	Adult	Trr	9
X-B-01	Arthropoda	Malacostraca	Isopoda		Isopoda1	Adult	Trr	1
X-B-06	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-11	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	12
X-B-25	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	1
X-B-27	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	5
X-B-30	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	2
X-B-36	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	3
X-B-37	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	1
X-B-38	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	3
X-B-39	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	15
X-B-40	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	1
X-B-53	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	2
X-B-54	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	4
X-B-55	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	11
X-B-57	Arthropoda	Malacostraca	Isopoda		Isopoda4	Adult	Trr	1
X-B-43	Arthropoda	Malacostraca	Isopoda		Isopoda5	Adult	Trr	1
X-B-01	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-07	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-09	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-11	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	3
X-B-13	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	16
X-B-20	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-22	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	4
X-B-23	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	10
X-B-27	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	2
X-B-28	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-31	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-32	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-36	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	2
X-B-39	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-43	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	4
X-B-48	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	19
X-B-54	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	2
X-B-55	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	2
X-B-56	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-57	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	5
X-B-60	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	2
X-B-65	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	3
X-B-66	Arthropoda	Malacostraca	Isopoda	Philosciidae	Isopoda2	Adult	Trr	1
X-B-49	Mollusca	Gasteropoda	Basommatophora	Planorbidae			Aqu	2
X-B-64	Mollusca	Gasteropoda	Basommatophora	Planorbidae			Aqu	1
X-B-65	Mollusca	Gasteropoda	Basommatophora	Planorbidae			Aqu	1
X-B-29	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	Tricladida1		Trr	1
X-B-35	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	Tricladida1		Trr	3
X-B-41	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	Tricladida1		Trr	1

BROM.	PHYLUM	CLASS	ORDER	FAMILY	MORPHOSPECIES	STAGE	HABIT	Nº
X-B-42	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	Tricladida1		Trr	1
X-B-38	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	Tricladida3		Trr	1
X-B-54	Platyhelminthes	Rhabditophora	Tricladida	Geoplanidae	Tricladida3		Trr	1
X-B-41	Platyhelminthes	Rhabditophora	Tricladida		Tricladida2		Trr	1



Sara-Xaali O’Reilly Berkeley, 2022