Experimental eco-evolutionary dynamics in floating aquatic plants

Mark Davidson Jewell

Department of Biology
McGill University, Montreal
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Spring pond deep and wide

Time for the vessel's return

Slow the duckweed flows together

Willows draw them apart again

-Wang Wei, 8C CE

Thesis abstract

Communities of organisms that experience a change in environmental conditions must modify their phenotypes in order to persist. The matching of phenotypes to the environment can be driven by physiological plasticity within individuals, by genetic evolutionary change within populations, or by ecological shifts in species composition within communities. However, how these three nested scales of phenotypic change combine is not well understood. To address this question, I use communities of small free-floating aquatic plants, dominated by species within the family *Lemnaceae*, focusing on *Lemna minor*, the common duckweed. The highly reduced morphology and rapid reproduction of *Lemnaceae* make these plants well-suited to highly replicated, multi-generational experimental manipulation, a powerful tool for hypothesis testing and identifying underlying mechanisms of phenotypic change. I investigate the drivers of change in simple morphological traits: frond area and root length, akin to the root-shoot ratio in land plants, in response to resource availability. I explore this question experimentally across multiple scales, from controlled laboratory microcosms to semi-natural mesocosms to natural field surveys, with the plant microbiome removed and intact, and in single-species populations to full multi-species communities.

I observed strong phenotypic plasticity in *Lemna minor* in both a controlled laboratory experiment, and in a regional survey of natural populations. The most plastic trait was root length, which varied widely as a function of nutrient availability. This phenotypic response was in part mediated by the presence of the microbiome which interacted with the environment to drive phenotypic change. The microbiome also systematically suppressed host fitness, across several genotypes and environments, suggesting that for *Lemna minor*, the microbiome is made up primarily of parasites, pathogens, and competitors.

In the field, both frond area and root length were correlated with natural gradients of resource availability, such that plants invested more biomass into the tissue responsible for the uptake of the limiting resource. Although most phenotypic variation I measured in the field was environmental, there were persistent differences in a common garden experiment, suggesting genetic differentiation among populations. In addition to variation among sites, I found

substantial amounts of genetic variation within sites. This was surprising given the low rates of gene flow, mutation and recombination reported in the literature. To better understand the life history of *Lemna minor*, I monitored a natural population over winter using experimental enclosures and found that >90% of plants survived, despite the lack of any specific overwintering structure such as those found in closely related species. The absence of a seasonal genetic bottleneck helps explain the maintenance of the high levels of observed genetic diversity.

In addition to high intraspecific diversity, *Lemna minor* often coexists with other species of *Lemnaceae* in multi-species communities. In a field survey, I found evidence of niche differentiation based on non-random distributions of species in relation to nutrient availability. This finding was supported by an experimental mesocosm experiment designed to partition the relative contributions of deterministic and stochastic ecological processes to changes in community composition. Species sorting was the dominant ecological process responsible for structuring communities, although the balance between sorting and drift was species-specific, within a single community. In a follow-up mesocosm experiment, I integrated these ecological processes with evolutionary and physiological processes and measured their contributions to changes in community mean phenotype after a dozen generations of growth over an environmental gradient. I found that physiological change was idiosyncratic among species and environmental treatments, and overall was not a primary driver of community phenotypic change. In contrast, strong species sorting and natural selection were equally responsible for phenotypic modification, driving changes in frond size. However, these two processes, selection among species (ecological change) and selection with species (evolution), tended to act in opposite directions.

This work helps to illuminate how populations and communities manage to appropriately modify their phenotypes to match environmental conditions. Aspects of this fundamental question are often difficult to test experimentally with higher plants and animals due to practical constraints. This work also highlights the strengths of using *Lemnaceae* as a model system and strongly supports its future development in ecology and evolution.

Résumé de la thèse

Les communautés écologiques qui subissent un changement de conditions environnementales doivent modifier leurs phénotypes afin de persister. L'adéquation des phénotypes avec l'environnement peut être induite par la plasticité physiologique des individus, par des changements génétiques évolutifs au sein des populations, ou par des changements écologiques dans la composition des communautés. Cependant, la façon dont ces trois échelles imbriquées de changement phénotypique se combinent n'est pas bien comprise. Pour aborder cette question, j'utilise des communautés de petites plantes aquatiques flottantes, surtout de la famille des *Lemnaceae*, en me concentrant sur *Lemna minor*, la lentille d'eau commune. La morphologie très réduite et la reproduction rapide des *Lemnaceae* se prêtent à des manipulations expérimentales hautement répliquées et multigénérationnelles, constituant un outil puissant pour tester des hypothèses et identifier les mécanismes sous-jacents. J'étudie les mécanismes de changement des traits morphologiques simples : la taille des frondes et la longueur des racines, semblables au ratio racine-pousse chez les plantes terrestres, en réponse à la disponibilité des ressources. J'explore cette question de manière expérimentale à plusieurs échelles, des microcosmes de laboratoire contrôlés aux mésocosmes semi-naturels, en passant par les observations sur le terrain, avec le microbiome végétal retiré ou intact, en monocultures comme en mélanges de toutes les espèces.

J'ai observé une forte plasticité phénotypique chez *Lemna minor* à la fois dans une expérience contrôlée en laboratoire et dans des populations naturelles. Le trait le plus plastique était la longueur des racines, qui variait considérablement en fonction de la disponibilité des nutriments. Cette réponse phénotypique était en partie médiée par la présence du microbiome, qui interagissait avec l'environnement pour générer des changements phénotypiques. Le microbiome a également systématiquement supprimé la valeur adaptative de l'hôte, sur plusieurs génotypes et environnements, ce qui suggère que pour *Lemna minor*, le microbiome est composé principalement de parasites, de pathogènes et de compétiteurs.

Sur le terrain, la taille des frondes et la longueur des racines étaient corrélées aux gradients naturels de disponibilité des ressources, de sorte que les plantes investissaient davantage de

biomasse dans le tissu responsable de l'absorption de la ressource limitante. Bien que la majorité de la variation phénotypique que j'ai mesurée sur le terrain soit d'origine environnementale, des différences persistantes ont été observées dans une expérience de jardin commun, suggérant une différenciation génétique entre les populations. En plus de la variation entre les sites, j'ai trouvé une quantité substantielle de variation génétique au sein des sites. Ceci est surprenant étant donné les faibles taux de flux génétique, de mutation et de recombinaison rapportés dans la littérature. Pour mieux comprendre le cycle de vie de *Lemna minor*, j'ai suivi une population naturelle pendant l'hiver en utilisant des enclos expérimentaux et j'ai constaté que >90% des plantes survivaient, malgré l'absence de toute structure d'hivernage spécifique comme celles que l'on trouve chez des espèces étroitement apparentées. L'absence d'un "goulot d'étranglement génétique" saisonnier contribue à expliquer le maintien des quantités élevées de diversité génétique observées.

En plus d'une grande diversité intraspécifique, *Lemna minor* coexiste souvent avec d'autres espèces de *Lemnaceae* dans des communautés multi-espèces. Lors d'une étude sur le terrain, j'ai trouvé des preuves de différenciation de niche basée sur des distributions non aléatoires des espèces en fonction de la disponibilité des nutriments. Cette constatation a été confirmée par une expérience en mésocosme conçue pour partitionner les contributions relatives des processus écologiques déterministes et stochastiques aux changements dans la composition de la communauté. "Le tri des espèces" (sélection) était le processus écologique dominant responsable de la structuration des communautés, bien que l'équilibre entre "le tri" et dérive soit spécifique à chaque espèce, au sein d'une même communauté. Dans une expérience de suivi en mésocosme, j'ai intégré ces processus écologiques aux processus évolutifs et physiologiques et mesuré leurs contributions aux changements du phénotype moyen de la communauté après une douzaine de générations sur un gradient environnemental. J'ai découvert que les changements physiologiques étaient idiosyncrasiques entre les espèces et les traitements environnementaux et que, dans l'ensemble, ils n'étaient pas le principal moteur des changements phénotypiques de la communauté. En revanche, un fort "tri des espèces" et la sélection naturelle étaient également responsables de la modification phénotypique, entraînant des changements dans la taille des frondes. Cependant, ces deux processus, la sélection entre les espèces (changement écologique)

et la sélection au sein des espèces (évolution), avaient tendance à agir dans des directions opposées.

Ces travaux contribuent à éclairer la manière dont les populations et les communautés parviennent à modifier leurs phénotypes pour s'adapter aux conditions environnementales. Certains aspects de cette question fondamentale sont souvent difficiles à tester expérimentalement avec des plantes et des animaux de grande taille en raison de contraintes pratiques. Ce travail souligne également les avantages des *Lemnaceae* comme modèle en écologie et évolution.

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There are many people to which I hold deep gratitude, without whom, the completion of this thesis would not have been possible. First and foremost, I thank my supervisor Graham Bell, who has been my primary mentor throughout the course of my graduate work, has both pushed and supported me, and who always found the time for me when I showed up unannounced at his office.

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I am extremely fortunate to have had the help of several undergraduate research assistants who not only have contributed critically to this work by helping me with data collection, but who have also engaged intellectually with the research concepts, explored ideas with me, and become in many cases good friends. I thank Elizabeth Hirsch, who's tireless work building the LEAP mesocosm experiment and countless hours photographing and measuring duckweed was nothing

short of heroic. I thank Rachel Takasaki who agreed to join me on a three-week duckweed hunt across Quebec without much of a plan and without any accommodations reserved in advance. This field survey was not only extraordinarily successful, it was also a great adventure. Finally, I thank Isabel Fernandez McAuley and Julia Kossakowski, who together, during the beginning of the pandemic, agreed to isolate together at McGill's research station and form an incredible research team, flexible to the ever-changing situation, and motivated to run an ambitious experiment. Our honest and provoking conversations during our many hikes up Mount Saint Hilaire to sample duckweed enclosures have been equally valuable to me as the tens of thousands of duckweed we counted.

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and council me as I navigate my career, who have continually lent out their cabin to use for writing retreats, and most importantly, have always shown me endless love. I thank my siblings Kirsten Jewell and Adam McClure and their children, who have provided me wonderful distractions from duckweed. I thank my uncle Carl Turner who is always eager to learn about and discuss my research, and who has taught me about the balance between creativity and rigour in science. Most of all, I thank my wife, Marion Bouchard, for sharing this adventure with me, for trusting me and my decisions, and showing endless patience and love.

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I am grateful to McGill University and its benefactors including Lorne Trottier, Arthur Willey, and the Vineberg family for a number of scholarships that have supported my research.

Table of Contents

List of tables and figures

List of tables

List of figures

Preface

Thesis format

The present thesis is submitted as a compilation of publications of which I am the lead author. Each chapter is a distinct publication that has either been published, accepted for publication or is in revision. Manuscripts have been slightly edited for inclusion in the current thesis. The six manuscripts and associated bibliographic information are as follows:

Chapter 1: Mark Davidson Jewell, Sofia van Moorsel, Graham Bell. Presence of the microbiome decreases fitness and modifies phenotype in the aquatic plant *Lemna minor*.
This manuscript has been accepted for publication in AoB Plants (March 2023).

Chapter 2: Mark Davidson Jewell, Graham Bell. Environmental and genetic variation in an asexual plant.
This manuscript is currently in revision at Aquatic Botany.

Chapter 3: Mark Davidson Jewell, Graham Bell. 2023. Overwintering and re-emergence in *Lemna minor*. Aquatic Botany 186:103633.
This manuscript has been published in Aquatic Botany.

Chapter 4: Mark Davidson Jewell, Graham Bell. Geographical distribution of floating aquatic plants in relation to environmental conditions in southern Quebec, Canada.
This manuscript has been accepted for publication in Aquatic Botany (April 2023).

Chapter 5: Mark Davidson Jewell, Graham Bell. 2022. A basic community dynamics experiment: disentangling deterministic and stochastic processes in structuring ecological communities. Ecology and Evolution 12:1-8.
This manuscript has been published in Ecology and Evolution.

Chapter 6: Mark Davidson Jewell, Graham Bell. Eco-evolutionary contributions to community trait change in floating aquatic plants.
This manuscript is currently in revision at Ecology.

Author contributions

With the guidance of my supervisor, I helped develop the ideas for all chapters, conducted or directed the research for all experiments, analyzed the data (with the exception of the eco-evo Anova in Chapter 6), produced the figures, and wrote the manuscripts that make up this thesis.

Sofia van Moorsel helped with data collection in Chapter 1 and contributed to revisions of the manuscripts that make up Chapters 1 and 4.

Graham Bell conceived the general idea of the thesis as well as the designs and analyses for Chapters 5 and 6. He helped develop the ideas and designs for all chapters, advised me in data analysis, conceived and performed part of the analysis in Chapter 6 (the eco-evo Anova), and provided suggestions on all manuscripts.

Several undergraduate research assistants, Elisabeth Hirsch, Rachel Takasaki, Julia Kossakowsi, and Isabel Fernandez-McAuley, provided technical assistance and helped with data collection.

Statement of original scholarship

All chapters constitute contributions to original knowledge. The following are, to the best of my knowledge, the most novel aspects of this thesis:

Chapter 1

- I performed the first reported experiment testing the effect of the presence/absence of a whole plant epiphytic microbiome on host fitness over a range of environmental conditions.
- I provide the first experimental evidence that the *Lemna minor* microbiome is dominated by pathogens, parasites, and competitors.
- I show that the presence of the microbiome modifies the phenotype in *L. minor* and mediates plasticity.

Chapter 2

- I separate environmental and genetic components of variation in frond area and root length in *L. minor* for the first time.
- I provide the first estimate of genetic variation in fitness within and among natural populations in an aquatic plant.

Chapter 3

- I provide the first estimate of seasonal frond re-emergence of *L. minor* in the field.

Chapter 4

- I report the first field survey of floating aquatic plants in Quebec describing species distributions as a function of water chemistry and nutrient availability.

Chapter 5

- I report the first mesocosm competition experiment using more than two species of floating aquatic plants.
- I applied a statistical design conceived to measure the effects of evolutionary processes to ecological data for the first time.
- I analytically separate selection from drift in semi-natural communities of floating aquatic plants for the first time.

Chapter 6

- I performed the first community multi-generational reciprocal transplant.
- I provide a novel statistical approach to dissecting ecological, evolutionary, and physiological components of community trait change.
- I provide the first experimental evidence of species sorting and natural selection acting on a trait systematically in opposite directions.

Introduction

Part 1 - Eco-evolutionary dynamics

Today, ecology and evolution are seen as two fields so closely intertwined that it would be impossible to consider one without considering the other. This was not always the case, since it was assumed that the two acted over such different time scales that what happened in one domain was largely irrelevant to the other (Slobodkin 1961). Long-term ecological factors, namely abiotic environmental conditions and stable ecological interactions, are what drives natural selection and therefore evolutionary change (Darwin 1859). Since evolutionary change was thought to play out only over millions of years, it would be unaffected by ephemeral short-term ecological change. Consequently, ecological phenomena like changes in species interactions (community structure, food webs, diversity, succession, etc.) could be studied without considering evolution. This older view changed as the result of substantial work in the second half of the 20th century that has shown that important evolutionary change can happen relatively quickly, sometimes over just dozens of generations or less (Hendry and Kinnison 1999, Carroll et al. 2007, Bell 2008). This rapid evolutionary change is comparable with the scale of short-term ecological processes, and therefore evolution must be considered when dealing with classical ecological questions. These realisations have led to the growth of the new conceptual synthesis referred to as Eco-evolutionary dynamics (Fussmann et al. 2007, Pelletier et al. 2009, Hendry 2017), that considers how the two groups of processes act and interact on contemporary timescales.

At this point it would be helpful to define a few key terms. By evolutionary change I'm referring to changes in the genetic makeup of a population, namely changes in allele frequencies. By ecological change, I'm referring to demographic processes, (the changes in birth and death rates in a population), species interactions resulting in changes in community structure, and changes in the abiotic environmental conditions. I also consider a third group of processes, physiological change, that I consider apart from ecology. This is primarily phenotypic plasticity, the ability of an individual genotype to express different phenotypes depending on the environment, either through changes in development, physiological acclimation, or maternal effects. These three

groups of processes can all influence each other on comparable timescales (Fig. 0-1). Ecological change will change the selection environment and may lead to rapid evolution (Bell 2008). Rapid evolution can result in trait change which can affect population dynamics (Lavergne et al. 2010) and community structure (Kinnison and Hairston 2007, Pelletier et al. 2009). Ecological change (both abiotic and changes in species interactions) often result in physiological change which can modify an organism's phenotype via plasticity (Sultan 2000), and this phenotypic change can influence ecological processes in the same ways as phenotypic change via evolution. Phenotypic plasticity must evolve and may be adaptive if it leads to increased mean fitness over a range of environmental conditions (Rago et al. 2019). Finally, physiological plasticity modifies the phenotypes that natural selection acts on, and as such can influence evolutionary change by either masking genotypes from natural selection, (Ghalambor et al. 2007), or allowing genotypes to persist in otherwise inhospitable environments which subjects them to new selection regimes (Schlichting and Wund 2014).

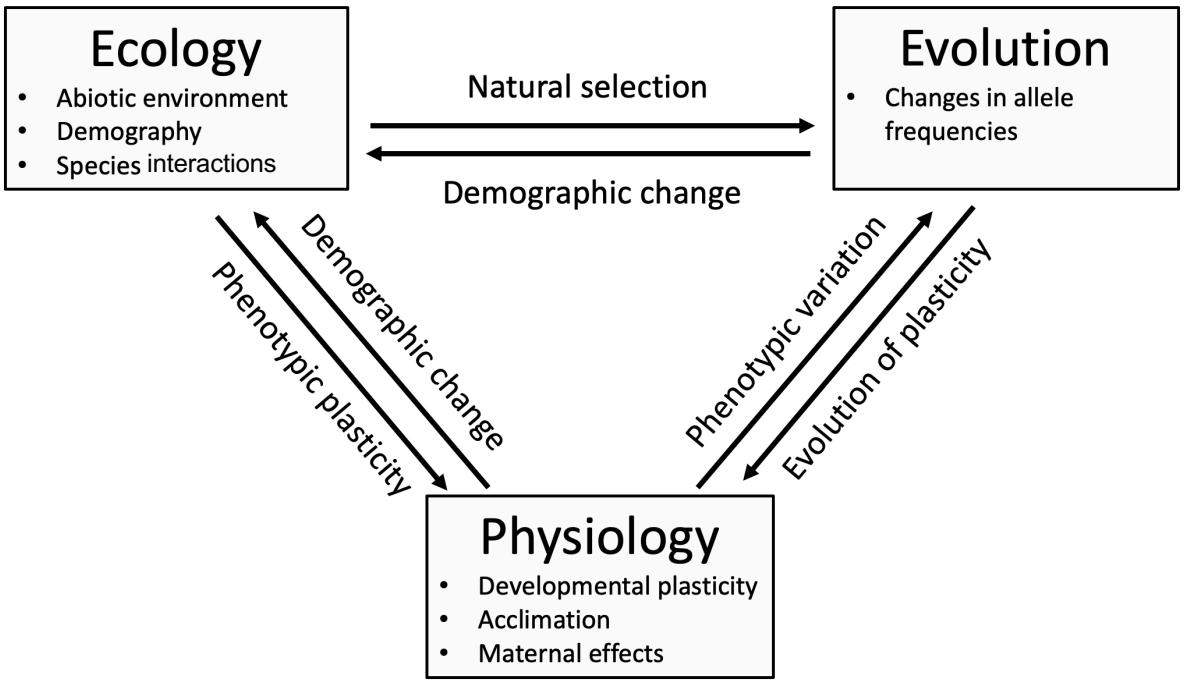

Fig. 0-1. Conceptual framework of eco-evolutionary dynamics

How communities respond to their environment to produce suitable phenotypes capable of maintaining positive fitness and therefore persistence may be the result of a combination of

stochastic and deterministic processes happening simultaneously at several levels. Population genetics has synthesized all evolutionary change into four fundamental processes: mutation, gene flow, natural selection, and genetic drift, (plus recombination and horizontal transfer). Mutation and gene flow represent the processes responsible for adding genetic variation into a population, the precondition for adaptive evolution. By contrast, both selection and drift reduce genetic variation, causing shifts in allele frequencies in a population either systematically (natural selection), or stochastically (genetic drift). These four processes combine to explain a population's overall evolutionary change (Bell 2008).

The mechanisms of ecological change are much more varied and numerous, especially when considering communities consisting of multiple species and trophic levels, all interacting. The seemingly irreducible complexity of these processes responsible for determining the patterns in the diversity, abundance, and composition of species in communities was perhaps best expressed when Lawton famously wrote that community ecology was "a mess" (Lawton 1999). Vellend's theory of community ecology (Vellend 2010, 2016), attempts to impose some order by reducing these into four broad groups, akin to those in population genetics. Whereas population genetics aims to describe the patterns of allele frequencies in a population, community ecology aims to describe the patterns of species frequencies in a community. Thus, a direct analogy can be made where community ecology is governed by just speciation, dispersal, selection, and drift. Speciation and dispersal introduce variation into an ecological community, and selection (also called species sorting) and (ecological) drift structure it. By reducing the mechanisms of ecological change in this way, we can consider the range of possible processes that can modify community phenotypes in response to environmental change (Fig. 0-2).

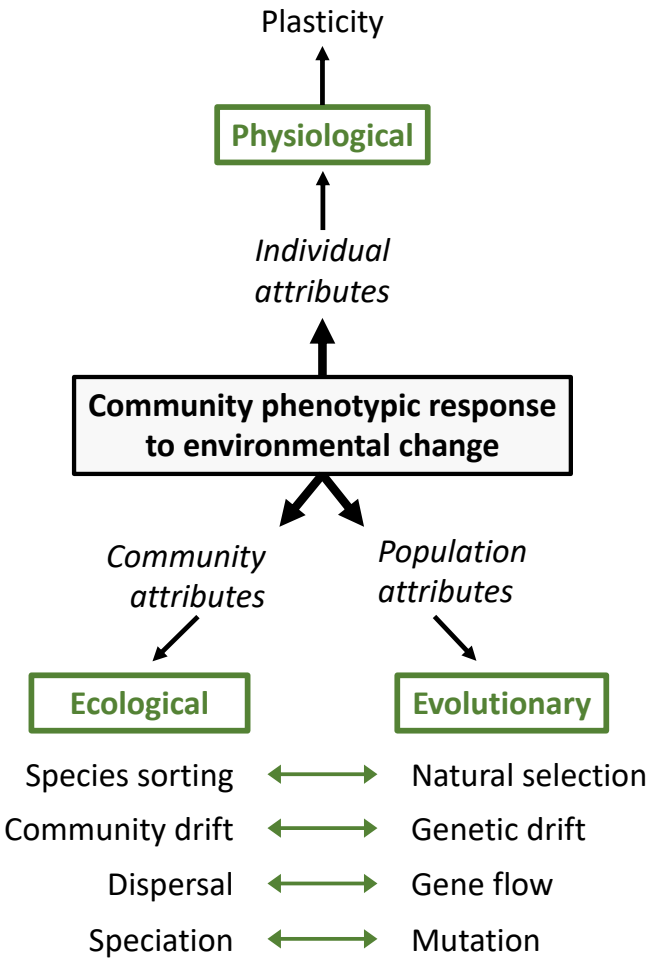

Fig. 0-2. Hierarchical description of all meta processes responsible for driving community phenotypic change

In this thesis I integrate this large array of processes into a unified conceptual framework, and through the work of field surveys and experimental manipulation, quantify how they act separately and together to drive phenotypic change. The first three chapters use single-species populations to estimate contributions of physiological and evolutionary change to phenotypic variation, whereas the last three chapters focus on multi-species communities and ask how ecological processes operate in isolation and in combination with intraspecific evolutionary and physiological change. The system I use throughout are free-floating aquatic plant communities, those dominated by plants in the family *Lemnaceae*, the duckweeds.

Part 2 - *Lemnaceae* as a model in ecology and evolution

The *Lemnaceae* are the simplest, smallest, and fastest-growing of all flowering plants (Hillman 1961a, Ziegler et al. 2015). Consisting of just a single leaf-like frond, usually only a few millimetres across, and often with a single submerged root, they float on the surface of shallow freshwater bodies worldwide. Their common name, duckweed, derives from their functional role as a food source for waterfowl, and their prolific growth. Their extraordinary simplicity has made them a subject of great interest in plant biology. First named by Linnaeus in 1753, and extensively described one hundred years later (Hegelmaier 1868), by the early 20th century they had become a popular early model in plant physiology. Throughout the 20th century, *Lemnaceae* continued to be used as a model to study such diverse subjects as vegetative growth, chemical growth regulation, flower induction, and developmental physiology (Hillman 1961a, Landolt 1986). Although *Arabidopsis thaliana* largely replaced *Lemnaceae* as the primary model in plant biology in the genetic era, recent years have seen a resurgence in interest in *Lemnaceae* (Acosta et al. 2021), in part due to their many applications with potential economic and environmental importance, and because their large population sizes, short generation times, and experimental manipulability make them particularly well-suited for laboratory experimental work. For experimental ecology and evolution, *Lemnaceae* provide a useful system to investigate fundamental questions of how species respond to their environment and enable a high degree of replication and long-term multi-generational studies within a reasonable time frame.

Systematics

Lemnaceae are an important example of how evolution can seemingly work backwards, reverting derived taxa to more primitive states. Descending from highly differentiated and morphologically complex flowering terrestrial plants, these monocotyledonous angiosperms have moved back to water from land, in addition to returning to a morphologically simplified form. The family *Lemnaceae* consists of 37 species distributed in 5 genera: *Lemna* (Lm., 13 species), *Landoltia* (La., 1), *Spirodela* (Sp., 2), *Wolffia* (W., 10) and *Wolfiella* (Wo., 11) (Les et

al. 2002, Sree et al. 2016). *Lemnaceae* make up a monophyletic group whose sister taxon is *Araceae* (Cabrera et al. 2008, Cusimano et al. 2011, Nauheimer et al. 2012), with which they share important morphological features, especially with the genus *Pistia* (water lettuce). Of the five duckweed genera, *Spirodela* is basal, which is of particular interest since it consists of the largest and most morphologically complex species, providing evidence for an evolutionary trajectory of continual morphological reduction and a decrease in differentiation within *Lemnaceae* during the adaptation to aquatic life (Landolt 1986). *Spirodela*, *Lemna* and *Landoltia* are sometimes grouped as the polyphyletic subfamily *Lemnoideae*, whereas the highly reduced *Wolffia* and *Wolffiella*, are grouped as the subfamily *Wolffioideae* (Fig. 0-3). The taxonomic relationships of this unique family have been clarified with the use of improved recent molecular evidence with nuclear DNA data corroborating earlier plastid sequences (Les et al. 2002, Wang et al. 2010, Sree et al. 2016).

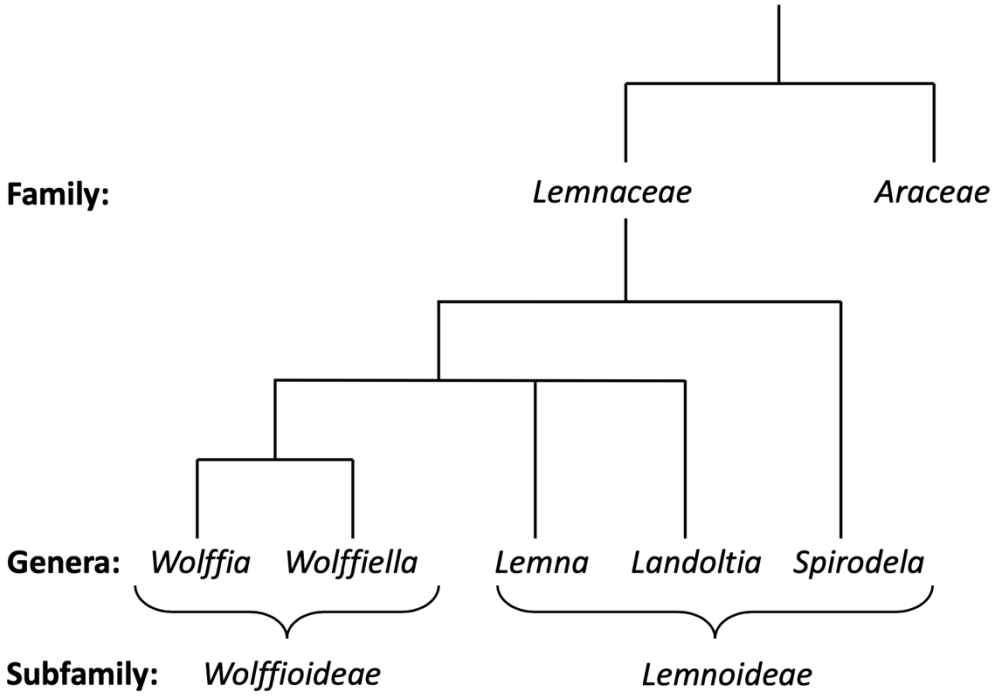

Fig. 0-3. Phylogeny of the family *Lemnaceae*

Morphology

The structures of the five genera of *Lemnaceae* are extremely reduced, all consisting of just a single frond (sometimes called a thallus), most likely a fusion of the leaf and stem, representing the complete reduction of an entire vascular plant. Attached to the lower surface of the frond may be any number of roots (completely absent in *Wolffia* and *Wolfiella*, a single root in *Lemna*, and between 1-20 roots in *Spirodela* and *Landoltia*). These tissues are only somewhat differentiated, since fronds can uptake nutrients directly from the water (Cedergreen and Madsen 2002), and vascular tissue (absent in *Wolffia* and *Wolffiella*) is reduced to tracheids in veins of the frond and the roots of some species (Daubs 1965).

Fronds differ slightly in shape between genera, and can range between 0.5mm (*Wolffia*) to about 1.5cm (*Spirodela*). Fronds consist of 1-10 layers of parenchyma between the upper and lower epidermis, which is fortified by a transparent waxy cuticle which protects it against mechanical damage and solar radiation (Borisjuk et al. 2018). Parenchyma cells have a central vacuole and are rich in chloroplasts on the dorsal side. Aerenchyma, present in all groups except *Wolffia*, allow for gas exchange across the layers of parenchyma, and are of ecological importance since they determine the buoyancy of the frond, which may be actively manipulated to provoke sinking and re-emerging in winter climates (Landolt 1986).

The root, when present, is attached at the basal node, from which 1-16 veins (in *Spirodela*, *Landoltia* and *Lemna*) run to the distal end of the frond. Veins are completely absent in *Wolffia* and *Wolffiella*. Roots consist of a single elongated strand which is surrounded by a root sheath and enclosed at the tip with a root cap. Roots are unbranching, lack root hairs, and are generally between 2-10cm in length. In addition to enhancing nutrient uptake, it has been suggested that the root(s) may function as a pendulum or keel that help to attenuate wind and wave disturbance and keep the frond floating upright (Landolt 1986).

Flowers, barely visible to the naked eye, may be bisexual (in *Lemna*) or unisexual, (though plants are always monecious), and may be pollinated by wind or small arthropod vectors such as

aphids, flies, mites, and spiders (Landolt 1986). Flower morphology is simplified, lacking a corolla and calyx, and consisting of only one or two stamens and a pistil per frond (Landolt 1998).

Despite the ability to produce flowers, fruits and seeds, most reproduction is asexual and vegetative, as daughter fronds emerge from meristematic tissue located within a reproductive or vegetative pouch (sometimes called the meristematic pocket) located on the ventral surface next to the node (Lemon and Posluszny 2000, Sree et al. 2016). In *Lemnoideae*, daughter fronds are produced in pairs from two lateral reproductive pouches, whereas in *Wolffioideae*, a single daughter emerges from a basal pouch. After budding from the mother's meristem, daughter fronds remain physically attached to the mother frond by an elongated and vascular stipe, which is hypothesized to transfer nutrients from the mother to daughter frond (Kim 2016). Fronds may remain attached for a period of time forming clonal colonies of anywhere from two (in *Wolffioideae*) to 20 fronds, before breaking apart at an abscission zone (Landolt 1986, Kim 2016). In one species, *Lemna trisulca*, abscission is delayed such that the persisting stipes result in even larger colonies consisting of tangled chains of dozens of fronds.

Several species produce vegetative winter buds known as "turions" that help the plant persist in unfavourable environmental conditions including low temperatures and desiccation stress. These seed-like structures emerge from the vegetative pouch and are actually modified fronds, high in starch and lacking aerenchyma, which sink through the water column once they detach from the mother frond (Jacobs 1947, Kim 2013). They are densely packed with starch, which can exceed 70% dry weight (Dölger et al. 1997) and provide a rich source of sugars once the turions re-emerge when conditions are favourable, resulting in the rapid growth of newly germinated fronds (Appenroth et al. 1996, Landolt 1998, Appenroth and Adamec 2015). Turion production is stimulated by environmental stress such as nutrient deficiency and attenuating day length and is an important adaptation that helps species in colder temperate climates like *Spirodela polyrhiza* survive winter (Landolt 1986, Appenroth et al. 1989).

Life history

One of the more curious features of *Lemnacea*'s life history, is their evolutionary reversion from sexual to mainly asexual reproduction (Bell 1982, Fourounjian et al. 2021). In most species of *Lemnaceae*, sexual reproduction is so rare that it is seldom observed in the field (Landolt 1998). Despite extensive work on the chemical and hormonal induction of flowering (Hillman 1961b, Cleland 1985, Wang 1990, Fourounjian et al. 2021), the environmental conditions that promote flowering in the wild are still poorly understood (Fourounjian et al. 2021). Instead, vegetative growth is the norm. An individual may produce up to a couple dozen daughter fronds over its lifetime, which is typically about a few weeks (Landolt 1986). The quality of these daughter fronds changes over a mother frond's life as birth order influences frond size and fitness (Ashby et al. 1949, Barks and Laird 2015). In favourable conditions, growth rates can be extremely rapid, with populations doubling every two to three days in most species and generations can be as short as 24h in *Lemna aequinoctialis*, *Wolffiella hyalina*, and *Wolffia microscopica* (Sree et al. 2015a, Ziegler et al. 2015). This maximisation of growth rate has led some to describe them as examples of "Darwinian demons" (Kutschera and Niklas 2015). The dispersal of plants between sites is thought to be straightforward, since their small size allows them to adhere to waterfowl that travel between water bodies (Jacobs 1947, Hillman 1961a). The smallest species of the genus *Wolffia*, have even been found viable after passing through the gut of waterfowl, which could permit longer distance dispersal (Silva et al. 2018).

Distribution and ecology

Lemnaceae are cosmopolitan, thriving in all regions of the world except in arctic and desert climates. With the exception of *Wolffiella*, all genera exist broadly across most continents, with the most diversity in the tropics (Landolt 1998). Among the 37 species, *Lemna minor*, the "common" or "lesser" duckweed stands out, as it has the widest distribution and is the most common and abundant species across a wide range of climates. This is partly explained by substantial intraspecific variation, often described as ecotypes (Landolt 1986, Ziegler et al. 2015).

Lemnaceae often coexist in species-rich communities made up of several species of duckweed, as well as liverworts in the family *Ricciaceae* (genera *Ricciocarpus* and *Riccia*) and water ferns in the family *Salviniaceae* (genera *Salvinia* and *Azolla*) (Fig. 0-4). Ecological specialization to different nutrient and light levels may explain their coexistence given the temporal variability of seasonal change, in addition to negative density dependent selection (Armitage and Jones 2019).

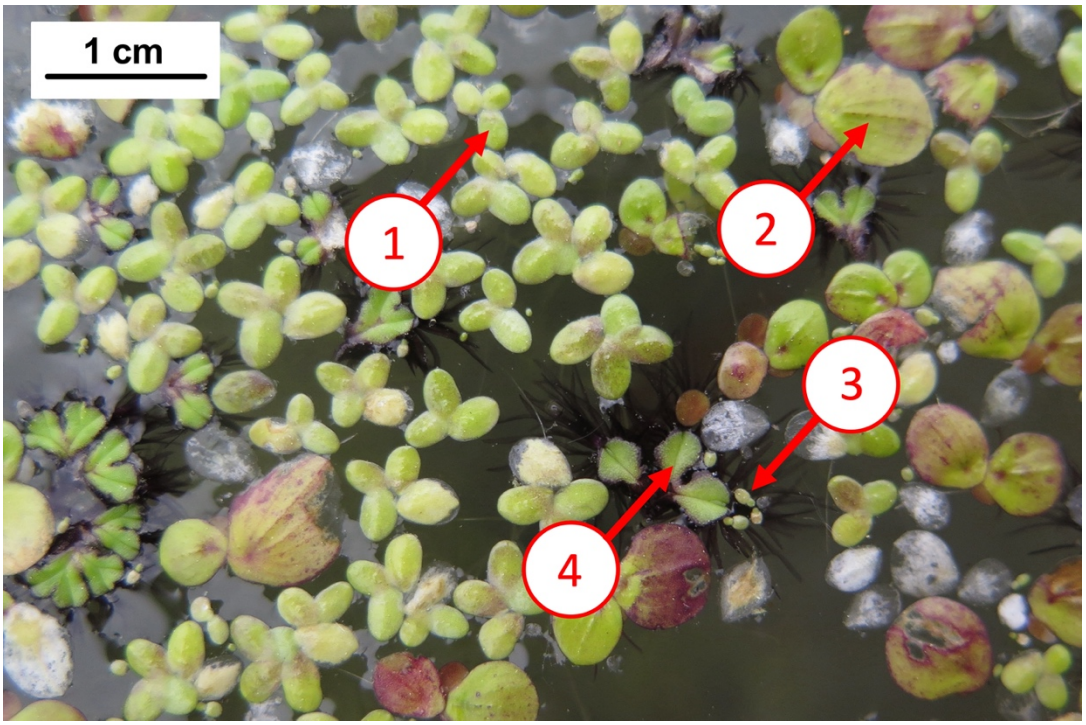

Fig. 0-4. A community of free-floating aquatic plants. Shown are three species of *Lemnaceae* (1. *Lemna minor*, 2. *Spirodela polyrhiza*, 3. *Wolffia columbiana*) and one liverwort (4. *Ricciocarpus natans*).

When abundant, *Lemnaceae* may act as keystone species, fundamentally modifying the environment for other species. In favourable conditions, *Lemnaceae* may grow to completely cover the water surface, and in highly eutrophic conditions, overgrowth may occur several fronds thick (Landolt 1986). This can have a profound effect on the ecosystem since they effectively shade out competitors including rooted macrophytes and phytoplankton (Portielje and Roijackers 1995), preventing algal blooms. As fronds senesce, they sink to the bottom of the water column where their decomposition may represent an important input of nutrients to the benthic

environment (Laube and Wohler, 1973), especially since cyanobacteria in the *Lemnaceae* microbiome can fix atmospheric nitrogen, providing nitrogen enrichment in oligotrophic environments (Duong and Tiedje 1985). Finally, *Lemnaceae* can be an essential food source for many animals including waterfowl, fish, and other herbivores (Landolt 1986).

Like all plants, a diverse assemblage of microbes colonise every available surface of the *Lemnaceae* fronds and roots, making up what is known as the plant microbiome or phyllosphere. For *Lemnaceae*, this consists of dense populations of diatoms, green algae, rotifers, stalked ciliates, and bacteria (Coler and Gunner 1969, Ishizawa et al. 2017a, Acosta et al. 2020). In addition to plant-microbe interactions, many insects including flies, beetles and aphids may feed on the fronds of *Lemnaceae*, or in some cases such as the parasitic leaf-mining Lemna fly (*Lemnaphila scotlandae* Cre.), lay eggs on these plants (Scotland 1940, Buckingham 1989).

Genetics

The size of the *Lemnaceae* genome varies 15-fold ranging from 150 Mbp in the oldest genus *Spirodela* to 2,203 Mbp in the most phylogenetically recent genus *Wolffia* (Wang et al. 2011, Hoang et al. 2019), representing a general expansion of the genome over the evolution of the family. There is no obvious correlation between genome size and chromosome number however, which is most often diploid and varies from 2n = 36 to 82 (Hoang and Schubert 2017, Hoang et al. 2019). In addition to interspecific variation, there is considerable variation in genome size among clones of some species (Wang et al. 2011). The relatively small genome of *Spirodela*, consisting of 19,623 protein-coding genes (Wang et al. 2014), includes most core gene families of land plants. Given the basal position of *Spirodela*, not just within *Lemnaceae*, but also within monocots, it provides an important resource for plant geneticists to investigate the highly conserved and ancestral genome structures of the common ancestors of many grain crops, and given their pronounced evolutionary reduction, reveal how gene presence and absence affect the gain and loss of function in traits. This accounts for the push to fully sequence the genomes of *Lemnaceae*, starting with clones of *Spirodela polyrhiza* (Wang et al. 2014), then *Lemna minor* (Van Hoeck et al. 2015), with the draft genomes of several other species in progress.

Studies investigating intraspecific genetic variation (primarily in *Lemna minor*) have documented high levels of genetic differentiation, even between populations in close geographic proximity (Vasseur et al. 1993, Cole and Voskuil 1996, Xue et al. 2012, El-Kholy et al. 2015), suggesting limited gene flow between sites. Surprisingly, there also seems to exist considerable intraspecific genetic variation within sites with studies of allozymic analysis documenting on average between 4-20 genotypes per site in *L. minor* based on 13 polymorphic loci (Vasseur et al. 1993, Cole and Voskuil 1996). How this genetic diversity is generated and maintained remains a subject of debate, considering the low estimates of gene flow (Cole and Voskuil 1996), per base pair mutate rate (Xu et al. 2019, Sandler et al. 2020), and frequency of sexual reproduction (Hillman 1961a, Landolt 1986, Vasseur et al. 1993, Ho 2018) in *Lemnacaea*.

Applications

Much of the current resurgence in duckweed research is due to the potential economic importance of several practical applications. Due to their fast growth rates and high protein content (Cheng and Stomp 2009), *Lemnaceae* are being developed as a human food source (Appenroth et al. 2017). Already consumed traditionally in parts of South-east Asia (van der Spiegel et al. 2013), *Lemnaceae*, particularly *Wolffia*, has the potential to replace soya as a plant-based protein source. Since production does not require arable land and has 5 to 10 times higher protein yields than conventional land-grown crops like soya (Roman et al. 2021), *Lemnaceae* has the potential to be much more environmentally sustainable and economically viable than most crops (Roman and Brennan 2019). For the same reasons, there is much optimism surrounding the development of *Lemnaceae* for biofuels since production would not compete with other agricultural crops (Su et al. 2014, Cui and Cheng 2015).

Lemnaceae are also used as feed for domestic animals including cattle, poultry, fish and swine (Sońta et al. 2019). This is of particular interest for small-scale farmers, who can grow *Lemnaceae* in runoff water to remove organic pollution before using it as feed to close the loop of sustainable agriculture (Devlamynck et al. 2021). Due to its ability to grow over wide ranges

of temperature, pH, and nutrient level (Landolt 1986), and to quickly uptake organic pollution, *Lemnaceae* has been used at larger scales by municipalities, especially in developing countries, as an effective and affordable way to treat municipal sewage and industrial waste water (Oron 1994). Harvesting wastewater-grown duckweed helps to remove surplus nutrients, which might otherwise be released into aquatic environments by conventional wastewater treatment plants (Oron et al. 1988). Beyond organic pollution, *Lemnaceae* are being studied as a potential candidate to use in phytoremediation because of their general stress tolerance and ability to quickly uptake pollutants like metals and pesticides. This is shown by their long history of use in ecotoxicology and biomonitoring (Wang 1990, Ziegler et al. 2016, Liu et al. 2021).

Although the idea of closed-loop resource recovery emerged from sustainable agriculture, it also applies to space-based life-support systems required for long-duration exploration missions, and as such, *Lemnaceae* has caught the attention of NASA (Escobar and Escobar 2017). At this point, the applications for *Lemnaceae* seem extensive.

In experimental community ecology and evolution

The unique life history traits of *Lemnaceae* that make them suitable for many industrial applications also make them an ideal experimental model in ecology and evolution (Laird and Barks 2018, Acosta et al. 2021). Their cosmopolitan distribution and high local abundance make them easy to study in the field, and their tractability means that they are easy to grow in the laboratory in artificial growth media, or to manipulate in semi-natural conditions. It is possible to remove the epiphytic microbiome to achieve aseptic cultures, either to reduce unwanted variation, or to study plant-microbe interactions (Zhang et al. 2010, Ishizawa et al. 2020). The extensive literature resulting from their long history as a model in plant biology combined with the growing genetic resources provide a framework to study more complex questions that would not be possible without these tools. The work of Elias Landolt, often considered the father of modern duckweed research, has produced an extensive collection of well over a thousand *Lemnaceae* clones collected from around the world, originally based in Zurich and now maintained at Rutgers University in the United States (Rutgers Duckweed Stock Cooperative,

www.ruduckweed.org). The possibility to order samples of these cultures simplifies studies on clonal divergence and permits simple manipulation of intraspecific diversity (van Moorsel 2022).

Perhaps the most advantageous traits are their small size and short generation time. Although these traits reduce the complexity of manipulative experiments pertaining to countless research questions, here I focus on their potential to advance the fields of experimental community ecology, experimental evolution, and the intersection between the two: experimental eco-evolutionary dynamics.

Experimental community ecology largely deals with species competition dynamics, competitive exclusion, coexistence, and the maintenance of biodiversity. Any experimental investigation of these processes must allow for several generations to pass to allow for any competitive advantage to manifest itself as change in community structure. Many of the 20th century's most important ecological experiments dealt with this by either running over many years or decades (such as the experimental grass plots of Cedar Creek or Rothamsted) (Tilman and Downing 1994, Silvertown et al. 2006), or by using microbial organisms with short generation times (for example, Gauss' competitive exclusion experiments with *Paramecium*) (Gause 1935). Furthermore, experiments must use communities consisting of sufficiently large populations so that ecological drift does not mask the other processes under study, and with enough replication of communities to retain sufficient statistical power to test hypotheses with multifactorial designs. If using large organisms, the study site must be large, which is often impractical or impossible. On the other hand, using microbes grown in flasks, although experimentally powerful, is sometimes met with the criticism of being overly reductionist and not relevant to natural environments (Hendry 2017).

The use of *Lemnaceae* permits highly replicated, multigenerational experiments that can be performed in a controlled laboratory situation, semi-natural mesocosms, or natural environments with the use of enclosures. Such recent experiments have for example identified negative frequency-dependent selection as a fundamental mechanism of species coexistence of *Lemna minor* and *Spirodela polyrhiza* (Armitage and Jones 2019), two species that often coexist in the wild. By manipulating intraspecific variation, additional work found that interspecific

competition between these same two species drives rapid genotypic change, which in turn feeds back to change the population trajectories of the competing species, showing that coexistence can be mediated by rapid evolution (Hart et al. 2019). Likewise, looking at interactions between *Lemna minor* and its associated microbiome, Tan et al. (2021) found that rapid evolution in *Pseudomonas*, an important constituent of the plant microbiome, modified the composition of the microbial community with important effects of the fitness of the host plants. These studies not only show the ability of rapid evolution to feed back on ecological parameters like population dynamics and species interactions, but highlight the strengths of using *Lemnaceae* as a model in experimental evolution. The field of experimental evolution involves measuring the phenotypic or genotypic consequences of prolonged growth in controlled selection environments. Studying natural selection in real time is a powerful approach to uncover the mechanisms of adaptive evolution, that goes beyond the traditional approach of inferring past processes based on current patterns (Kawecki and Ebert 2004, Kawecki et al. 2012). The field is relatively young (Kawecki et al. 2012), with most work up until now relying on microbial systems like *Pseudomonas*, *Saccharomyces*, and *Chlamydomonas*. The use of *Lemnaceae* as a model in experimental evolution not only provides a macroscopic extension of traditional microbial systems, but has additional potential strengths thanks to the availability of hundreds of clones, genomic tools including full genome sequencing, the ability to artificially induce flowering and perform crosses, and the possibility to track individuals manually over generations. Future work using *Lemnaceae* could extend what we know about the capacity of rapid evolution to save species or whole communities from extinction (evolutionary or community rescue) (Bell and Gonzalez 2009, Low-Décarie et al. 2015), how higher plants may respond to warming (Rodríguez-Trelles and Rodríguez 1998) and increased atmospheric CO_2 (Collins and Bell 2004, Low-Décarie et al. 2013) and the evolution of resistance to herbicide (Fugère et al. 2020, Gaines et al. 2020). The return of *Lemnaceae* may just be the stimulus that helps move the field of experimental evolution out of the laboratory flask and outside into nature.

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Preamble to Chapter 1

The aim of this chapter was two-fold. The main objective was to assess the influence of the epiphytic microbiome on host fitness for *Lemna minor*. There is growing interest in plant-microbe interactions with an abundance of work aiming to identify and characterise microbe-plant mutualism with potential applications for agriculture. Although the vast majority of work has been with terrestrial plants, a number of studies have used *Lemna minor* as a model to identify plant growth-promoting bacteria. However, there has not yet been a basic study assessing the fitness and phenotypic implications of the full natural microbiome on *Lemna minor*. By growing plants from several natural sites in the lab, with and without their associated microbes, we were able to quantify how the microbiome influenced plant fitness and mediated phenotype. The secondary objective, important in the context of this thesis, was to determine the degree of plasticity in the basic morphological traits that we use across most subsequent chapters. We manipulated resource levels in controlled growth chambers and measured plastic change in frond area and root length. This helped inform subsequent work assessing phenotypic plasticity in the field.

This chapter has been accepted at AoB Plants.

Chapter 1 - Presence of the microbiome decreases fitness and modifies phenotype in the aquatic plant *Lemna minor*

Mark Davidson Jewell[1], Sofia van Moorsel[2], & Graham Bell[1,3]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.
[2]Department of Geography, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[3]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Summary

Much recent work has focused on characterising the mutualistic elements of the plant microbiome, often aiming to identify bacterial strains that can increase plant fitness. Although most work has focused on terrestrial plants, *Lemna minor*, a floating aquatic angiosperm, is increasingly used as a model in host-microbe interactions. Here we assess the fitness and phenotypic consequences of the full microbiome for *L. minor* by assaying plants from eight natural sites, with and without their microbiomes, over a range of environmental conditions. We find that the microbiome suppresses plant fitness, for all genotypes and across all environmental conditions. This decrease in fitness was accompanied by phenotypic changes, with plants forming smaller colonies and producing smaller fronds and shorter roots with the microbiome present. Although the *L. minor* microbiome clearly includes important symbionts, our findings suggest that we cannot discount the important pathogenic, parasitic, and competitive interactions, whose influence can override that of mutualists.

Introduction

Recent decades have seen considerable interest in the relationship between plants and their associated microorganisms, the plant microbiome (Bahram et al. 2018, Arias-Sánchez et al. 2019, Schmid et al. 2019, Tan et al. 2021). It is well known that a taxonomically rich assemblage of microbes colonises every accessible plant tissue and often have important effects on plant functioning and fitness. Plant-associated microbiomes may confer fitness advantages to the plant

host, via increased nutrient uptake, stress tolerance (Smith et al. 2010, Zhu et al. 2010, Lau and Lennon 2012, Kivlin et al. 2013), resistance to pathogens (Pieterse et al. 2014, Compant et al. 2019), reduced herbivory (Hubbard et al. 2019), and increased nutrient uptake resulting from, for example, arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria, whose symbiotic associations with terrestrial plants often release their hosts from severe nitrogen or phosphorus limitation (Smith and Read 2008, Mahmud et al. 2020). Even associations with non-AMF were shown to improve plant performance in a controlled inoculation experiment (Hahl et al. 2020). Associations with plant growth-promoting bacteria (PGPB) and other microbial organisms are of specific interest given their potential application to increase the yields of agricultural crops (Glick 2012, de Souza et al. 2015). However, many reported plant–microbe interactions are negative (Bever 2003, Kulmatiski et al. 2008, Van der Putten et al. 2013), and are thought to play a role in promoting plant coexistence. (Bever et al. 1997, Bever 2003).

Although the vast majority of work on the plant microbiome focuses on terrestrial plants, there is a growing literature investigating the consequences of microbiota for floating aquatic plants (Crump and Koch 2008, Xie et al. 2015). Much of this work has focused on *Lemna minor*, a tiny floating aquatic plant in the family *Lemnaceae* which is increasingly used as a model system for host-microbe interactions (Zhang et al. 2010, Acosta et al. 2021). Among the smallest of all angiosperms*, L. minor* consists of only a single floating leaf-like frond to which a single unbranched root is attached. Its reproduction is almost exclusively asexual and vegetative with daughter fronds budding out of the mother frond's two meristematic pouches located on the frond's lower surface. Daughter fronds remain attached to the mother for a certain period of time by a stipe, a stem-like bundle of vascular tissue, resulting in colonies of varying sizes, before splitting apart after abscission severs the stipe (Landolt 1986, Lemon et al. 2001). They are widespread and abundant, often found growing on the surface of eutrophic ponds, wetlands, and slow-moving rivers. In the wild, the fronds and roots are covered in a species rich assemblage of microbes (Gilbert et al. 2018, Acosta et al. 2020), which can be removed by sterilisation in the lab when used as an experimental model (Bowker et al. 1980). Interest in the *L. minor* microbiome dates back to the early 20th C, with the observation of an association with N-fixing bacteria (Bottomley 1920), and has accelerated in recent years (Ishizawa et al. 2017b, 2017a, 2019, Gilbert et al. 2018, Chen et al. 2019, Acosta et al. 2020, Iwashita et al. 2020, O'Brien et al.

2020a, 2020b, Tan et al. 2021) with a general consensus that plant-microbe interactions play an important role in mediating plant fitness and function. Although most of this research focuses on identifying specific PGPB strains, recent work has characterised the complete core bacterial assemblage associated with *L. minor* (Acosta et al. 2020), which consists of largely Proteobacteria (*Pseudomonas* and Actinobacteria) and bears a close resemblance to the leaf microbiome in terrestrial plants like *Arabidopsis* and rice. Although certain select strains of microbes are important in promoting *L. minor* growth, it remains unclear how the full natural assemblage, which also includes countless microbial pathogens, parasites and competitors, impacts plant fitness. The small size of this plant makes it well-suited to highly replicated experiments, and its fast generation time and vegetative asexual reproduction means that the life-time fitness can be measured across multiple generations within a single experiment simply as population growth rate.

The effect of the microbiome on host fitness and phenotype may both depend on the environment and the host genotype. Plant genotypes often differ in their responses to abiotic environmental conditions (Rehfeldt et al. 2002, Wilczek et al. 2014). These GxE (genotype by environment) interactions have been shown in some cases to depend on the microbiome, whose composition may vary among plant genotypes (Wagner et al. 2016, O'Brien et al. 2020a), or whose impact may mediate plant phenotypic responses to the environment. Just like the abiotic environment, the biotic environment can affect expression of phenotypically plastic traits and fitness in terrestrial plants (Friesen et al. 2011, Wagner et al. 2014), and these microbially-mediated shifts in plant phenotype have been shown to effect plant tolerance to environmental stress (Wagner et al. 2014, Hubbard et al. 2019, O'Brien et al. 2019). Furthermore, certain environmental conditions can lead to the decoupling of plant-microbe mutualisms (Shantz et al. 2016). Thus, the traits and fitness of the host plant depend on the host genotype, its microbiome, the abiotic environment, and the interactions between these three factors.

In this study we have three aims. First, we ask how the presence of the natural *L. minor* microbiome affects plant fitness. Second, we ask whether fitness effects of the microbiome depend on the specific environmental conditions and are associated with changes in plant

phenotypic plasticity. Thirdly, we ask if different genotypes and their associated microbiomes differ in terms plant fitness and phenotypic plasticity (GxE).

Materials and methods

Sampling

Eight natural rivers, ponds, and swamps supporting populations of *L. minor* were located within a 10km radius of Montreal, Canada (Fig. 1-1, Table S1-1 in Appendix 1). From each site large samples of plants were taken, brought back to a research greenhouse at McGill University, and maintained in samples of natural pond water, also collected from each site. Microscopy revealed that the plant microbiome included a large assemblage of diverse groups of epiphytic protists, in addition to bacteria and fungi.

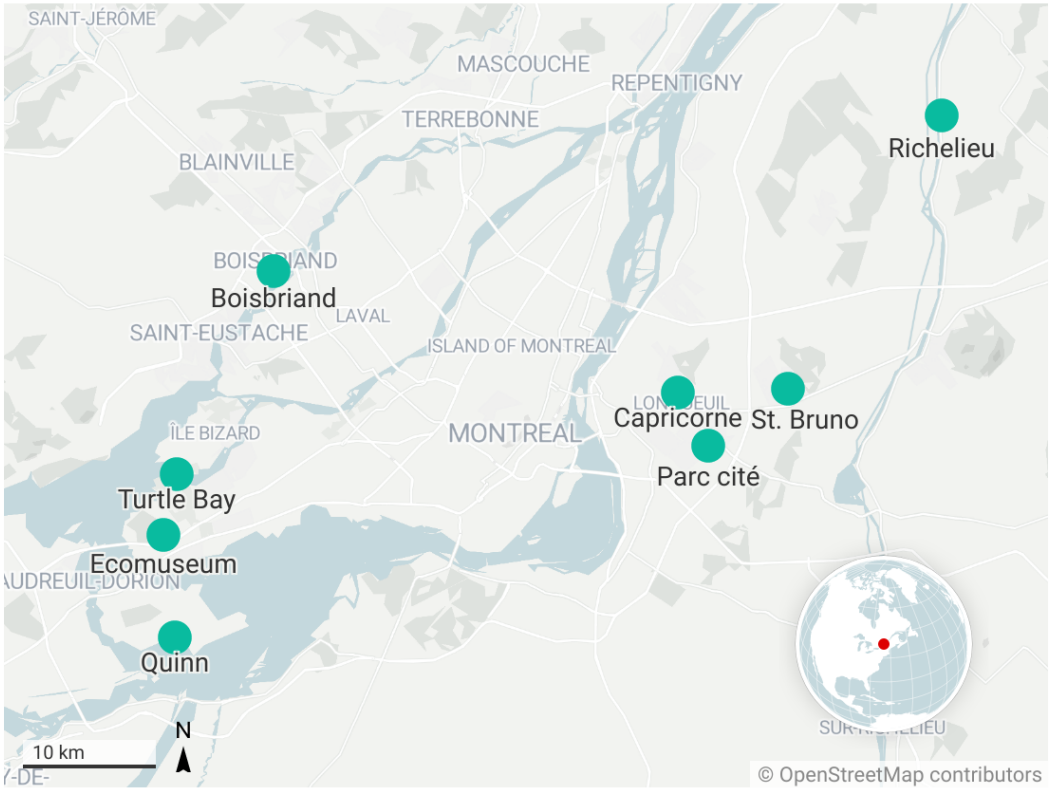

Fig. 1-1. Map of sampling sites around Montreal, Quebec, Canada. Created with Datawrapper.

Removal and reintroduction of the microbiome

To test the effect of the microbiome on plant performance, we first removed the microbiome from all fronds, and then reintroduced it to a subsample from each site as a way to control for the process of microbiome removal. To sterilize the plants, individual fronds were thoroughly rinsed in deionised water, submerged for approximately 3 minutes in 10% bleach and then transferred to sterile high-nutrient Hoagland's E-medium (recipe in Appendix 1, Table S1-2). After two weeks, surviving cultures were examined with microscopy, and those that appeared to be axenic were transferred to agar plates made with Bold's basal medium (Stein 1973) to promote algal growth, and to agar plates made with Yeast extract-peptone-dextrose growth medium (YEPD) to promote yeast and bacterial growth. After an additional two weeks of growth in Bold's and YEPD, cultures were again examined by microscopy. This process was repeated until axenic fronds were obtained for each site.

Once sterility was confirmed, for each of the eight sites, a single *L. minor* frond (here on in referred to as a genotype) was used to found a clonal population that would serve as the ancestor for all assay cultures. After one month of expansion in sterile conditions, each population (one per genotype), consisting of several hundred fronds, was split in two, one which would remain axenic, the other to which we would reintroduce the microbiome. The original samples from all sites consisting of untreated *L. minor* fronds with their intact microbiome, growing in their natural pond water were maintained in open 12L containers in the greenhouse (DI water added weekly to replace that lost from evaporation), and were used to reintroduce the microbiome to the experimental populations. This was done by culturing axenic fronds in their natural pond water in 1.5L culture tubs, surrounded by untreated plants from that site with intact microbiomes for an additional two weeks, or about four generations. We used a floating circular boom (10 cm diameter) to physically isolate the target fronds (of which there were roughly 50) from the others, but submerged roots were allowed to intermingle (Fig. 1-2). This allowed sufficient time for the reacquisition of the microbiome, which, for *L. minor* in similar experimental conditions, has been shown to begin within 24 hours, and to reach a stable community after 5-14 days (Acosta et al. 2020). This extra step ensured that we did not have a confounding effect of the sterilization-induced selection on more robust individuals.

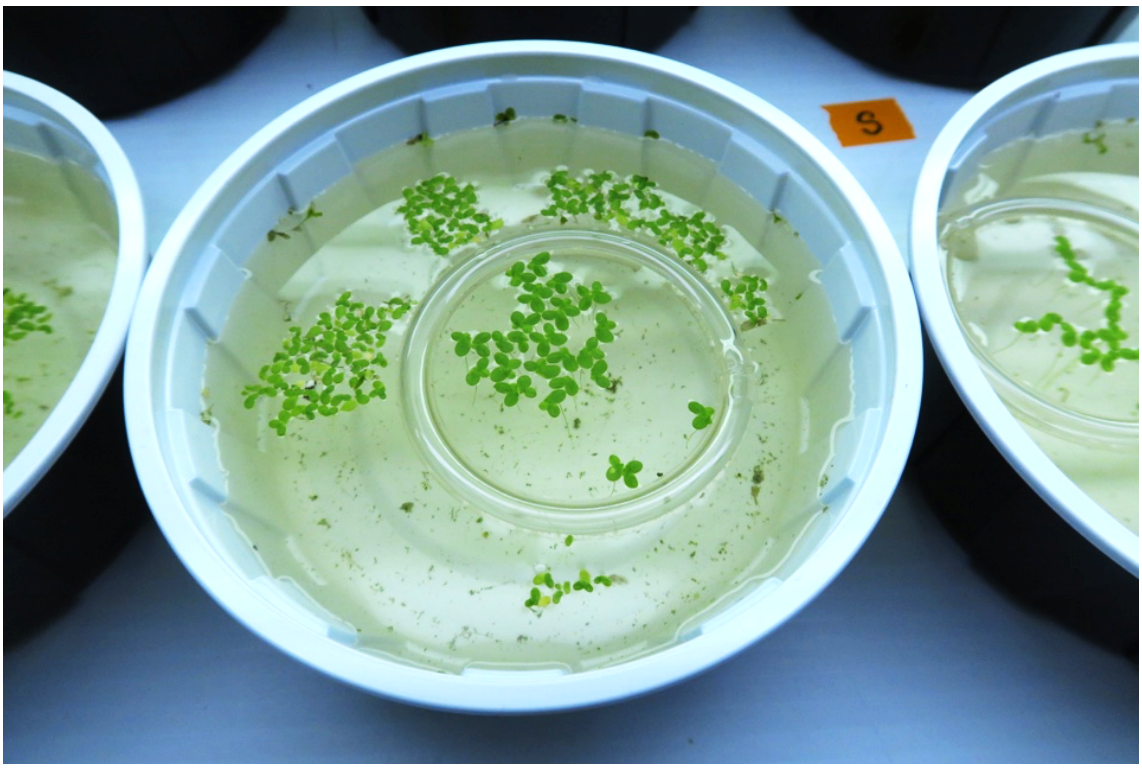

Fig. 1-2. Reinoculation of the microbiome back to axenic *Lemna minor* fronds. The container, filled with natural pond water is filled with fronds from the same site, with their microbiome intact. The floating circular boom isolates the sterilised target fronds. Although the fronds are spatially separated at the water's surface, the roots intermingle. To reinoculate the microbiome back to the axenic fronds, the target fronds were cultured here for two weeks (about four generations).

Acclimation

Once two populations, (one axenic, the other with its microbiome, both founded from the same common ancestor,) were obtained for each genotype, all cultures were acclimated for an additional two weeks in a controlled common garden setting to remove any maternal effects and ensure an equal physiological starting point for all plants. Each population, consisting of ~150 individuals, was grown in a stoppered 500mL Erlenmeyer flask filled with 350mL of diluted Hoagland's E-media ([N]=2750 µg L^{-1} and [P]= 423.5 µg L^{-1}) placed in controlled growth

chambers of the McGill phytotron (165 µmols m^{-2} s^{-1} light, 20^0C, 70% relative humidity, with a 14/10 light-dark cycle).

Growth assay

The main experiment consisted of a growth assay of all 16 populations (8 genotypes, each with the microbiome either present or absent) in four distinct abiotic environments, a 2 x 2 crossed treatment of light level and nutrient concentration. Each assay was replicated in three flasks. This fully factorial design results in a total of 192 assays (8 genotypes x 2 microbiome treatments x 4 environment treatments x 3 replicates). Ten random individual fronds were used to inoculate each 500mL Erlenmeyer flask filled with 350mL of sterile Hoagland's E-media, modified to obtain the desired treatment levels, either low nutrients ([N]=500 µg L^{-1}, [P]= 77 µg L^{-1}), or high nutrients ([N]=5000 µg L^{-1}, [P]= 770 µg L^{-1}). The flasks were then placed in four growth chambers in the McGill phytotron (20^0C, 70% RH, 14/10 light-dark cycle), two of which were set at low light conditions (30 µmols m^{-2} s^{-1}) the other two at high light conditions (300 µmols m^{-2} s^{-1}). The initial common garden conditions were at intermediate light and nutrient conditions in relation to the low and high treatments in the experimental growth assay. Flasks were plugged with foam stoppers and all transfers were done using sterile techniques. The 48 flasks in each growth chamber were randomly positioned, leaving a 15cm boundary from the chamber wall.

The growth assay lasted for a total duration of four weeks after which population growth rates and plant phenotypes were measured. However, to maintain populations in a state of exponential growth, the growth assay was broken into two two-week assays. After the first two weeks of growth in the treatment environments, before fronds reached complete surface cover (on average ~100 fronds/ flask), 10 randomly sampled fronds from each flask were transferred to an identical treatment flask of fresh media. Furthermore, since inoculating sterile growth media with natural fronds (with intact microbiomes) would likely create conditions with phytoplankton present but important zooplankton grazers absent, prolonged growth could result in plant-algal competition (van Moorsel 2022). By transferring the cultures after only two weeks, phytoplankton remained

sparse. The flasks were repositioned randomly in the growth chambers following the mid-assay transfer.

At the end of the experiment the total number of fronds and the number of colonies (groups of attached fronds) were recorded for each flask. From each flask, we randomly sampled 10 individuals (on average ~10 % of the population) for whom we measured frond area and root length by imaging (plants were pressed onto a sheet including a reference ruler and photographed at a standard 20cm distance) and subsequent image analysis using Image J. Only mature individuals (those from which a daughter frond was budding) were included.

Statistical analysis

The experiment had four response variables: population growth rate, colony size, frond area, and root length. Growth rate was calculated for each flask during the final two-week growth period using the standard formula for exponential growth $r = \ln\left(\frac{Nt}{N0}\right)/t$ where N_0 is initial population size, t is time in days, and N_t is population size at time t. For each response variable we used a linear mixed-model Anova to test the effects of the microbiome and environmental conditions, (two fixed factors), and genotype (one random factor). Expected Mean Squares and estimates of F were evaluated as described by Sokal and Rohlf (1981), Box 12.1 pg.383 'Mixed Model'. Since there is only a single measure of growth rate per flask, replicate flask was used as the error variance, and similarly, an average value of frond area and root length of the 10 measured individuals was used as a single measure per flask.

Results

Growth rate (fitness)

The plants grew rapidly over the four-week growth assay with an average doubling time of 6 days across all treatments. Growth rate was affected by environmental conditions ($F_{3,21}$=362.6,

$p<0.001$), with plants growing 1.23x faster in high nutrient conditions than in low nutrient conditions, and more than 1.95x faster in high light conditions than in low light (Fig. 1-3). In the most favourable environmental conditions (high light - high nutrients), average doubling time was 4 days, whereas in the most stressful environmental conditions (low light – low nutrients), it was 10 days.

There was considerable variation in growth rate among genotypes, all of which responded to light and nutrients in the same direction (increased growth rate with higher resource levels). The extent of this increase varied among genotypes however, leading to a significant genotype x environment interaction ($F_{21,128}=2.92$, $p<0.001$), indicating the presence of a genotype by environment (GxE) effect. Presence of the microbiome had a strong and consistent effect on growth rate ($F_{1,7}=32.3$, $p<0.001$), reducing growth rate in all environmental conditions (Fig. 1-3) and for seven of the eight genotypes (Fig. 1-4a). Again, the magnitude of this negative effect varied among genotypes and environmental conditions leading to significant interactions between microbiome x genotype ($F_{7,128}=3.39$, $p=0.002$), and microbiome x environmental condition ($F_{3,128}=5.63$, $p=0.001$). Full Anova tables for all analyses can be found in appendix 1 (Table S1-3).

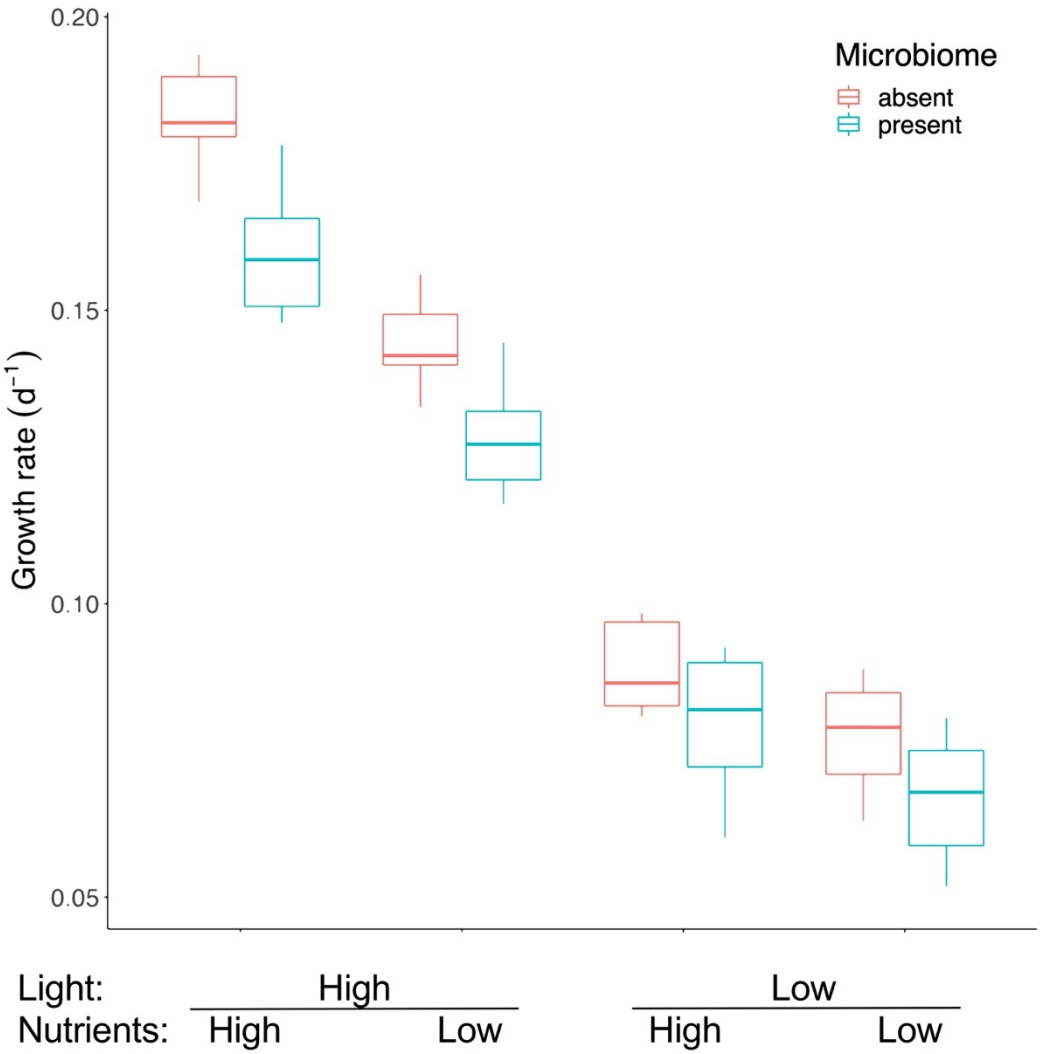

Fig. 1-3. Population growth rate for *Lemna minor* assayed in four modified environmental conditions, with and without its natural microbiome. Each box and whisker represent the variation among 8 independent populations (3 replicate flasks were averaged for each of the 8 genotypes). Boxes represent the upper and lower quartiles and whiskers represent max and min values.

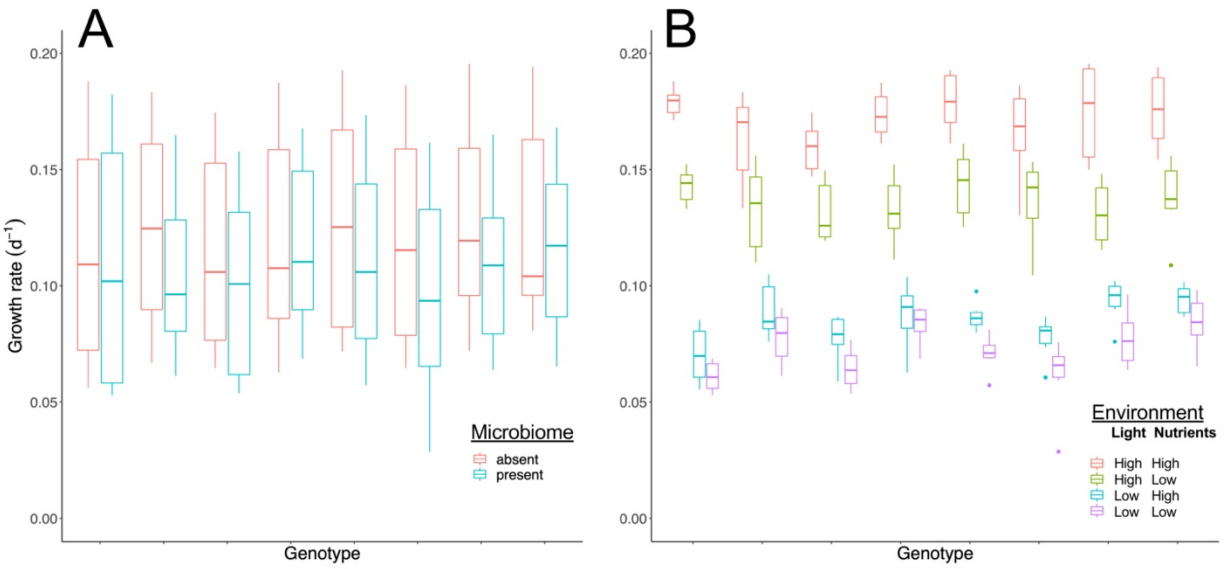

Fig. 1-4. Growth rates for eight genotypes of *Lemna minor*, grown in four different environmental conditions, with and without their microbiomes. A) Variation is the result of 3 replicate assays in each of four environments all grouped together. B) Variation is the result of 3 replicate assays with and without the microbiome all grouped together.

Phenotypic plasticity

Plant phenotype was modified by both the abiotic environment and the presence of the microbiome. Root length responded strongly to the abiotic environment ($F_{3,21}$=80.09, $p<0.001$), increasing in length by 2.1x in low nutrient conditions, although this response was stronger in high light conditions (Fig. 1-5b). There was considerable variation in root length among genotypes ($F_{7,128}$=12.06, $p<0.001$), which interacted with environmental condition ($F_{21,128}$=3.47, $p<0.001$), indicating that the plastic response in phenotype to environmental conditions differed among genotypes (GxE). Systematically across all environmental conditions, roots were shorter when the microbiome was present ($F_{1,7}$=52.45, $p<0.001$), (Fig. 1-5b).

Frond phenotype was also modified by the environmental condition. Fronds grown in low light conditions were visibly darker green in colour than those grown in high light (Fig. 1-6). Frond

area responded to environmental condition ($F_{3,21}$=21.20, p<0.001) and was on average 1.1x times larger when grown in high nutrient conditions compared to low nutrient conditions (Fig. 1-3b). There was considerable variation in frond area among genotypes ($F_{7,128}$=19.44, p<0.001) although these all responded similarly to the environment, (no genotype x environment interaction) ($F_{21,128}$=1.27, p>0.05). The presence of the microbiome resulted in systematically smaller fronds ($F_{1,128}$=50.05, p<0.001) across all environmental treatments (Fig. 1-5a), and for all genotypes. The extent of this varied among genotypes resulting in a marginally significant microbiome x genotype interaction ($F_{7,128}$=2.09, p<0.049). Furthermore, the GxE was mediated by the microbiome resulting in significant 3-way interactions ($F_{21,128}$=2.19, p=0.004).

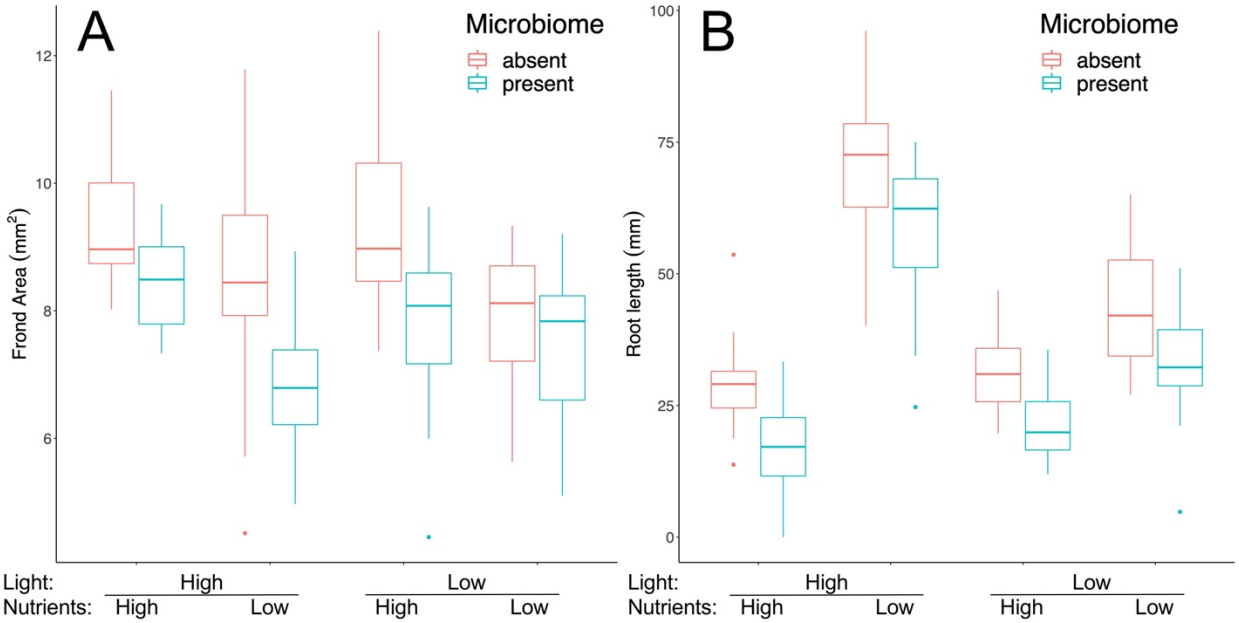

Fig. 1-5. Phenotypic consequences of growth in four modified environmental conditions for *Lemna minor*, with and without its natural microbiome. A) Variation in frond area (mm^2) B) Variation in Root length (mm). Each box and whisker represent the variation among 8 independent populations (3 replicate flasks were averaged for each of the 8 genotypes). Boxes represent the upper and lower quartiles, whiskers represent max and min values, and outliers are shown as points.

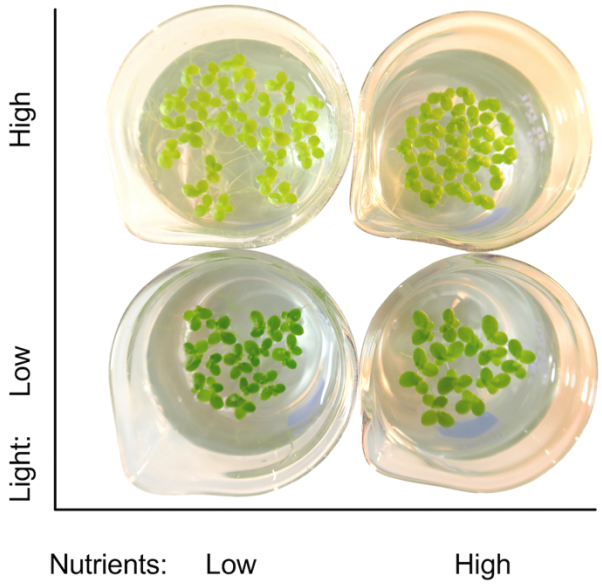

Fig. 1-6. Phenotype of a single genotype of *Lemna minor*, grown in four environmental conditions without the microbiome

Average colony size, i.e., the number of attached fronds, changed markedly with the abiotic environment ($F_{3,128}$=162.49, p<0.001), with smaller colonies in high nutrient and light conditions and larger colonies when these resources are in shorter supply. We regressed colony size on growth rate and found that the slower growing the population, the greater the number of fronds that remain attached ($F_{3,188}$=96.7, p<0.001, m= -36.7, R^2=0.60) (Fig. 1-7). However, this also depended on the presence of the microbiome. In general, the presence of the microbiome decreased colony size. Although the slope of the relationship between colony size and growth rate was the same whether the microbiome was present or absent, the intercept was significantly different (p=0.001), such that for the same growth rate, plants with their intact microbiome exhibited smaller colony sizes (Fig. 1-7).

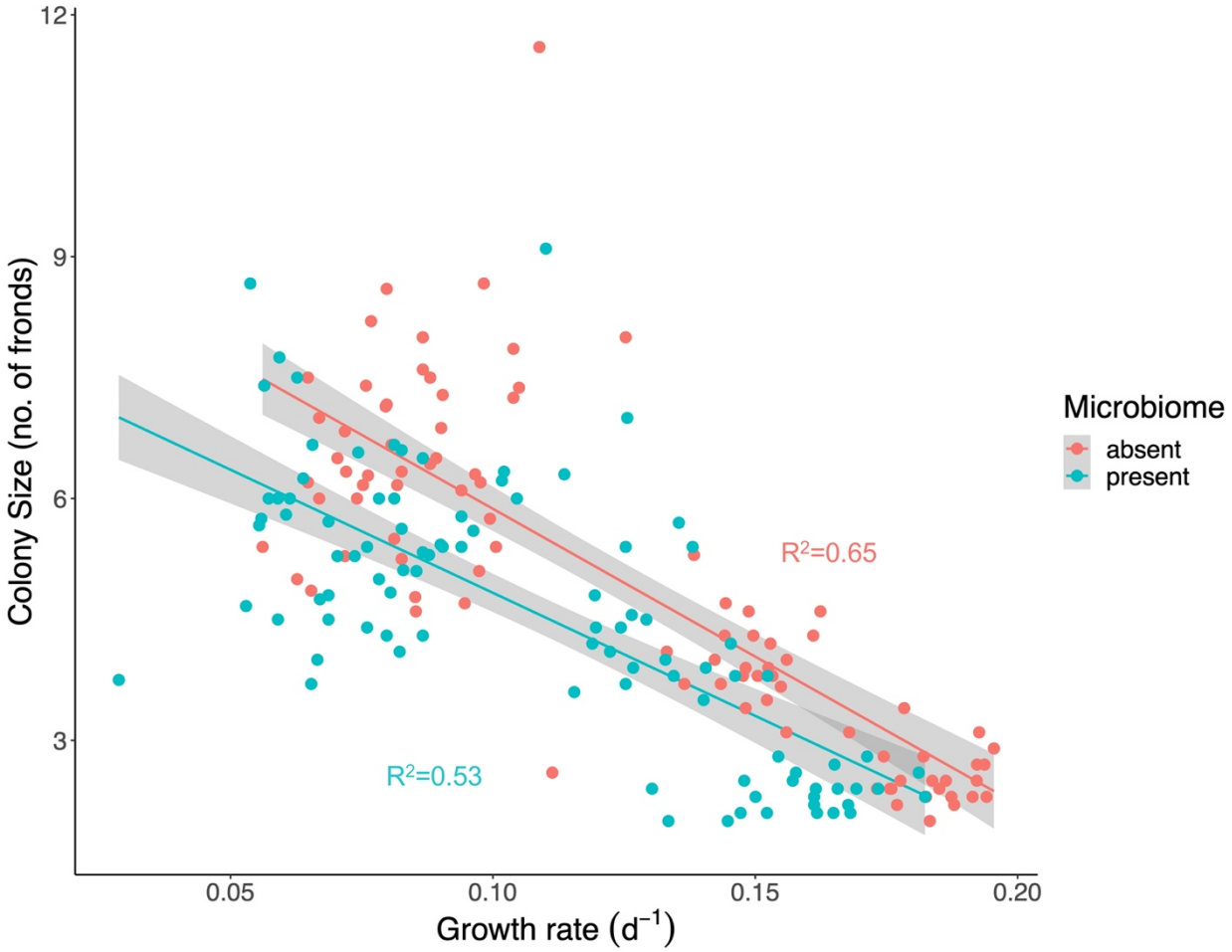

Fig. 1-7. *Lemna minor* colony size as a function of its growth rate for populations with and without their microbiome. Each regression is the result of 96 points (8 genotypes x 4 environments x 3 replicates). Shading around each regression line are 95% confidence intervals.

Discussion

We assessed the impact of the microbiome on the fitness and phenotype of *Lemna minor.* Since host-microbe interactions are often dependent on environmental conditions, plant genotype and microbial community structure, we performed a fully factorial growth assay for eight different *L. minor* genotypes, with and without their microbiome, and grown in a range of environmental conditions.

Effect of the microbiome on host phenotype and fitness

The main aim of our experiment was to assess the fitness and phenotypic consequences of the microbiome for *L. minor*. Although considerable recent work has investigated the importance of certain microbes (mostly bacteria) for *L. minor* growth (Ishizawa et al. 2017b, 2017a, 2019, Gilbert et al. 2018, Chen et al. 2019, Acosta et al. 2020, Iwashita et al. 2020, O'Brien et al. 2020a, 2020b, Tan et al. 2021), the aim has largely been to isolate certain PGPB that increase plant fitness, with few studies characterising the impact of the entire intact natural microbiome on plant performance. Here we isolate plants from eight different genotypes with their full natural microbiomes and assess the impact of the microbiome on host fitness and phenotype.

The effect of the microbiome on plant growth rates was strong and consistent. Contrary to our expectation, the presence of the microbiome decreased plant fitness, on average by 12%. This was the case across all environmental conditions (Fig. 1-3), and for seven of the eight genotypes (Fig. 1-4a). Although several important plant-bacteria and plant-fungi mutualisms have been identified for *L. minor* that increase plant fitness (Acosta et al. 2020, O'Brien et al. 2020a, 2020b, Tan et al. 2021), our results suggest that the importance of pathogens, parasites, and competitors in the microbial assemblage far surpass that of any mutualistic microbes. This is not necessarily surprising given the rich literature documenting the importance of fungal and bacterial pathogens (Rejmankova et al. 1986, Underwood and Baker 1991, Zhang et al. 2010, Ishizawa et al. 2017a, 2017b) and algal competition (van Moorsel 2022), on *L. minor* growth. In land plants, assemblages of PGPB are often unstable in the field (Parnell et al. 2016), and in *L. minor*, the effects of fitness-enhancing strains can be lost with the inclusion of additional strains due to non-additive effects (Ishizawa et al. 2017a).

The effects of the microbiome on plant phenotype were equally clear. The presence of the microbiome resulted in plants with shorter roots and smaller fronds across all genotypes (Fig. 1-5). One explanation for smaller fronds would be the presence of many microbes, including photosynthetic algae, that decrease nutrient availability through direct competition with *L. minor*. However, if this were the main mechanism through which the microbiome modified *L. minor* phenotype, then it would result in increased root length, the ubiquitous plastic response to

decreased nutrient availability. However, we found the opposite, i.e. shorter roots, perhaps a plant response to limit the available surface area for microbes to colonize. In addition, although decreased nutrient availability results in an increase in colony size, we find that the presence of the microbiome increases frond abscission resulting in smaller colonies (Fig. 1-7). This is consistent with other work that has found microbially-mediated shifts in average colony size in *L. minor* (O'Brien et al. 2020a). We therefore conclude that the mechanism by which the microbiome suppressed plant fitness in our experiment goes beyond changes in resource levels. Frond abscission in response to heavy metals has been extensively studied in *L. minor* in the ecotoxicology literature and it is well known that toxic stress generally decreases colony size (Severi 2001, Li and Xiong 2004a, 2004b, Henke et al. 2011, Topp et al. 2011, O'Brien et al. 2020a). The decrease in colony size we observe when the microbiome was present could be due to a similar phenomenon, resulting from toxic microbial secondary metabolites.

One reason for the apparent inconsistency of our results with studies that report a fitness enhancing effect of many microbes (O'Brien et al. 2020a, 2020b, Tan et al. 2021), is that most of these studies limit the microbial assemblage to bacteria (that can be cultivated on yeast mannitol), and exclude many important and ubiquitous microbes such as diatoms, filamentous chlorophytes, and parasitic fungi (Rejmankova et al. 1986, Desianti 2012, Kohout et al. 2012, Moora et al. 2016). Furthermore, many studies are specifically seeking to identify only these mutualistic associations with PGPB (Gilbert et al. 2018; Chen et al. 2019). Due to *L. minor*'s extremely rapid growth rate, among the fastest of all plants, research is often in the context of the plant's many industrial applications which include waste water remediation (Landesman et al. 2011; Iqbal and Baig 2016), biomass production as biofuel (Verma and Suthar 2015), animal feed (Islam et al. 2004; Cheng and Stomp 2009), and human consumption (Sree et al. 2016; Appenroth et al. 2017). For all these applications, there is a keen incentive to further enhance growth rate. Much research has focussed on identifying and selecting the most productive genetic strains of *L. minor* (Bergmann et al. 2000), and much of the work on the microbiome has been done in the same vein, aiming to identify and isolate specific strains of PGPB (Yamaga et al. 2010, Tang et al. 2015, Appenroth et al. 2016). This bias in the literature could lead to a general impression that the microbiome is dominated by mutualistic fitness-enhancing associations, despite a general lack of evidence. Most studies intentionally isolate strains of

bacteria that are good candidates to promote plant growth, which are then artificially inoculated to the axenic plants. Here we take the opposite approach, to estimate the overall effect on their host of the large and diverse assemblages of microbes that make up the microbiome. There are few studies that have tested the effect of entire *L. minor* microbiome on plant fitness instead of just a small subset of bacteria, and those that did found conflicting results. The study that most resembles ours in design, reinoculated the full microbial community to axenic *L. minor*, and concluded that the microbiome increased frond senescence (Underwood and Baker 1991).

A limitation of this study is the fact that we did not characterize the microbial community and thus, we can only speculate on the mechanisms responsible for our results. Variation in the phenotypic and fitness consequences of the microbiome was surprisingly consistent across all genotypes. This is notable since our genotype treatment included not just different plant clones, but also independent microbiomes from each genotype. Despite the possibility of strong differences in microbial community composition among genotypes, their overall effect on each plant genotype was overwhelmingly uniform. This is consistent with work that has shown the absence of plant-microbe specialization among genotypes in *L. minor* by manipulating plant genotype and microbial community source independently (O'Brien et al. 2020a). It appears that in our experiment, the eight independent microbial communities were of similar composition, at least in terms of broad functional groups and their interactions with the plant host.

Effect of environment on host phenotype and fitness

The largest source of variation in our experiment was environmental, namely light and nutrient availability, which impacted growth rates, frond area, root length and colony size in a strong and consistent way for all genotypes, with or without the presence of the microbiome. Both light and nutrients were limiting at low levels (Fig. 1-3).

In low nutrient conditions the main phenotypic response was an increase in root length. This response to low light was combined with a decrease in frond area, as plants invested a larger portion of their biomass to root tissue (Fig. 1-5b). Although in *L. minor*, nutrient uptake takes

place via both the roots and fronds, longer roots increase rates of Nitrogen uptake due to the increased surface area, and an increase in root length in low nutrient conditions is consistent with other studies (Cedergreen and Madsen 2002, 2004). This plastic response in root length was strengthened in high light conditions, perhaps since the increase in plant growth resulted in more severe nutrient limitation.

Fronds became smaller in low nutrient conditions (Fig. 1-5a), which is a common response to stress in *L. minor* (Mohan and Hosetti 1999, Naumann et al. 2007, O'Brien et al. 2020a). In low light conditions, fronds were also visibly darker green in colour (Fig. 1-6), another standard plant response to light limitation due to an increase in leaf chlorophyll content (Björkman 1981, Minotta and Pinzauti 1996).

Colony size is controlled by the abscission of the stipe, vascular tissue connecting the mother and daughter fronds (Landolt 1986). Here we find that higher resource levels resulting in increased growth led to increased abscission and therefore smaller colony size. One possible interpretation is that in low resource environments, daughter fronds act as a sink by continuing to receive fixed carbon from the rest of the colony through prolonged attachment.

Genetic variation in phenotype and fitness

In addition to the large and consistent plastic effects of the environment on phenotype and fitness, there were also genetic differences among the populations from the eight different water bodies. Although we cannot be sure that samples taken from different sites represent different genotypes, studies on natural populations of *L. minor* have shown considerable among-site genotype diversity at similar geographical scales to ours (Vasseur et al. 1993, Cole and Voskuil 1996, Xue et al. 2012), and it's reasonable to assume that samples taken from different sites represent different genotypes (Ho 2018). By removing environmental variation through common garden growth assays, we can estimate the variation in fitness due to genetic differences. With the microbiome absent, we found small but significant differences in fitness and phenotype among genotypes, indicating some genetic control of these traits. This is consistent with previous

work that find large differences in fitness among clones, for example, Ziegler et al. (2015), who, in a common garden assay of 13 species of *Lemnaceae* detected a greater amount of variation in growth rate among genotypes of the same species than variation among species or even genera. Finally, we also detected small genotype by environment (GxE) interactions for both fitness and phenotype in the absence of the microbiome. Although plants from all sites responded in a similar direction to light and nutrients, the magnitude of these responses differed among genotypes indicating the presence of variation in the genetic control of phenotypic plasticity.

Conclusions

To return to our initial three questions we set out to address, we conclude that the full *L. minor* microbiome unequivocally suppresses fitness of the host plant. This was the case in all environment treatments for seven of the eight genotypes. The decrease in fitness was accompanied by phenotypic changes, with plants producing smaller fronds and shorter roots with the microbiome present. There was some variation in the magnitude of the effect of microbiome on plant fitness among genotypes perhaps because of differences in microbial composition among sites. Likewise, there was variation among genotypes in the phenotypic response to environment, but this was independent of the microbiome. Although the *L. minor* microbiome has been shown to include important mutualistic associations with many bacteria, their influence seems to be overridden by pathogenic, parasitic, and competitive interactions in our system. Future work should focus on characterising the eukaryotic microbiome and understanding how the abiotic environment mediates shifts in host-microbial associations.

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Preamble to Chapter 2

In Chapter 1 we found considerable plasticity in frond area and root length for *Lemna minor* as a function of irradiance and water nutrient availability. In Chapter 2, a regional field survey, we ask if the same traits vary in the field as a function of resource availability, and whether this variation is the result of plasticity or local adaptation. After measuring the phenotypes of 1020 plants from 34 sites in the field as well as several environmental correlates, we brought samples back to the lab to use in a common garden experiment. This common garden was designed to determine the contributions of environmental and genetic variance to phenotypic variation. A secondary objective of this chapter was to measure genetic variation in fitness which enabled us to estimate the strength of purifying selection and the evolution of fitness.

This chapter is currently in revision at Aquatic Botany.

Chapter 2 - Environmental and genetic variation in *Lemna minor*

Mark Davidson Jewell[1] and Graham Bell[1,2]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.
[2]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Summary

Species may respond to variation in environmental conditions by modifying their phenotype to match local levels of resource availability. This phenotypic response can be driven by plastic physiological change, or by adaptive genetic change. Here we use *Lemna minor* (lesser duckweed), a small aquatic macrophyte that is increasingly used as a model in ecology and evolution, to investigate the source and maintenance of phenotypic variation in natural environments. We found substantial phenotypic variation in *L. minor* in the field, with its frond area and root length changing predictably over natural environmental gradients of resource availability. Separating environmental and genetic variation in these traits in a common garden, we attribute the majority of phenotypic variation we observed in the field to phenotypic plasticity. Despite this, there was substantial within-site genetic variation. We found evidence of strong purifying selection in the field, that is necessarily balanced by mutation and migration. Using measures of environmental and genetic variation in phenotype and fitness, we estimate rates of evolution of fitness, and dispersal necessary to sustain the observed levels of genetic variation.

Introduction

Separating environmental from genetic contributions to phenotypic variation is central to evolutionary ecology since it illuminates how species respond to their local environment and

produce phenotypes capable of maintaining positive fitness and thus population persistence. When a population experiences new environmental conditions, either by environmental change or range expansion, existing genotypes may shift their phenotypic expression via physiological change (aka. adaptive plasticity), or evolution may shift genotype frequencies leading to local adaptation via genetic change (Sultan 2000, Kingsolver et al. 2002). Natural selection, acting on novel mutation and standing genetic variation, should reach beyond the limits of plasticity and maximize population mean fitness (Auld et al. 2010). The rate of evolutionary change is however dependent on the amount of genetic variation in a population, namely genetic variation in fitness (Burt 1995). Like phenotype, fitness itself is comprised of environmental and genetic components (Schoen et al. 1994). Genetic variation in fitness is the raw material natural selection acts on, and determines a population's rate of adaptive evolution in the face of environmental change and genetic degradation caused by deleterious mutation, maladaptive gene flow, genetic drift and inbreeding depression (Burt 1995, Hendry et al. 2018). Despite the critical role of intraspecific genetic variation to species adaptation and survival (Booy et al. 2000), the amount of genetic diversity in natural populations remains largely unknown in some systems. Whereas much recent work has focused on the genetic structure of endangered populations, it is equally important to understand how genetic variation may contribute to the successes of populations that thrive.

In this study we quantified environmental and genetic variation in morphological traits and in fitness for the plant *Lemna minor* (lesser duckweed), a tiny floating aquatic plant in the family *Lemnaceae* found in eutrophic ponds and wetlands. Among the smallest of all angiosperms*, L. minor* consists of only a single leaf-like frond, a few mm across, to which a single unbranched root is attached. Its reproduction is almost exclusively asexual and vegetative with daughter fronds budding out the mother frond's lower surface (Landolt 1986, Lemon and Posluszny 2000). Daughter fronds remain attached to the mother for a certain period of time before splitting apart after abscission (Landolt 1986, Lemon et al. 2001). Their generation time may be as short as just a few days, and their small size results in populations of hundreds of thousands to millions of individuals in a single pond. Because they are widespread and abundant, are easily maintained and manipulated in the laboratory, and possess highly reduced morphology and simplified

physiology, they are being increasingly used as a tractable model system in ecology and evolution (Laird and Barks 2018, Hart et al. 2019, Vu et al. 2019).

We quantified phenotypic variation in two morphological traits: frond area and root length. The extremely simplified morphology of *L. minor* means that these two traits essentially capture the totality of biomass allocation between shoot and root tissue, responsible for the capture of light and nutrients. The frond is essentially a photosynthetic sheet whose area may fluctuate to balance light capture and photosynthesis (growth) with the production of daughter fronds (reproduction) (Vasseur et al. 1995). Root length on the other hand has been shown to vary depending on nutrient levels, since uptake rates are proportional to root surface area (Cedergreen and Madsen 2002). Optimal phenotype in *L. minor*'s root-shoot ratio should then vary in the field as a function of local availability of light and nutrients, with the plant investing more biomass into the tissue responsible for the uptake of the limiting resource. Such phenotypic variation could arise via plasticity or local adaptation, or both, with consequences for within and among site genetic diversity. By measuring phenotypic expression in a common garden assay (Kawecki and Ebert 2004) we can quantify environmental and genetic components of variation in frond area and root length to determine if these traits have a genetic basis and result from local adaptation.

Our study had three main objectives. First, we ask if phenotypic variation in *L. minor* is correlated with natural gradients of resource levels. We hypothesize that biomass allocation and phenotype should match the environment to increase uptake rates of limiting resources, such that low light environments produce plants with larger fronds, and low nutrient environments produce plants with longer roots. Secondly, we ask if such phenotypic variation is due primarily to plasticity or local adaptations. We do this by quantifying the environmental and genetic contributions to phenotypic variation. Thirdly we aim to estimate adaptive potential in natural populations of *L. minor* by quantifying the standing genetic variation in fitness.

Materials and Methods

Field survey

In July 2019, we conducted a regional survey of natural populations of *Lemna minor*. We located 34 sites consisting of lakes, wetlands, ponds, and roadside ditches broadly distributed across southern Quebec, Canada (Fig. 2-1). Within each site, we sampled from three microsites, situated at least 10m apart and supporting a minimum of 10 *L. minor* colonies. From each microsite, we collected 10 free-floating colonies of *Lemna minor*. Colonies consisted of between 2-10 attached fronds. Since daughter fronds emerge from two meristematic pockets located on the frond's lower surface, frond genealogy is easily inferred. For each colony we measured both frond area and root length of the oldest frond by photographing the plant against a standard ruler and later analysing the photos using ImageJ. This resulted in 30 measurements of each trait per site.

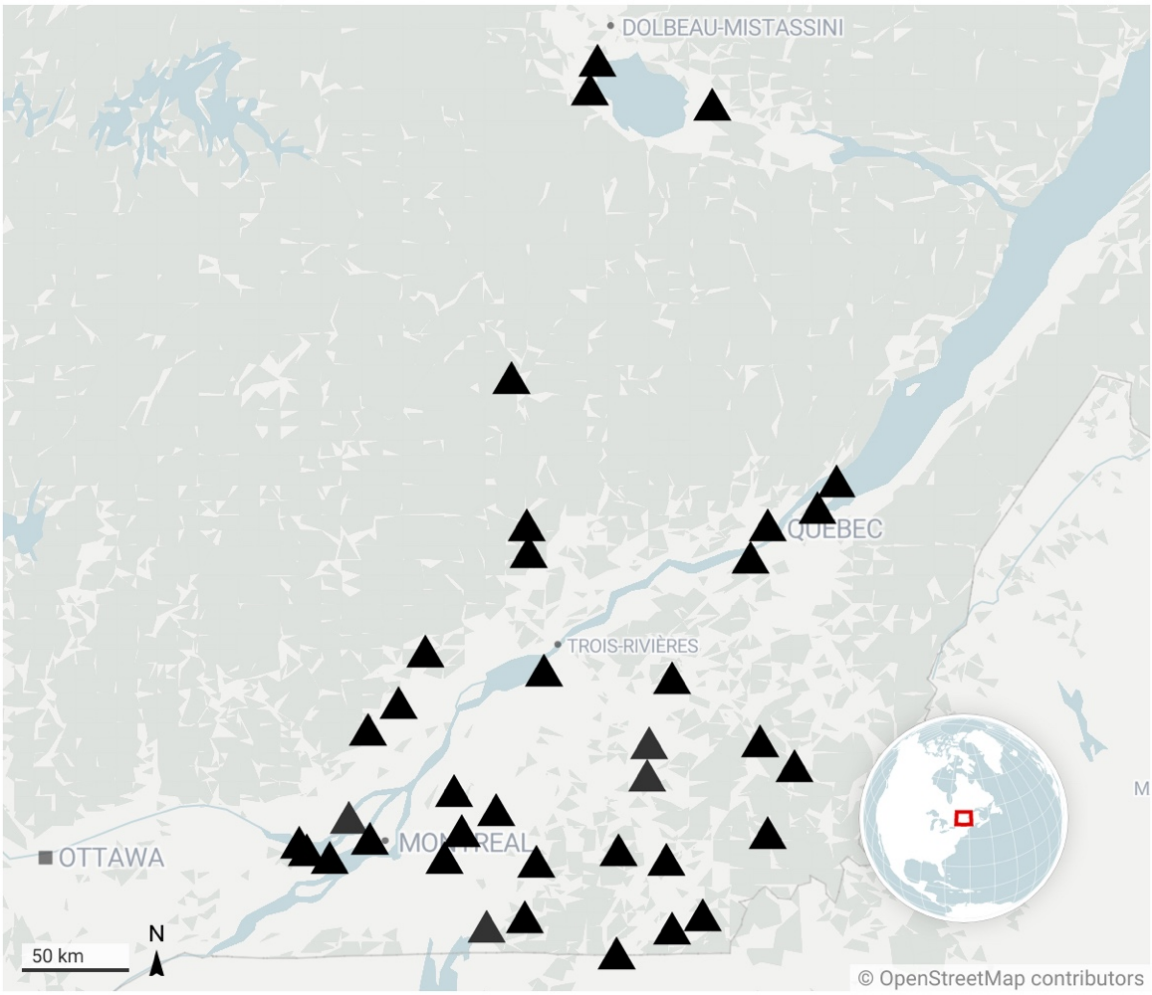

Fig. 2-1. Thirty-four sites distributed across southern Quebec, supporting natural populations of *Lemna minor*. Map was produced with Datawrapper.

For each site we measured several environmental variables that we expected to be correlated with plant phenotype. Light availability, as percent transmittance of photosynthetically active radiation (PAR), was estimated *in situ* with the use of BF5 Sunshine Sensor (Delta-T, Burwell, Cambridge, UK) (Paquette et al. 2007). This instrument consists of an array of seven quantum sensors under a semi-shaded hemispherical dome to give estimates of diffused light under any meteorological condition. We took two simultaneous paired measurements, one at the sampling site in question, and a second reference point at a nearby open site (field or road) under full sun. Percent transmittance PAR was then estimated as the ratio of diffused light between the site and the reference measurement. This method has been demonstrated as a reliable and practical

alternative to more standard measurement techniques including hemispherical image analysis (Rich et al. 1993, Paquette et al. 2007). Percent transmittance of PAR was estimated at each of the three microsites as well as the center of the site. All measurements were taken 1.5m above the water level, above any aquatic macrophytes or riparian herbaceous plants to obtain an estimate of shading from the canopy cover. These four measurements were then averaged to produce a single estimate of light availability for each site. To measure water nutrient content, we took eight water samples from the center of each site at a depth of 30cm. Total Nitrogen (TN), total Phosphorus (TP), dissolved Nitrogen (DN) and dissolved Phosphorus (DP) were estimated each from two replicate samples. Acid-washed tubes were first rinsed, and then filled with sample water, unfiltered for TN and TP samples, and sterile filtered at 0.45um for DN and DP samples. After sampling, all tubes were stored in a cooler on ice and brought back to the lab for analysis. Samples were then stored at 4^{o}C and processed within 14 days. Water samples were analysed for TN and DN with a continuous flow analyser (OI Analytical Flow Solution 3100 ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor (Patton and J.R. 2003) and for DP using a standard protocol (Wetzel and Likens 2000). TP was measured using colorimetric detection with a spectrophotometer at 890 nm, after digestion with potassium persulfate and the addition of an ammonium molybdate solution (Wetzel and Likens 2000). All samples were analysed at the GRIL, Université du Québec à Montréal (UQAM) analytical laboratory. We used a YSI probe (YSI professional plus, Xylem Inc., Yellow Sprigs, OH, USA.) to measure water temperature and pH at the centre of each site at a depth of 30cm. Full list of environmental correlates can be found in the Appendix 2 (Table S2-1).

Common garden assay

To determine the sources of phenotypic variation in the field, we brought samples back to McGill University to use in a common garden growth assay. Whereas phenotypic variation in the field is due to a mixture of environmental and genetic sources, growth in a common garden removes environmental variation, isolating genetic variation. A single colony of *L. minor* was collected from each of 3 microsites for each site and preserved in tubes filled with natural sample water and stored in the dark during transport.

Back in the lab, all tubes were placed under artificial grow lights (100 µmols/m^2/s) until the plants had doubled in number, consisting of at least two detached colonies which would be used to found two clonal replicates for each microsite. The common garden assay was done using 500mL Erlenmeyer flasks. There was a total of 204 flasks (34 sites x 3 microsites x 2 replicate flasks). Flasks were filled with 350mL of growth media, diluted Hoagland's E-media ([N]=5000 µgL^{-1}, [P]= 780 µgL^{-1}, pH=7.0 +/-0.05) (recipe in Appendix 2, Table S2-2), plugged with a foam stopper, and then autoclaved. A single colony (3-4 attached fronds) was used to inoculate each flask. These initial fronds were first marked on their dorsal surface with a small dot with a permanent marker to later track generations. This was to ensure that phenotypes were only measured on fronds at least two generations younger than those sampled from the field.

All flasks were placed in one of two identical controlled growth chambers of the McGill phytotron (200 µmols/m^2/s light, 20^0C, 70% relative humidity, with a 14/10 light-dark cycle). The two replicates were blocked, with one replicate of each microsite in each chamber. The 102 flasks in each growth chamber were randomly positioned, leaving a 15cm boundary from the chamber wall on all sides. The common garden assay was broken into three 10-day phases, separated by two transfers. Transferring the plants to fresh media every 10 days prevented nutrient depletion, all-the-while limiting the growth of phytoplankton whose differential abundance among flasks could influence nutrient availability and plant growth. To remove any maternal or carry over effects, we tracked generations to ensure that we only measured the phenotypes of plants at least two generations younger than the initial plants brought back from the field. The first, 10-day preliminary acclimation phase served to ensure an equal physiological starting point of all plants before we began to track population growth rates, and to ensure the removal of all fronds initially present in the assay. After these 10 days of growth, all fronds marked with a black dot were discarded, and a single younger colony was randomly selected and used to inoculate identical flasks with fresh growth media, after the oldest frond in this colony was again marked. After a second 10 days of growth, flasks were again removed, and all plants were transferred to fresh media before being returned to the chambers for a final 10 days of growth. After each of the two transfer dates, all flasks were returned to the same growth chambers, but their positions within each were independently randomized. At the end of the

experiment, the total number of fronds was counted in each flask and used to calculate rates of exponential population growth (over the final 20 days). From each flask, we randomly sampled 10 individuals (on average ~15% of the population) for whom we measured frond area and root length by imaging (plants were pressed onto a sheet including a reference ruler and photographed at a standard 20cm distance) and subsequent image analysis using Image J (Abràmoff et al. 2004). Second generation fronds (marked with a black dot) were excluded, as were immature fronds (that didn't yet have two daughter fronds budding from them).

Statistical analysis

To test whether there were differences in phenotype among sites and microsites in the field, we used a 2-way nested analysis of variance (Anova) with microsite nested within site. Both site and microsite were analysed as type II random factors. This was done for both response variables (frond area and root length). Since the environmental correlates were measured at the site level, all 30 measures of phenotype (10 individuals x 3 microsites) were averaged to produce a single value per site for frond area and root length. We then regressed site mean phenotype (both frond area and root length) against the environmental correlates (light availability, TN, TP, DN, DP, and pH) using linear regression and simplified the models by removing non-significant terms. To test whether there were differences in phenotype among sites and microsites after the common garden assay we used a similar nested Anova as that used for the field data, but with a 3rd level (replicate flask), nested within microsite, using the 10 individuals per flask as the error variance. Growth rate (fitness) was calculated for each flask over the final 20 days of the common garden assay using the standard formula for exponential growth $r = \ln\left(\frac{Nt}{N0}\right)/t$ where N_0 is initial population size, t is time in days, and N_t is population size at time t. To test for differences in fitness among sites and microsites we used a similar nested Anova with microsites nested within sites. However, since there is only a single measure of fitness per flask, replicate flask was used as the error variance.

Results

We observed substantial phenotypic variation in the field for both frond area and root length. Frond area varied significantly among sites ($F_{33,68}$=5.39, p<0.0001) and among microsites within sites ($F_{68,918}$=15.26, p<0.0001) with roughly equal contributions to variation from each level (Table 2-1). Similarly, root length varied significantly among sites ($F_{33,68}$=14.12, p<0.0001) and among microsites within sites ($F_{68,918}$=3.46, p<0.0001), although the majority of this variation was at the among site level (Table 2-1).

Table 2-1. Variation in frond area (mm^2) and root length (mm) measured in the field. Ten individual plants were sampled in each of three microsites, for 34 sites situated broadly across southern Quebec, Canada.

Trait	Source	df	SumSq	MeanSq	*F*	*p*	Variance Component
Frond Area	Site	33	13844	419.52	5.39	<0.0001	11.389
	Microsite	68	5294	77.853	15.26	<0.0001	7.2753
	Error	918	4682	5.1002			5.1002
	Total	1019	23820				
Root Length	Site	33	335062	10153.39	14.12	<0.0001	314.47
	Microsite	68	48861	718.54	3.46	<0.0001	51.10
	Error	918	190561	207.58			207.58
	Total	1019	239422				

Variation in frond area was correlated with natural levels of light availability ($F_{1,32}$ = 16.99, p=0.0002, β= -9.663, R^2=0.35), with plants growing in more heavily shaded sites with thicker canopy cover expressing larger fronds (Fig. 2-2A). Variation in root length was correlated with water nutrient levels (both dissolved and total Nitrogen and Phosphorus), with plants growing longer roots in lower nutrient conditions (Fig. 2-2B). The variable with the most explanatory power was total Phosphorus content ($F_{1,32}$ = 27.59, p<0.0001, β= -0.10, R^2=0.46). PH failed to explain additional variation in these traits.

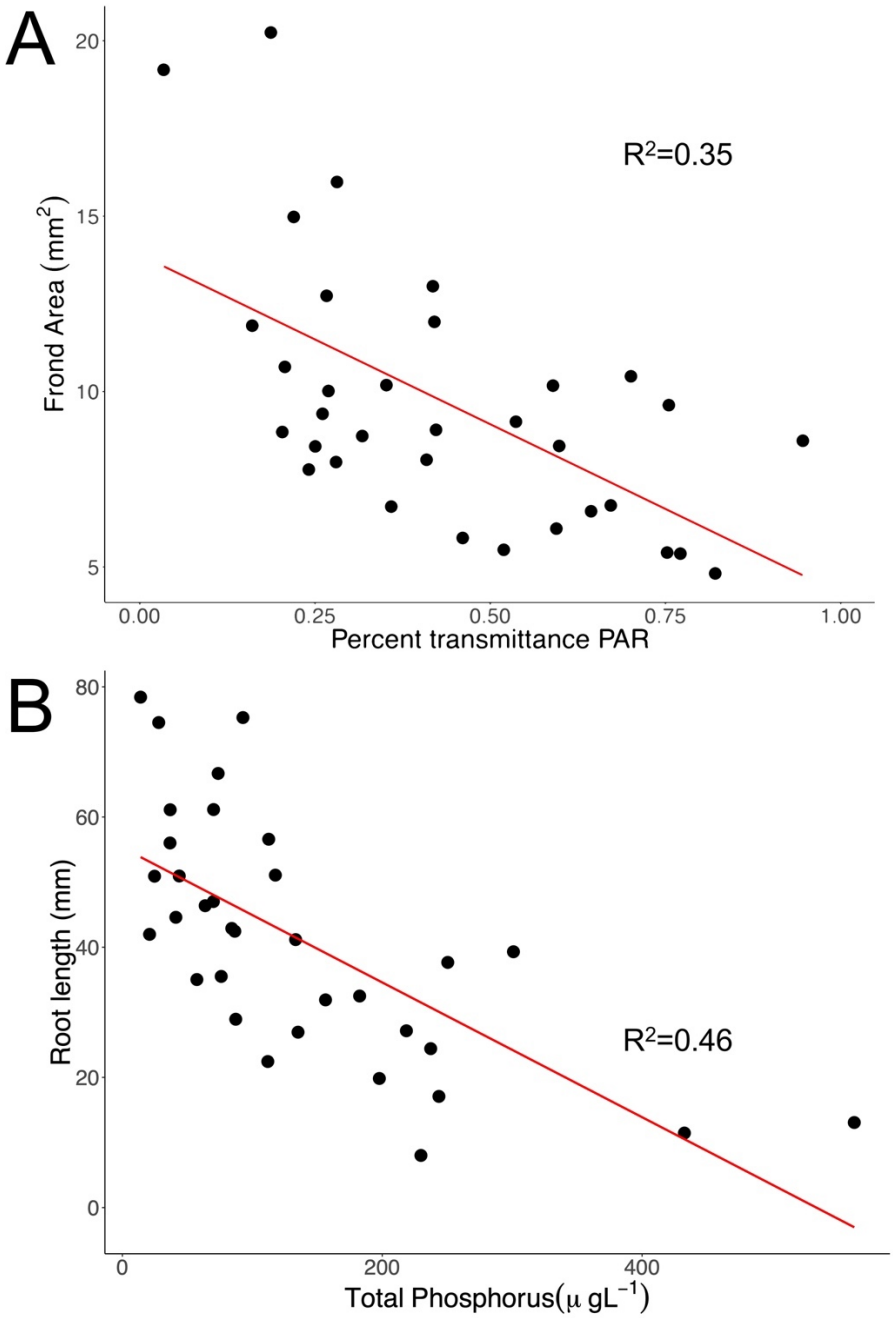

Fig. 2-2. Correlations between phenotypes measured in the field and natural levels of resource availability. A. Average frond area (mm^2) as a function of local light availability. Each point is the average of 10 individuals in each of 3 microsites to give a single value of average frond area per site. Light availability is measured as percent transmittance of photosynthetically active radiation. B. Average root length (mm) as a function of water total Phosphorus (μgL^{-1}). Each point is the average of 10 individuals in each of 3 microsites to give a single value of average root length per site.

Plants from the field were taken back to the lab and grown in a common garden assay. Mean generation time in the common garden was 4.1 days which resulted in a total of ~7 generations for the full 30-day common garden assay. Although diverse protists and cyanobacteria were observed in the flasks with microscopy, their densities remained low as the growth media never became green to the naked eye.

Whereas phenotypic variation in the field is due a mixture of environmental and genetic sources, any persistent variation in the common garden can be attributed to genetic differences. There was a major reduction in phenotypic variation among sites, comparing measurements from the field to those in the common garden, for both frond area (Fig. 2-3 A&B), and root length (Fig. 2-3 C&D). Frond area generally increased in the common garden compared to field measurements, likely due to the vastly lower irradiance provided by artificial light in the growth chambers (200 μmols/s/m^2) compared to natural irradiance, even in shaded sites.

Despite the overall marked decrease in phenotypic variation, variation among sites and microsites persisted in the common garden assay. Frond area varied significantly among sites ($F_{33,68}$=1.71, p=0.03) and among microsites within sites ($F_{68,102}$=2.15, p=0.0002) with roughly twice as much variation within sites among microsites, than among sites (Table 2-2). Similarly, root length varied significantly among sites ($F_{33,68}$=1.98, p=0.009) and among microsites within sites ($F_{68,102}$=1.44, p=0.05), with roughly equal amounts of variation among sites and among microsites (Table 2-2).

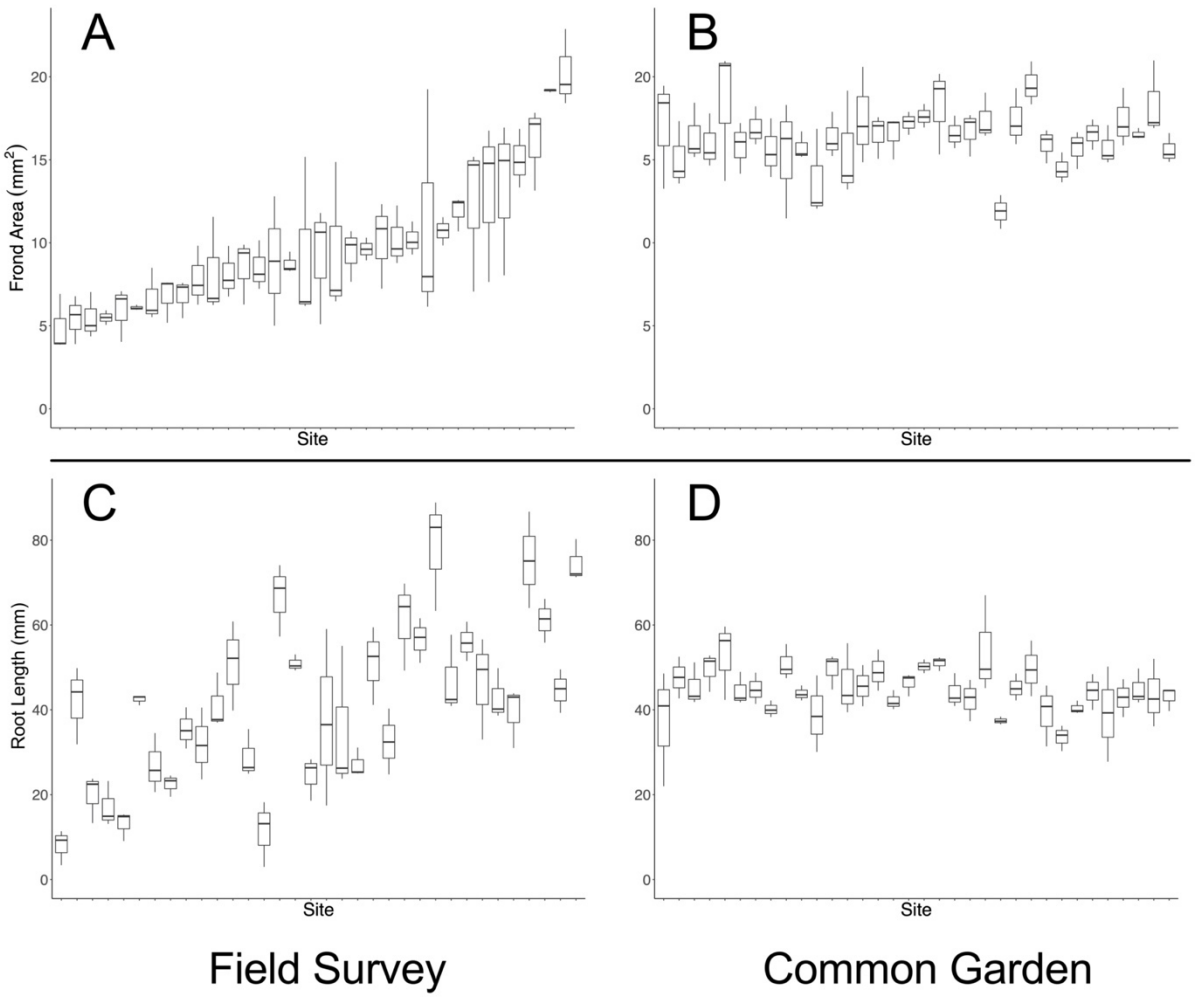

Fig. 2-3. Phenotypic variation for plants in the field and grown in a common garden assay. Boxes and whiskers show among microsite variation for each site. Sites are ordered by mean frond area and are consistent for the four panels. A&C. Phenotypic variation in the field. Phenotype was measured on 10 plants per microsite and averaged to produce a single estimate per microsite. B&D. Phenotypic variation in the common garden. Phenotype was measured on 10 plants in each of two replicate flasks for each microsite and averaged to produce a single estimate per microsite.

Table 2-2. Variation in frond area (mm^2) and root length (mm) among plants grown in a common garden assay. Ten individual plants were measured in each of two replicate flasks for each of three microsite, for 34 sites situated broadly across southern Quebec.

	Source	df	SumSq	MeanSq	*F*	*p*	Variance Component
Frond Area	Site	33	4028	122.05	1.711	0.0313	0.845
	Microsite	68	4851	71.34	2.154	0.0002	1.911
	Replicate	102	3379	33.12	5.862	<0.0001	2.747
	Residuals	1836	10373	5.65			5.65
Root Length	Site	33	45204	1369.8	1.979	0.008923	11.295
	Microsite	68	47063	692.1	1.440	0.0472	10.58
	Replicate	102	49010	480.5	3.076	<0.0001	32.43
	Residuals	1836	286724	156.2			156.2

Comparing variance components calculated in the field and then in the common garden, we estimate that 93% of the among site variation in frond area in the field was environmental, with only 7% genetic, which persisted in the common garden (Fig. 2-3 A&B, Table 2-3). Likewise, 96% of the among site variation in root length in the field was environmental, with only 4% due to genetic variation, which persisted in the common garden (Fig. 2-3 C&D). Whereas the vast majority of among site phenotypic variation was environmental in origin, within site phenotypic variation had a more substantial genetic component (Frond area: 26%, Root length: 21%), (Table 2-3).

Table 2-3. Environmental and genetic components of phenotypic variation. Variation in the field survey (FS) consists of the combined contributions of environmental and genetic variance, whereas variation in the common garden (CG) isolates the genetic component.

Trait	Source	Variance Component		% Environmental	% Genetic
		(FS)	*(CG)*	*(FS-CG)/FS*	*CG/FS*
Frond Area	Site	11.389	0.845	0.926	0.074
	Microsite	7.275	1.911	0.737	0.263
Root Length	Site	314.47	11.295	0.964	0.036
	Microsite	51.10	10.58	0.793	0.207

Fitness was estimated for each replicate flask in the common garden assay as population exponential rate of increase. There was added variance in fitness among sites ($F_{33,68} = 2.03$, $p=0.007$) and among fronds within sites ($F_{68,102} = 3.26$, $p<0.0001$) (Table 2-4). The variance among fronds within sites was roughly twice as large as the variance among sites.

Table 2-4. Variation in fitness measured in the common garden assay

Source	df	SumSq	MeanSq	*F*	*p*	Variance Component
Site	33	0.0383	0.001160	2.0314	0.0069	0.000098
Microsite	68	0.0388	0.000571	3.2561	<0.0001	0.000198
Error	102	0.0179	0.000175			0.0001753
Total	203	0.0950				

Discussion

Environmental and genetic variation in phenotype

In this study we set out to explain the nature, origin, and maintenance of phenotypic variation in *L. minor* in the field. Phenotype varied widely among sites, with mean frond area varying by a factor of two (Fig. 2-2A), and mean root length by a factor of more than eight (Fig. 2-2B). This variation was overwhelmingly the result of phenotypic plasticity. Although there were persistent differences in phenotype among sites in the common garden assay, the reduction of variation in frond area by 93% and in root length by 96% (Fig. 2-3, Table 2-2) reveals that among site phenotypic variation is almost exclusively environmental. This is consistent with previous work that has shown a large degree of plasticity in these traits, (Vasseur and Aarssen 1992, Cedergreen and Madsen 2002) and the absence of local adaptation (Vámos and van Moorsel 2022). Both among and within sites, the environmental contribution to phenotypic variation was larger for root length than frond area, which is also consistent with previous work reporting root length as *L. minor*'s most plastic trait (Vasseur and Aarssen 1992). Phenotypic variation in *L. minor* in the field is largely explained as a plastic response to the abiotic environment, shifting its phenotype to levels of resource availability. 35% of among site variation in frond area is explained by light availability, with plants producing larger fronds in more heavily shaded environments. The

production of larger leaves in low light environments is a standard ecophysiological response in plants (Meziane and Shipley 1999, 2001), that influences fitness through photosynthesis, transpiration and thermoregulation (Anten et al. 1995, Hirose et al. 1997). Similarly, 46% of among site variation in root length is explained by nutrient availability with a dramatic increase for plants growing in sites with low levels of dissolved N and P. This is consistent with previous experimental work that has documented a plastic increase in root length in *L. minor* in response to nutrient limitation (Cedergreen and Madsen 2002). Although *L. minor* can uptake inorganic nutrients through both the root and the frond (Landolt 1986, Cedergreen and Madsen 2002), this balance shifts depending on both nutrient availability (Cedergreen and Madsen 2002), and irradiance (Cedergreen and Madsen 2004) with the production of longer roots resulting in an increase in root N uptake and NO_3 reduction. Variation in frond area and root length in *L. minor* can be conceptualised as a simplified root-shoot ratio (Cedergreen and Madsen 2002). A well-studied trait in land plants (Brouwer 1962, Poorter and Nagel 2000), *L. minor* seems to respond to resource limitation by investing more biomass into increasing the surface area of the tissue responsible for the uptake of the limiting resource.

In addition to among-site phenotypic variation, we observed significant phenotypic variation within sites. Whereas frond area varied substantially both among sites and among microsites within sites, the majority of variation in root length was at the among site level. Given the largely environmental origin of this variation, it is perhaps unsurprising that frond area would vary within sites due to the patch-like variation in light availability caused by fine-scale shading from macrophytes and riparian plants (Bell et al. 1991). In contrast, water nutrient availability is likely much more homogenous within sites due to mixing and diffusion resulting in most variation in root length manifesting among and not within sites. For both frond area and root length, the proportion of phenotypic variation with a genetic origin was much higher within sites (26% and 21%) than among sites (7% and 4%). The larger contribution of environmental variation to phenotype among sites can be explained by the greater environmental variation at the higher geographical resolution. However, we observed a surprisingly large amount of within site genetic variation. Environmental variation aside, the absolute amount of genetic variation in frond area was twice as large within sites than among sites, and equal within and among sites for root length. Whereas among site genetic variation is easily explained by adaptation to local conditions

or genetic drift given limited gene flow, the large amount of within site genetic diversity is surprising, especially in the absence of sexual reproduction.

In the common garden assay, the contribution of replicate flask to overall phenotypic variation was significant and second only to residual variation. This is perhaps surprising since replicate flasks consisted of clones, descending from the same ancestor sampled from the field. However, replicate flasks confounded several sources of variation including flasks effect, chamber effect (from the blocked design), and birth order effects from the original parental frond which have been shown to persist over several generations (Barks and Laird 2015, 2016, Mejbel and Simons 2018). Removing this variation from the residuals enabled us to detect the higher-level effects of microsite and site.

Genetic variation in fitness and evolutionary potential

In asexual, clonal populations, fitness can be directly measured as the population's exponential rate of increase (Bell 2008). Like phenotype, fitness also consists of environmental and genetic components that can be separated in a common garden assay. There is strong evidence for a large amount of genetic variation in fitness among different genotypes of *L. minor*, and in some cases, even greater variation among genotypes of the same species of *Lemnaceae* than among closely related species (Ziegler et al. 2015). However, how this variation maps onto the landscape remains unclear (Xu et al. 2015). Although many studies have reported considerable among-site genotype diversity (Vasseur et al. 1993, Cole and Voskuil 1996, Xue et al. 2012, Xu et al. 2015, Ho 2018), it is sometimes thought that *L. minor* possess poor levels of within site genetic diversity (William C . Jordan 1996, Xu et al. 2015). To our surprise, we found that there was twice as much genetic variation in fitness within sites (among microsites) than among sites (Table 2-4). This is consistent with studies quantifying intraspecific genetic variation in *L. minor* using allozymes (Vasseur et al. 1993, Cole and Voskuil 1996, El-Kholy et al. 2015) and amplified fragment length polymorphisms (Bog et al. 2022) that have reported between 4-20 genotypes per site. The source of this genetic variation remains unclear given the low estimates

of gene flow (Cole and Voskuil 1996), mutation rates (Sandler et al. 2020), and frequency of sexual reproduction (Hillman 1961a, Landolt 1986, Vasseur et al. 1993, Ho 2018) in *L. minor*.

Genetic variation in fitness is arguably the most important parameter in evolutionary biology since it is what natural selection acts upon, and is therefore directly related to the adaptive potential of a population (Burt 1995). Fisher formalised this relationship in his 1930 fundamental theorem of natural selection (Fisher 1930, Crow 2002) by equating the standardized additive genetic variance in fitness (SV_A) with the per generation change in ln mean fitness, $\overline{w}$ (Equation 1.1).

$$SV_A = \frac{var(w)}{\overline{w}^2} = \Delta \ln(\overline{w}) \quad (1.1)$$

In a constant environment, all populations experience genetic degradation due to deleterious mutations (Lynch and Gabriel 1990), maladaptive gene flow (Lenormand 2002), and genetic drift (Barton and Partridge 2000). The amount of genetic variation in fitness then represents the population's ability to counteract these processes and predicts the per-generation increase in mean fitness expected to result from natural selection (Fisher 1930). Likewise, this rate of evolution of fitness, represents the evolutionary potential of a population to respond to maladaptation caused by environmental change. Despite nearly 100 years since Fisher first recognized the crucial importance of this relationship, how much genetic variation in fitness exists in natural populations is a question that still sees considerable debate (Burt 1995, Shaw and Shaw 2014, Hendry et al. 2018).

Although genetic variation in fitness is the result of dominance and epistatic variance in addition to additive variance (Burt 1995, Matsui et al. 2022), this coarse measure can be used to approximate the upper limit of SV_A and therefore rates of evolutionary change. Taking the microsite variance component from the common garden analysis of variance (Table 2-4), and standardizing it by dividing it by the square of mean fitness, we estimate SV_A as 0.0094. This means that fitness is degraded by about 1% each generation by mutation and immigration, and then restored via purifying selection. Empirical estimates of SV_A in wild populations are exceeding scarce. From the 30 estimates in the literature, including just five on plants, SV_A

seems to range from 1-10% (Burt 1995, Hendry et al. 2018), which is consistent with our findings.

Migration-selection balance

Whereas genetic variation is constantly removed each generation via purifying selection, it is continually renewed by mutation and migration. A multi-niche polymorphism describes how genetic variation can be maintained in a population though spatially-variable selection, where low-fitness alleles persist in a population given gene flow between niches that favour different optimal phenotypes (Maynard Smith 1970, Bulmer 1972). Having obtained estimates of environmental variance (V_E) and additive genetic variance (V_A) for traits in addition the genetic variance in fitness (γ), we can estimate the rate of migration (m) necessary to sustain these observed levels of variation given a range in the selection difference among niches (Bulmer 1985). If θ_1 is the optimal phenotype in niche 1, and θ_2 is the optimal phenotype in niche 2, then we can solve for m, the proportion of the population that must migrate between niches each generation to maintain the polymorphism (Equation 1.2, from Bulmer 1985, Eq 10.65, pg. 181):

$$[V_A + 2m(V_E + \gamma)^2] = m(1 - m)(\theta_1 - \theta_2)^2(V_A + V_E + \gamma) \quad (1.2)$$

We calculated the rate of dispersal necessary to maintain the observed variation in frond area (Fig. 2-4 A) and root length (Fig. 2-4 B), over a range of selection differences ($\theta_1 - \theta_2$), in the absence of mutation. The hyperbolic function indicates that, in the absence of mutation, the rate of dispersal of about 1% that is sufficient to sustain the observed diversity given a selection difference of 7mm^2 for frond area, and 15mm for root length). In a study on the genetic structure of *L. minor* populations in central Minnesota, Cole and Voskuil (1996) estimated much lower rates of gene flow, Nm =0.3, which suggests that mutation must play a critical role in maintaining the genetic variation we observed.

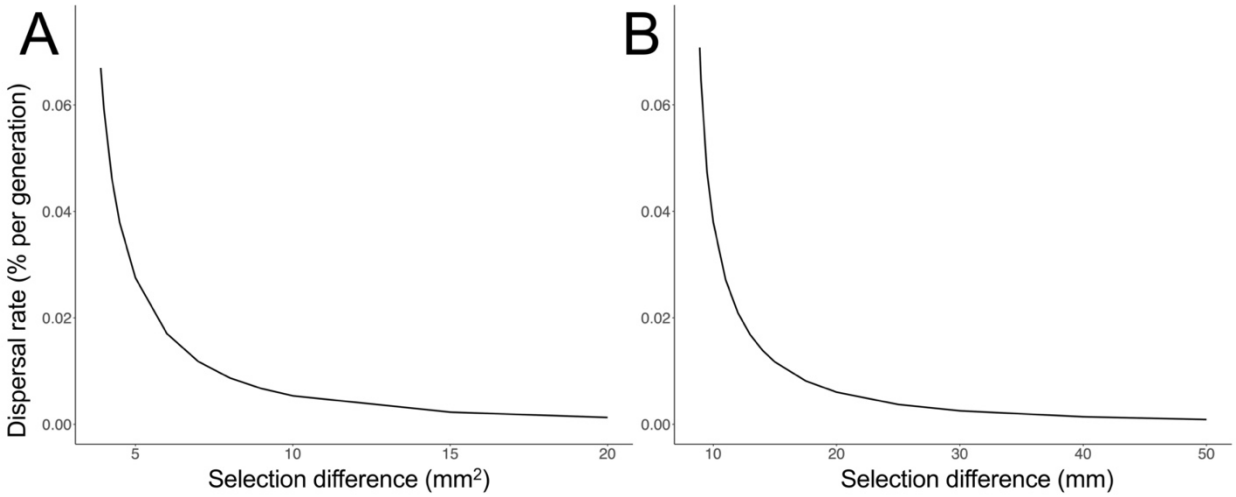

Fig. 2-4. Migration-selection balance. Dispersal rate (% per generation) between two environments required to maintain the observed average within site phenotypic variation in the absence of mutation, as a function of selection difference (the difference in optimal phenotype between the two environments). A. Migration-selection balance for to maintain variation in frond area (mm^2). B. Migration-selection balance to maintain variation in root length (mm).

Conclusions

Frond area and root length varied widely in the field and were correlated with natural levels of resource availability, with plants investing more biomass into the tissue responsible for the uptake of the resource that is in short supply. This was most striking for root length, for which variation among sites was more than sixfold, and strongly correlated with levels of dissolved phosphorus. This large phenotypic variation in the field was overwhelmingly a result of phenotype plasticity, and not local adaptation. Despite the predominance of environmental variation in both traits, there was also a genetic basis to these traits that persisted when environmental variation was removed. We recorded surprisingly high levels of genetic variation in phenotype and fitness within sites, which itself indicates the presence of strong purifying selection of about 1% per generation and the potential to counter environmental change. Future work should focus on uncovering mechanisms responsible for maintaining such high levels of genetic variation in *L. minor.* The continued development of *Lemnaceae* as a model system in experimental population genetics (Acosta et al. 2021), community ecology (Laird and Barks

2018) and eco-evolutionary dynamics (Hart et al. 2019) promises illumination in understanding the larger mechanisms responsible for maintaining diversity more generally.

Acknowledgments

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Preamble to Chapter 3

In Chapter 2 we measured environmental and genetic variation in *Lemna minor* in the field. Phenotypic variation was correlated with resource availability and was mostly the result of phenotypic plasticity. Despite a failure to detect local adaptation, we observed substantial genetic variation among sites in *Lemna minor*. This is consistent with the literature which reports high levels of genetic differentiation, likely due to low rates of gene flow combined with genetic drift. Surprisingly there was even more genetic variation within sites, than among sites. The source of this variation is unknown. Although *Lemna minor* has been used as a model in plant biology for well over one hundred years, its overwintering strategy is not well understood. It is often assumed that winter months in colder climates lead to a seasonal bottleneck in the population due to mass senescence. However, there are no estimates of frond re-emergence or percent survival in the literature. To better understand the life history of this model plant, we ran a simple field experiment designed to estimate the percent survival in a natural duckweed pond that freezes over completely for four months of the year. This is critical in the context of understanding how *Lemna minor* manages to sustain within site genetic variation.

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Chapter 3 - Overwintering and re-emergence in *Lemna minor*

Mark Davidson Jewell[1] & Graham Bell[1,2]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.
[2]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Summary

In cold climates, aquatic plants employ a variety of strategies to survive winter, usually by either going to seed, or by producing resistant vegetative tissue that can withstand freezing. Although *Lemna minor*, the common duckweed, lacks any specific overwintering structure, it thrives over a vast geographic distribution including in northern climates. It is often thought that populations are subjected to a seasonal bottleneck with only a small proportion of plants surviving to found the following year's population. Almost exclusively asexual and with rapid rates of vegetative growth, one would expect populations to be mostly clonal, descending from a small number of individuals each spring. Despite this, several studies have reported surprisingly high levels of within site genetic variation. In this study we use experimental enclosures to measure re-emergence of *L. minor* in a forested pond in Quebec, Canada, after four months of ice cover. To our surprise, 92% of fronds survived the winter, indicating the virtual absence of any bottleneck. Immigration was negligible as there was no difference in recruitment between closed and open enclosures. High levels of within site genetic variation are then understood to be maintained by a balance between mutation and purifying selection.

Introduction

Lemna minor is the most common and ubiquitous of all species in the family of duckweed, *Lemnaceae*, abundant over a wide range of environments and on all continents except Antarctica (Landolt 1986). It is a small floating aquatic angiosperm with a reduced morphology consisting

of only a single leaf-like frond a few mm across, and a single unbranching root (Hillman 1961a). Flowering is extremely rare, and instead, nearly all reproduction is vegetative and clonal, where daughter fronds bud from two meristematic pockets on the lower surface of the mother frond to produce a small colony. This simple reproductive strategy can lead to extremely short generation times of just a few days in favourable conditions, giving the plant the reputation of having one of the fastest population growth rates of all angiosperms (Ziegler et al. 2015). A population of just a few individuals can expand exponentially until there is complete surface cover, and in favourable conditions, dense overgrowth can result in millions of individuals in even a small pond.

It is often thought that populations are largely clonal, with the dense populations resulting primarily from the rapid vegetative growth of a small number of founding fronds each spring. This would especially be the case in cold climates where populations might experience a seasonal bottleneck due to winter senescence (Tang et al. 2014). This would result in a recurring seasonal reduction of intraspecific variation, which would lead to low genetic diversity unless it was balanced by high rates of mutation and gene flow. However, mutation accumulation experiments suggest slow rates of per base pair mutation in *Lemnaceae* compared to other multicellular plants (Xu et al. 2019, Sandler et al. 2020). Despite this, some authors have reported surprisingly high levels of within site genetic variation based on allozymic analysis, amplified fragment length polymorphisms, and common gardens (Vasseur et al. 1993, Cole and Voskuil 1996, El-Kholy et al. 2015, Bog et al. 2022, Jewell and Bell 2022a). For example, Vasseur et al. (1993) identified 157 different genotypes of *L. minor* in just eight ponds within a 12km radius. In a field survey of 34 sites, we found twice as much genetic variation in fitness within sites than among sites (Jewell and Bell 2022b). This paradox has often been explained by suggesting that genetic diversity is replenished by high levels of dispersal, likely via waterfowl (Jacobs 1947, Keddy 1976, Cole and Voskuil 1996), although this is rarely observed.

Although the life history of *L. minor* has been studied for at least 150 years (Hegelmaier 1868, Guppy 1894, Caldwell 1899), its overwintering strategy is still not fully understood. Within *Lemnaceae*, there exist a wide variety of strategies to resist periods of harsh environmental stress, which appear to be a driving force behind the diversification of the group (Crawford et al. 2006). Whereas some species produce resistant seeds via the sexual phase, or resistant vegetative

"winter buds" called turions, *L. minor* doesn't possess any apparent specialized structure to survive freezing or desiccation. Since *L. minor* is cosmopolitan, its overwintering strategy will depend on local climate conditions, and as such, there appear to be diverse ecotypes adapted to varying degrees of winter severity (Landolt 1986, Crawford et al. 2006). In general, *L. minor*, is capable of maintaining growth in lower temperatures than other species of *Lemnaceae*, such that growth can continue over winter in milder climates (Reddy and DeBusk 1985). In colder climates, fronds can avoid becoming trapped in surface ice by sinking to the bottom where they remain dormant until temperatures warm. Sinking is facilitated by turions, starch-rich vegetative buds, denser than usual fronds due to a reduction in aerenchyma tissue (Jacobs 1947, Hillman 1961a, Landolt 1986, Kim 2013, Appenroth and Adamec 2015). Turion production has been reported in certain strains of *L. minor* (Jacobs 1947, Dudley 1987), although modern classification now places them in the species *L. turionifera*. Whereas species like *Spirodela polyrhiza* produce "true turions", proto-turions, or simply seasonal shifts in frond nutrient balance resulting in denser tissue, may be more widespread and facilitate the seasonal sinking as a viable overwintering strategy. As colonies of *L. minor* sink, or become trapped in the ice, outer fronds and tissue may senesce, protecting the innermost meristematic tissue capable of regenerating the colony when conditions improve. However, it is unknown what proportion of fronds survive the winter in different climates, with important implications for genetic diversity.

In this study we estimate the severity of the seasonal genetic bottleneck in a population of *L. minor*, located in Quebec, Canada, close to the species' northern range limit. We measure the proportion of fronds from the autumn population that successfully overwinter to found the following year's population.

Materials and methods

The site of study was a small pond located close to the summit of Mont Saint Hilaire on McGill University's Gault Nature Reserve, Quebec, Canada (45° 32' N, 73° 08' W) (Fig. S3-1 in Appendix 3). January average daily low temperatures are -15^0C and still freshwater lakes and ponds remain frozen for about four months of the year with ice cover up to 80cm thick. The

study pond is heavily shaded by the forest canopy and sustains a population of *Lemna minor* that forms a dense mat covering the entire surface of the pond by mid-summer. Although *L. minor* sometimes grows in species-rich communities with other floating aquatic plants, this pond supports a monoculture population. In mid-October 2020, we installed four experimental enclosures measuring 120cm by 120cm, consisting of a wooden structure wrapped with transparent plastic allowing the passage of light, and perforated to permit the flow of water and micro-organisms, but not of *L. minor* (Fig. 3-1). These were pounded into the sediment of the pond until the walls were flush against the bottom substrate. Two of the enclosures were erected near the boundary of the pond where there was dense cover and overgrowth of *L. minor*, and two were erected near the centre of the pond where the population was sparser (since wind blows the fronds to the ponds' edge). All four locations had equal depths of 1m +/-15cm. To inhibit the possibility of immigration (via birds or otherwise), two of the four enclosures (one in the center, one near the boundary) were capped with a layer 50% shade cloth whose mesh size was small enough to prevent the passage of *L. minor* fronds. The two remaining enclosures were left open. In mid-October 2020, before water temperatures fell below 10^0C, we estimated the total number of fronds in each enclosure by manual counting.

We monitored the enclosures for frond re-emergence in the spring from the ice melt onwards. Every 4-5 days, the enclosures were sampled, fronds were counted and then removed. This gave an idea of the phenological pattern of frond re-emergence as well as the total proportion of fronds that survived the winter. On each sampling date, water temperature was measured at a depth of 30 cm. Since plants may have reproduced between sampling dates, the actual percentage of frond re-emergence could have been lower than estimates based on total frond counts. To remove the contribution of reproduction, we measured population growth rates in two 19 L tubs situated on the bank of the pond. The tubs were filled with pond water and seeded with the fronds removed from the enclosures which were then recounted each sampling date to obtain estimates of population growth rates. These were calculated for each tub on each sampling date using the standard formula for exponential growth $r = \ln\left(\frac{Nt}{N0}\right)/t$ where N_0 is initial population size, t is time in days, and N_t is population size at time t. To test whether there were differences in percent re-emergence between the closed and open enclosures, we used a two-sampled student's t-test.

Fig. 3-1. Four experimental enclosures situated in a small, forested pond, photographed here in late October.

Results

Fronds in all four enclosures began to appear on the surface in early April with water temperatures of 5^0C, and continued to re-emerge for three months until early June with a water temperatures of 18^0C (Fig. 3-2A). For each enclosure, the proportion of surviving fronds (percent re-emergence) was calculated as the total number of re-emerged fronds divided by the total

number of fronds from the previous autumn. Percent re-emergence was high for all enclosures, ranging from 80%-103%, (mean=92%) (Fig. 3-2B). There was no difference in percent re-emergence between closed and open enclosures (t_2=0.1026, p=0.93), suggesting that immigration played an insignificant role in establishing the spring population. This is consistent with observations as there were no sightings of any waterfowl visiting the pond.

Measuring rates of population increase of re-emerged fronds (Table 3-1), we observed little growth in April and May, with virtually no growth when water temperature was below 10^0C and only modest growth when water temperature rose above this, although doubling times were consistently greater than 50 days. This changed for the final two sampling dates in early June when water temperatures rose above 15^0C resulting in a sharp increase in growth with doubling times of around 5 days. By this point however, re-emergence had already slowed dramatically, since on average 96% of all plants to re-emerge had done so already.

The total number of fronds sampled at any given date is the sum of the fronds that re-emerged since the previous sampling date and the fronds resulting from reproduction during this period. Since we don't know the pattern of re-emergence between any two dates, we are unable to make a precise estimate of the contribution of reproduction to frond number. By making the unrealistic assumption that all the fronds to re-emerge between two sampling dates did so on the first day, then by incorporating the measured exponential growth rates, we can obtain a conservative estimate of re-emergence that sets the upper limit for the contribution of reproduction to total frond number that we can contrast with the raw count data. Comparing the total number of fronds counted in the spring to those from the previous year's autumn population, when reproduction is unaccounted for, average percent re-emergence was 92%, and fell only slightly to 87% when reproduction was included.

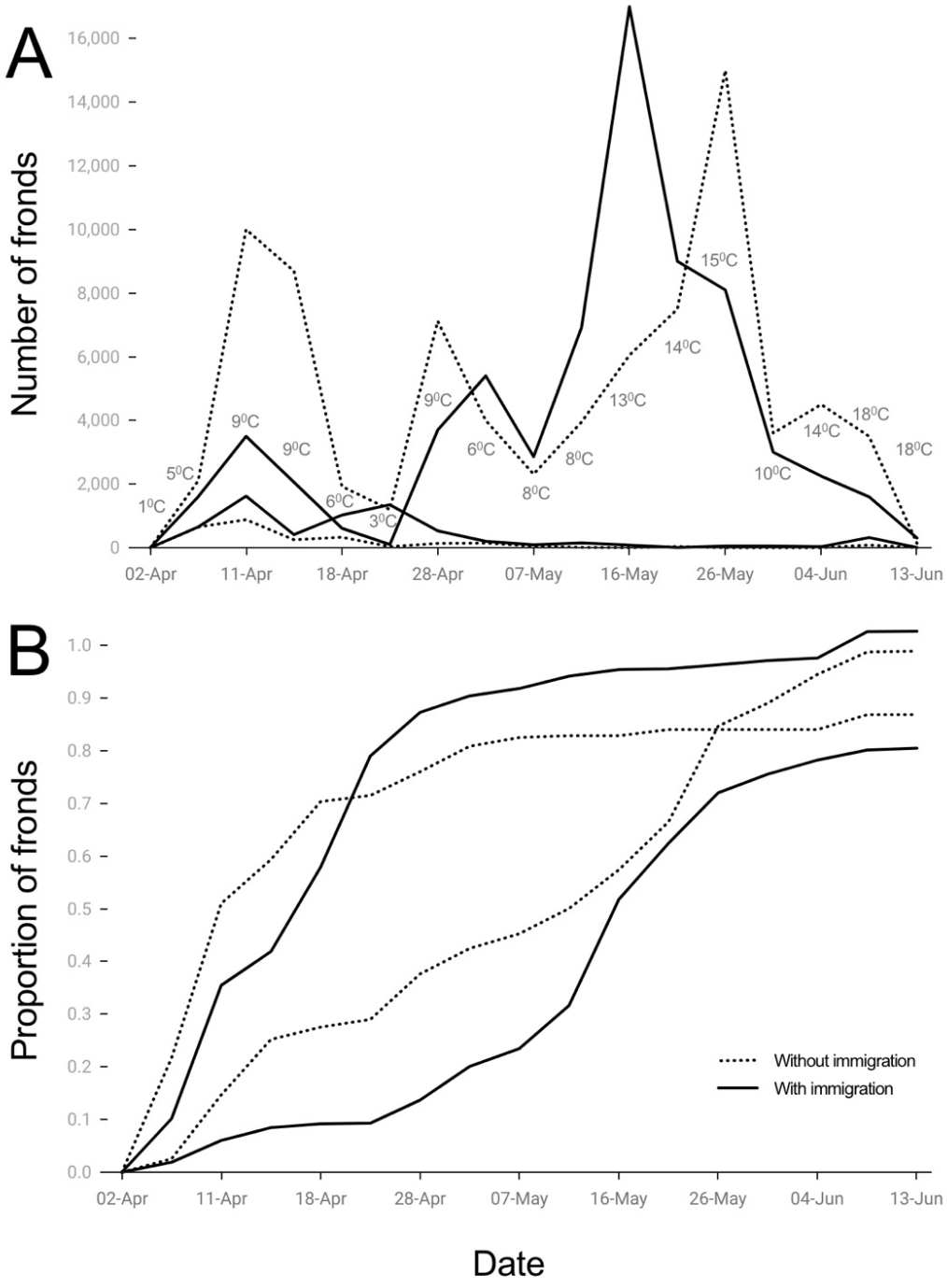

Fig. 3-2. Re-emergence of *Lemna minor* after winter dormancy. Each of the four lines shows frond re-emergence in one of the four enclosures. Dotted lines represent enclosures capped with shade cloth to prevent immigration, and solid lines represent open enclosures. A) Frond re-emergence shown as absolute number of fronds counted on each sampling date. Water temperature measured at 30 cm is shown for each sampling date. B) Frond re-emergence shown as the proportion of the previous year's population size.

Table 3-1. *Lemna minor* population growth rates. Means for each sampling date are based on two independent growth assays.

Sampling Date	Water temperature (^{0}C)	Exponential growth rate, r (d^{-1})		Doubling time (d)
		mean	SD	
02-Apr	2			
06-Apr	5	0.0066	0.0059	107
11-Apr	9	0.0132	0.0037	53.0
13-Apr	9	0.0164	0.0124	42.6
18-Apr	6	0.0125	0.0025	55.8
23-Apr	3	0.0065	0.0077	108
28-Apr	9	0.0072	0.0041	96.8
03-May	6	0.0047	0.0073	149
07-May	8	0.0006	0.0053	1188
11-May	8	0.0044	0.0045	158
16-May	13	0.0049	0.0041	144
21-May	14	0.0117	0.0017	59.7
26-May	15	0.0136	0.0059	51.4
31-May	10	0.0114	0.0007	61.6
04-Jun	14	0.0113	0.0103	61.7
09-Jun	18	0.1328	0.0039	5.27
13-Jun	18	0.1403	0.0189	4.99

Discussion

Frond re-emergence and growth appear to be strongly synchronized with water temperature (Bornkamm 1966), with fronds beginning to float to the surface once water temperature reached about 5^{0}C. To our surprise, percent re-emergence was extremely high, with about nine out of every ten fronds surviving the winter. That plants successfully survive four months in winter conditions is striking given the average lifespan of *L. minor* is just 3-5 weeks in summer conditions (Laird and Barks 2018). There were no fronds visible in the surface ice, suggesting that most fronds sank to the bottom of the pond over winter. Ice thickness on the adjacent Lac Hertel reached up to 80cm, meaning that there was likely a shallow layer of liquid water at

bottom the pond (~1m deep). In addition, the thick sediment layer of mud and leaf litter could act as a refuge to protect fragile plant tissue.

The near absence of any seasonal bottleneck in the population helps to explain how *L. minor* can sustain high genetic diversity despite the near absence of sexual reproduction and possible low rates of mutation and gene flow. This diversity could be maintained over the season by variable selection arising from any spatial or temporal environmental variability that confers a competitive advantage to different genotypes (Chesson 2000, Adler et al. 2007). Likewise, negative frequency-dependent selection that has been identified as a diversity maintenance mechanism among different species of *Lemnaceae* could act among genotypes (Armitage and Jones 2019, Jewell and Bell 2022c).

Part of the fascination with *L. minor* is its overwhelming ecological success despite, or perhaps because of its morphological, physiological, and ecological simplicity. Without any elaborate overwintering mechanism, it is the most successful of all *Lemnaceae* species in cold climates. Given substantial genetic diversity, combined with exceedingly short generation times, it is possible that *L. minor*'s success is in part due to rapid adaptation to the wide range of conditions it experiences. This is supported by work that shows ecological specialization among genotypes (Ziegler et al. 2015, Roubeau Dumont et al. 2019, van Moorsel 2022), high genetic differentiation among sites (Cole and Voskuil 1996, Xue et al. 2012, Xu et al. 2015), and experimental evolution studies reporting rapid evolutionary change in *L. minor* (Hart et al. 2019, Tan et al. 2021, Jewell and Bell 2022a).

Future work should focus on identifying the ecophysiology of frond quiescence in *L. minor*. Comparing frond starch content, photohormonal profiles, and rates of photosynthesis and respiration between autumn, winter and spring fronds would help determine the mechanisms that promote sinking and winter survival. Whereas substantial work has investigated the ecophysiological cues that induce turion production and sinking in species like *S. polyrhiza* (Appenroth et al. 1996; Appenroth and Adamec 2015), similar studies are lacking for *L. minor*. Such work may reveal that the distinction between true turions and resistant winter fronds or proto-turions may be blurry, and although *L. minor* lacks overwintering vegetative structures that

are morphologically distinct from summer fronds, the mechanisms governing winter quiescence in *L. minor* and dormancy in related turion-producing species may be similar.

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Preamble to Chapter 4

In the first three chapters we studied *Lemna minor* in isolation. We observed a genetic basis to frond area and root length, although most of the variation in these traits was the result of phenotypic plasticity and not local adaptation. Despite this, there was substantial genetic differentiation among sites and genetic diversity within sites, partially explained by the absence of any seasonal genetic bottleneck.

In the remaining chapters we study communities dominated by *Lemna minor*, but also sustaining populations of other species of free-floating plants. In Chapter 4 we use data from the Chapter 2 field survey, but instead of focusing on phenotypic variation in *Lemna minor*, we ask whether species distributions are a function of resource availability or other environmental factors.

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Chapter 4 - Ecological specialization and not dispersal limitation governs the distributions in floating aquatic plants

Mark Davidson Jewell[1] & Graham Bell[1,2]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.
[2]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Summary

Communities of free-floating aquatic plants dominated by *Lemnaceae* (duckweeds) are common in ponds and wetlands globally with important ecological functions. In addition to *Lemna minor*, the common duckweed, many other species of *Lemnaceae*, liverworts (*Ricciaceae*) and water ferns (*Salviniaceae*), often coexist over broad geographic areas. Despite these species all sharing a similar mode of life and resource requirements, important morphological and physiological differences exist among species. However, the degree to which these differences play a role in shaping their distributions is unclear. We report a regional field survey of floating aquatic plants in southern Quebec, Canada. We find that species distributions are influenced by ecological specialization to water phosphorus levels. High nutrient specialization also followed a continual reduction in body size and morphological complexity, with smaller, simpler species found in higher nutrient sites. We also found a latitudinal gradient in species richness with more northerly sites supporting less diversity. The distribution of these plants appears to be the result of competitive sorting of suitable species from a regional species pool without any substantial effect of dispersal limitation.

Introduction

A major goal of ecology is to understand the mechanisms responsible for the distribution and abundance of species. Where species are found across a landscape may be the result of niche differentiation and habitat filtering (Maire et al. 2012). Niche differentiation involves the

ecological specialization to local environmental conditions (Zuppinger-Dingley et al. 2014, Meilhac et al. 2020). At the same time, species from the regional pool are filtered by the local environment which favours the strongest competitors in each set of conditions (Shipley et al. 2006). In the absence of habitat filtering, the distributions of ecologically equivalent species will vary stochastically, but with spatial aggregation resulting from dispersal limitations (Freestone and Inouye 2006). A basic way to detect niche differentiation is by asking if sites where a species is present differ in their environmental conditions from sites where it is absent. This is particularly relevant for species that occupy a (seemingly) similar ecological niche, such as floating aquatic plants. Free-floating aquatic plants have similar modes of life and resource requirements, but variation in co-occurrence patterns and species distributions may point towards some degree of ecological specialization.

We report a regional field survey of free-floating aquatic plants across southern Quebec, Canada. These are communities of morphologically reduced, unrooted plants that either float on or just under the surface of lakes, ponds and wetlands. They are usually dominated by species in the family *Lemnaceae* (duckweeds), but may also consist of liverworts (*Riccia* and *Ricciocaropus*), waterferns (*Azolla* and *Salvinia*), stoneworts (*Chara*) and the morphologically similar hornwort *Ceratophyllum*. Many of these species have global distributions and similar surveys have been reported across North America (Docauer 1983, McIlraith 1988, Smith 2014, Mccann 2016), Europe (Karttunen and Toivonen 1995, Kočić et al. 2008, Cvijanović et al. 2018, Paolacci et al. 2018) and Asia (Tang et al. 2014, Chen et al. 2022).

Free-floating plant communities are ecologically important as they are one of two alternative stable states in shallow, lentic water (Scheffer et al. 2003, de Tezanos Pinto and O'Farrell 2014), (the other being communities dominated by submerged rooted macrophytes and phytoplankton), and as keystone species their presence fundamentally changes the features of the ecosystem (Landolt 1986, de Tezanos Pinto and O'Farrell 2014). Present in mesotrophic and eutrophic environments, under favourable conditions these plants can completely cover the water's surface, shading out phytoplankton and submerged, rooted plants, and can lead to altered nutrient cycling, anoxic conditions, and changes in biodiversity (Janse and Van Puijenbroek 1998). We characterised 37 natural communities supporting free-floating aquatic plants, broadly distributed

across the northern temperate zone of Quebec, Canada, and measured water pH and levels of resource availability (nutrients and light). The objective of this study was to determine if ecological specialization contributed to the distribution of these plants in the field.

Materials and methods

Field Survey

In July 2019, we conducted a regional survey of natural communities of free-floating aquatic macrophytes. These are communities usually dominated by plants in the family *Lemnaceae* (duckweeds) and sometimes include species of liverworts. Because they are unrooted, these plants generally require lentic water protected from wind, such as bays of lakes and rivers, forested ponds and wetlands. We located 37 sites supporting such populations or communities, broadly distributed across southern Quebec, Canada (Fig. 4-1).

Communities were characterized by recording the identity of all species present. This was done by a general survey of the pond followed by sampling in three microsites, at least 10 m apart along a transect following the perimeter of the water body. These unrooted plants tend to accumulate by the water edges because of wind and currents and are rarely found in open water unless they form a complete cover. Species were identified based on their morphology, meaning that we may have missed cryptic species of *Lemnaceae* (Senevirathna et al. 2021). In addition to presence/absence data, for each site we recorded the most abundant species, that is, with a relative abundance clearly greater than 50%. If not immediately clear, this was done by exhaustively counting abundances for three samples for each microsite. *Lemna minor* was present at all sites where any floating aquatic plants were observed, thus, this species was used in this study to define the conditions necessary to support floating aquatic plant communities. Six other species of floating aquatic macrophytes were identified in at least one of the sampling sites: the angiosperms *Lemna trisulca*, *Spirodela polyrhiza*, *Wolffia columbiana*, and *Ceratophyllum demersum*, and the liverworts *Ricciocarpus natans* and *Riccia fluitans*.

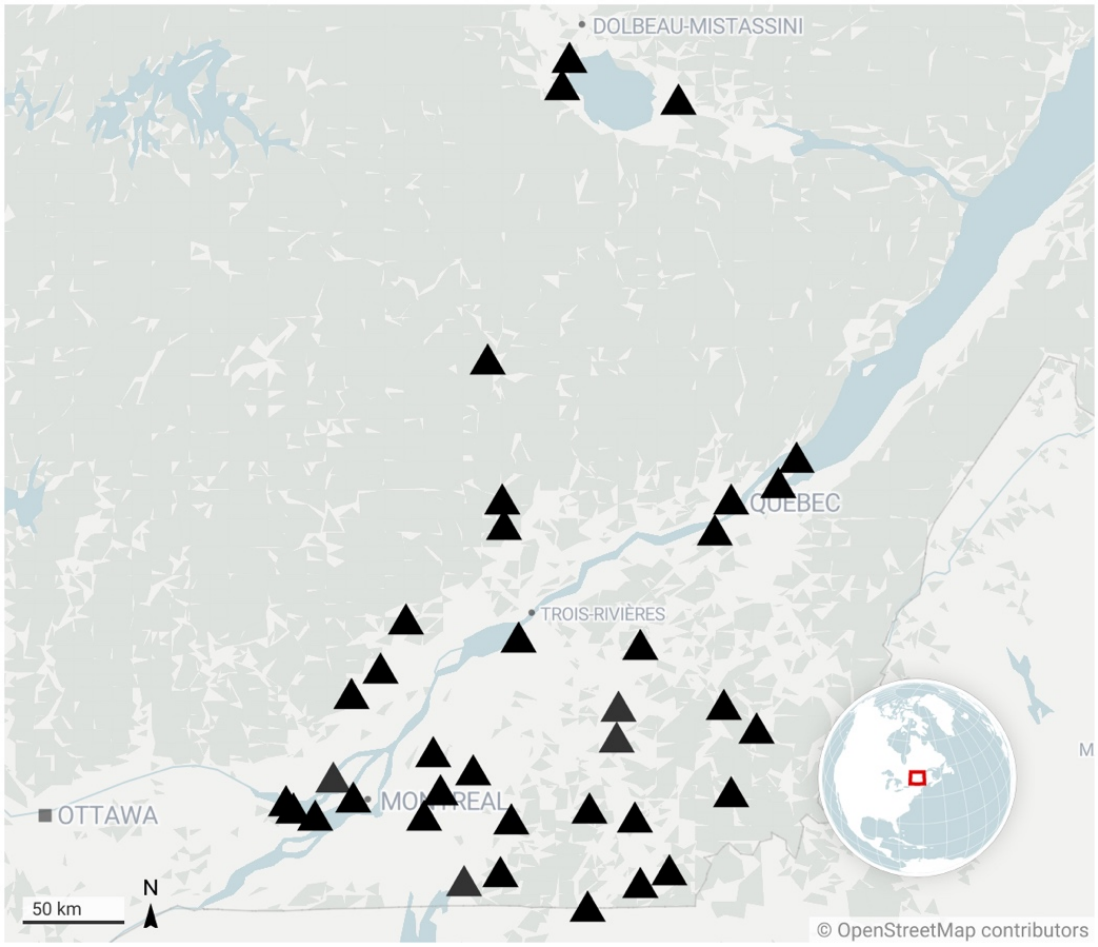

Fig. 4-1. Thirty-seven sites supporting communities of free-floating aquatic macrophytes, distributed across southern Quebec.

Environmental variables

For each site we measured several environmental variables that we expected to be correlated with ecological specialization. Light availability, as percent transmittance of photosynthetically active radiation (PAR) was estimated *in situ* with the use of BF5 Sunshine Sensor (Delta-T, Burwell, Cambridge, UK) (Paquette et al. 2007). This instrument consists of an array of seven quantum sensors under a semi-shaded hemispherical dome to give estimates of diffuse light under any meteorological condition. We took two simultaneous paired measurements, one at the sampling site over water, and a second reference point at a nearby open site (field or road) under full sun. Percent transmittance PAR was then estimated as the ratio of diffused light between the

site and the reference measurement. This method has been demonstrated as a reliable and practical alternative to more standard measurement techniques including hemispherical image analysis (Rich et al. 1993, Paquette et al. 2007). Percent transmittance of PAR was estimated at each of the three microsites as well as the center of the site. All measurements were taken 1.5m above the water level, above any rooted aquatic macrophytes or riparian herbaceous plants, to obtain an estimate of shading from the canopy cover. These four measurements were then averaged to produce a single estimate of light availability for each site.

To measure water nutrient content, we took four water samples from the center of each site at a depth of 30cm. Total Nitrogen (TN) and total Phosphorus (TP), were estimated each from two replicate samples. Acid-washed glass tubes were first rinsed, and then filled with unfiltered sample water. All tubes were stored on ice and brought to the lab for analysis. Samples were then stored at 4^{o}C and processed within 14 days. Water samples were analysed for TN with a continuous flow analyser (OI Analytical Flow Solution 3100 ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor (Patton and J.R. 2003). TP was measured using colorimetric detection with a spectrophotometer at 890 nm, after digestion with potassium persulfate and the addition of an ammonium molybdate solution (Wetzel and Likens 2000). All samples were analysed at the GRIL, Université du Québec à Montréal (UQAM) analytical laboratory.

We used a YSI probe (YSI professional plus, Xylem Inc., Yellow Springs, OH, USA.) to measure pH at the centre of each site at a depth of 30cm. A full list of environmental measurements can be found in the supplementary information (Table S4-1 in Appendix 4).

Data analysis

The range of values in each environmental variable where a species was present was compared to that when it was absent. Since the number of sites were different in each category we used Welch's t-test for unequal variances. To control for inflated type 1 error caused by using

independent tests for each species, we used the Bonferroni correction which lowered the critical alpha from 0.05 to 0.008.

Results

L. minor was present in all sites, such that we did not find any site in our survey that supported any free-floating aquatic plants that did not include *L. minor*. Although *L. minor* was in some cases growing in monospecific stands, more commonly it coexisted with at least one other species (Fig. 4-2A).

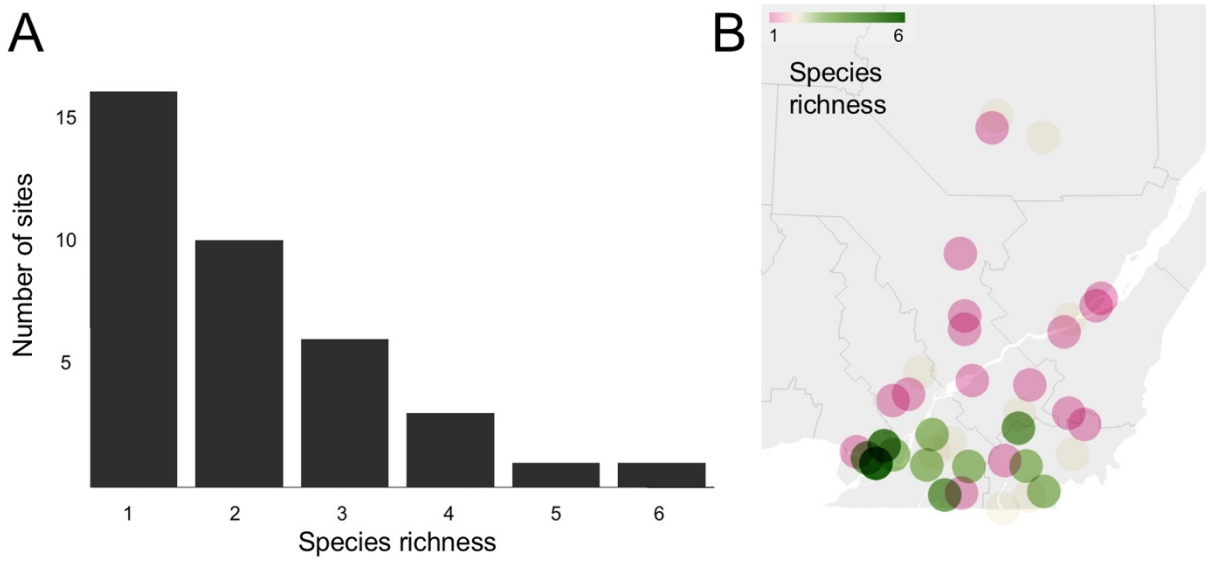

Fig. 4-2. Species richness for all 37 sites. A. Species richness-frequency plot. B. Geographical distribution of site species richness (alpha diversity).

In order of abundance, *L. minor* occurred in all 37 sites, *S. polyrhiza* in 16, *W. columbiana* in 9, *L. trisulca* in 6, *R. natans* in 4, *C. demersum* in 3 and *R. fluitans* in 2 sites. Not only was *L. minor* present in all sites, it was also most often the dominant species, contributing >50% relative abundance to the community. Species distributions did not show an obvious geographical

signature as all species were evenly spread across the landscape with the exception of the northern limit of the survey which was generally species poor (Fig. 4-3). Taken together, there was a strong latitudinal gradient of species richness, with the number of species per site diminishing further north (Fig. 4-2B).

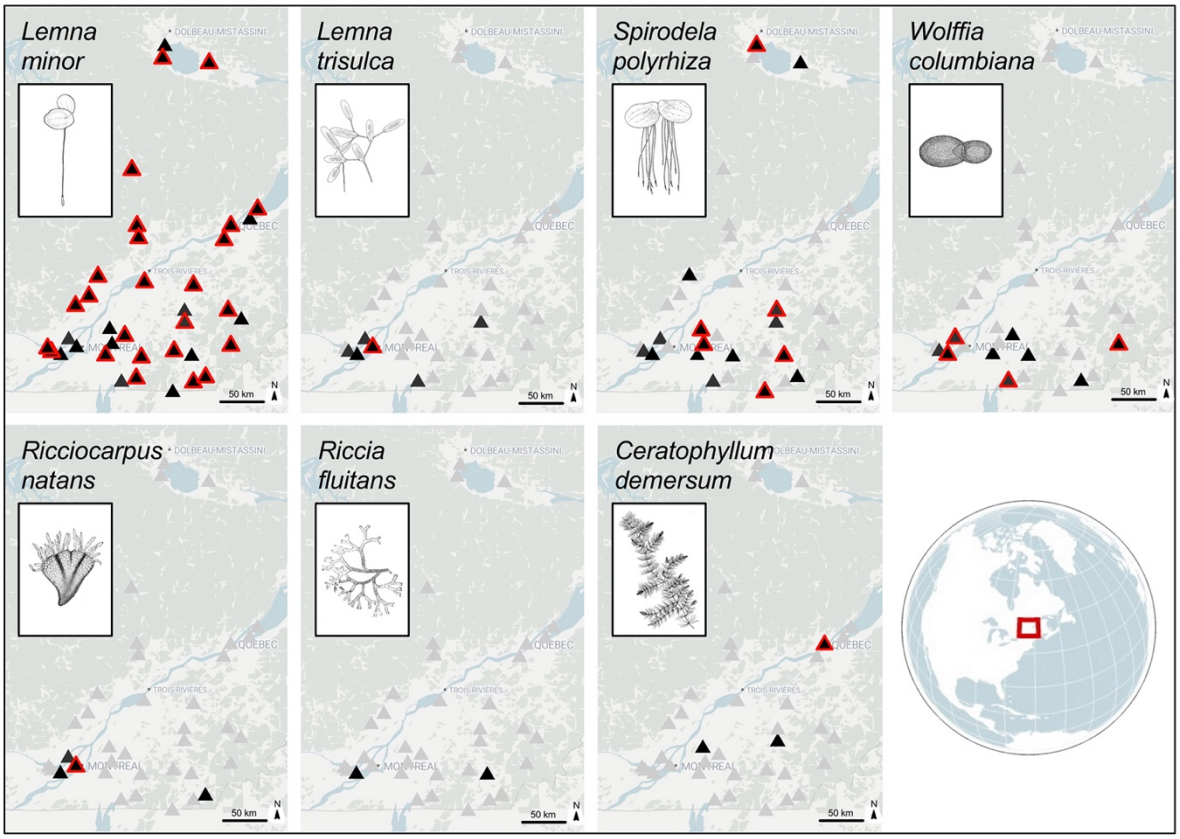

Fig. 4-3. The distributions of seven species of free-floating aquatic plants across southern Quebec. Light grey triangles are sites where the species is absent, black triangles are where the species is present, black triangles outlined in red are where the species is the most abundant in the community. *Lemna minor* was present in all 37 sites.

To determine if species distributions were the result of ecological specialization, we compared sites where a species was present to sites where it was absent. We did this for each species and each environmental variable. Species varied in their distributions as a function of environment, with pronounced differences in mean values of the four environmental variables (Fig. 4-4). Sites

where *R. fluitans* was present were more heavily shaded, with lower % PAR than sites where it was absent ($t_{34.74} = 5.251$, $p<0.0001$, Fig. 4-4). *W. columbiana* was found in high nutrient sites, with both total N and total P higher in sites where it was present than absent (TN $t_{12.17} = 2.353$, $p = 0.036$, TP $t_{9.318} = 3.434$, $p = 0.007$, Fig. 4-4). The two liverworts *R. natans* and *R. fluitans* were found in nutrient-poor sites: TP was lower in sites where they were present than where they were absent (Rn $t_{12.60} = 2.265$, $p=0.042$, Rf $t_{34.33} = 2.837$, $p = 0.008$, Fig. 4-4). Full results from Welch's t-tests can be found in the supplementary information (Table S4-2 in Appendix 4). With perhaps the exception of nutrients (TN and TP) which were lower further north, there was no spatial aggregation of the environemntal varaibles (Fig. 4-5).

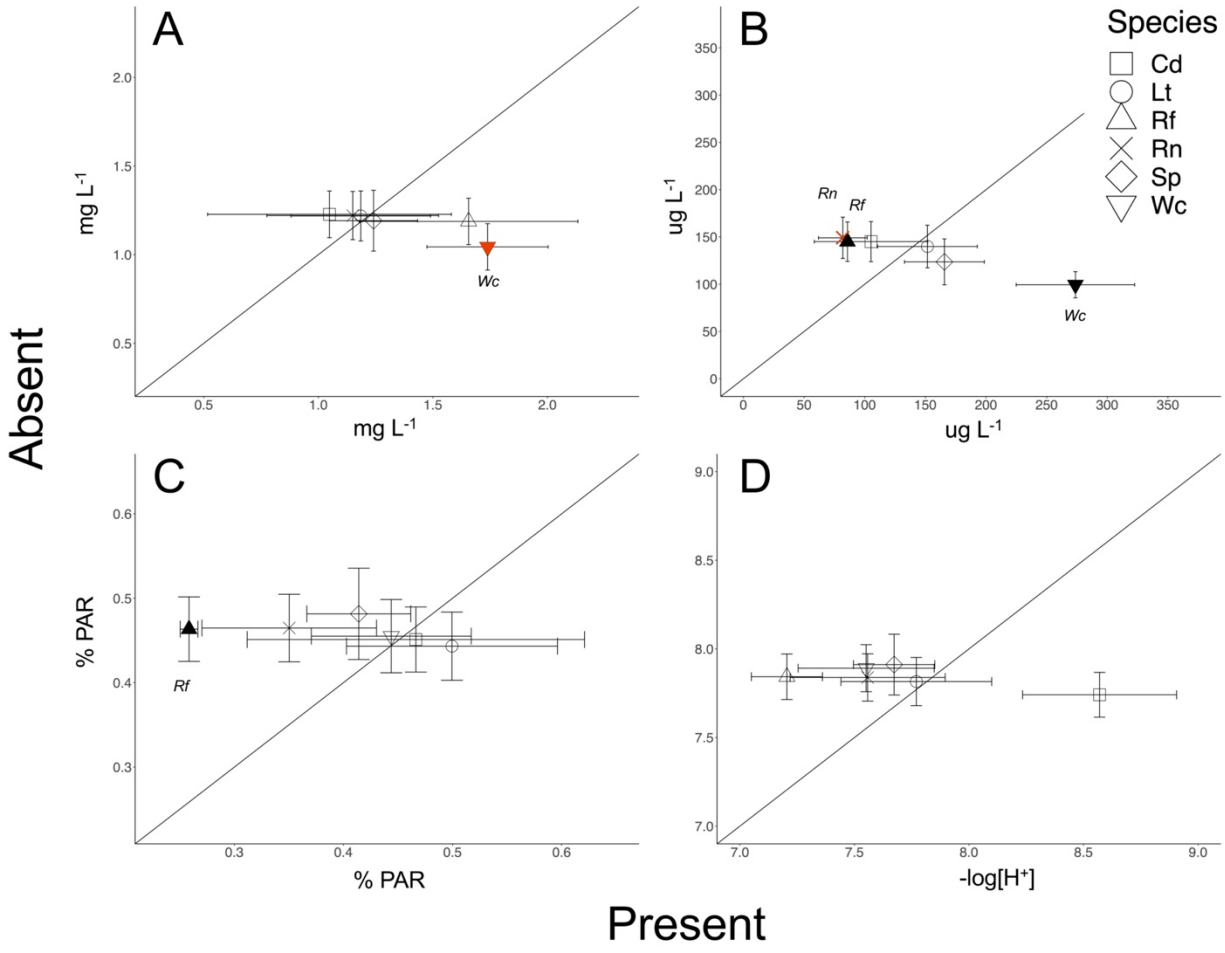

Fig. 4-4. Presence and absence of the six species, excluding *Lemna minor*, as a function of the abiotic environment. A. Species distributions as a function of total Nitrogen (mgL^{-1}). B. Species distributions as a function of total Phosphorus (μgL^{-1}). C. Species distributions as a function of

light availability (% PAR). D. Species distributions as a function of pH. In all panels, error bars show the standard error of the means. Significant deviations from the 1:1 line indicating ecological specialization are shown with filled symbols. Black filled symbols are significant at the Bonferroni critical $p<0.008$, red filled symbols are marginally significant at $p<0.05$ but >0.008.

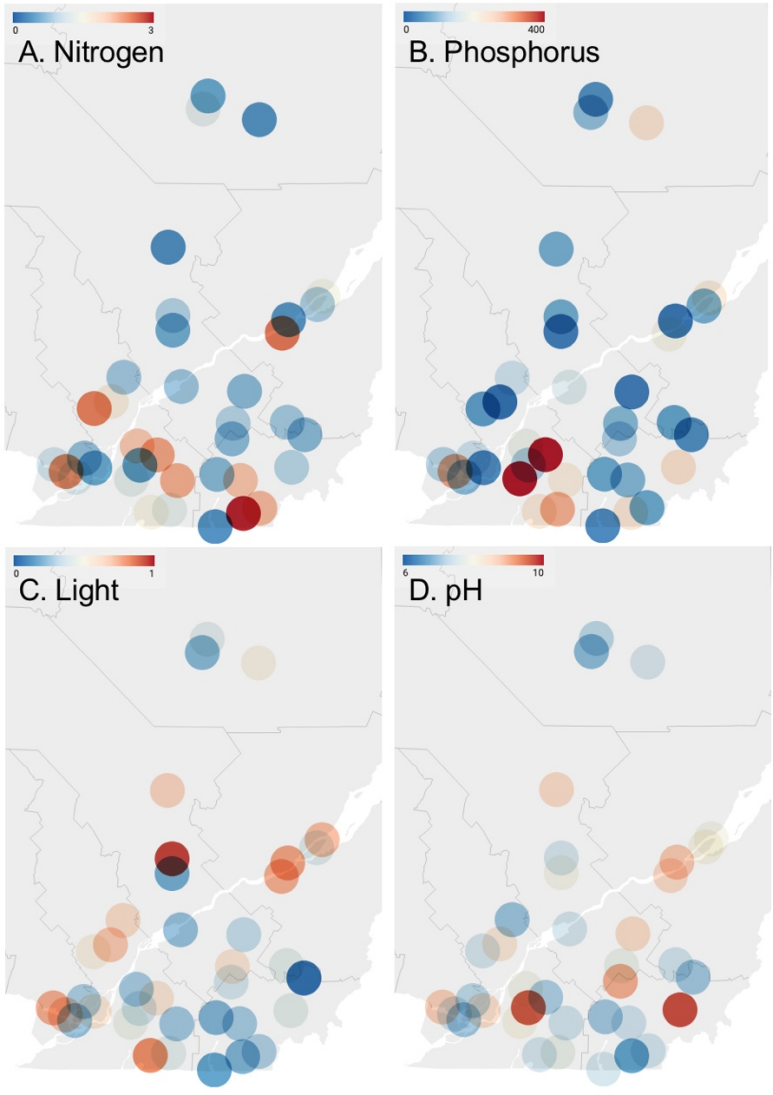

Fig. 4-5. Geographical distribution of four environmental variables. A. Total Nitrogen (mgL^{-1}). B. Total Phosphorus (μgL^{-1}). C. Light (percent photosynthetically active radiation) D. pH ($-\log[H^+]$).

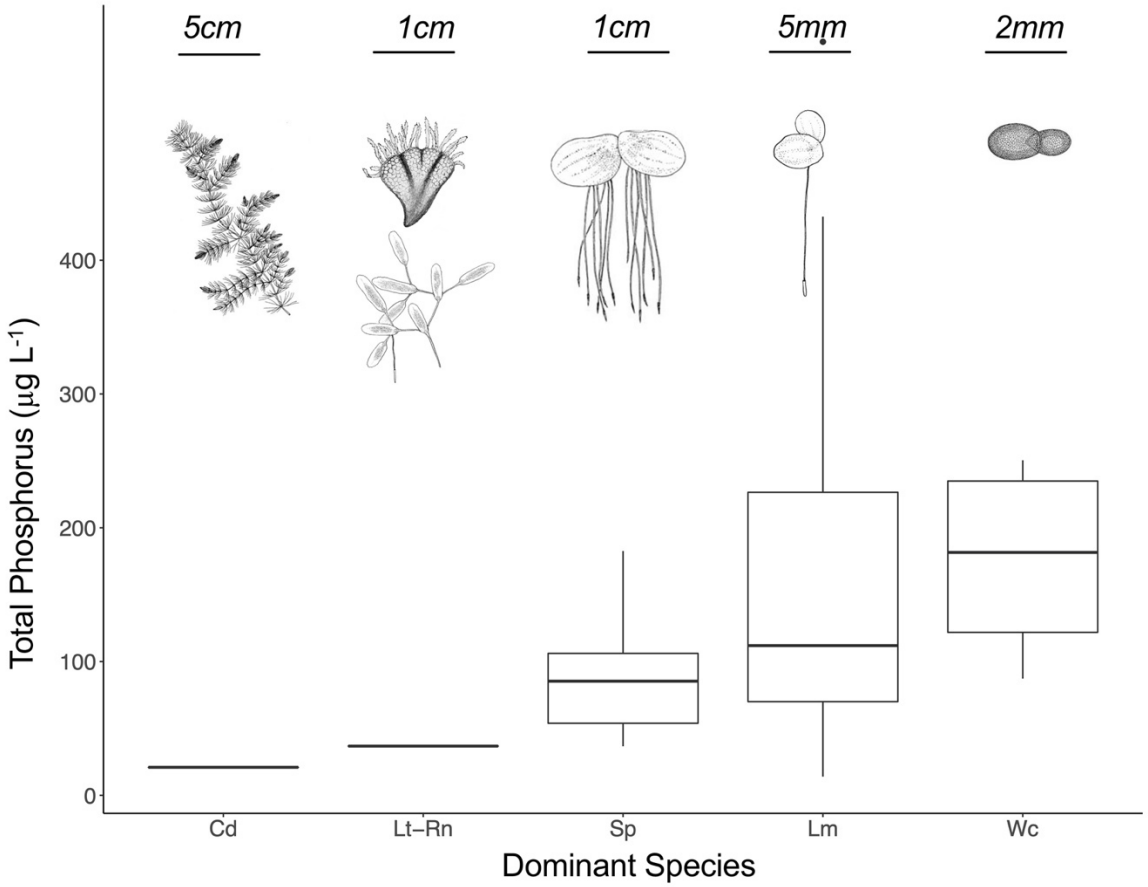

Fig. 4-6. Total water phosphorus content in sites dominated by different species. Dominant species refers to a species with a relative abundance of at least 50%. Cd = *Ceratophyllum demersum,* Lt = *Lemna trisulca*, Rn = *Ricciocarpus natans*, Sp = *Spirodela polyrhiza*, Lm = *Lemna minor*, Wc = *Wolffia columbiana*.

Discussion

Ecological specialization

We found evidence of specialization for three species: *W. columbiana*, among the smallest of *Lemnaceae* and of all angiosperms, and the two liverworts, *R. natans* and *R. fluitans*. Whereas *W. columbiana* was found primarily in high N and P environments, the liverworts were found in low nutrient and low light environments (Fig. 4-4). *L. minor* was present in all sites, thus we know that all sites were potentially suitable habitat for free-floating plants. The absence of any

obvious spatial aggregation of the environmental variables among sites (Fig. 4-5) suggests that any significant result is the result of ecological specialization and not due to dispersal between more closely situated water bodies, i.e., a confounding of site and environmental conditions.

The primary environmental variable to which species showed specialization was nutrient availability, mostly of phosphorus. Phosphorus is often the limiting resource in wetlands (Bedford et al. 1999) and has been found to structure aquatic plant communities in most (Smith 2014, Peeters et al. 2016) but not all cases (Paolacci et al. 2018). *Lemnaceae* are adapted to eutrophic environments, and often outcompete other angiosperms, liverworts and phytoplankton in high nutrient conditions (Landolt 1986, Feuchtmayr et al. 2009, Peeters et al. 2013). Within *Lemnaceae*, the genus *Wolffia* has the highest P requirements (Docauer 1983, Landolt 1986), and has a competitive advantage in high-P environments in the field (Kočić et al. 2008), mesocosm experiments (Smith 2014, Jewell and Bell 2022a), and laboratory growth assays (Mccann 2016). These results are consistent with a mesocosm experiment that competed four of the five most common species found here over a range in environmental conditions and found that *W. columbiana* was the most competitive in high N and P conditions, and *R. natans* the most competitive in nutrient poor conditions (Jewell and Bell 2022a). Likewise, a comprehensive field survey of 122 sites in Croatia reported similar results, with assemblages of liverworts (*R. natans* and *R. fluitans*), *C. demersum*, and *L. trisulca* common in low nutrient sites, with these species being displaced by *L. minor* and *W. arrhizal* (a close European relative of *W. columbiana*) with increasing water phosphorus (Kočić et al. 2008).

Although all seven species of free-floating plants found in this study consist of simple, morphologically reduced fronds/thalli with similar resource requirements, all species possess distinct morphological, physiological and ecological characteristics. Plants can be divided into two main functional groups: those that float on the water's surface (*L. minor, S. polyrhiza, W. columbiana, R. natans*), and those submerged just under the surface (*L. trisulca, C. demersum, R. fluitans*). This difference in strategy can reduce interspecific competition for space (McIlraith 1988, Gopal and Goel 1993), but in highly productive environments (for example in eutrophic conditions), the thick mats formed by the floating species can lead to competitive exclusion of submerged species by shading, sometimes called "overtopping" (McIlraith 1988, McIlraith et al.

1988). This was the case in our survey where *L. minor*, *W. columbiana* and *S. polyrhiza* tended to dominate communities in high P and N sites.

Plant size, evolutionary reduction and nutrient specialization

Presence/absence data was strengthened by data of species dominance, defined as a species constituting >50% relative abundance in a community. In response to TP, species fell generally in the same order for both variables, with the hornwort C. *demersum* and liverworts most often present and dominant in low-P environments, followed by the *Lemnaceae* (Fig. 4-6). Within *Lemnaceae*, *L. trisulca*, specializes in low nutrient environments, followed by *S. polyrhiza*, and finally *W. columbiana*, who specializes in high nutrient environments. Although *L. minor* is more competitive in high nutrient environments (Landolt 1986), it is a generalist that can persist over a broad range of conditions. This ordering of species follows a general reduction in plant body size and the simplification of morphology, such that the smaller and simpler the plant, the greater the competitive advantage it has in more eutrophic conditions (Fig. 4-6). Even within *Lemnaceae*, the evolutionary trajectory has been one of simplification and reduction with the larger genera (*Spirodela* and *Landoltia*) more basal in the phylogeny (Cabrera et al. 2008, Cusimano et al. 2011), with the family undergoing simplification in *Lemna*, and further simplification in the tiny *Wolffia* (Landolt 1986, Sree et al. 2015a). In addition to a reduction in size and the fusion of the stem and leaf into a frond, *Lemnaceae* show increasing morphological and physiological simplification such that there is a general reduction in root number and vasculature from *Spirodela* to *Lemna* to *Wolffia* (Landolt 1986). Since all species can absorb nutrients directly through the frond, the root function decreases in importance when nutrients are less limiting. Although roots in *L. minor* increase rates of nutrient uptake by increasing the total plant surface area and vary in length as a function of nutrient gradients (Cedergreen and Madsen 2002, Jewell and Bell 2022b, Jewell et al. 2022), this advantage diminishes the smaller the plants become given the scaling of the surface area to volume ratio (Eissenstat 1992). In the extreme, species in the genus of *Wolffia,* the smallest and fastest growing of all flowering plants (Sree et al. 2015a, 2015b), consist of just single green frond about 1mm across, and lack roots and all vasculature completely (Daubs 1965, Landolt 1986, Sree et al. 2015a).

Latitude-diversity gradient

There was a clear latitudinal gradient in species richness. In the southern temperate region, species often coexisted in communities of two to six species in a single site, whereas north of the Saint Lawrence river, *L. minor* was most often in monoculture, or with S. *polyrhiza*, with species richness never greater than two) (Fig. 4-2B). This diversity gradient is possibly driven by average temperature which is thought to be a key factor in constraining distributions in *Lemnaceae* (Landolt 1986, Feuchtmayr et al. 2009). Species distributions did not show clustering suggesting that dispersal limitation was not an important factor for species distributions. This is especially relveant for rare species, whose presence was widely distributed across just a few sites.

Lemna minor, the "Darwinian demon"

Despite substantial ecological specialization among species, *L. minor* dominated most communities across a broad range of environmental conditions supporting its classification as an effective generalist. This is consistent with other field surveys of free-floating plants reporting ubiquitous and abundant populations of *L. minor* (Landolt 1986, Mccann 2016, Paolacci et al. 2018) and experimental work reporting a major fitness advantage over other species over a broad range of environmental conditions (Hillman 1961a, McLay 1976, Portielje and Roijackers 1995, Jewell and Bell 2022a). Although often dominant in temperate and tropical sites (Landolt 1986), *L. minor* can maintain growth at lower temperatures than any other species of *Lemnaceae* (Hillman 1961a, Hodgson 1970), and has near 100% winter survival in cold climates (Jewell & Bell, manuscript in preparation) which explains its prevalence in sites north of the limits of other species (Daubs 1965). *L. minor*'s broad geographic distribution and success could be due to substantial intraspecific variation among clones, sometimes described as ecotypes (Landolt 1986, Ziegler et al. 2015, van Moorsel 2022). Its ability to maintain high absolute and relative fitness across a broad range of environmental conditions, in every region of the world with the exception of desert and artic biomes, has led some to describe *L. minor* as an example of a "Darwinian demon" (Kutschera and Niklas 2015).

Statistical considerations and limitations

To test for ecological specialization, we compared sites where a species was present to sites where it was absent. A significant deviation from the 1:1 line indicates ecological specialization. However, a species could still be ecologically specialized even if it does not differ from the 1:1 line. If a species were specialized for intermediate conditions of an environmental variable, and absent from both very high and low conditions, the means for presence and absence would be comparable (although the variances would be different), and no statistical difference would be detected. Thus, the lack of significant results does not exclude specialization to intermediate nutrient availability. Consequently, our approach is rather conservative.

Another point to keep in mind is that since *R. natans*, *R. fluitans* and *C. demersum* were rare, (only present in 4, 2 and 3 sites respectively), the statistical power to detect significant differences between presence and absence was low. For example, although *C. demersum* was found uniquely in high-pH and Rf in low-pH sites, these results were not statistically significant. More sites will have to be added to future surveys that include more water bodies with *C. demersum* present. In contrast, to control for inflated type 1 error caused by using independent tests for each environmental variable, the Bonferroni correction lowered alpha from 0.05 to 0.008. Even when applying this highly conservative correction, specialization in *W. columbiana* to high TP, and *R. fluitans* to low TP and low light remain significant.

Conclusions

Despite these above-mentioned limitations, this survey of aquatic floating plants in 37 sites distributed across southern Quebec shows clear evidence of ecological specialization in a subset of the surveyed species and a dominance of *L. minor* across all surveyed sites. Although all seven species of free-floating aquatic plants possess similar modes of life and resource requirements, morphological and physiological simplification has resulted in some plants becoming adapted to eutrophic conditions. Furthermore, we observed little evidence that dispersal limitation influenced species distributions. Consequently, we conclude that the

distribution of these plants is the result of competitive sorting of suitable species from a regional species pool without any substantial effect of dispersal limitation.

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Preamble to Chapter 5

In Chapter 4, we detected a non-random distribution of species in natural communities in relation to nutrient availability. This evidence of ecological specialization suggests that species sorting should play a role in structuring communities. This provided the backdrop to Chapter 5 which describes a mesocosm experiment designed to measure the relative contributions of different ecological processes to changes in community structure. We used communities consisting of three species of *Lemnaceae*, and one liverwort. These four species were among the five most common free-floating plants we observed in the field and have important morphological and ecological differences. Run over about a dozen generations, we monitored changes in species composition in 48 large experimental communities and estimated the contributions of species sorting and ecological drift.

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Chapter 5 - A basic community dynamics experiment: Disentangling deterministic and stochastic processes in structuring communities of floating aquatic plants

Mark Davidson Jewell[1] and Graham Bell[1,2]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.
[2]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Summary

Community dynamics are governed by two opposed processes: species sorting, which produces deterministic dynamics leading to an equilibrium state, and ecological drift, which produces stochastic dynamics. Despite a great deal of theoretical and empirical work aiming to demonstrate the predominance of one or the other of these processes, the importance of drift in structuring communities and maintaining species diversity remains contested. Here we present the results of a basic community dynamics experiment using floating aquatic plants, designed to measure the relative contributions of species sorting, ecological drift to community change over about a dozen generations. We found that species sorting became overwhelmingly dominant as the experiment progressed, and directed communities towards a stable equilibrium state maintained by negative frequency-dependent selection. The dynamics of any particular species depended on how far its initial frequency was from its equilibrium frequency, however, and consequently the balance of sorting and drift varied among species.

Introduction

Patterns in the composition and diversity of species in a community are the result of many interacting processes. Borrowing concepts from population genetics, Vellend (2010, 2016) distilled these down to four fundamental processes in a conceptual synthesis of community

ecology: selection, ecological drift, speciation, and dispersal. Since speciation and dispersal are responsible for the introduction of new variation into the community, the dynamics of a closed community is essentially governed by selection (also referred to as species sorting) and ecological drift alone. Species sorting is natural selection at the level of species, which will produce distinct assemblages of species in different habitats, each local community consisting of those species best adapted to their local conditions of growth. This classical "niche-based" view asserts that species coexistence is due to functional differences between species and predicts deterministic dynamics. Ecological drift is genetic drift at the level of species, which will produce a distinctive assemblage of species in any given place whose composition is unrelated to local conditions. This "neutral-based" view assumes the functional equivalency of species and predicts stochastic community dynamics. The relative importance of these two processes in structuring communities has been vigorously debated in the last two decades and many attempts have been made to show that one of these processes is much more important than the other (Wright 2002, Hubbell 2006, Rosindell et al. 2011, Wennekes et al. 2012). It would be difficult to support either extreme view, however, because both processes will be active at all times everywhere, and the main goal of community ecology should be to understand how the balance between them depends on the underlying physical and biotic characteristics of sites. Many recent developments have been proposed to resolve the niche-neutral controversy (Leibold and McPeek 2006, Adler et al. 2007, Haegeman and Loreau 2011, Chase 2014, Fisher and Mehta 2014, Matthews and Whittaker 2014, Shoemaker et al. 2020, Siqueira et al. 2020), and there is a growing body of empirical experimental work aimed at disentangling stochastic from deterministic processes in community assembly (Chase 2010, Gilbert and Levine 2017, Ron et al. 2018).

Species sorting and ecological drift will have directly opposed effects on the species composition of communities under a given set of environmental conditions. Under species sorting, communities that initially differ in composition will converge on the same composition, which represents the stable equilibrium community for that set of conditions. Under ecological drift, communities that are initially identical in composition will diverge over time. The relative contributions of these two processes to community dynamics (changes over time in species composition) can therefore be estimated by setting up replicated communities with different

initial composition. Ecological drift will cause divergence of replicate communities of any given initial composition. Species sorting will cause convergence of communities that initially differ in composition.

A third factor which might influence how a community changes over time is its initial state, as both drift and sorting may be historically contingent (Chase 2003, Fukami 2015). A species' initial frequency may influence its sensitivity to ecological drift due to the tendency of stochasticity to increase in importance in smaller effective populations. Likewise, species sorting might depend on a mechanism that favoured abundant species, such as priority effects or growth inhibition by exudates. The contributions of sorting, drift and initial state will sum to the overall change in composition observed over a given period of time.

Such experiments have been done for single-species populations to estimate the contributions of natural selection, genetic drift and ancestry to the evolution of fitness and of phenotypes such as cell size in bacteria and the evolution of heterotrophy in *Chlamydomonas* (Travisano et al. 1995, Bell 2013). Despite the clear analogy of these processes in population genetics to community ecology (Vellend 2010), no similar work has yet been done in multi-species communities. Here we extend experimental evolution into ecology to estimate the relative contributions of species sorting (the ecological equivalent of natural selection), ecological drift (genetic drift), and initial state (ancestry) to community species dynamics.

We assembled experimental communities of floating aquatic macrophytes from the family *Lemnaceae* that frequently coexist in the field. These are highly reduced angiosperms that consist of a single leaf-like frond which may or may not bear a submerged unbranched root, depending on the species. Reproduction is nearly always asexual and vegetative, which results in extremely short generation times of less than a week in eutrophic conditions. Many species are widespread and abundant in lentic ecosystems and often coexist in multi-species communities consisting of hundreds of thousands to millions of individuals. Because of their small size and short generation time, they are being increasingly used as a model system in ecology and evolution (Laird and Barks 2018, Hart et al. 2019, Vu et al. 2019) and enable us to run highly-replicated experiments lasting more than a dozen generations in a single season. Here we report the results of a basic

community dynamics experiment using semi-natural communities consisting of four such species of *Lemnaceae*. By manipulating initial relative abundances of species and following changes in species composition over time, we can estimate the relative contributions of these opposing processes to community species dynamics.

Materials and Methods

Source community

The source plant community was isolated from a eutrophic pond adjacent to fallowed agricultural fields on the Macdonald campus of McGill University, Quebec, Canada (45° 42' N, 73° 94' W). The pond sustains a diverse community of floating macrophytes, the four most abundant being *Lemna minor* (Lm), *Lemna trisulca* (Lt), *Spirodela polyrhiza* (Sp) *and Wolffia columbiana* (Wc), all in the family *Lemnaceae*. Large samples consisting of hundreds of thousands of individuals were taken in June 2020 and manually separated into the constituent species.

Experimental design

The experiment was conducted at the LEAP (Large Experimental Array of Ponds) facility at Gault Nature Reserve of McGill University in Quebec, Canada (45° 32' N, 73° 08' W), (Fugère et al. 2020) (Fig. 5-1). Forty-eight large mesocosms (surface area=2.43m^2) were filled each with 500L of water piped from Lac Hertel, a mesotrophic lake on the reserve, 1km upstream of the experiment. The water was sieved to remove fish, tadpoles, macroinvertebrates, and macrophytes, but contained intact communities of zooplankton and phytoplankton. Material from the source community was used to assemble four community types defined by the initial relative abundance of each species (10%, 20%, 30% or 40%) (Table 5-1). Relative abundance was calculated as mass-weighted frequencies using an average value of individual mass for each species. Each community was seeded with a total of 1g wet mass of community biomass, which works out to between ~2,000-3,000 individuals, depending on the community type (Table 5-1). Abundances of the larger species were determined by manual counting, while Wc, only ~0.5mm

wide, was weighed and added in bulk, using an estimate of mean frond mass. Each of the four community types were replicated in 12 mesocosms (total number of mesocosms = 48) which were arranged in six blocks of eight mesocosms, each block containing two replicates of each community type, with community type randomized within block. All mesocosms received a one-time initial addition of inorganic Nitrogen and Phosphorus (KNO_3 and H_2KPO_4), to obtain initial dissolved concentrations of these nutrients in the mesocosms comparable to those of the pond from which the source community was taken (800 μgL^{-1} N and 40 μgL^{-1} P). The mesocosms were covered with 70% shade cloth to mimic canopy cover. Although this minimized the input of wind-carried debris like leaf litter, rainwater could pass through the mesh cloth, which roughly balanced water lost due to evaporation. Communities were then left to grow for 12 weeks, from the beginning of July to the end of September, ending shortly before the first frost. All mesocosms were randomly sampled every two weeks to estimate species relative abundances. This was done by first mixing the communities to break up species clustering, then removing a fixed percentage of the surface area (~5%) with a net. Although mixing modified the spatial structure of the community, it allowed us to efficiently obtain representative samples. These samples were exhaustively counted before being returned to the mesocosm.

Table 5-1. Initial composition of the four community types by mass-weighted relative abundances. The four were species: *Lemna minor* (Lm), *Lemna trisulca* (Lt), *Spirodela polyrhiza* (Sp), and *Wolffia columbiana* (Wc).

Community type	Species	Initial relative abundances (%)	Initial no. of individuals
A	*Lm*	*40*	*1081*
	Lt	*30*	*150*
	Sp	*20*	*72*
	Wc	*10*	*625*
	Total	*100*	*1928*
B	*Lm*	*30*	*811*
	Lt	*40*	*200*
	Sp	*10*	*36*
	Wc	*20*	*1250*
	Total	*100*	*2297*
C	*Lm*	*20*	*541*
	Lt	*10*	*50*
	Sp	*40*	*145*
	Wc	*30*	*1875*
	Total	*100*	*2611*
D	*Lm*	*10*	*270*
	Lt	*20*	*100*
	Sp	*30*	*109*
	Wc	*40*	*2500*
	Total	*100*	*2979*

Fig. 5-1. The experimental setup. Forty-eight mesocosms arranged in six blocks of eight on the LEAP platform at McGill's Gault Nature Reserve. Each block is covered with shade cloth which serves to mimic canopy cover and minimize the input of debris by wind.

Statistical analysis

The main goal of this experiment was to estimate the contributions of species sorting, ecological drift, and initial state to community change. Overall variation in final species composition among communities can be broken into these three components, whose contributions to variation can be partitioned using an Anova framework (Travisano et al. 1995, Bell 2013). If Y_{ij} is the final frequency of the focal species in community type i and replicate j, then its deviation from that species' mean initial frequency, $Y_{initial}$, can be partitioned into three additive components representing the three sources of variation:

$$Y_{ij} - Y_{initial} = (Y_{ij} - Y_i) + (Y_i - Y) + (Y - Y_{initial})$$

where Y_i is the mean final frequency of the focal species in community type i, and Y is the grand mean final frequency of the focal species across all community types and replicates. For n community types (communities with different initial species composition) each replicated m times, the total variation attributable to sorting, drift and initial state can be calculated as follows:

- $nm\ S\ (Y - Y_{initial})^2$, the shift in grand mean representing an overall convergence to an equilibrium composition (sorting),
- $m\ S\ (Y_i - Y)^2$, the variance among community types around the grand mean representing the influence of a community's initial state, and
- $S\ S\ (Y_{ij} - Y_i)^2$, the variance among replicates of same community type representing neutral variation (drift).

Such a partition was done for each species at the end of the experiment to estimate the overall contributions of sorting, drift and initial state, as well as for each intermediate census to assess how the contributions changed over time.

We calculated a normalized value of the change in relative abundance of each species at each census in each mesocosm as the difference between the relative abundance of that species in the current and immediately preceding census, divided by its relative abundance in the preceding census. For each species, we used the regression of this normalized change in relative abundance on its preceding relative abundance to determine whether species dynamics were frequency-dependent. We used the X-intercept (at which change in relative abundance is zero) as an estimate of the equilibrium abundance of that species in a stable community.

Results

There was an initial sharp drop in the relative abundances of *Lemna trisulca* (Lt) and *Wolffia columbiana* (Wc) because of transfer shock, with a fraction of plants immediately sinking to the bottom of the mesocosms. Because of this, we have used week 2 as the initial time point. After

this initial settling time, community composition continued to change during the 12-week experiment with *Lemna minor* (Lm) coming to dominate most communities, regardless of its initial frequency, at the expense of Lt and Wc which became rare (but not extinct) in most communities (Fig. 5-2). *Spirodela polyrhiza* (Sp), maintained moderate abundances in most communities. The overall trajectory of community composition seemed to be largely independent of initial composition and was consistent among replicates. This was the case regardless of whether relative abundance was weighted by mass (as for the main analysis), or left as un-weighted individual counts (Fig. S5-1 & S5-2 in Appendix 5).

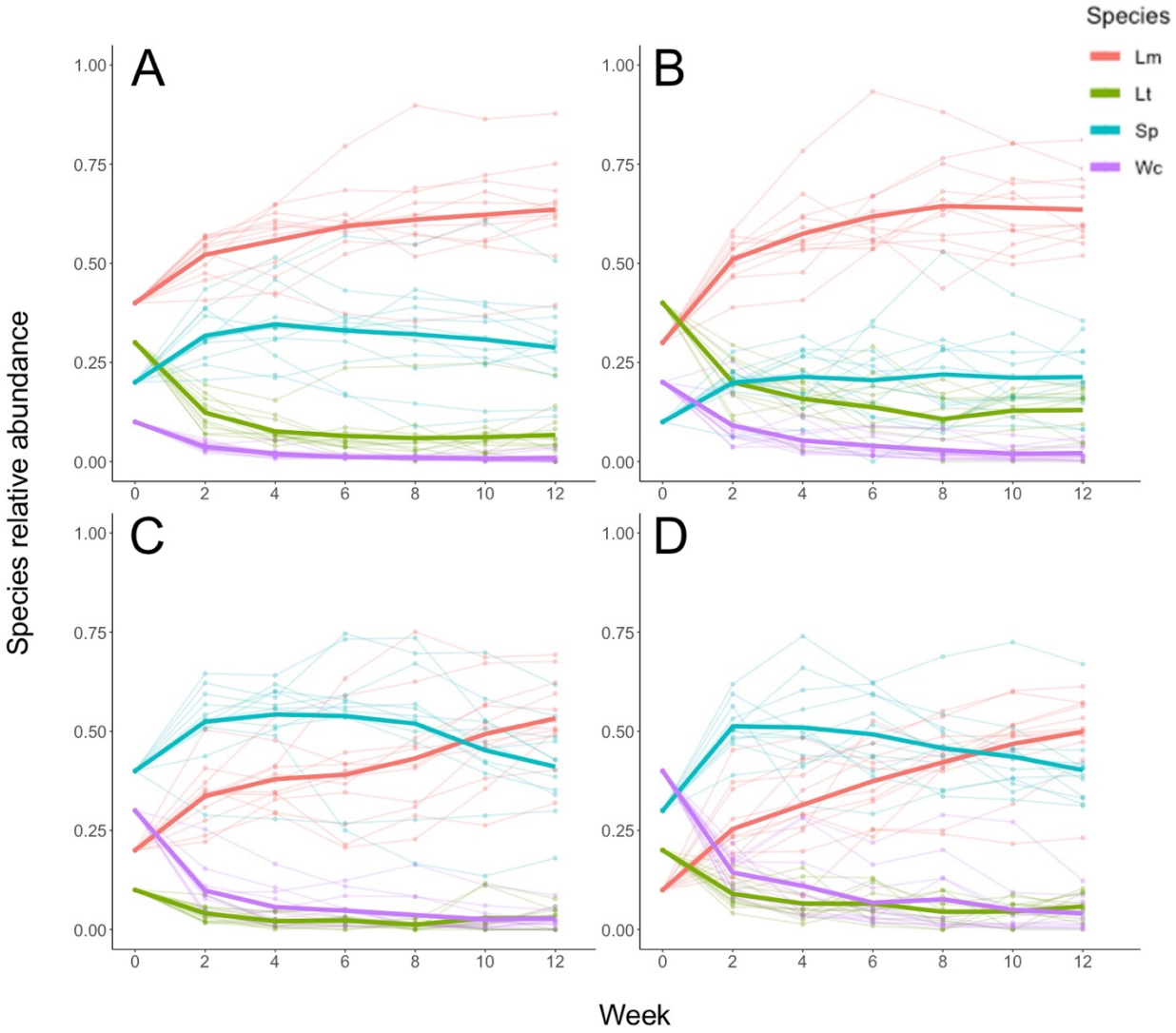

Fig. 5-2. Community dynamics over 12 weeks of growth. All four community types (A, B, C & D) consist of the same four species, which differ in their initial relative abundances depending on the community type (Table 5-1). The four species were *Lemna minor* (Lm), *Lemna trisulca* (Lt), *Spirodela polyrhiza* (Sp), and *Wolffia columbiana* (Wc). Each community type was replicated in 12 mesocosms, the means of which are shown as bold lines.

By the end of the experiment, each community type had diverged substantially from its initial composition. The extent of this divergence has three components: sorting as the source of directional change, drift as idiosyncratic divergence among replicates, and initial state as retention of differences among community types. To quantify the relative contributions of

sorting, drift and initial state to final community composition we partitioned the overall sum of squares into components representing these three processes (Fig. 5-3). This analysis was done for each time point (see Table S5-1 in Supporting information), to evaluate how these three sources of community change varied over the course of the experiment (Fig. 5-4).

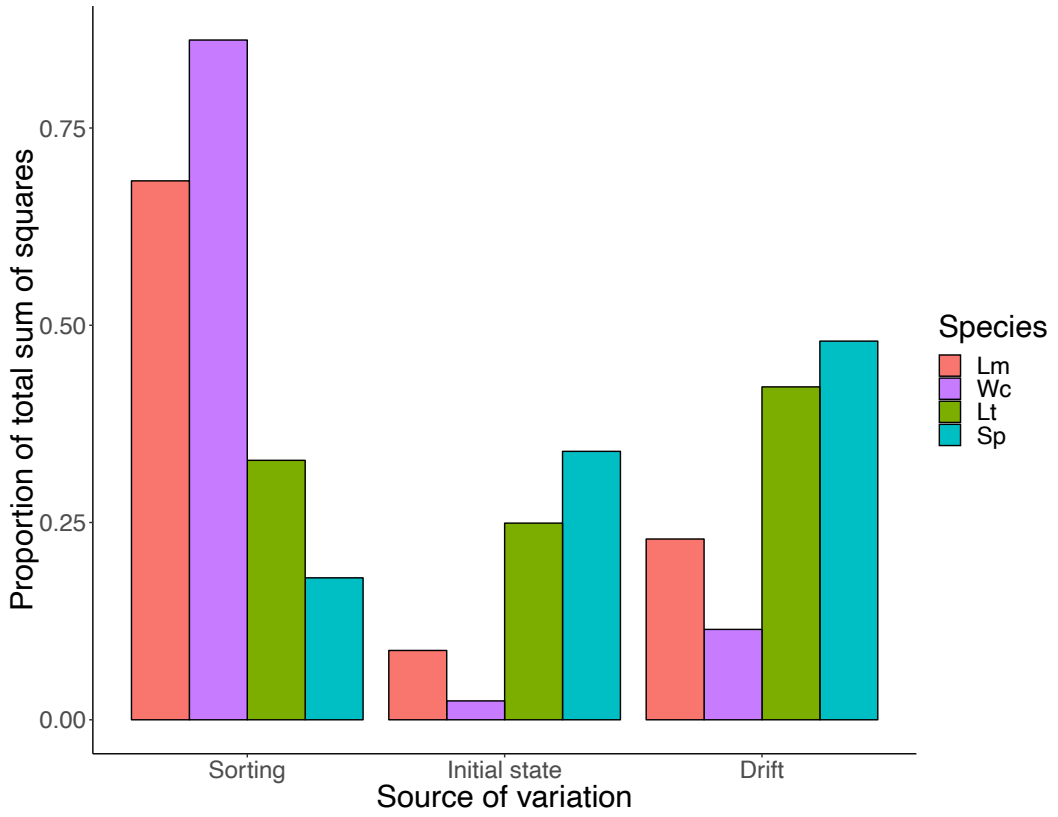

Fig. 5-3. Contributions of sorting, drift and initial state as proportions of the total sum of squares to overall community change. Contributions were calculated separately for each of the four species: *Lemna minor* (Lm), *Lemna trisulca* (Lt), *Spirodela polyrhiza* (Sp), and *Wolffia columbiana* (Wc). The underlying Anova table is in Appendix 5 (Table S5-1).

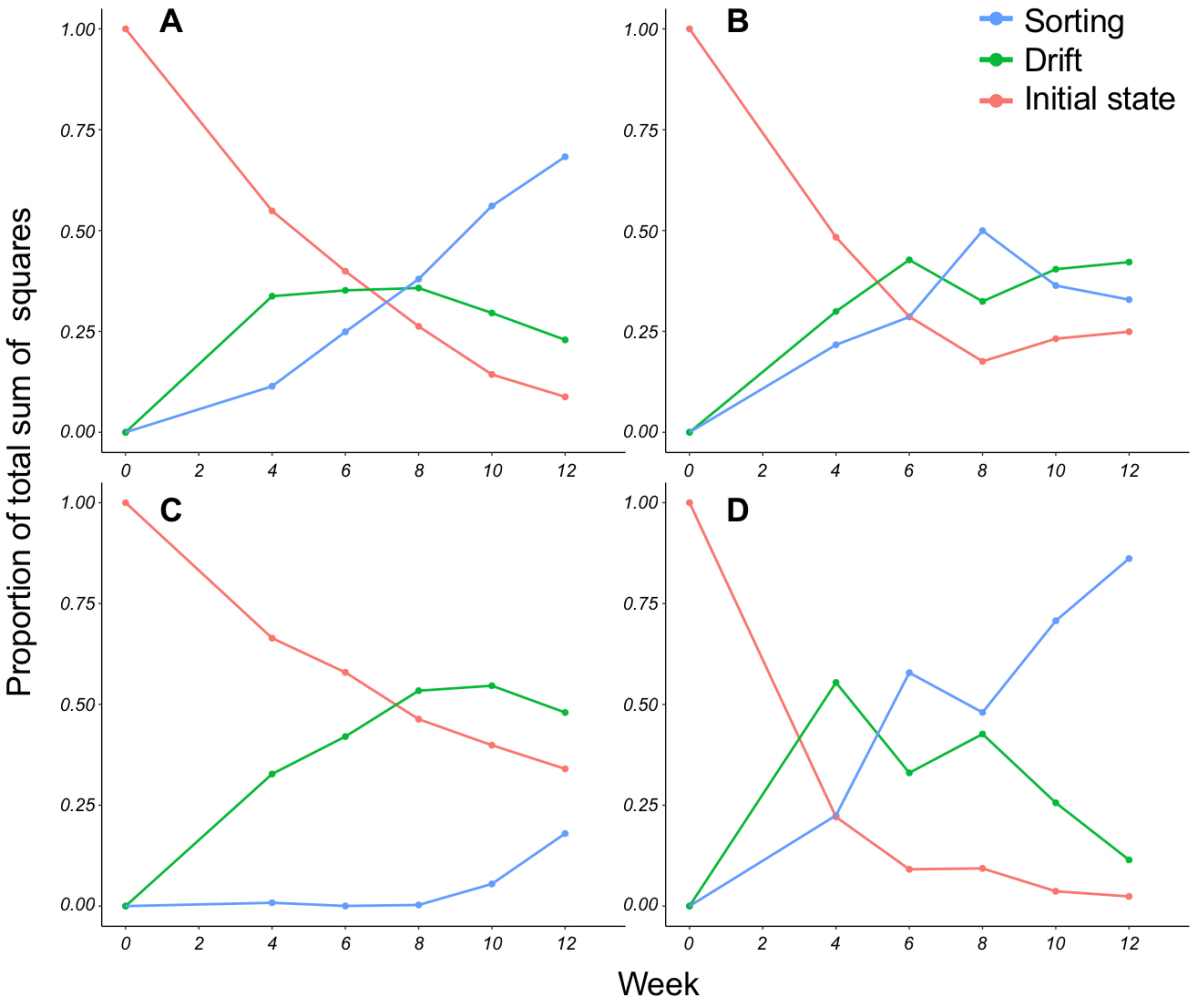

Fig. 5-4. Contributions, measured as proportion of total sum of squares, of sorting, initial state, and drift to overall community change over the course of the experiment for A) *Lemna minor*, B) *Lemna trisulca*, C) *Spirodela polyrhiza* and D) *Wolffia columbiana*. Sums of squares are from the Anova shown in Table S5-1 in Appendix 5.

We regressed the normalized change in relative abundance between time points against previous relative abundance for each species to obtain estimates of equilibrium species frequencies (Fig. 5-5). These regressions are autocorrelated, but they can be used to estimate the equilibrium frequency of a species as the X-intercept, yielding 0.76 for Lm, 0.05 for Lt, 0.27 for Sp and 0.01

for Wc. When constrained to sum to 1, these frequencies are 0.70 for Lm, 0.04 for Lt, 0.25 for Sp and 0.01 for Wc.

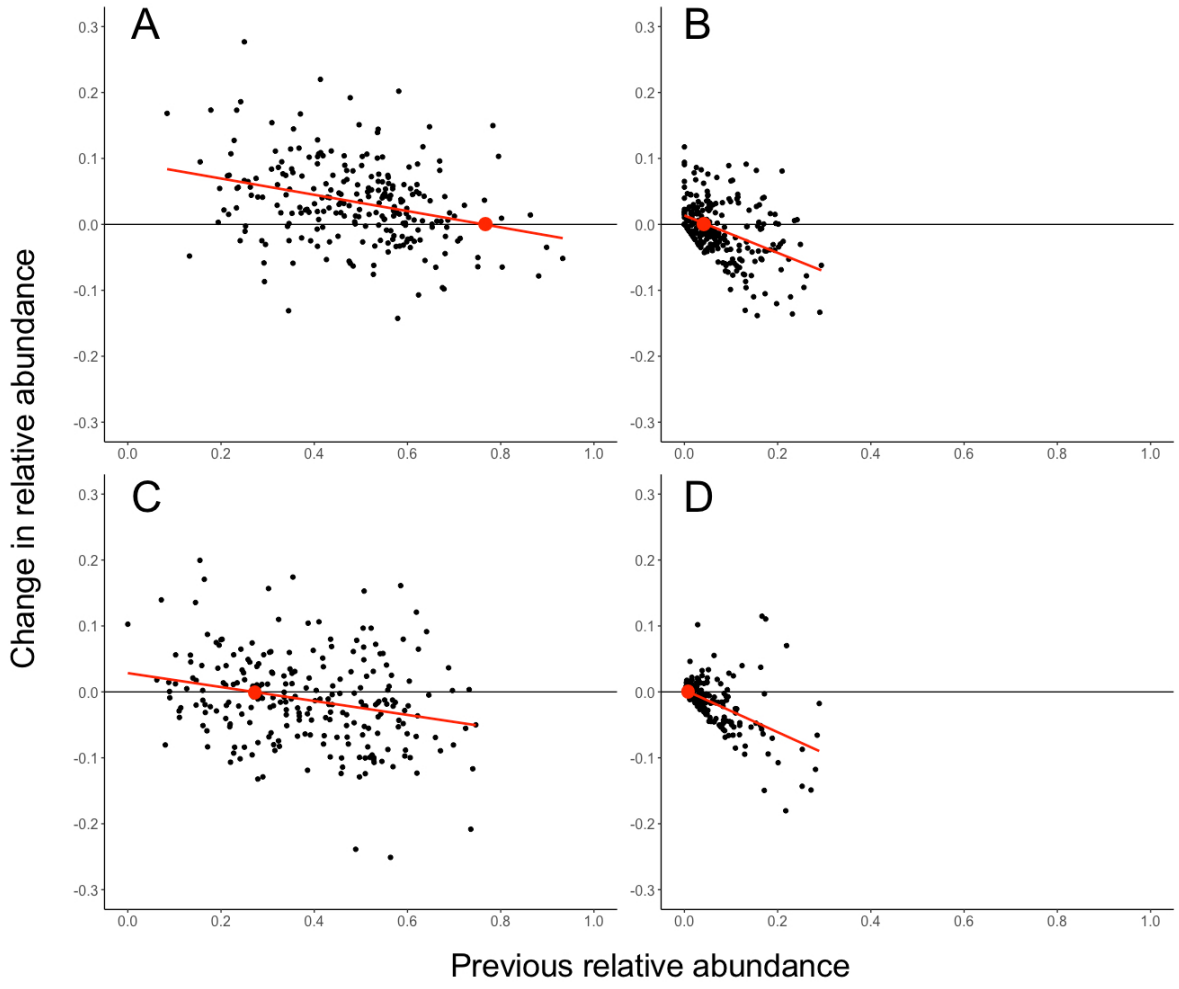

Fig. 5-5. Change in species relative abundance as a function of previous relative abundance for A) *Lemna minor*, B) *Lemna trisulca*, C) *Spirodela polyrhiza*, and D) *Wolffia columbiana*. Y=0 indicates no change in species relative abundance and thus where the regression line intersects this gives an estimate for the equilibrium frequency of that species.

Discussion

By the end of the experiment, the initial state of the community had ceased to be a principal determinant of composition for all species (Fig. 5-3). A large contribution of initial state would indicate either that species frequencies had not changed, or that change was contingent on history, either through drift (for example, because of the increased risk of extinction when a species becomes rare) or sorting (for example, through facilitation, inhibition, or competitive intransitivity). The dominant process depended on the focal species, as the dynamics of Lm and Wc were governed predominantly by sorting, while those of Lt and Sp were governed largely by drift (Fig. 5-3). Overall, Lm benefited at the expense of Wc, while Lt and Sp remained largely static with a moderate amount of stochastic variation around their mean state. A modest contribution of sorting (and therefore a large relative contribution of drift) could be caused either by weak competition or because the equilibrium frequency of a species fell close to its average initial frequency over all community types (as was the case for Sp, whose initial and equilibrium relative abundances were both 0.25). Conversely, the dynamics of the species whose equilibrium frequencies are the furthest from their average initial frequencies (Lm and Wc) were most dominated by species sorting.

The strong effect of sorting and the absence of any strong effect of initial state suggests that the community tends towards an equilibrium composition, either through competitive exclusion or stable coexistence. Given a relatively constant and spatially homogeneous environment (May 1973, Chesson 2017), the distance from equilibrium which an actual community lies is determined by the balance of sorting and drift. Whether or not this equilibrium community involves the stable coexistence of several species can be determined by considering how the frequency of a species changes as a function of its current value (Fig. 5-5). The presence of a large negative correlation for all four species is evidence of negative frequency-dependent selection acting as a stable coexistence mechanism (Chesson 2000, Adler et al. 2007). The slope of this correlation indicates the strength of the frequency-dependence, and its intercept with zero change in frequency indicates the equilibrium frequency of that species. This negative frequency-dependence is probably a widespread coexistence mechanism in natural communities of floating freshwater macrophytes (Barrat-Segretain and Elger 2004, Gérard and Triest 2018,

Armitage and Jones 2019, Hart et al. 2019) responsible for maintaining local diversity. Any real community will deviate from the ideal equilibrium composition through ecological drift.

The balance of sorting, drift and initial state shifted in a simple and predictable way over time. At the beginning of the experiment, since no change has yet occurred, all variation in community composition is due to initial state (Fig. 5-4). As time progresses, the relative contribution of initial state diminishes for all species, indicating the lessening contribution of initial state to community dynamics. Both species sorting and ecological drift increase in importance, and as the community nears equilibrium, roughly balance each other. This balance is however species-specific, due to the strength of competition. The further away a species' frequency is from its equilibrium value, the stronger species sorting will act to bring it closer. Thus, both sorting and drift are acting on all species at all times, but how they combine depends on the species and will be determined by its competitive advantage and how far it is from its equilibrium frequency.

The standard approach to quantify stochastic effects on community structure is with the use of pairwise beta-diversity indices (eg. Bray-Curtis) (Anderson et al. 2011). Compositional dissimilarity is calculated for all pairwise combinations of sites in the same conditions and averaged to produce an estimate of among site (beta) diversity due to stochasticity, ranging from 0 to 1 (Gilbert and Levine 2017, Ron et al. 2018). Our approach of using an Anova framework allows us to partition the contribution stochastic processes into those due to ecological drift (variation in vital rates among individuals of the same species) and priority effects (stochastic variation in order of colonisation), and allows us to compare these numerically with the contribution from species sorting. Furthermore, obtaining estimates for each species separately can reveal how these fundamental processes may operate distinctly for different species in a community. The weakness of such an approach is that the contribution of sorting is highly sensitive to the initial frequencies (and their distances from the equilibrium frequencies). Consequently, estimates of the proportion of the total sum of squares due to stochasticity will also be sensitive to an arbitrary initial frequency. To work around this, it may also be informative to consider the contributions of stochastic processes as the raw within-group (drift) and among-group (initial state) sums of squares. We compared these measures to the more commonly used Bray-Curtis beta-diversity, calculated among replicate communities of each community type, at

each time point, and found that both methods captured largely the same variability in species frequencies (Fig. 5-6). Equating this variability to drift of course assumes that the experiment is perfectly controlled, since any unintentional biotic and abiotic variation among replicate mesocosms contributes to our estimates of drift.

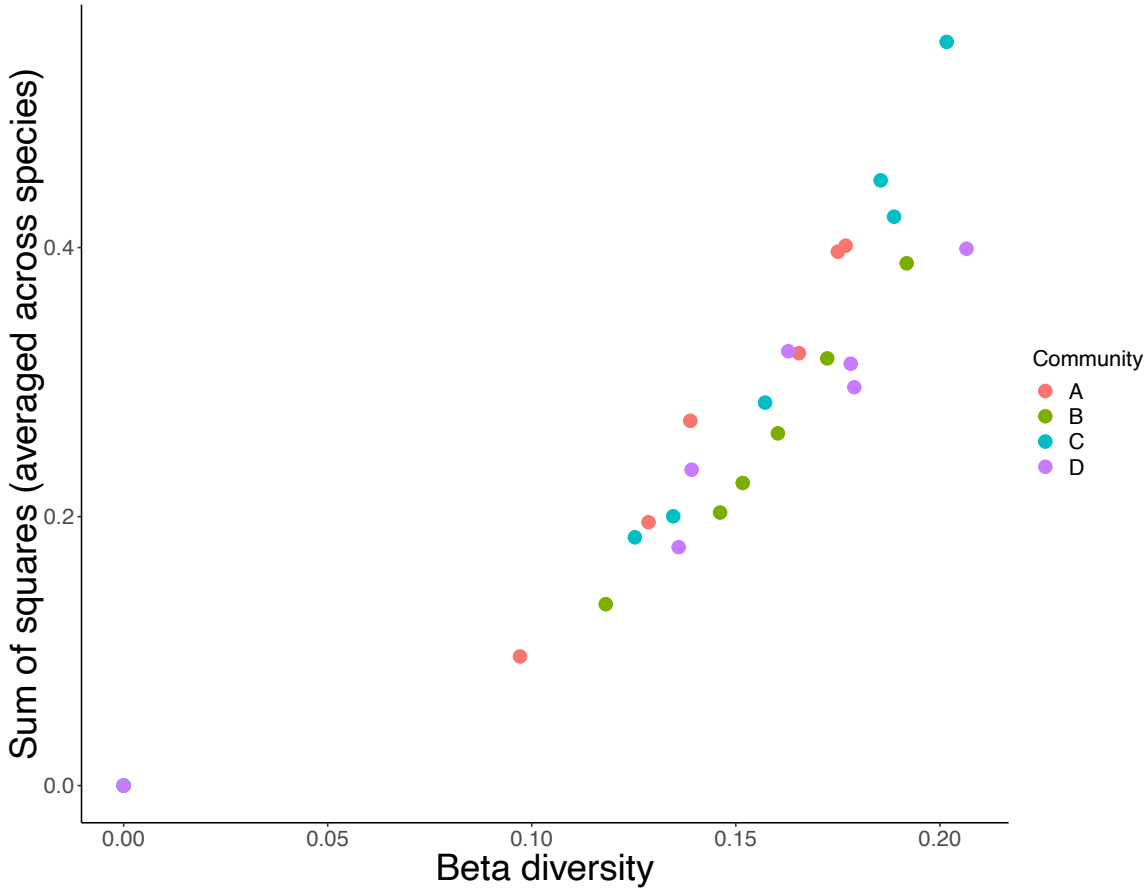

Fig. 5-6. A comparison of two measures of ecological drift. Sums of squares were calculated for each community type, time point and species, and then averaged across the four species to allow comparison with beta diversity. Beta diversity was calculated as mean pairwise Bray-Curtis pair-wise dissimilarity for each community type at each time point.

We conclude that the dynamics of our experimental communities shifted over time but were eventually dominated by species sorting, which resulted in the predictable and more or less deterministic shift towards an equilibrium state. The contribution of initial state declined

consistently over time, but the balance between species sorting and ecological drift varied among species because, as in any community, some species were closer than others to their equilibrium frequency.

Acknowledgments

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Preamble to Chapter 6

In the preceding chapters we have investigated the roles of phenotypic plasticity (physiology), adaptation (evolution) and species sorting and genetic drift (ecology) in modifying phenotypes and community structure in floating aquatic plants, in the lab, field and in semi-natural mesocosms. However, we have only considered these processes in isolation or in pairwise combination. For example, in Chapter 1 we focused on phenotypic plasticity alone. Scaling up in complexity, in Chapter 2 we assessed the relative roles of plasticity and local adaptation together in determining phenotypes in the field, in Chapter 4 we focused on evolved ecological differences among species and how habitat filtering (species sorting) determines species distributions and diversity, and in Chapter 5 we assessed the roles of species sorting and ecological drift in determining community structure. In the final chapter we describe a mesocosm experiment designed to partition total community phenotypic change into contributions representing the three classes of mechanisms: physiological, evolutionary, and ecological.

This chapter is currently in revision at Ecology.

Chapter 6 - Eco-evolutionary contributions to community trait change in floating aquatic plants

Mark Davidson Jewell[1*] and Graham Bell[1,2]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.
[2]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Summary

An entire community of organisms may become modified when its environment changes. These modifications can happen through physiological process (plasticity), evolutionary processes (adaptation) or shifts in species composition (sorting). The outcome of these three sources of change constitutes the community's phenotypic response, but how they combine to drive community trait dynamics is not currently well understood. We have conducted a community selection experiment in which communities of short-lived floating aquatic plants were grown in a range of stressful conditions, and measured changes in their body size. Determinants of phenotypic change were assessed with a full community reciprocal transplant which led to estimates of the contributions of plasticity, adaptation, and sorting. Species were modified during the experiment by both plasticity and adaptation, but in either case the magnitude and direction of change differed among species. Sorting and adaptation were of equal magnitude, but tended to act in opposite directions: in conditions where species with large fronds prevailed, each species evolved smaller fronds, and vice versa. We conclude that community trait dynamics cannot be understood simply by extrapolating the adaptive response of any single species to the whole community.

Introduction

A community of organisms that experiences prolonged exposure to a new environment may become altered in response to the new conditions. The community response is characterized by a shift in mean phenotype for a trait common to all species. Such a shift in community mean phenotype may be due to three distinct kinds of process: the physiological response of individuals to a change in the conditions of growth (plasticity), the demographic response of the community through shifts in the relative abundance of species (sorting), and the genetic evolutionary response of each species (adaptation) (Fig. 6-1). Plasticity, sorting and adaptation, as well as their interactions, may all contribute to any phenotypic change in a multi-species community, and acting collectively they drive community trait dynamics (Guimarães et al. 2017, van Moorsel et al. 2019, Hall et al. 2020).

	Community trait change		
Level of change	Interspecific trait change	Intraspecific trait change	
Type of process	Ecological	Physiological	Evolutionary
Process	**Sorting**	**Plasticity**	**Adaptation**
	Change in community composition through shifts in species relative abundances	*Environmentally induced phenotypic change of individuals due to physiological modification during development or over the course of their lifetime*	*Phenotypic change within species attributable to shifts in genotype frequencies, where genetic variation may be either pre-existing or novel*

Fig. 6-1. Constituent processes of community trait change.

Historically, ecological and evolutionary processes have been studied in isolation (Slobodkin 1961), but it is increasingly clear that they have the potential to occur on overlapping timescales and can feed back on each other (Thompson 1998, Hendry and Kinnison 1999, Kinnison and Hendry 2001, Hairston et al. 2005, Saccheri and Hanski 2006). For example, ecological change including changes in community composition will often shape the selection environment which drives rapid evolution (Hendry and Kinnison 1999, Reznick and Ghalambor 2001, Carroll et al.

2007) and rapid evolutionary change can feed back to influence ecological parameters like population dynamics (Turcotte et al. 2011) and community structure (Johnson et al. 2009, Hart et al. 2019). The evolution of increased phenotypic plasticity may further alter demographics by promoting persistence in stressful environments (Ghalambor et al. 2007) and either inhibit or promote further evolutionary change by modifying phenotypic variation and its link to genetic variation (Ghalambor et al. 2007, Schlichting and Wund 2014). These findings have stimulated research in the growing field of eco-evolutionary dynamics (Fussmann et al. 2007, Urban et al. 2008, Pelletier et al. 2009, Post and Palkovacs 2009, Schoener 2011), which has as a central goal to understand the relative contributions and interactions of ecological, physiological, and evolutionary processes to community change (Schoener 2011).

Growing interest in the importance of evolutionary change over ecologically relevant timescales has led to the development of partitioning metrics to separate evolutionary from non-evolutionary processes in affecting different properties of populations, communities and ecosystems (Hairston et al. 2005, Ellner et al. 2011, Merilä and Hendry 2014, van Benthem et al. 2017, De Meester et al. 2019). Focusing on community mean phenotype, sometimes referred to as functional identity, is of particular interest given its inclusion of both genetic and non-genetic determinants, its response to environmental change (Garnier et al. 2004, Guittar et al. 2016, Bjorkman et al. 2018), and its direct link to determining ecosystem processes (Grime 1998, Garnier et al. 2004, Mokany et al. 2008).

Estimating the contributions of plasticity, sorting and adaptation to community change is not a straightforward task (van Benthem et al. 2017). Community trait change can be easily partitioned into inter- and intra-specific components, but the intra-specific component may combine both non-genetic and genetic change (Fig. 6-1). Separating plasticity from adaptation either requires detailed genetic information about the populations, or trait data from large-scale transplant experiments that measure lineage trait expression across environments. A variety of analytical procedures have been used to partition overall community phenotypic change into components that represent these processes (Collins and Gardner 2009, Govaert et al. 2016, van Benthem et al. 2017, Govaert 2018). Collins & Gardner (2009) adapted the Price equation (Price 1970, 1972) to partition community phenotypic change into that between species, between lineages and within

lineages. First designed to measure evolutionary change within a population from one generation to the next, the Price equation is readily extended to measure change within multi-species communities over longer time scales and has been used to describe changes in toxin resistance within microbial communities, and carbon uptake by marine phytoplankton in high-CO_2 environments (Collins and Gardner 2009). However, this method requires detailed data on the dynamics of different lineages within species, which is often difficult to obtain if lineages are indistinguishable from one another. Furthermore, Govaert et al. (2016) pointed out that the Price equation approach cannot determine the cause of phenotypic change within lineages, lumping together both non-genetic change due to phenotypic plasticity and genetic change due the introduction of novel genetic variation via mutation, immigration, or horizontal gene transfer.

The most rigorous method to distinguish between phenotypic plasticity and evolutionary change is with the use of a classical reciprocal transplant experiment, where populations from two environments are cultured in both their 'home' and 'away' environments (Miller and Fowler 1993, Kawecki and Ebert 2004). Fitness and/or phenotype are then measured on the second or third generation of growth in the transplanted environment, minimizing maternal effects and allowing plastic physiological change to be fully expressed, but before shifts in genotype frequencies become relevant. This reaction norm approach has been used to identify local adaptation (Kawecki and Ebert 2004, Hargreaves et al. 2020) and to partition observed differences in traits between populations into contributions from plasticity and evolutionary processes (Govaert et al. 2016, Stoks et al. 2016). Although reciprocal transplants are usually done with a single species, the concept can be extended to a whole community (Govaert et al. 2016). Despite being proposed more than 5 years ago, a multi-generational community reciprocal transplant has to our knowledge yet to be carried out. Here we describe a community selection experiment where whole communities are exposed to modified environments and whose effects are assessed using a full community reciprocal transplant assay.

Floating aquatic plant communities

We assembled experimental communities of four species of floating aquatic plants: the angiosperms *Lemna minor* (here designated Lm), *Spirodela polyrhiza* (Sp) and *Wolffia*

columbiana (Wc), and the liverwort *Ricciocarpus natans* (Rn). These are small, morphologically simplified plants that generally consist of no more than a flattened leaf-like frond that may bear one or more submerged roots. The plants reproduce vegetatively in most conditions by releasing a daughter frond every few days from a meristem on the lower surface of the parental frond in the case of the three angiosperms, and by fragmentation in the case of the liverwort. Because they are widespread and abundant, are easily maintained and manipulated in the laboratory or outdoors, and possess highly reduced morphology and simplified physiology, they are being increasingly used as a tractable model system in ecology and evolution (Laird and Barks 2018, Hart et al. 2019, Vu et al. 2019). They are particularly well suited for a community selection experiment since their small size allows for large populations and high replication, and their rapid reproduction permits more than a dozen generations within a single season.

We use community mean frond area as our measure of phenotype since it is a simple and easily measurable trait common to all four species that has ecological relevance, and one that should respond to environmental conditions via physiological, ecological and evolutionary processes. The frond is essentially a photosynthetic sheet whose area may fluctuate to balance light capture and photosynthesis (growth) with the production of daughter fronds (reproduction) (Vasseur et al. 1995). Average frond area varies widely among the four species (Rn has fronds roughly twice as big as Sp, 5x bigger than Lm, and 66x bigger than Wc), and therefore shifts in species composition in a community will greatly change mean frond area as well as the total number of individuals in the community.

Optimal leaf size in plants depends on the interaction of temperature, light, water and nutrient availability and influences fitness through its effect on total light capture and photosynthesis, thermoregulation and transpiration (Parkhurst and Loucks 1972, Anten et al. 1995, Hirose et al. 1997). In land plants, low irradiance tends to lead to the production of larger leaves. This is the case for shade versus sun leaves of the same plant (Rozendaal et al. 2006), mean leaf size for plants within species along environmental gradients (Petritan et al. 2009, Kichenin et al. 2013), and among species adapted to different environments (Hamann 1979, Ackerly and Reich 1999). In species consisting of only a single leaf or frond, this standard physiological response should be compounded since it will also capture shifts in biomass allocation away from roots and into

shoots when light is limiting (Brouwer 1962; Poorter & Nagel 2000). This is the case for Lm whose root:frond area ratio shifts in response to both light and nutrient availability (Cedergreen and Madsen 2002). In addition to these ecological and plastic responses, there is evidence that frond size in Lm has a genetic basis (Vasseur and Aarssen 1992, Vasseur et al. 1995), and that populations in the field sustain a surprisingly large amount of genetic variation (Vasseur et al. 1993, Cole and Voskuil 1996). Furthermore, frond (or more generally leaf) area has been identified as both a response and effect trait due to its correlations with both environmental variables and rates of photosynthesis and growth (Lavorel and Garnier 2002). That variation in mean frond area can be influenced by several processes, respond to multiple environmental variables, and affect community and ecosystem properties, justifies its use as a focal trait in our community selection experiment.

Design of a community selection experiment

A community selection experiment begins with a source community of several species, collected from its natural environment. The community should ideally be well-adapted to its environment and in a state of evolutionary and ecological equilibrium. The experiment is conducted in two phases. Phase 1 is the selection phase, in which communities are cultured in modified environmental conditions. Phase 2 is the relaxation phase, in which the original conditions are restored to all communities (Fig. 6-2). In Phase 1, a sample of the ancestral source community is transferred to a modified environment and propagated for several or many generations, leading to a derived community. At the same time, a replicate sample is maintained in the original environment, so that it retains the attributes of the ancestral community. The average value of a character may become modified in the derived community relative to the ancestral community. The processes responsible for this modification are evaluated by a reciprocal transplant assay at the end of the selection phase. To perform this assay, samples from both the ancestral and derived communities are transplanted into both the original and modified environments. After a lag of two or three generations, to allow any carry-over or maternal effects to decay, the phenotypes of all species from the community are scored. The results of the assay can then be used to partition the contributions of sorting, plasticity and adaptation, and their interactions, to overall phenotypic change.

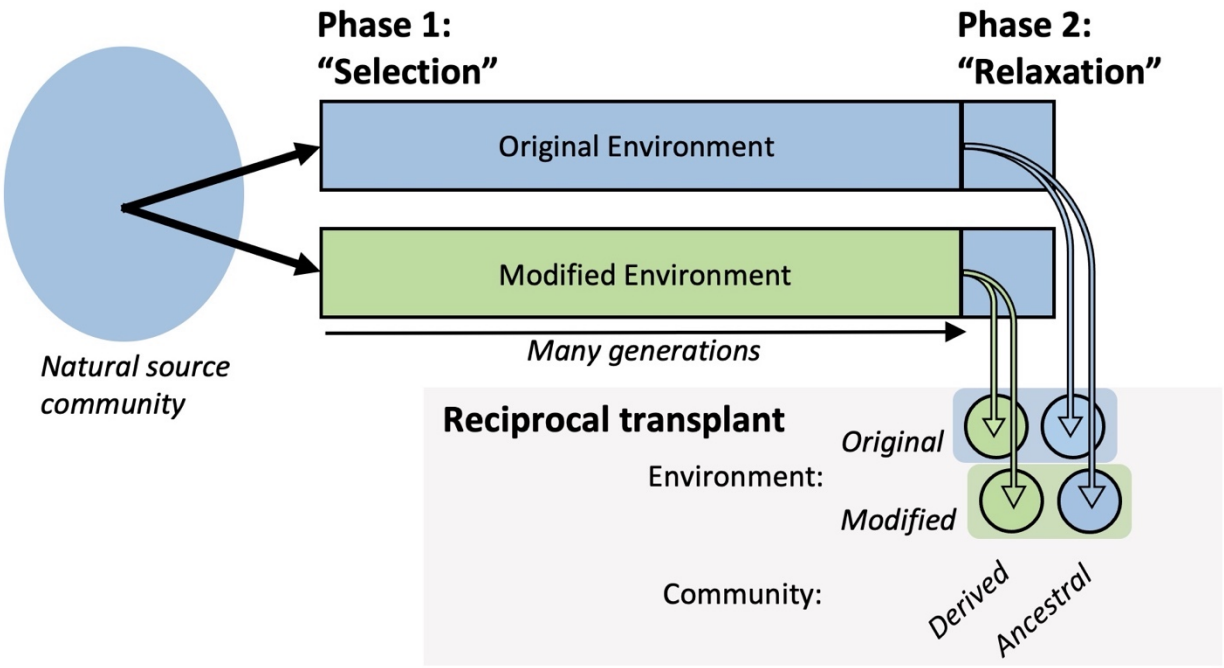


Fig. 6-2. The design of a basic community selection experiment.

The results of the reciprocal transplant assay can be compared with data from the experiment itself. First, measurements at the beginning of Phase 1 correspond with the ancestral community in the original environment and express the initial state of any given character. Secondly, the equivalent measurements at the end of Phase 1 correspond to the derived community in the modified environment, and express the combined effects of plasticity, sorting and adaptation. Thirdly, any change that has occurred in the modified environment by the end of the second generation in Phase 1 can be confidently attributed to plasticity, because there has not yet been enough time for sorting or adaptation to cause substantial change. Hence, phenotypes at this point are expected to be similar to those expressed by the ancestral community in the modified environment in the reciprocal transplant assay. Finally, Phase 2 corresponds to the derived community cultured in the original environment, where any persistent change must be attributed to sorting or adaptation. This approach is less rigorous, because it compares the state of the same communities at different times, but it will highlight any unexpected, and potentially questionable, outcome of the reciprocal transplant assay.

In this report, we describe the outcome of a community selection experiment using four species of floating aquatic plants, and measure how mean frond area responds to changes in light and nutrient availability. The objective of our experiment was to monitor phenotypic change in a whole community over several generations and then evaluate the contributions of plasticity, sorting and adaptation.

Materials and methods

Source community

The source plant community was isolated from a eutrophic pond adjacent to fallowed agricultural fields on McGill University's Macdonald campus, Quebec, Canada (45° 42' N, 73° 94' W). The pond sustains a diverse community of floating macrophytes consisting of three species of duckweed (Lm, Sp and Wc) and one liverwort (Rn). In June of 2018, we took large samples consisting of hundreds of thousands of individuals, taken from 10 microsites around the pond to ensure that our samples were representative of the pond's overall intraspecific genetic diversity. Samples were then combined, thoroughly mixed, and then sorted into the constituent species which would be used to inoculate the experimental communities.

Experimental design

Our community selection experiment consisted of propagating samples isolated from the source community in outdoor mesocosms under a range of environmental conditions. Whereas the simplified description of a general community selection experiment outlined above involves propagating the ancestral community in both original and modified environmental conditions, here we use eight distinct modified conditions in addition to the original environment, essentially running eight separate community selection experiments, allowing us to generalise our results.

The experiment was conducted at the LEAP research facility at McGill's Gault Nature Reserve in Quebec, Canada (45° 32' N, 73° 08' W) (Fugère et al. 2020). 18 180L mesocosms were filled with water piped from Lac Hertel, a pristine mesotrophic lake on the reserve, 1km upstream of the experiment. The water was sieved to remove fish and tadpoles but contained intact communities of zooplankton and phytoplankton. Mesocosms were then seeded with identical mixtures of the four species of macrophytes isolated from the source community and left to settle for one week. The four species were added in equal abundances by wet mass, 35g per species per mesocosm (which translates to roughly 23,000 individuals for Lm, 5,400 for Sp, 87,500 for Wc, and 4,000 for Rn in each mesocosm). A factorial gradient of light and nutrients was then applied to the mesocosms with three levels of each factor. This gives two replicate mesocosms for each of nine unique sets of environmental conditions. The mesocosms were arranged in a split-plot design with nutrient level and replicate randomly positioned within each light level. Light (% shading) and nutrients (dissolved Nitrogen and Phosphorus, DN and DP) were measured at the site of the source community at the time of sampling and the experimental treatment levels were determined so that the intermediate treatment (medium light, medium nutrients) mimicked these conditions. We refer to this treatment level as the "original" environment and the other eight as "modified" environments. The gradient in light availability was established with the use of varying layers of 50% shade cloth, quadrupling between levels (Low= 3%, Medium = 12%, High = 50%, in reference to an unshaded site). The nutrient gradient was established by the addition of inorganic nitrates and phosphates (KNO_3 and H_2KPO_4), maintaining a constant ratio of DN and DP. The natural water from Lac Hertel served as the low nutrient level (DN=200 μgL^{-1}, DP=10 μgL^{-1}), nutrients were quadrupled for the medium level (DN=800 μgL^{-1}, DP=40 μgL^{-1}), and quadrupled again for the high level (DN=3200 μgL^{-1}, DP=160 μgL^{-1}). DN and DP were measured in all mesocosms every two weeks and topped off to maintain the treatment nutrient levels throughout the experiment. Nutrient samples were analysed for DN with a continuous flow analyser (OI Analytical Flow Solution 3100 ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol (Patton and J.R. 2003) and for DP using a standard protocol (Wetzel and Likens 2000). All samples were analysed at the GRIL-Université du Québec à Montréal (UQAM) analytical laboratory.

The experiment was conducted in two phases: Phase 1 which applied the nine treatment combinations of light and nutrients to the mesocosms over 12 weeks ("Selection phase", Fig. 6-3A), and Phase 2 where all mesocosms were reverted to the original (intermediate) conditions for an additional two weeks ("Relaxation", Fig. 6-3C). Communities were randomly sampled every two weeks to measure frond area and estimate the relative abundance of each species. Communities were first mixed to eliminate spatial aggregation, then sampled by taking three blind scoops using a small net (diameter = 3cm) which yielded hundreds of individuals. From this sample, individuals were sorted by species and exhaustively counted to obtain species relative abundances. Phenotypes were then measured for ten individuals of each species. In the case that samples included fewer than ten individuals for a rare species, we continued to blindly sample, and sort out the species until we obtained sufficient material. The ten were selected again by blindly scooping into each species-specific sample, this time using a bacterial loop which isolates a single individual at a time. These ten individuals of each species were then photographed and analyzed in imageJ to obtain frond area. To minimize variation due to frond age, only mature individuals were included, using only those that already had a daughter frond budding from them. From estimates of species' mean frond area and relative abundances we calculated community mean frond area for each mesocosm as $\sum_{i=1}^{s}(\overline{FA}_i \times p_i)$, where $\overline{FA}_i$ is mean frond area for species *i* and p_i is the proportion of species *i* in the community.

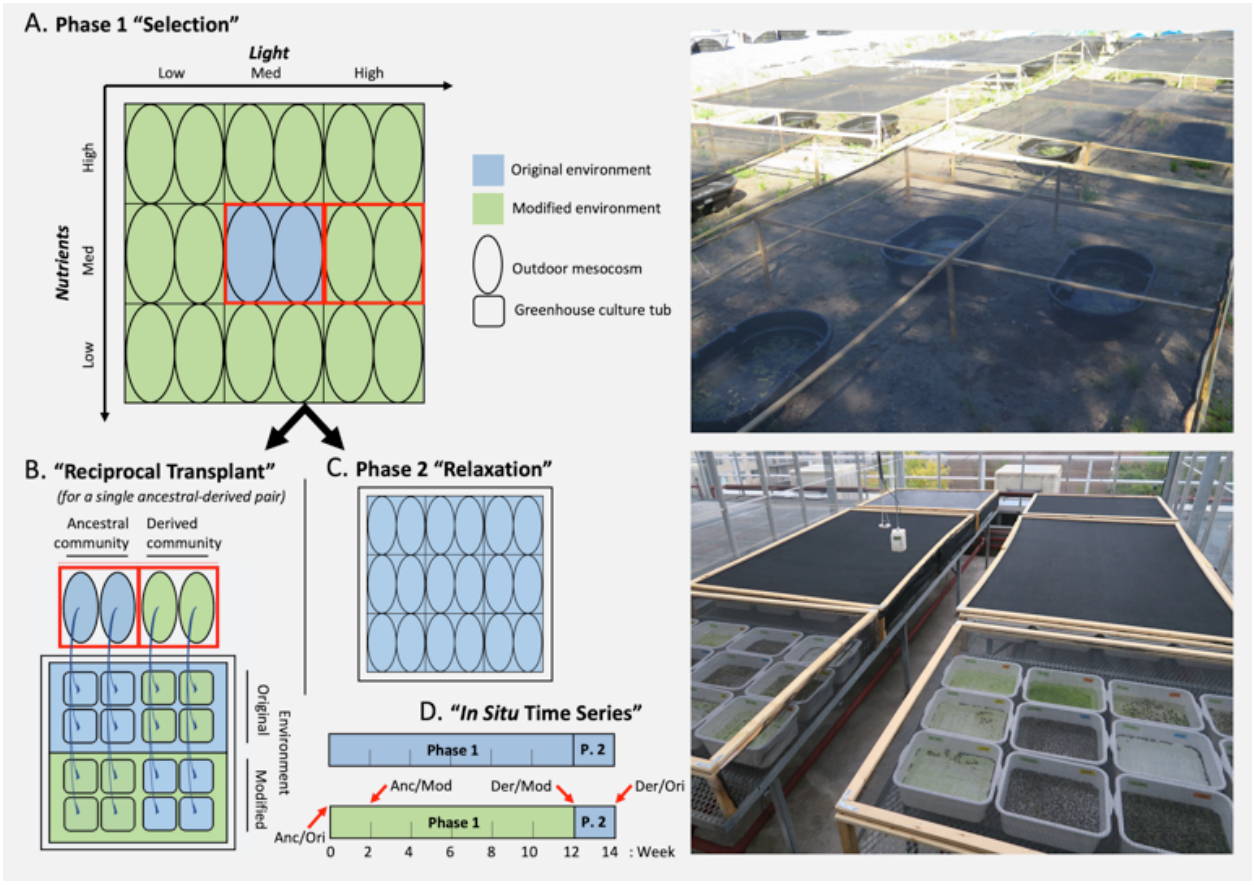


Fig. 6-3. Experimental design. A) 18 mesocosms with identical initial species compositions were subjected to a crossed gradient of light and nutrients. Two replicate mesocosms were kept in each of eight unique modified environments as well as the original environment (medium light-medium nutrients) which was designed to mimic the environmental conditions of the natural source community from which the plants were collected. After 12 weeks of growth (Phase 1), phenotypic change was assessed using both a reciprocal transplant trial and an in situ time series. B) At the end of Phase 1 samples were taken from all mesocosms to inoculate a reciprocal transplant trial in a research greenhouse. Communities from the original environment are referred to as ancestral and communities from modified environments are referred to as derived. Only one ancestral-derived pair is shown here. C) Phase 2 consisted of returning all mesocosms to the original environmental conditions for an additional two weeks. D) Using both Phase 1 and Phase 2 measurements, we can obtain an in situ time series with an identical structure as the reciprocal transplant data to use as an independent source of evidence.

Reciprocal transplant trial

At the end of Phase 1, we used a reciprocal transplant trial to assess the consequences of 12 weeks of growth in modified environments on community mean phenotype. At this point we refer to communities grown in Phase 1 modified environments as "derived", and communities grown in the Phase 1 original environment as "ancestral". By assaying all communities, both ancestral and derived, in both original and modified environments, we were able to quantify the contributions of plasticity, sorting and adaptation to overall community change.

Random samples (5% of the mesocosm surface) were taken from each mesocosm at the end of Phase 1 and used to inoculate the reciprocal transplant, located in a research greenhouse in McGill University's Phytotron. For each of the eight derived communities, a pair-wise reciprocal transplant assay was conducted with the ancestral community, assaying both communities in both modified and original environments. Each of the eight derived-ancestral pairs resulted in 16 growth assays – two replicate mesocosms per community, each assayed in two environments, replicated twice (Fig. 6-3B).

These assay environments were assembled in the greenhouse in 10L tubs filled with natural water and plankton communities from Lac Hertel, the same as in Phase 1. Nutrient and shading treatments were applied in the same way as for Phase 1. The mesocosm samples were used to inoculate the assay tubs at half of the density as that in the mesocosms at the end of Phase 1 to allow for rapid population growth. After two weeks (roughly one to two generations), we measured frond area on 10 randomly sampled individuals of each species. Total number of fronds of all species in all assays were counted at the beginning and end of the reciprocal transplant to obtain the average number of generations.

In situ time series

Phase 2 of the experiment consisted of reverting all mesocosms to the original environmental conditions for an additional two weeks. At week 12, after samples had been taken from the mesocosms to be used in the reciprocal transplant trial, the mesocosms were all reverted to

medium light and nutrient levels (Fig. 6-3C). Light levels were obtained by adding or removing shade cloth. Since during Phase 1, dissolved nutrient levels of all mesocosms consistently dropped below the medium treatment level by the end of each two-week period, Phase 2 levels could be obtained by modifying the final bi-weekly nutrient addition.

The objective of Phase 2 was to obtain a second set of measurements *in situ* to compare with the reciprocal transplant. As for the reciprocal transplant, phenotypes were obtained for both the ancestral and derived communities in both original and modified environments. Measurements of the ancestral community in the original environment were obtained from the week 1 readings at the beginning of Phase 1; measurements of the derived communities in the modified environments were obtained from the week 11 readings at the end of Phase 1; measurements of the ancestral community in the modified environments were obtained from week 3 readings, two weeks (roughly one to two generations) after treatments were first applied; and measurements of the derived communities in the original environment were obtained at the end of Phase 2, two weeks (roughly one to two generations) after all mesocosms were reverted to the original environmental conditions. We refer to this heterogeneous set of measurements as the "*in situ* time series" (Fig. 6-3D) which serves as a check on the more rigorous reciprocal transplant assay and a separate source of evidence.

Statistical Analysis

At the end of Phase 1, we used a simple 1-way Anova to evaluate if community mean frond area had significantly diverged among the 9 environments. Environment was the fixed factor, and given that there were only two mesocosms per level of environment, mesocosm represents the error variance. To help visualise shifts in species relative abundances over Phase 1, we calculated competition coefficients for each species in each environment using abundance at the final Phase 1 time point. These were calculated based on the classical method for selection coefficients (Bell 2008, p.62) when measuring competition between genotypes or species, extended to full communities. The competition coefficient of species 1, c_1, is given by

$$c_1 = \frac{r_1 - r_2}{r_2} \ln(2) = \frac{1}{g} ln \left(\frac{\frac{f_{1\,final}}{f_{2\,final}}}{\frac{f_{1\,initial}}{f_{2\,initial}}} \right)$$

where r is the growth rate in doublings per day of either the focal species (1) or the total community (2), g is the number of generations of the total community, f_1 is the relative frequency of the focal species, and $f_2 = 1 - f_1$ is the relative frequency of all other species bar the focal species.

Although we aimed to replicate the treatment environmental conditions in the reciprocal transplant, given that it took place in smaller volume tubs in a greenhouse as opposed to outdoor mesocosms, other aspects of the environment may have differed that could have affected plant growth. We therefore calculated standardized deviations in mean frond area to compare Phase 2 phenotypes with those from the reciprocal transplant (derived communities in original environment) for all communities. For each species, the deviation in mean frond area from the overall mean was calculated for each treatment combination and standardized by dividing it by the overall mean. These standardized deviations are independent of size and allow the species to be combined in the same analysis. They were calculated separately for the Phase 2 and the reciprocal transplant communities and then compared using linear regression, calculating the coefficient of correlation.

Eco-Evo Anova

The outcome of the community selection experiment was evaluated with a reciprocal transplant consisting of assaying the two community types (ancestral and derived) in each of two environments (original and modified) at the end of which phenotypes were scored on a random sample of individuals from each assay. The phenotype Y of any individual is assumed to be governed by the additive effects of i^{th} Environment E, the j^{th} Community C, and the k^{th} Species S, plus their interactions, plus error.

$$Y_{ijkl} = constant + E_i + C_j + S_k + (EC)_{ij} + (ES)_{ik} + (CS)_{jk} + (ECS)_{ijk} + e_{ijkl}$$

The contribution of any source of variation can then be estimated by a three-way factorial Anova. This will enable the contribution of physiological, ecological and evolutionary processes leading to the overall response to be evaluated (Table 6-1). There are two complications, however. First, the number of individuals may differ among species, giving rise to an unbalanced data structure. Secondly, the relative abundance of the species may differ between communities, giving rise to an unbalanced and disproportional data structure. If these were merely nuisances, the analysis could be rescued by some statistical procedure such as resampling. In fact, both are essential features of the data, representing the ecological structure of the community and how it is altered by exposure to a novel environment.

Such a preliminary three-way Anova would give a rough idea of the structure of the data, but is inadequate given the difficulties we have pointed out. For a more detailed analysis, the three-way classification is broken up into three two-way analyses: 1. The Community-Environment analysis is straightforward because the data structure is balanced. 2. The Species-Environment structure is unbalanced but proportional, because the species have the same abundances in the two assay environments. 3. The Species-Community analysis is more difficult when the species composition of the ancestral and derived communities differ, because the data are then both unbalanced and disproportionate. This inflates the differences between the Community means because of the difference in frequency of the species, and leads to an underestimate of the Species x Community interaction, which may even yield a negative Sum of Squares (SS). One way out of this difficulty is to use an appropriate uniform weighting for each species, which yields an unbiased estimate of the Species x Community term (see Snedecor & Cochran 1967 section 16.6 p 484; the analysis of unbalanced data is reviewed by Hector et al. 2010). The effect of this procedure, however, is to remove the effect of the change in species composition, whereas we wish to retain it. This can be done by using this adjusted Species x Community SS, from which the effect of any shift in species composition has been removed, while partitioning the Community SS into additive components that represent ecological and evolutionary processes. The mean phenotypes for the two communities are:

$$Ancestral: \overline{Y}_{anc} = \frac{\sum (n_{i,anc}\ Y_{i,anc})}{\sum n_i}$$

$$Derived: \overline{Y}_{der} = \frac{\sum (n_{i,der}\ Y_{i,der})}{\sum n_i}$$

where the abundance of the i^{th} species is n_i and its mean phenotype in the j^{th} community is Y_{ij}. Hence the difference in mean phenotype is:

$$N(Y_{der} - Y_{anc}) = \sum \left(n_{i,der}\ Y_{i,der} - n_{i,anc}\ Y_{i,anc}\right) = \sum Y_{i,der}\ \Delta n_i + \sum n_{i,anc}\ \Delta Y_i$$

where $N = \Sigma\ n_i$, $\Delta n_i = (n_{i,der} - n_{i,anc})$, and $\Delta Y_i = (Y_{i,der} - Y_{i,anc})$. The first term on the right-hand side is the ecological effect, generated by a shift in species composition, and the second term is the evolutionary effect, generated by a change in species mean phenotype independently of assay environment. The parallel to the Price decomposition of phenotypic change is clear (Price 1970, Collins and Gardner 2009). The first term is a covariance: the change in species abundance Δn_i is caused by differences in growth rate, with $\frac{1}{2}$ EN $(\Sigma\ Y_{i,der}\ \Delta n_i)/(S-1) = Cov(Y_{i,der}, \Delta n_i)$. The second term is the weighted change in mean species phenotype, caused in this case by natural selection (or some other evolutionary process); any physiological change (plasticity) is captured by the Environment main effect. The overall unadjusted Community SS is equal to $\frac{1}{2}$ EN $(Y_{der} - Y_{anc})^2$, so this can be partitioned into three components:

$$Community\ Ecology: Eco = \frac{1}{2} EN \left(\sum Y_{i,der}\ \Delta n_i\right)^2$$

$$Community\ Evolution: Evo = \frac{1}{2} EN \left(\sum n_{i,anc}\ \Delta Y_i\right)^2$$

$$Community\ Interaction: Eco \times Evo = EN \left(\sum Y_{i,der}\ \Delta n_i\right)\left(\sum n_{i,anc}\ \Delta Y_i\right)$$

The Community Ecology term expresses the contribution of shifts in the relative abundance of species (sorting) to the Community SS. The Community Evolution term expresses the contribution of any consistent shift in mean species phenotype. The third term, the Eco x Evo interaction, is a sum of products that is positive if abundance and phenotype score change in the

same direction, and negative otherwise. It represents a covariance that might be substantial if, for example, those species that have adapted more successfully (through an increase or decrease of phenotype score) have thereby increased in frequency in the community. These three terms do not lead straightforwardly to estimates of variance components, but a rough measure of the relative contribution of ecological and evolutionary effects can be calculated by neglecting the covariance-like interaction term, expressing the other two as fractions of their total, and multiplying this fraction by the Community variance component.

The Species x Community interaction expresses how the overall phenotypic difference between communities varies among species, independently of Environment. The highest-order interaction of Species x Community x Environment expresses variation among species in the extent of specific adaptation to environment, and is estimated by difference.

In practice, any real experiment may differ from this ideal model. The sample of species taken from each Community may not be proportional to its relative abundance, for example because it is desired to measure equal numbers of individuals from each species, or because some species have become so rare that only very few individuals are available for measurement. We have mitigated these shortcomings by randomly resampling (with replacement) a fixed number of individuals from each species in proportion to its known relative abundance and analysing this random sample. The values of parameters (such as SS and variance components) are then estimated as averages over a large number of independent resamples.

Our community selection experiment used eight distinct modified environments and as such, we analysed each ancestral-modified pair separately. Since the ancestral communities assayed in the original environment are identical for each pair-wise transplant, the same assays were used for all pairings. Given that these analyses are not independent, we obtained estimates of the overall contributions of plasticity, sorting and adaptation to variance by taking the averages of all pair-wise reciprocal transplants. The same Eco-Evo Anova was used to analyse the *in situ* time series data whose results we then compared with those of the reciprocal transplant as a separate source of evidence.

Table 6-1. Interpretation of three-way Anova of a reciprocal transplant experiment.

Source	Factors	df	Interpretation
Environment E_i	Fixed: 2 states, Original and Modified	1	Physiological plasticity: variation in average individual phenotype between environments (overall reaction norm).
Community C_j	Fixed: 2 states, Ancestral and Derived	1	Eco-evolutionary dynamics: variation in average phenotypes of communities caused by evolution (natural selection within species causing change in species mean phenotype) or species sorting (selection among species causing shift in community composition) or both.
Species S_k	Random: S species	S – 1	Ecological statics: variation among average phenotypes of species attributable to ancestry.
Env x Com $(EC)_{ij}$	First-order interaction	1	The plastic response has become altered in the Derived community, perhaps by selection. This represents specific adaptation if the character measured is fitness and is greater in the Ancestral/Original and Derived/Modified than in the converse combinations. Species sorting is not responsible because species composition is balanced between one set of community-environment combinations (Ancestral/Original plus Derived/Modified) and the other (Ancestral/Modified plus Derived/Original).
Env x Spe $(ES)_{ik}$	First-order interaction	S – 1	Variation in degree and direction of plasticity among species (variation among species' reaction norms).
Com x Spe $(CS)_{jk}$	First-order interaction	S – 1	Variation of species mean phenotype between communities, caused by natural selection (not species sorting) varying among species.
Env x Com x Spe $(ECS)_{ijk}$	Second-order interaction	S – 1	Variation in the extent of specific adaptation among species; equivalently, the modification of the plastic response varies among species.
Residual e_{ijkl}	' Error '	4(N-S)	Idiosyncratic variation among N individuals per sample
Total		4N - 1	

Results

The plants reproduced vigorously during the growing season with initial doubling times of about 8 days for Lm, 15 days for Rn and 10 days for Sp. A large proportion of Wc initially sank to the bottom in all mesocosms due to transfer shock but recovered in following few weeks. After five weeks' growth all communities had expanded to cover the entire surface of each mesocosm, and further expansion involved overgrowth and the death of senescent individuals. There were strong and consistent changes in community mean frond area, which, as an average over all environmental treatments, fell by about 20%, and by the end of Phase 1, differed significantly between communities (Fig. 6-4) (ANOVA, $F_{(1,16)} = 89.6$, $p < 0.0001$).

These differences in community mean frond area were due to both shifts in species relative abundances and phenotypic change within species. By the end of Phase 1, there were large differences between environments in species competitive abilities (Fig. 6-5). Generally, Lm was the most competitive in all environments and dominated most communities. After Lm, Rn was most competitive in high light and low nutrient conditions, and Wc in high nutrient conditions, although there were strong interactive effects between light and nutrients making generalisations difficult (Fig. 6-5). Mean phenotypes shifted consistently for all species over Phase 1, with frond area increasing with increasing nutrient availability and decreasing light (Fig. 6-6).

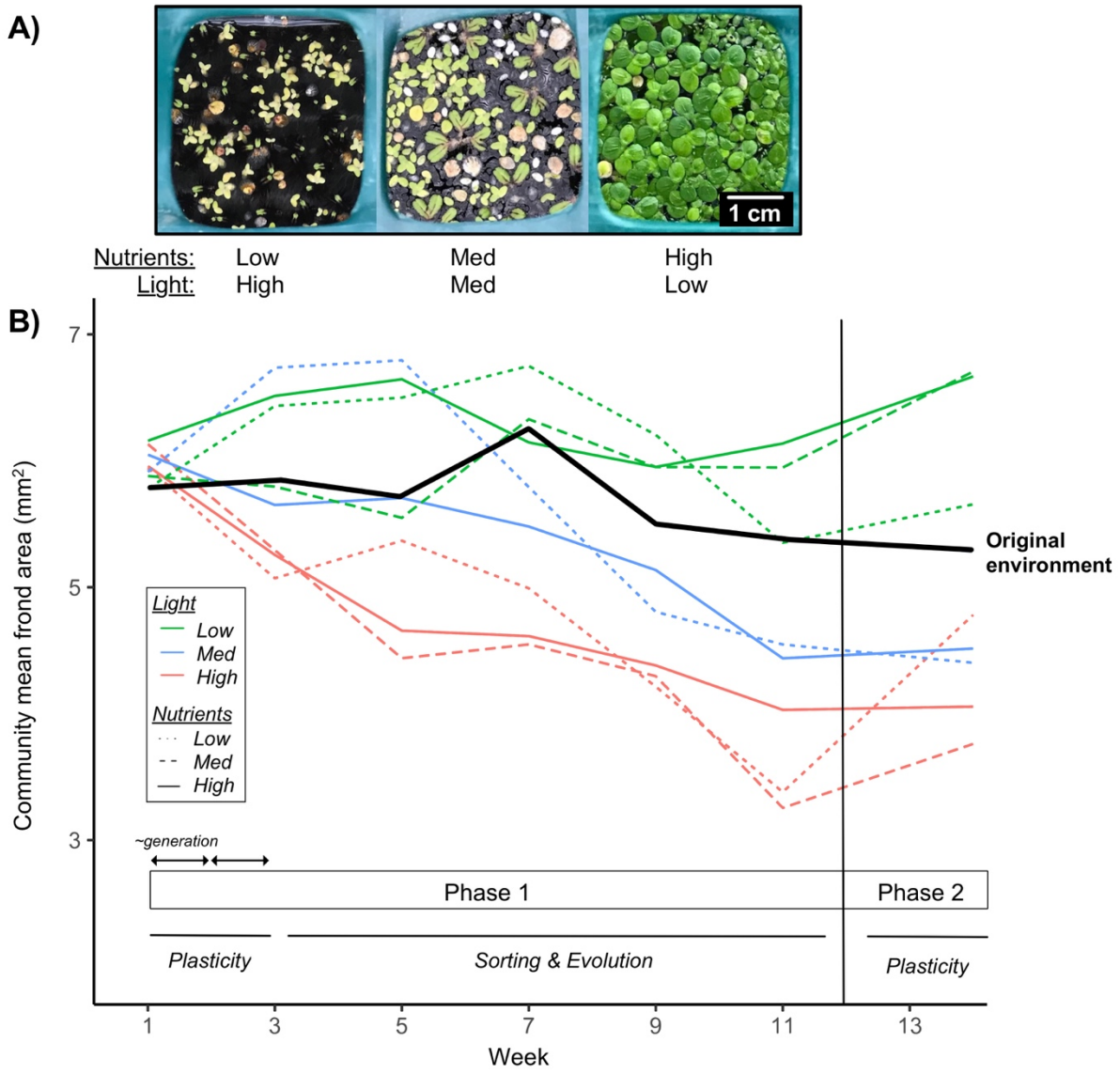


Fig. 6-4. Community mean phenotype dynamics. A) Photos of three communities at the end of Phase 1. B) Changes in community mean frond area over 12 weeks of growth in modified environments (Phase 1), followed by an additional two weeks after a reversion to the original environmental conditions (Phase 2). Each line is one of 9 unique environments: 8 modified environments and 1 original environment (medium light-medium nutrients), and the average of two replicate mesocosms. For each mesocosm, community mean frond area is calculated as a species' mean frond area weighted by its relative abundance in the community, summed across all species in the community. Variation in community mean frond area at week 1 is due to idiosyncratic senescence resulting from transfer stress during the 1-week settling time between the initial transfer of plants to the mesocosms (week 0), and when treatments were first applied (week 1).

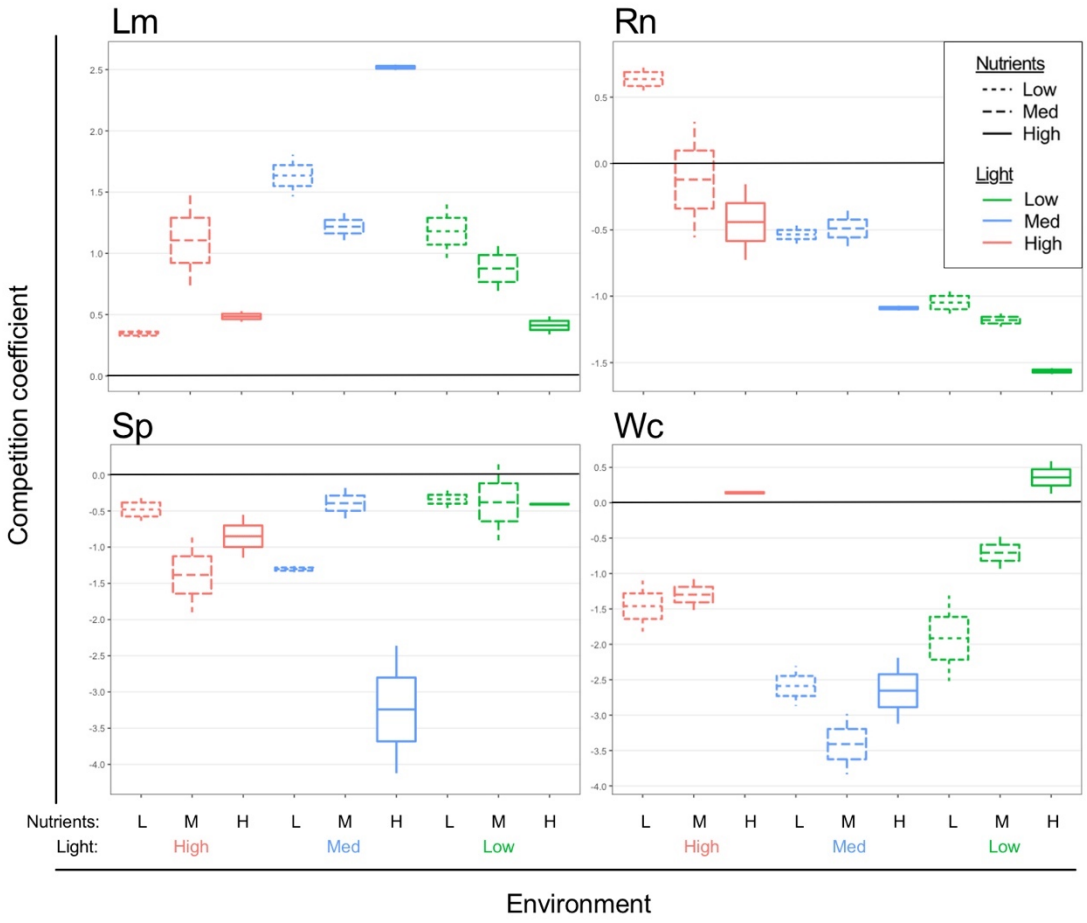


Fig. 6-5. Competition coefficients after 12 weeks of growth in the original and the eight modified environmental conditions. The horizontal line at 0 indicates no change in relative abundance over Phase 1. (Lm = *Lemna minor*, Rn = *Ricciocarpus natans*, Sp = *Spirodela polyrhiza*, Wc = *Wolffia columbiana*.)

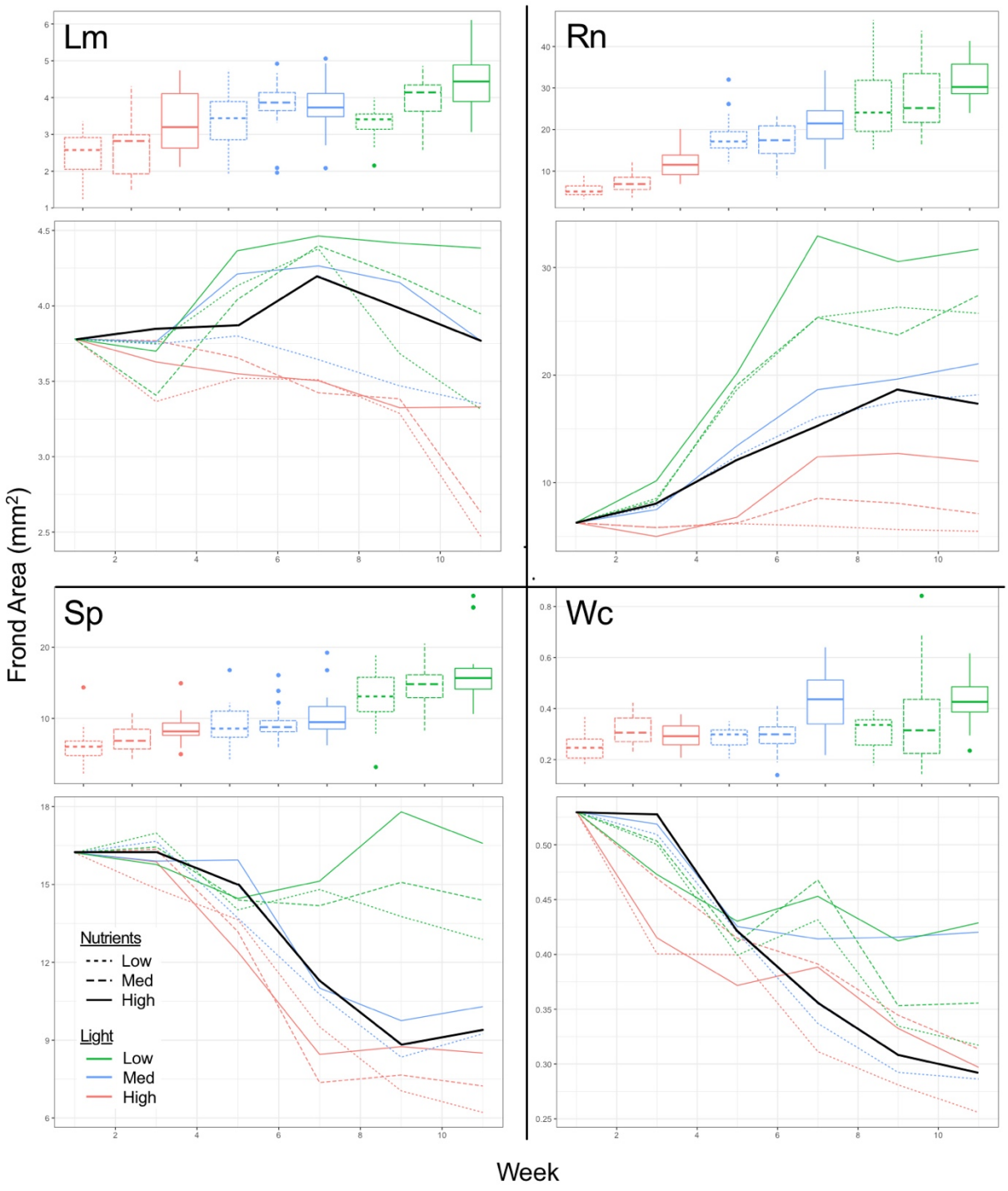


Fig. 6-6. Changes in frond area of the four species over 12 weeks of growth in the original and the eight modified environments (Phase 1) due to the combined effects of phenotypic plasticity and evolution. Lines are the average of two replicate mesocosms each from which 10 individuals of each species were sampled. The original environment is denoted with a bold black line. Box

plots show final differences in frond area among the nine environments for each species at the end of Phase 1 (week 11 measurements only). (Lm = *Lemna minor*, Rn = *Ricciocarpus natans*, Sp = *Spirodela polyrhiza*, Wc = *Wolffia columbiana*.)

For both the reciprocal transplant and the *in situ* time series, the overall phenotypic variance among plants is generated by three factors: Species (the four species composing each community), Environment (Original vs Modified) and Community (Ancestral vs Derived). The interpretation of these factors and their interactions is shown in Table 6-1. The Species effect is the extent to which the evolved differences among species are maintained when the conditions of life change. The main effect of Environment reflects the plastic modification of the phenotype of an individual by the conditions it experiences during its lifetime. The Community term expresses both ecological and evolutionary change and is partitioned into these two components and their interaction.

We used the Eco-Evo Anova to estimate the contributions of each source of variation to overall phenotypic variance for each community. This produces a separate set of estimates for each of the two replicate mesocosms in each of the eight modified environments (Table S6-1, Appendix 6). Although the reciprocal transplant and *in situ* time series data are arguably independent, the set of 16 estimates within each are not since the ancestral community assayed in the original environment was identical for each ancestral-derived pairing. For this reason, for both the data sets, we calculated the average contributions of each source of variation to overall phenotypic change across all eight modified environments (Fig. 6-7).

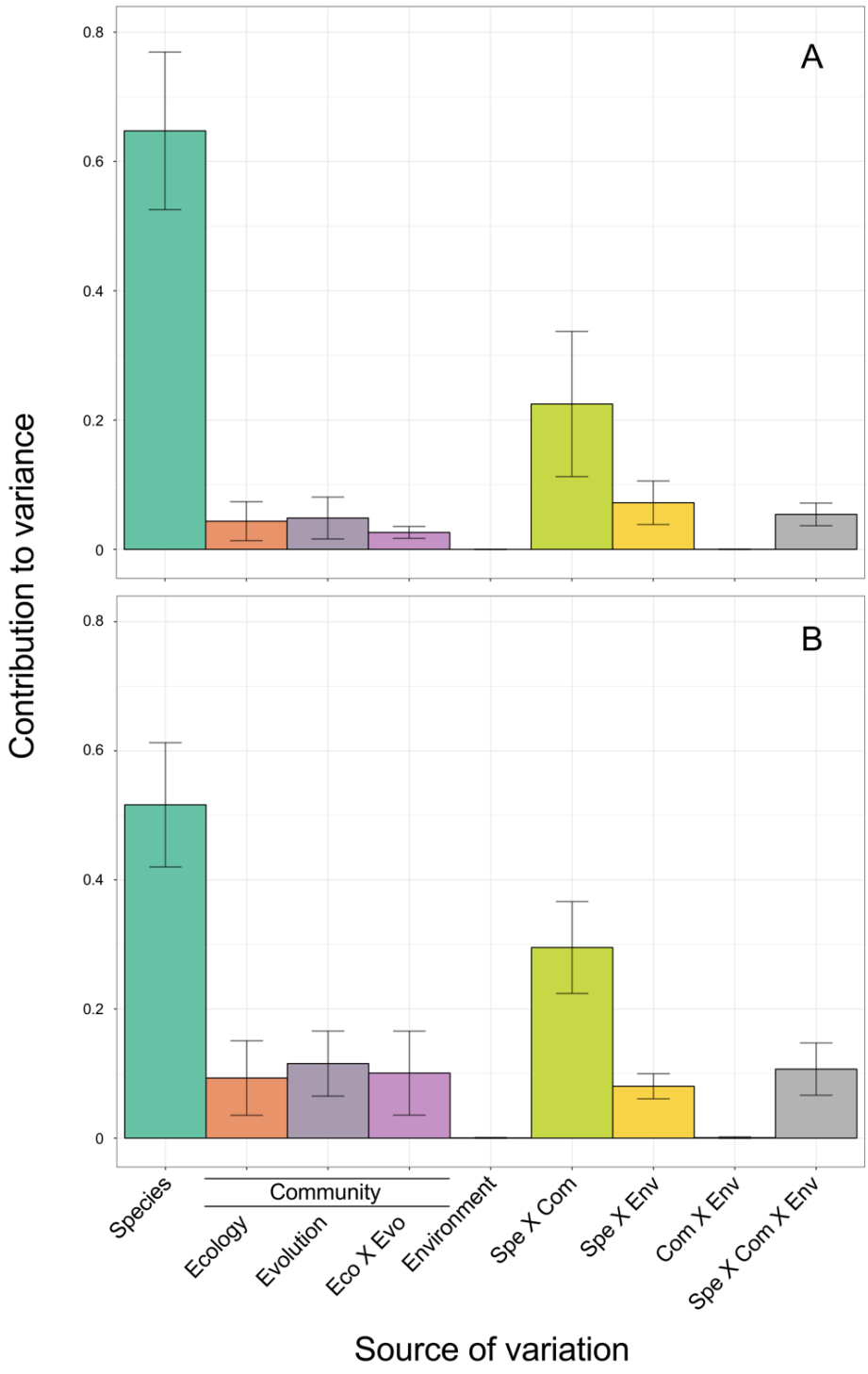


Fig. 6-7. Contributions of all sources other than residual variance to overall variation in community mean frond area for the **(A)** reciprocal transplant trial, and **(B)** in situ time series. Contributions are the result of averaging estimates for two replicate mesocosms for each of eight modified environments. The community term is partitioned into variation due to ecology,

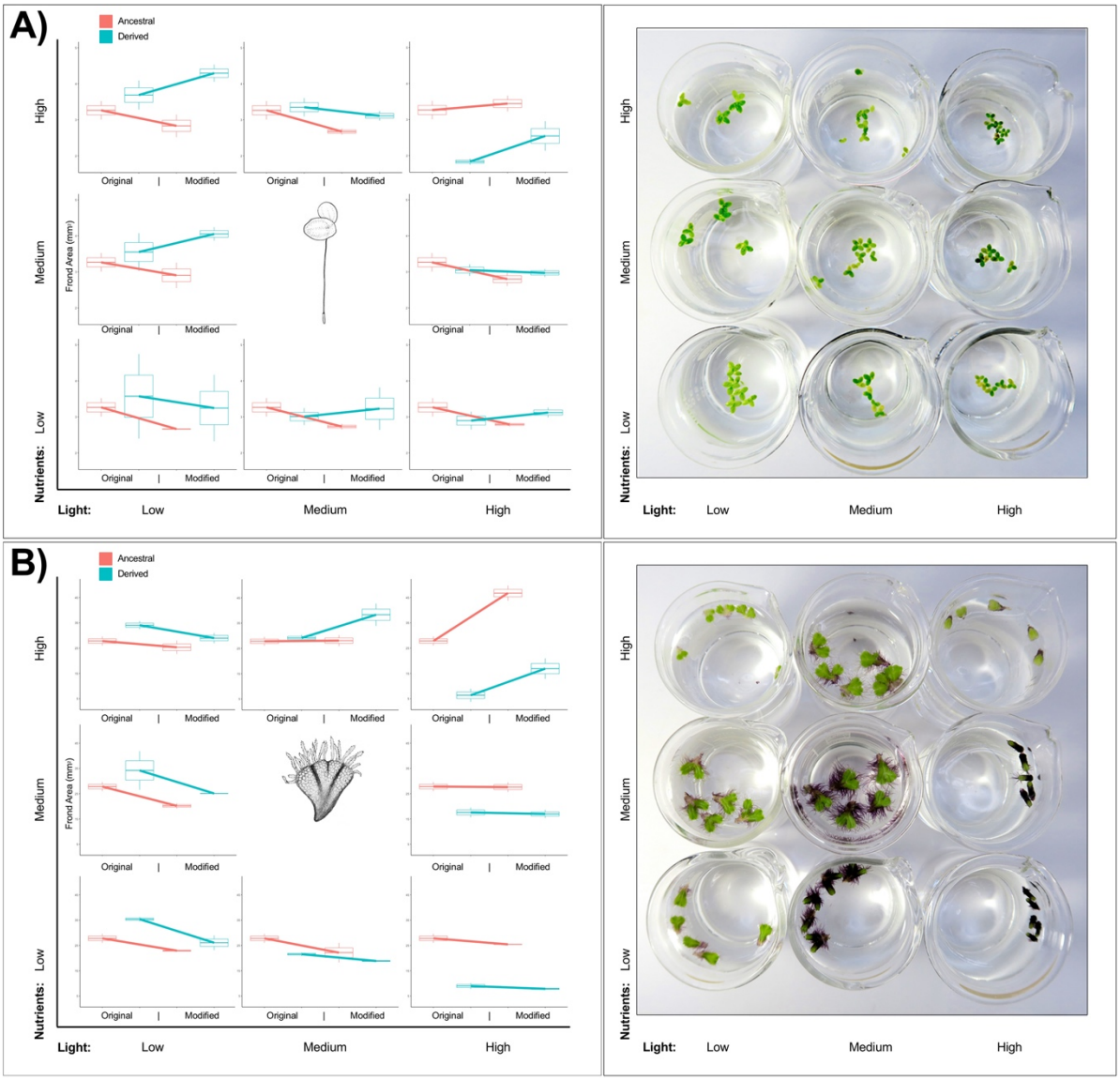
evolution, and their interaction. Error bars are 95% confidence intervals and show the variation in contributions among the eight modified environments.

To further compare these two sources of evidence, the reciprocal transplant and the *in situ* time series, we calculated standardised deviations in species mean frond area as a way to compare phenotypic variation among environments for each species in the outdoor mesocosms with those of the greenhouse tubs in the reciprocal transplant. These standardized deviations comparing phenotypes in Phase 2 and the reciprocal transplant (derived communities in original environment) are highly correlated ($r^2 = 0.80$) with a regression coefficient ($b = 0.91$) which overlaps unity (95% C.I. 0.75, 1.08). We conclude that the phenotypes expressed during Phase 2 in the outdoor mesocosms were consistent with those in greenhouse conditions of growth.

Discussion

In our community selection experiment, we found that community mean frond area responded strongly to changes in both light and nutrients (Fig. 6-4), driven by both inter- and intra-specific trait change. The primary source of variation in frond area is attributed to Species. The pronounced initial morphological differences between the four species largely persist when light and nutrients are manipulated, so that the Species term accounts for about half of the overall variance among individuals (excluding residual variance) (Fig. 6-7). Interactions between Species and both Environment and Community are also prominent. For example, there is a general tendency for fronds to become larger at low light levels and high nutrient levels, due to both plasticity and adaptation. This parallels the normal plastic response to light and nutrients of herbaceous terrestrial plants grown from seed (Meziane and Shipley 1999, 2001) and confirms that our observations are consistent with these well-established ecophysiological generalizations. However, different species do not invariably respond to the same extent. For example, Lm and Wc show this expected plastic response to nutrients at low and high light levels, but not at intermediate light, whereas Rn shows this plastic response at medium and high light levels, but not under low light (Fig. 6-8 & Fig. S6-1, Appendix 6). Likewise, Rn and Sp had

strong, but opposite plastic responses — whereas frond area increased in low light and low nutrient environments for Sp, it decreased for Rn (Fig. 6-8 & Fig. S6-1, Appendix 6). These Species x Environment interactions were so strong that the overall contribution of plasticity was negligible. Similar interactions have been reported for terrestrial plants (Meziane and Shipley 1999). Likewise, there was considerable variation among species in the extent to which frond size shifted due to adaptation. Whereas all species evolved larger fronds in low light and smaller fronds in high light, the evolutionary response to nutrients was extremely variable resulting in a strong Species x Community interaction (Fig. 6-8 & Fig. S6-1, Appendix 6).



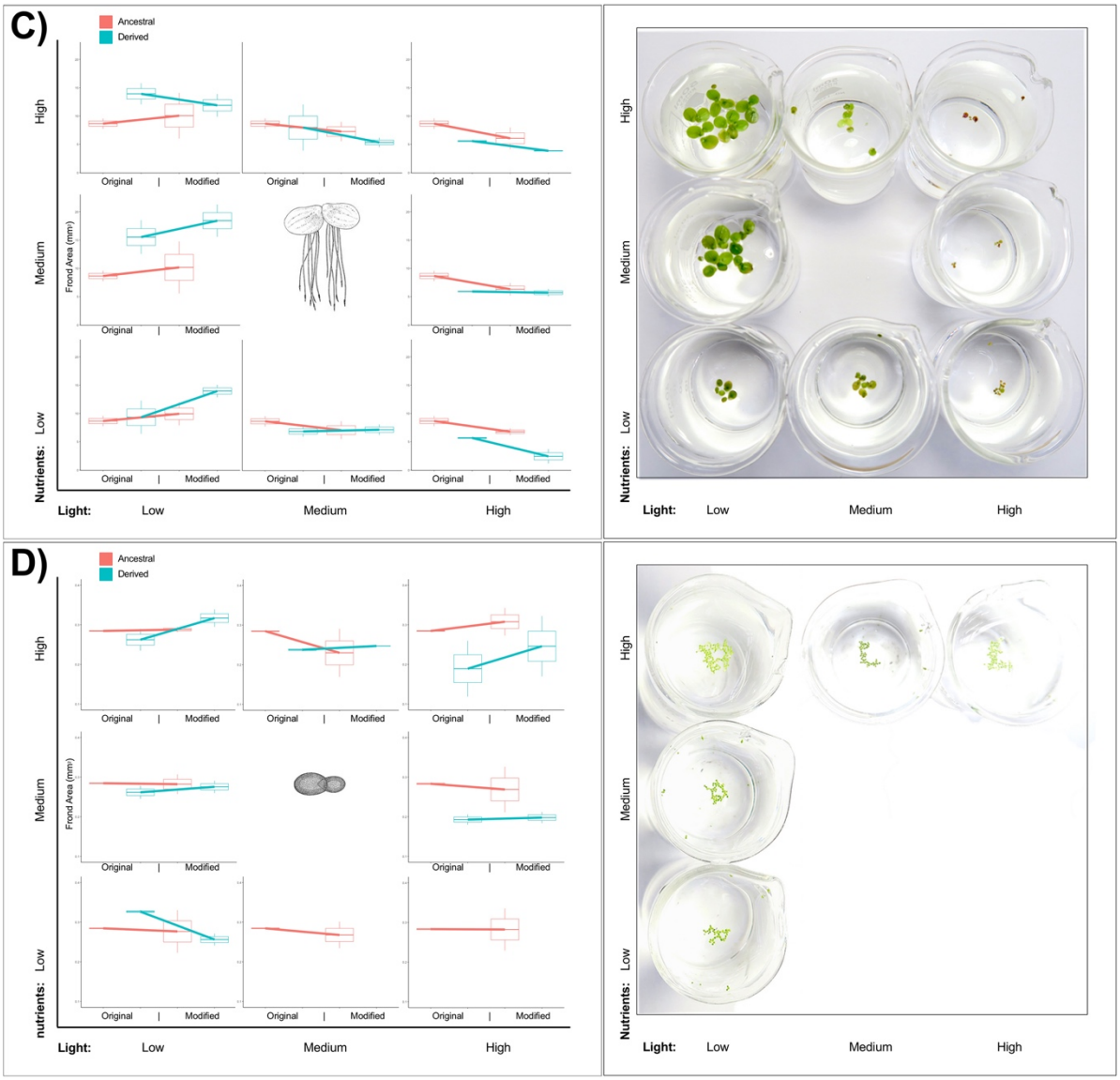


Fig. 6-8. Phenotypic consequences of 12 weeks of growth in modified environments (Phase 1), assessed with a reciprocal transplant experiment for A) *Lemna minor*, B) *Ricciocarpous natans,* C) *Spirodela polyrhiza*, and D) *Wolffia columbiana*. Community type can be either Ancestral or Derived, assay environment can be either Original or Modified. The Original environment is characterised by the medium light – medium nutrients combination. Each small panel is a reciprocal transplant for a single Ancestral-Derived pair, one for each of the eight unique Derived communities. Each of these is the result of 16 culture tub assays, (two replicate assay tubs × two replicate mesocosms per community × four community-environment combinations).

Box plot means are the result of 10 individual plants per culture tub, × two replicate culture tubs, × two replicate mesocosms = 40 measurements. Box plots whiskers represent the variation among the two independent replicate mesocosms. Since the reciprocal transplant was done with the entire intact community, and not with each species separately, the four large panels are not independent. Difference in frond area between assay environments indicates a plastic response whereas differences between community type indicates evolution. Difference in slope between community types indicates evolved differences in the plastic response. The absence of data indicates local extinction of that species in the community. Photographs were taken of individuals from the derived community in the modified environment at the end of the reciprocal transplant.

It was more surprising to find that the Community term, expressing both sorting (ecology) and adaptation (evolution), accounted for about one-quarter of the variance, with roughly equal contributions from each (Fig. 6-7). This result is in line with other studies that have found the rate and effect size of evolution to be of comparable magnitude to that of ecological processes in determining community structure and dynamics (Hairston et al. 2005, Palkovacs et al. 2009, Bassar et al. 2010, Pantel et al. 2015) and further emphasizes the importance of including the possibility of rapid evolution when considering how communities respond to environmental change (Fugère et al. 2020). The interaction between Ecology and Evolution terms was both strong and unexpected. If selection within species (Evolution, representing adaptation) and selection between species (Ecology, representing sorting) act in the same direction, then fronds will evolve to become larger (or smaller) in all species, while larger (or smaller) species become more abundant. We found instead that sorting and adaptation tended to act in opposite directions (Fig. 6-9); in communities where species evolved smaller fronds, the larger species had a competitive advantage and increased in relative abundance, and vice versa. This response was largely dependent on community productivity — in increasingly stressful environmental conditions (high light and/or low nutrients) that resulted in lower overall community productivity, fronds of all species evolved to become smaller, whereas the larger species (Rn and Sp) outcompeted the smaller species (Lm and Wc). Likewise, in beneficial environmental conditions (low light and/or high nutrients) that resulted in higher overall community

productivity, species tended to evolve larger fronds, but the smaller species (namely Wc) had a competitive advantage. Possible explanations as to why selection may not act in the same direction within and among species include the presence of inter-specific allelopathic interactions, which have been identified for several species of duckweed (Wolek 1974, Jang et al. 2007, Bich and Kato-Noguchi 2012), or other species interactions resulting in negative-frequency dependence (Armitage and Jones 2019). Alternatively, within species selection may have altered frond area due to an environmentally induced covariance between phenotype and fitness (Rausher 1992), although the reciprocal transplant should theoretically disentangle this covariance by separating the genetic from plastic sources of frond size. Finally, it is possible that less stressful environments (low light, high nutrients) resulted in selection favouring an increase in frond size indirectly by acting on a genetically linked trait, and at the same time enabling the relative proliferation of the smaller species with higher potential growth rates.

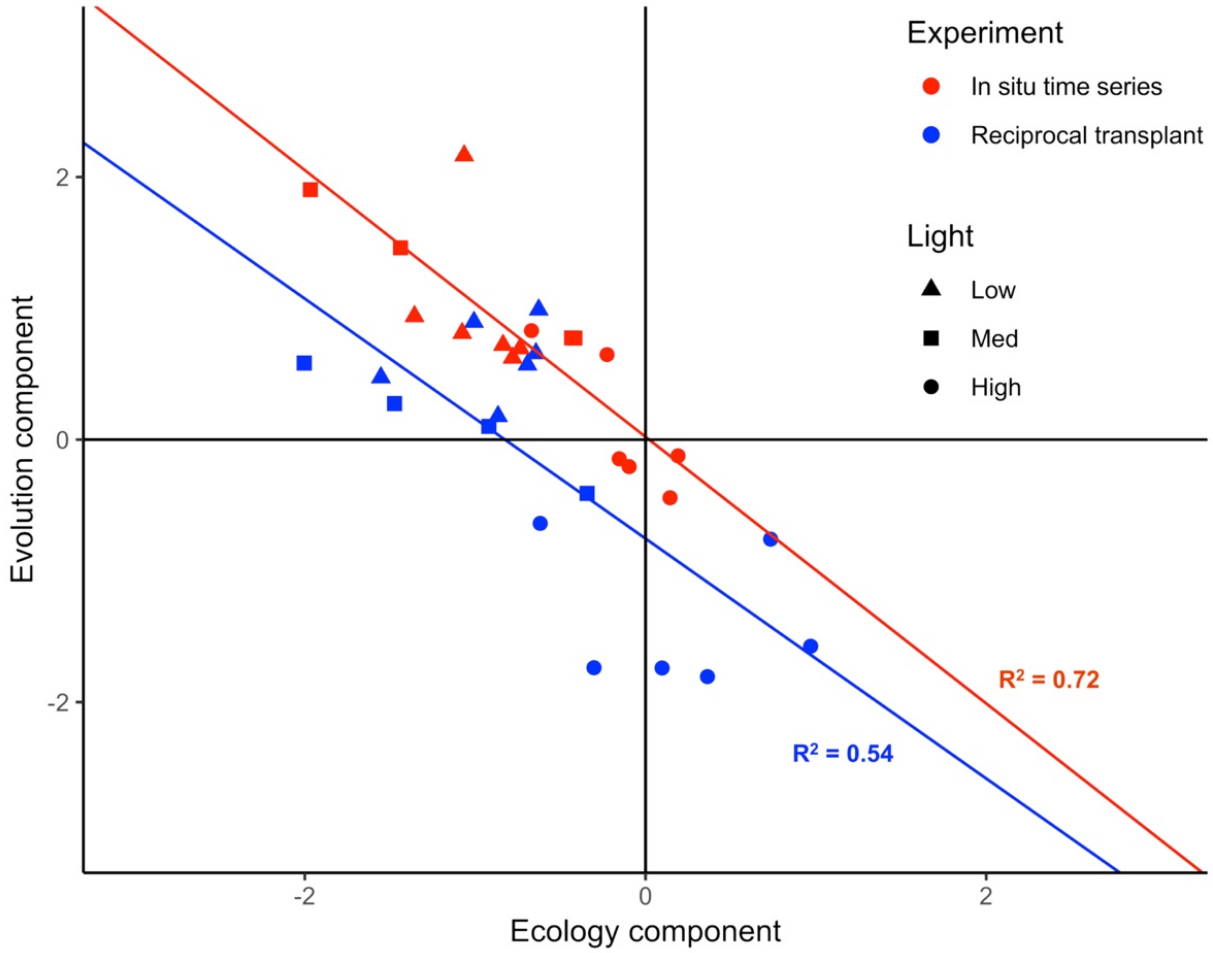


Fig. 6-9. Correlation between the contributions of species sorting (ecological component) and adaption (evolution component) to variation in phenotype for the reciprocal transplant and general reversion experiments. Each point is a single mesocosm.

Any real experiment will deviate from the ideal community selection experiment as outlined in the introduction. For example, it is unlikely that the source community is in a static state of ecological and evolutionary equilibrium, and therefore the community mean phenotype of the ancestral community in the original environment will undoubtedly change over the course of Phase 1 through seasonal species turnover, ongoing response to variables like day length and temperature, or imperfect replication of the source community's environmental conditions. This was the case for our experiment where mean frond area changed for three of the four species over the course of Phase 1 in the original environment (Fig. 6-6). In addition, we began Phase 1

with equal relative abundances of all species in each community and not with those of the source community, potentially throwing communities out of equilibrium, which further explains shifts in the community mean frond area over Phase 1 due to sorting. The reciprocal transplant and subsequent partition of variance into its components is based on a comparison between all communities at the end of Phase 1 and therefore does not incorporate any potential change in the ancestral community, but instead attributes variation in phenotype among communities accumulated over the course of Phase 1 to plasticity, sorting and adaptation. Reassuringly, the strikingly similar results between our reciprocal transplant (that discounts change in the ancestral community over Phase 1) and the *in situ* time series, indicates that the change in frond area in the ancestral communities was insignificant compared to the differences between ancestral and derived communities. Both tests produced extremely similar results, both in terms of the relative contributions to variance (Fig. 6-7), and the negative eco-evo relationship (Fig. 6-9). These results are further strengthened by the tight correlation of standardized deviations in frond area comparing phase 2 with the reciprocal transplant, despite obvious environmental differences between the source community, our outdoor mesocosms and greenhouse culture tubs.

The agency responsible for evolutionary change in our experiment is uncertain. Epigenetic changes might be transmitted over several generations because reproduction was exclusively vegetative (Verhoeven and Preite 2014). This would mimic genetic change and over the long-term lead to selection for adaptive plasticity. However, the main effect of Environment is very small, and we have shown that evolved phenotypes were conserved during the transition from Phase 1 to Phase 2, and from Phase 1 to the reciprocal transplant experiment. Alternatively, this evolutionary change could be caused by strong natural selection acting on genetic variation. This would require a large amount of pre-existing genetic variation in the populations given the short time span of the experiment. It could be assumed that populations of such fast growing, asexual species would be made up of only a small number of clones, especially in colder climates where populations likely go through an annual genetic bottleneck in the winter. There is however considerable evidence that duckweed populations maintain a surprisingly high level of within site genetic diversity. In *L. minor*, the most studied of the four species, allozymic and microsatellite sequence analysis of field populations showed in all cases a high degree of within population genetic diversity (Vasseur et al. 1993, Cole and Voskuil 1996, El-Kholy et al. 2015).

The most extensive survey (Vasseur et al., 1993) found on average 20 genotypes per site based on 18 loci, 13 of which were polymorphic. Furthermore, Ziegler et al. (2015) concluded from a common garden growth experiment using 39 clones of duckweed from 13 species that the majority of variation in growth rate was attributed to variation among ecotypes/clones and not species. This mirrors our own ongoing work using common garden growth assays where we find greater variation in fitness among individuals of Lm within sites than among sites (unpublished). Given this likely high degree of genetic variation within species in our source community, we conclude that strong natural selection acting on standing genetic variation, and not epigenetic change, is likely to have been the process responsible for phenotypic modification (van Moorsel et al. 2019). In similar work using two of the same species (Lm and Sp), Hart et al. (2019), also found that genotypic evolution over 10-15 generations resulted in phenotypic changes which altered competitive hierarchies and therefore community dynamics. Given the enormous population sizes and short generation times of such floating aquatic plants, it is perhaps not surprising that evolutionary processes should play an important role in structuring their communities.

Our experiment has shown how the average phenotype of a community may become modified over the course of several generations by sorting, plasticity, and adaptation. The overall community response, however, could not be reliably predicted from the response of any given species due to a negative correlation between the ecological and evolutionary effects on phenotypic change. This highlights the need to consider the effect of rapid evolutionary change when predicting community trait dynamics in response to environmental change. These results are in line with other recent studies that have demonstrated the importance of rapid evolution in structuring communities in ways which can alter eco-physiological responses and mediate species interactions (Becks et al. 2012, Pantel et al. 2015, Stoks et al. 2016, Hart et al. 2019, Fugère et al. 2020). We conclude that community trait dynamics cannot be understood simply by extrapolating the adaptive response of any single species to the whole community.

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General conclusions

As local environmental conditions change, communities of organisms must produce modified phenotypes capable of maintaining positive fitness to persist. As explored throughout this thesis, this change can be driven by a combination of physiological, evolutionary, and ecological processes. Communities of floating aquatic plants, primarily those in the family *Lemnaceae*, provide a useful experimental system to investigate how these different processes combine to drive phenotypic change.

Whereas my primary interest in *Lemnaceae* are in their extraordinary utility as a model system to test fundamental questions about how organisms respond to their environment, these ubiquitous plants have an enormous ecological impact on freshwater systems globally. Often considered keystone species, *Lemnaceae* fundamentally change the abiotic environment of other species around them by shading their submerged competitors (Landolt 1986, de Tezanos Pinto and O'Farrell 2014). In eutrophic systems, the presence of *Lemnaceae* may prevent algal blooms and effectively reduce water nutrient content (Cheng and Stomp 2009, Xu and Shen 2011). With current patterns of global change predicted to continue into the foreseeable future, both climate warming and continued eutrophication will further push shallow freshwater systems towards a state dominated by these floating aquatic plants (de Tezanos Pinto and O'Farrell 2014), making an understanding of their ecology and evolution increasingly important.

For aquatic plants, light and nutrients represent the two fundamental resources necessary for growth, and changes in their availability will result in plant phenotypic change suited to increase the uptake of the limiting resource. Given the highly reduced morphology of such plants, I focus on just two simple traits: frond area and root length, equivalent to the root-shoot ratio in terrestrial plants, and ask how they change in a community as a function of light and nutrient availability. Over six chapters I isolate different combinations of physiological, ecological, and evolutionary mechanisms, with the overall goal to understand how these processes combine to drive community phenotypic change.

Phenotypic change can occur at two fundamental levels: within individuals of a certain type (species or genotypes) via plasticity, or via the differential rates of reproduction among types via selection. In *Lemna minor*, phenotypic plasticity was the dominant mechanism responsible for phenotypic variation in field (Chapter 2). Strong correlations between traits and resource availability measured in the field shows that phenotype in this species is modified to match resource levels. When grown in a common garden assay, most phenotypic variation disappeared, indicating an environmental and not genetic origin of this variation. This was confirmed by a controlled laboratory experiment (Chapter 1) where I show considerable plasticity in both frond area and root length.

Selection was strong and deterministic, both among species (species sorting) and among genotypes (natural selection), which are similar processes in asexual organisms. In Chapter 5 I measured the contributions of sorting and ecological drift to changes in species composition and found that most change was directional, driven by clear differences in competitive advantages among species. This was supported by the field survey (Chapter 4) where I show that species distributions and abundances were influenced by ecological specialization and environmental filtering. Within species, I detected strong directional selection in a mesocosm experiment (Chapter 6) that resulted in a systematic reduction in frond area for four species. In *Lemna minor*, I detected substantial genetic variation in the field by growing plants in a common garden assay (Chapter 2), and estimated rates of purifying selection. Despite both strong directional and purifying selection, I measured considerable genetic variation in the field, both in morphological traits and in fitness (Chapter 2). In addition to clear genetic differentiation among populations, surprisingly, most variation was within sites (Chapter 2). The absence of any seasonal genetic bottleneck (Chapter 3) means that the estimated rates of purifying selection must be balanced by mutation and gene flow to maintain the high observed levels of within site genetic variation. Likewise, despite strong competition, species tended to coexist (Chapter 5), and in the field, most sites supported more than one species (Chapter 4). Like genetic variation, species diversity must be maintained by a balance between competitive exclusion and dispersal. Although we found little evidence of any dispersal limitation in the field (Chapter 4), we also found little evidence of competitive exclusion, since species tended to display negative frequency-dependent selection (Chapter 5), an important mechanism of diversity maintenance. In addition, spatially or

temporally variable selection within sites could lead to dynamic changes in competitive advantages among species, which, when paired with modest rates of dispersal, could prevent competitive exclusion and maintain species diversity.

Both frond area and root length responded to gradients in resource availability at a variety of scales. The root has the clear eco-physiological function to absorb nutrients and its length directly affects the rate of uptake. This trait responded to nutrient availability in the expected direction in all cases, both in experimental manipulations and in the field. In *Lemna minor*, this response had a strong physiological component, as was demonstrated in controlled laboratory conditions (Chapter 1), and in natural populations in the field (Chapter 2). There is also substantial variation in average root length and number among different species of *Lemnaceae*, which is understood to be the result of ecological specialization. In a mesocosm experiment (Chapter 6), I show that the only species to have completely lost its roots gains a competitive advantage in relative fitness in high nutrient conditions. This is consistent with what I found in the field, where species distributions are influenced by water phosphorus levels, with smaller plants with fewer roots more likely to be present and dominate communities in higher nutrient environments (Chapter 4). In this way, physiological, ecological, and evolutionary processes all act in the same direction to produce suitable root lengths of individuals in a community in response to levels of nutrient availability.

The response of frond area was more complex and idiosyncratic. Although physiological, ecological, and evolutionary processes all contributed to changes in average community frond area, they did not always act in the same direction. For example, although I found a correlation between environmental variation in frond area and light availability in the field (Chapter 2), in a controlled laboratory experiment plastic changes in frond area occurred only in response to nutrient availability and not light (Chapter 1). Although I found consistent evidence that species with smaller fronds were better competitors in high nutrient environments both in a mesocosm experiment (Chapter 6) and in the field (Chapter 4) suggesting both ecological specialization (evolution) and environmental filtering (ecology), the physiological response was in the opposite direction, with high levels of nutrients resulting in an increase in frond area in a controlled laboratory experiment (Chapter 1). Finally, although I found that evolution and sorting drove

changes in frond area in a mesocosm experiment (Chapter 6) these acted in opposite directions, and in the field, most variation in frond area was not due to local adaptation but instead to plasticity (Chapter 2).

There are many possible reasons for these patterns. Firstly, frond area is a complex trait, and unlike root length, has additional functional roles than just resource uptake. Although variation in frond area does influence light capture and rates of photosynthesis, growth must also be balanced with reproduction, since a plant can increase total colony frond area by limiting individual frond size. Frond thickness may also vary in response to light and temperature, with additional layers of parenchyma better able to maintain high photosynthetic rates at higher light intensities. Likewise, under environmental stress (nutrient, light or temperature), many species produce smaller, denser fronds capable of withstanding harsh environmental conditions. In this sense, frond area, although functionally informative, does not tell the whole eco-physiological story. There are two main inconsistencies in our results. First, why do we see plasticity in frond area in response to light in the field (Chapter 2), but not in the lab (Chapter 1) or in mesocosms (Chapter 6)? Second, why do we observe evolution in frond size in response to light in the mesocosms (Chapter 6), but not local adaptation in the same trait in the field (Chapter 2)?

It is possible that the absence of plasticity in frond area in the lab (Chapter 1) is due to the vastly different levels of irradiance between artificial fluorescent lights in the lab (max 300 µmols m^{-2} s^{-1}) and that in the field (over 2000 µmols m^{-2} in full sun). Comparing frond area in *Lemna minor* in the Chapter 6 mesocosm study to that in the lab (Chapter 1) or field (Chapter 2), we find that average frond area across all treatments is roughly one third of the size. It is possible that this extreme reduction in frond size was due not to light (since irradiance in the outdoor mesocosms would be comparable to that in the field), but to temperature stress due to the relatively small size (1000L) and black colour of the plastic mesocosms (Hodgson 1970). Since light (percent shading) and temperature were confounded, we cannot be sure of the cause of reduction in frond size. Finally, development in epigenetics has demonstrated that transgenerational plasticity may be of greater importance in clonal plants than previously thought since traits acquired from changes in DNA methylation are more likely to be inherited in the absence of meiotic recombination (Verhoeven and Preite 2014, Latzel et al. 2016, Wilschut et al. 2016). It could be

that persistent phenotypic change in the reciprocal transplant assay (Chapter 6) was not due to changes in the DNA sequence resulting from natural selection, but instead to epigenetic transgenerational plasticity. Recent work has identified stress-induced differential methylation as a potentially important mechanism of plastic stress tolerance in plants (Groot et al. 2016, Huber et al. 2021), a full appreciation of which could further integrate our understanding of evolutionary and eco-physiological responses in driving heritable phenotypic change.

A central goal of ecology and evolution is to understand how organisms, populations and communities respond to changes in their abiotic environment. Using such a simple experimental system like floating aquatic plants can provide a unique opportunity to gain a deeper understanding of the different mechanisms responsible for driving phenotypic change. Such work is significant as it provides much-needed experimental verification of many fundamental concepts that are often difficult to demonstrate with larger organisms. The work presented here in this thesis is a small but important contribution to the larger goal of fully realising the integration of the fields of ecology and evolution into a unified conceptual framework.

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Appendices

Supplementary material for Chapter 1

Supplementary material for Chapter 2

Supplementary material for Chapter 3

Supplementary material for Chapter 4

Supplementary material for Chapter 5

Supplementary material for Chapter 6

Supplementary material for Chapter 1

Table S1-1. Overview of the eight sites from where the duckweed populations were sampled.

Sampling date	Site name	Latitude	Longitude	Type	Conductivity	pH
27.10.19	Boisbriand	45.605142	-73.829298	river	172.7	7.04
27.10.19	Capricorne	45.522952	-73.441432	swamp	238.9	6.94
27.10.19	St. Bruno	45.525435	-73.33423	pond	198.1	6.97
27.10.19	Parc cite	45.486928	-73.410892	swamp	189	7.17
27.10.19	Richelieu	45.709192	-73.187892	river	336.8	6.39
27.10.19	Ecomuseum	45.42687	-73.935591	pond	284.3	7.08
27.10.19	Quinn	45.357844	-73.924407	pond	335.5	7.01
27.10.19	Turtle Bay	45.468912	-73.92244	river	438.1	6.53

Table S1-2. Recipe for Hoagland's E Medium used in low, medium and high nutrient treatments. The pH was set to 5.8 before autoclaving the media.

	Low	Med	High
$MgSO_4$	1.230 mg/L	6.765 mg/L	12.300 mg/L
$Ca(NO_3)$ x 4 H_2O	2.714 mg/L	14.93 mg/L	27.140 mg/L
KH_2PO_4	0.435 mg/L	2.394 mg/L	4.3530 mg/L
KNO_4	1.263 mg/L	6.944 mg/L	12.625 mg/L
H_3BO_3	7.150 µg/L	39.33 µg/L	71.50 µg/L
$MnCl_2$ x $4H_2O$	4.550 µg/L	25.03 µg/L	45.50 µg/L
$ZnSO_4$ x 7 H_2O	0.550 µg/L	3.025 µg/L	5.500 µg/L
$NaMoO_4$ x 2 H_2O	0.225 µg/L	1.238 µg/L	2.250 µg/L
$CuSO_4$ x 5 H_2O	0.350 µg/L	1.925 µg/L	3.500 µg/L
$FeCl_3$ x 6 H_2O	0.048 mg/L	0.266 mg/L	0.484 mg/L
EDTA	0.150 mg/L	0.825 mg/L	1.500 mg/L

Table S1-3. Mixed model Anovas for four response variables: A. Fitness, B. Frond area, C. Root length, and D. Colony size. Microbiome and environment were evaluated as fixed factors, and genotype as a random factor. Expected Mean Squares and estimates of F were evaluated using Sokal & Rohlf Box 12.1 pg.383 'Mixed Model'. ns = not significant at $\alpha = 0.05$.
Sokal, R.R. & Rohlf, F.J. 1981. *Biometry* (second edition). W.H. Freeman & Company, New York.

A. Fitness (r, day^{-1})

Source	**Df**	**Sum Sq**	**Mean Sq**	***F***	***p***
Microbiome	1	0.0103	0.0103	32.3	< 0.001
Environment	3	0.3047	0.1016	362.6	< 0.001
Genotype	7	0.0045	0.0006	6.74	< 0.001
Mic x Env	3	0.0016	0.0005	5.63	0.001
Mic x Gt	7	0.0022	0.0003	3.39	0.002
Env x Gt	21	0.0058	0.0003	2.92	< 0.001
Mic x Env x Gt	21	0.0023	0.0001	1.15	ns
Residual	128	0.0121	0.0001		
Total	191	0.3465			

B. Frond area (mm^2)

Source	**Df**	**Sum Sq**	**Mean Sq**	***F***	***p***
Microbiome	1	71.07	71.07	50.05	< 0.001
Environment	3	54.69	18.23	21.20	< 0.001
Genotype	7	92.40	13.20	19.44	< 0.001
Mic x Env	3	11.65	3.88	5.72	0.001
Mic x Gt	7	9.94	1.42	2.09	0.049
Env x Gt	21	18.15	0.86	1.27	ns
Mic x Env x Gt	21	31.24	1.49	2.19	0.004
Residual	128	86.92	0.68		
Total	191	376.06			

C. Root length (mm)

Source	Df	Sum Sq	Mean Sq	*F*	*p*
Microbiome	1	5665	5665	52.45	< 0.001
Environment	3	50453	16818	80.09	< 0.001
Genotype	7	5113	730	12.06	< 0.001
Mic x Env	3	41	14	< 1	ns
Mic x Gt	7	755	108	1.78	ns
Env x Gt	21	4408	210	3.47	< 0.001
Mic x Env x Gt	21	1996	95	1.57	ns
Residual	128	7750	61		
Total	191	76181			

D. Colony size (no. of fronds)

Source	Df	Sum Sq	Mean Sq	*F*	*p*
Microbiome	1	9.7	9.70	11.02	0.013
Environment	3	404.6	134.87	162.49	< 0.001
Genotype	7	25.4	3.63	3.49	0.002
Mic x Env	3	12.7	4.24	4.07	0.008
Mic x Gt	7	6.2	0.88	< 1	ns
Env x Gt	21	17.5	0.83	< 1	ns
Mic x Env x Gt	21	44.7	2.13	2.05	0.008
Residual	128	133.3	1.04		
Total	191	654.1			

Supplementary material for Chapter 2

Table S2-1. Environmental variables measured at 34 sites in a regional field survey across southern Quebec. We measured pH, total and dissolved phosphorus (TP, TN, μgL-1), total and dissolved Nitrogen (TN, DN, mgL-1) and light availability (% PAR).

Site	Date	Latitude	Longitude	pH	DP	TP	DN	TN	Light
1	3.07	45.35321°N	73.50109°W	7.23	76.867	133.337	0.590	0.718	0.280
2	4.07	45.31410°N	74.12367°W	8.75	88.187	117.739	0.876	1.055	0.755
3	5.07	45.58946°N	73.54749°W	7.45	13.750	57.320	1.669	2.539	0.537
4	6.07	46.07681°N	73.30515°W	8.35	7.892	14.013	1.601	1.661	0.701
5	6.07	46.19259°N	73.21547°W	7.00	32.639	135.180	0.689	0.782	0.644
6	7.07	46.19258°N	73.21545°W	7.95	9.406	27.966	0.245	0.543	0.187
7	7.07	46.51430°N	72.46699°W	7.46	40.800	73.708	0.769	0.867	0.946
8	7.07	47.26608°N	72.46958°W	8.58	44.946	76.077	0.175	0.315	0.672
9	8.07	47.26608°N	72.46960°W	6.90	42.511	92.794	1.189	1.246	0.220
10	8.07	48.43040°N	72.24117°W	7.22	26.057	43.762	0.363	0.510	0.409
11	9.07	48.38147°N	71.43300°W	7.51	125.852	243.630	0.312	0.357	0.519
12	9.07	48.38146°N	71.43303°W	8.79	7.234	20.924	0.334	0.371	0.771
13	10.07	46.57517°N	70.58054°W	9.71	68.640	250.423	0.688	0.899	0.423
14	10.07	46.39409°N	71.34498°W	8.62	24.149	197.770	1.551	2.586	0.752
15	12.07	45.26175°N	73.19555°W	8.04	477.600	563.307	0.926	1.267	0.461
16	12.07	45.32396°N	73.08501°W	9.63	17.678	112.620	0.248	0.432	0.352
17	12.07	45.26176°N	73.19556°W	7.91	27.201	182.611	1.824	2.098	0.269
18	13.07	45.06233°N	72.58469°W	7.43	57.423	229.787	0.901	1.527	0.821
19	13.07	45.11318°N	72.46155°W	7.78	134.004	300.987	0.855	1.291	0.418
20	13.07	45.08803°N	72.38696°W	7.39	113.966	218.587	1.579	2.263	0.261
21	14.07	45.28217°N	72.10385°W	7.05	18.537	70.054	0.540	0.654	0.207
22	14.07	45.46537°N	72.00205°W	9.01	63.375	111.916	0.572	0.676	0.359
23	14.07	45.50369°N	72.01519°W	7.80	21.116	86.521	0.590	0.876	0.594
24	14.07	45.50546°N	72.02381°W	7.12	362.530	432.564	1.696	2.325	0.598
25	15.07	45.24932°N	71.53657°W	7.36	13.511	84.206	2.163	2.133	0.267
26	15.07	45.24799°N	71.53523°W	6.64	281.412	237.302	2.421	2.970	0.203
27	16.07	45.07227°N	71.58451°W	7.44	19.000	36.657	0.301	0.341	0.160
28	16.07	45.07507°N	71.59313°W	7.40	24.886	70.186	2.165	2.194	0.281
29	16.07	45.08341°N	71.53482°W	7.39	19.992	63.705	0.543	0.795	0.420
30	16.07	45.48730°N	71.10011°W	7.05	14.768	41.154	0.518	0.666	0.034
31	17.07	45.48744°N	71.10004°W	8.56	14.702	24.754	0.594	0.666	0.317
32	17.07	46.14832°N	72.34435°W	7.45	41.551	156.356	0.664	0.758	0.241
33	18.07	45.28924°N	73.44792°W	7.05	23.431	87.248	0.809	1.178	0.250
34	18.07	45.28931°N	73.44771°W	8.55	19.529	36.790	0.405	0.510	0.589

Table S2-2. Recipe for Hoagland's E Medium used in the common garden assay. The pH was set to 7.0 before autoclaving the media.

	Concentration
MgSO4	12.300 mg/L
Ca(NO3) x 4 H2O	27.140 mg/L
KH2PO4	4.3530 mg/L
KNO4	12.625 mg/L
H3BO3	71.50 µg/L
MnCl2 x 4H2O	45.50 µg/L
ZnSO4 x 7 H2O	5.500 µg/L
NaMoO4 x 2 H2O	2.250 µg/L
CuSO4 x 5 H2O	3.500 µg/L
FeCl3 x 6 H2O	0.484 mg/L
EDTA	1.500 mg/L

Supplementary material for Chapter 3

Fig. S3-1. Location of duckweed pond, Mont Saint Hilaire, Quebec, Canada.

Supplementary material for Chapter 4

Table S4-1. Environmental variables measured at 37 sites in a regional field survey across southern Quebec. We measured pH, total phosphorus (TP, µgL-1), total Nitrogen (TN, mgL-1) and light availability (% PAR).

Site	**Date**	**Waterbody type**	**Lat**	**Long**	**pH**	**TP**	**TN**	**Light**
1	3.07	Large pond	45.35321°N	73.50109°W	7.23	133.337	0.718	0.280
2	4.07	Wetland	45.28174°N	74.03322°W	7.35	310.970	2.501	0.697
3	4.07	Roadside ditch	45.31410°N	74.12367°W	8.75	117.739	1.055	0.755
4	5.07	Wetland	45.58946°N	73.54749°W	7.45	57.320	2.539	0.537
5	6.07	Bay of Lake	46.07681°N	73.30515°W	8.35	14.013	1.661	0.701
6	6.07	Wetland	46.19259°N	73.21547°W	7.00	135.180	0.782	0.644
7	7.07	Small pond	46.19258°N	73.21545°W	7.95	27.966	0.543	0.187
8	7.07	Wetland	46.51430°N	72.46699°W	7.46	73.708	0.867	0.946
9	7.07	Small pond	47.26608°N	72.46958°W	8.58	76.077	0.315	0.672
10	8.07	Wetland	47.26608°N	72.46960°W	6.90	92.794	1.246	0.220
11	8.07	Wetland	48.43040°N	72.24117°W	7.22	43.762	0.510	0.409
12	9.07	Bay of river	48.38147°N	71.43300°W	7.51	243.630	0.357	0.519
13	9.07	Large pond	48.38146°N	71.43303°W	8.79	20.924	0.371	0.771
14	10.07	Stream	46.52252°N	71.02543°W	8.04	77.394	0.829	0.398
15	10.07	Large pond	47.00466°N	70.51506°W	7.95	226.532	1.479	0.722
16	10.07	Large pond	46.57517°N	70.58054°W	9.71	250.423	0.899	0.423
17	10.07	Roadside ditch	46.39409°N	71.34498°W	8.62	197.770	2.586	0.752
18	12.07	Large pond	45.26175°N	73.19555°W	8.04	563.307	1.267	0.461
19	12.07	Bay of lake	45.32396°N	73.08501°W	9.63	112.620	0.432	0.352
20	12.07	Wetland	45.26176°N	73.19556°W	7.91	182.611	2.098	0.269
21	13.07	Large pond	45.06233°N	72.58469°W	7.43	229.787	1.527	0.821
22	13.07	Small pond	45.11318°N	72.46155°W	7.78	300.987	1.291	0.418
23	13.07	Wetland	45.08803°N	72.38696°W	7.39	218.587	2.263	0.261
24	14.07	Wetland	45.28217°N	72.10385°W	7.05	70.054	0.654	0.207
25	14.07	Wetland	45.46537°N	72.00205°W	9.01	111.916	0.676	0.359
26	14.07	Small pond	45.50369°N	72.01519°W	7.80	86.521	0.876	0.594
27	14.07	Roadside ditch	45.50546°N	72.02381°W	7.12	432.564	2.325	0.598
28	15.07	Wetland	45.24932°N	71.53657°W	7.36	84.206	2.133	0.267
29	15.07	Small pond	45.24799°N	71.53523°W	6.64	237.302	2.970	0.203
30	16.07	Small pond	45.07227°N	71.58451°W	7.44	36.657	0.341	0.160
31	16.07	Small pond	45.07507°N	71.59313°W	7.40	70.186	2.194	0.281
32	16.07	Small pond	45.08341°N	71.53482°W	7.39	63.705	0.795	0.420
33	16.07	Stream	45.48730°N	71.10011°W	7.05	41.154	0.666	0.034
34	17.07	Small pond	45.48744°N	71.10004°W	8.56	24.754	0.666	0.317
35	17.07	Small pond	46.14832°N	72.34435°W	7.45	156.356	0.758	0.241
36	18.07	Large pond	45.28924°N	73.44792°W	7.05	87.248	1.178	0.250
37	18.07	Small pond	45.28931°N	73.44771°W	8.55	36.790	0.510	0.589

Table S4-2. Welch's t-test for samples with unequal variances. Lt = *Lemna trisulca*, Sp = *Spirodela polyrhiza,* Wc = *Wolffia columbiana*, Rn = *Ricciocarpus natans*, Rf = *Riccia fluitans*, Cd = *Ceratophyllum demersum.*

Species	Environmental Variable	t	df	p
Lt	Light	0.5364	6.863	0.6086
Sp	Light	0.9364	34.99	0.3555
Wc	Light	0.1323	14.10	0.8966
Rn	Light	1.2786	4.660	0.261
Rf	Light	5.2509	34.74	**<0.0001**
Cd	Light	0.0960	2.256	0.9314
Lt	pH	0.1287	6.820	0.9013
Sp	pH	0.9652	33.92	0.3413
Wc	pH	1.0451	11.38	0.3177
Rn	pH	0.7732	3.995	0.4826
Rf	pH	3.1687	2.806	0.0554
Cd	pH	2.3088	2.601	0.1176
Lt	TN	0.0992	7.318	0.9237
Sp	TN	0.1906	32.81	0.8500
Wc	TN	2.3531	12.17	**0.0362**
Rn	TN	0.1771	3.837	0.8684
Rf	TN	0.9458	1.156	0.5000
Cd	TN	0.3267	2.252	0.7718
Lt	TP	0.2512	8.326	0.8078
Sp	TP	1.0276	29.27	0.3125
Wc	TP	3.4336	9.318	**0.0071**
Rn	TP	2.2649	12.60	**0.0419**
Rf	TP	2.8371	34.33	**0.0076**
Cd	TP	0.7762	2.901	0.4959

Supplementary material for Chapter 5

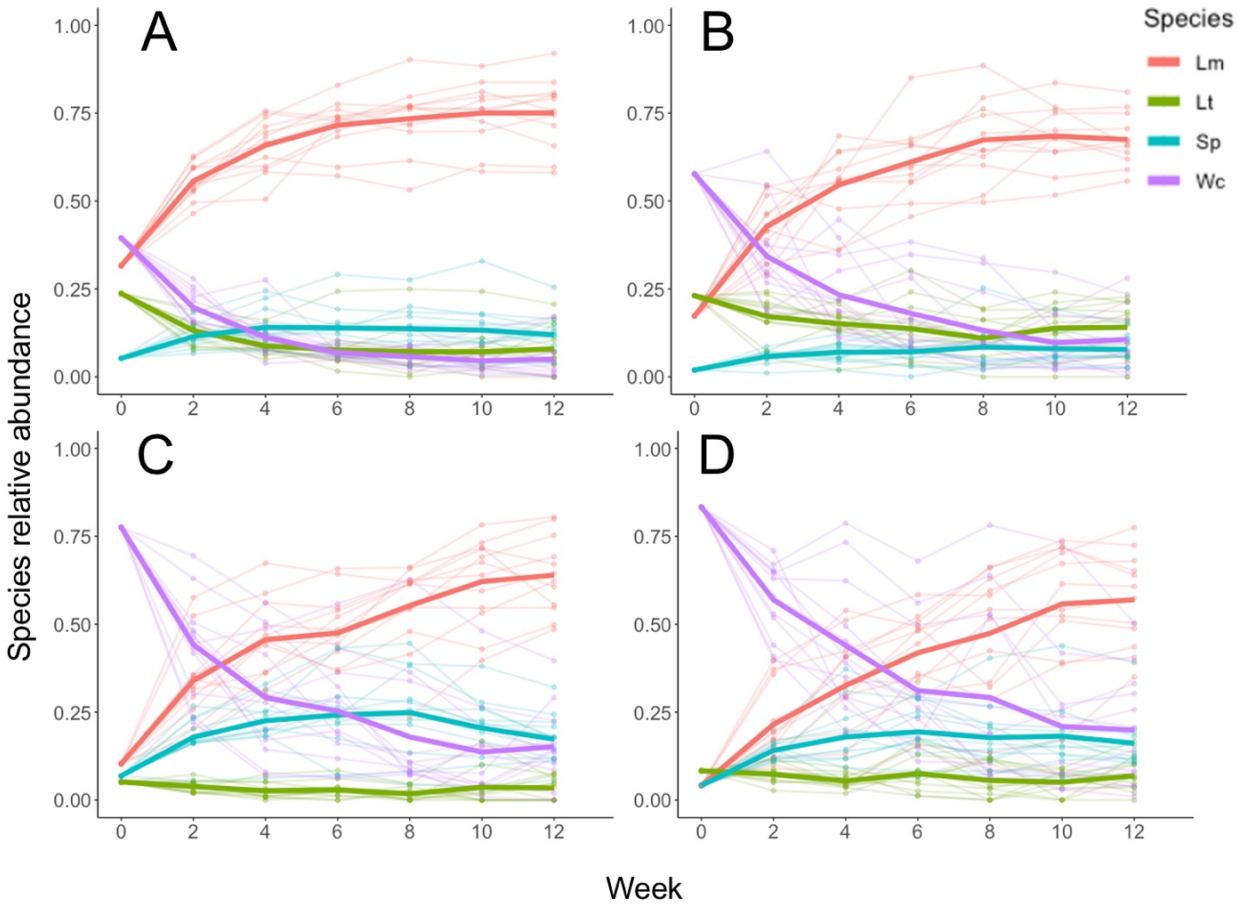

Fig. S5-1. Community dynamics over 12 weeks of growth. Here, species relative abundance is measured as proportion of total individuals in the community, and is not weighted by species average mass. All four community types (A, B, C & D) consist of the same four species, which differ in their initial relative abundances depending on the community type. The four species were *Lemna minor* (Lm), *Lemna trisulca* (Lt), *Spirodela polyrhiza* (Sp), and *Wolffia columbiana* (Wc). Each community type was replicated in 12 mesocosms, the means of which are shown as bold lines.

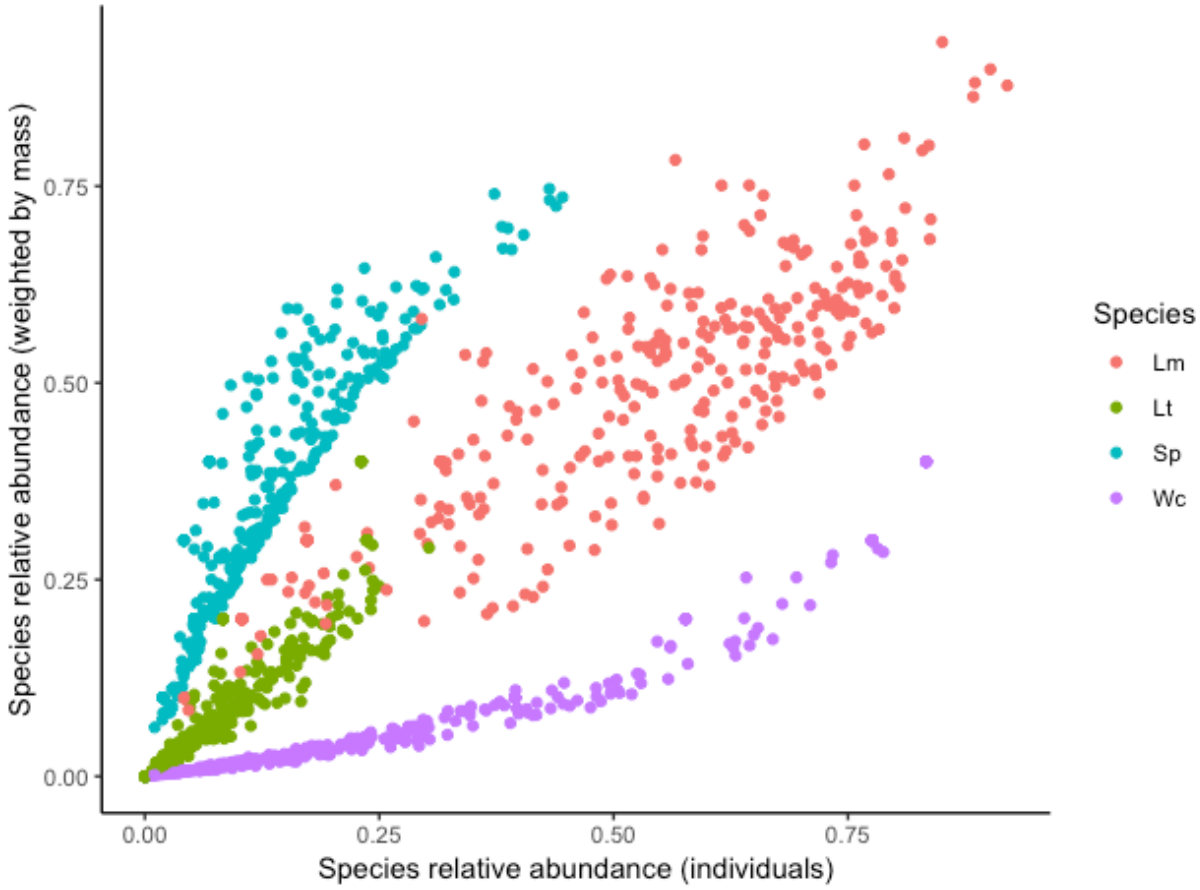

Fig. S5-2. A comparison of two measures of species relative abundance, weighted and unweighted by species average mass. Points included all data from the experiment (all community types, replicates, and species).

Table S5-1. Anova tables. The partition of variance in change in species frequency into components representing sorting, drift, and initial state. Analyses are done for each species separately, and for each time point over the course of the experiment.

Week: 4, Species: Lm				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.125*	*0.125*	*0*
Initial state	*3*	*0.603*	*0.201*	*0.016*
Drift	*44*	*0.371*	*0.008*	*0.008*
Total	*48*	*1.099*	*0.023*	

Week: 4, Species: Lt				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.053*	*0.053*	*0.000*
Initial state	*3*	*0.118*	*0.039*	*0.003*
Drift	*44*	*0.073*	*0.002*	*0.002*
Total	*48*	*0.243*	*0.005*	

Week: 4, Species: Sp				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.010*	*0.010*	*0*
Initial state	*3*	*0.837*	*0.279*	*0.022*
Drift	*44*	*0.412*	*0.009*	*0.009*
Total	*48*	*1.260*	*0.026*	

Week: 4, Species: Wc				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.051*	*0.051*	*0.001*
Initial state	*3*	*0.050*	*0.017*	*0.001*
Drift	*44*	*0.126*	*0.003*	*0.003*
Total	*48*	*0.228*	*0.005*	

Week: 6, Species: Lm				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.377*	*0.377*	*0.004*
Initial state	*3*	*0.604*	*0.201*	*0.016*
Drift	*44*	*0.532*	*0.012*	*0.012*
Total	*48*	*1.512*	*0.032*	

Week: 6, Species: Lt				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.080*	*0.080*	*0.001*
Initial state	*3*	*0.081*	*0.027*	*0.002*
Drift	*44*	*0.120*	*0.003*	*0.003*
Total	*48*	*0.281*	*0.006*	

Week: 6, Species: Sp				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.001*	*0.001*	*0*
Initial state	*3*	*0.843*	*0.281*	*0.022*
Drift	*44*	*0.612*	*0.014*	*0.014*
Total	*48*	*1.455*	*0.030*	

Week: 6, Species: Wc				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.125*	*0.125*	*0.002*
Initial state	*3*	*0.020*	*0.007*	*0.000*
Drift	*44*	*0.071*	*0.002*	*0.002*
Total	*48*	*0.215*	*0.004*	

Week: 8, Species: Lm				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.708*	*0.708*	*0.011*
Initial state	*3*	*0.489*	*0.163*	*0.012*
Drift	*44*	*0.667*	*0.015*	*0.015*
Total	*48*	*1.864*	*0.039*	

Week: 8, Species: Lt				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.159*	*0.159*	*0.003*
Initial state	*3*	*0.056*	*0.019*	*0.001*
Drift	*44*	*0.103*	*0.002*	*0.002*
Total	*48*	*0.318*	*0.007*	*0.00*

Week: 8, Species: Sp				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.004*	*0.004*	*0*
Initial state	*3*	*0.654*	*0.218*	*0.017*
Drift	*44*	*0.754*	*0.017*	*0.017*
Total	*48*	*1.412*	*0.029*	

Week: 8, Species: Wc				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.144*	*0.144*	*0.003*
Initial state	*3*	*0.028*	*0.009*	*0.001*
Drift	*44*	*0.128*	*0.003*	*0.003*
Total	*48*	*0.301*	*0.006*	

Week: 10, Species: Lm				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*1.089*	*1.089*	*0.021*
Initial state	*3*	*0.278*	*0.093*	*0.007*
Drift	*44*	*0.574*	*0.013*	*0.013*
Total	*48*	*1.942*	*0.040*	

Week: 10, Species: Lt				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.107*	*0.107*	*0.002*
Initial state	*3*	*0.068*	*0.023*	*0.002*
Drift	*44*	*0.119*	*0.003*	*0.003*
Total	*48*	*0.295*	*0.006*	

Week: 10, Species: Sp				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.064*	*0.064*	*0*
Initial state	*3*	*0.466*	*0.155*	*0.012*
Drift	*44*	*0.638*	*0.014*	*0.014*
Total	*48*	*1.168*	*0.024*	

Week: 10, Species: Wc				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.214*	*0.214*	*0.004*
Initial state	*3*	*0.011*	*0.004*	*0.000*
Drift	*44*	*0.078*	*0.002*	*0.002*
Total	*48*	*0.303*	*0.006*	

Week: 12, Species: Lm				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*1.389*	*1.389*	*0.028*
Initial state	*3*	*0.179*	*0.060*	*0.004*
Drift	*44*	*0.466*	*0.011*	*0.011*
Total	*48*	*2.034*	*0.042*	

Week: 12, Species: Lt				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.086*	*0.086*	*0.001*
Initial state	*3*	*0.066*	*0.022*	*0.002*
Drift	*44*	*0.111*	*0.003*	*0.003*
Total	*48*	*0.263*	*0.005*	

Week: 12, Species: Sp				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.172*	*0.172*	*0.001*
Initial state	*3*	*0.326*	*0.109*	*0.008*
Drift	*44*	*0.460*	*0.010*	*0.010*
Total	*48*	*0.958*	*0.020*	

Week: 12, Species: Wc				
Source	***Degrees of freedom***	***Sum of squares***	***Mean Square***	***Variance component***
Sorting	*1*	*0.220*	*0.220*	*0.005*
Initial state	*3*	*0.006*	*0.002*	*0.000*
Drift	*44*	*0.029*	*0.001*	*0.001*
Total	*48*	*0.256*	*0.005*	

Supplementary material for Chapter 6

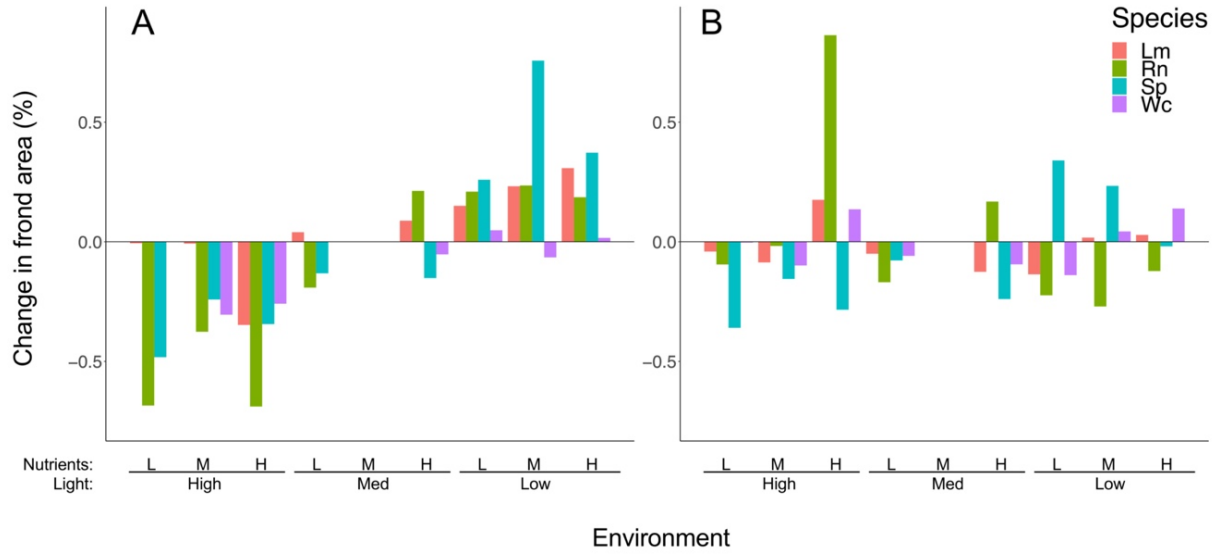

Fig. S6-1. Components of intra-specific trait change revealed by the reciprocal transplant. A) Change in frond area due to adaptation. B) Change in frond area due to plasticity. Change in frond area is quantified separately for each species in each ancestral-derived pair (for a single modified environment). Change in frond area due to adaptation is calculated as the difference among mean frond area of the derived and ancestral populations across both assay environments, and standardised by dividing by that of the ancestral population, ((derived – ancestral)/ ancestral). Change in frond area due to plasticity is calculated as the difference among mean frond area of populations assayed in the original and modified assay environments across ancestral and derived populations, and standardised by dividing by that in the original assay environment, [(modified – original) / original].

Table S6-1. Partition of variance in community mean frond area for the *reciprocal transplant* and the *In situ time series*. Negative variance components are set to 0.

Reciprocal Transplant **Environment: 1, High Light – Low Nutrients**								
Replicate mesocosm: 1					**Replicate mesocosm: 2**			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*6761*	*2254*	*4.02*	*3*	*4272*	*1424*	*2.53*
Community	*1*	*54*	*54*	*0*	*1*	*227*	*227*	*0*
Ecology		*101*	*101*	*0.48*		*17*	*17*	*0.06*
Evolution		*261*	*261*	*1.28*		*339*	*339*	*1.67*
Eco x Evo		*-308*	*-308*	*0*		*-129*	*-129*	*0*
Environment	*1*	*47*	*47*	*0*	*1*	*27*	*27*	
Spe x Com	*3*	*2502*	*719*	*5.12*	*3*	*3166*	*753*	*5.34*
Spe x Env	*3*	*167*	*56*	*0.36*	*3*	*139*	*46*	*0.31*
Com x Env	*1*	*15*	*15*	*0*	*1*	*49*	*49*	*0*
Spe x Com x Env	*3*	*169*	*56*	*0.37*	*3*	*172*	*57*	*0.38*
Error	*384*	*1739*	*5*	*4.53*	*384*	*1337*	*3*	*3.48*
Total	*399*	*11453*			*399*	*9389*		

Reciprocal Transplant **Environment: 2, High Light – Medium Nutrients**								
Replicate mesocosm: 1					**Replicate mesocosm: 2**			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*9653*	*3218*	*6.47*	*3*	*10910*	*3637*	*6.56*
Community	*1*	*171*	*171*	*0*	*1*	*11*	*11*	*0*
Ecology		*39*	*39*	*0.17*		*56*	*56*	*0.25*
Evolution		*56*	*56*	*0.25*		*65*	*65*	*0.29*
Eco x Evo		*77*	*77*	*0.13*		*-110*	*-110*	*0*
Environment	*1*	*31*	*31*	*0*	*1*	*25*	*25*	*0*
Spe x Com	*3*	*609*	*181*	*1.44*	*3*	*948*	*236*	*1.67*
Spe x Env	*3*	*228*	*76*	*0.58*	*3*	*230*	*77*	*0.52*
Com x Env	*1*	*48*	*48*	*0.01*	*1*	*15*	*15*	*0*
Spe x Com x Env	*3*	*124*	*41*	*0.30*	*3*	*75*	*25*	*0.14*
Error	*384*	*1671*	*4*	*4.35*	*384*	*1938*	*5*	*5.05*
Total	*399*	*12535*			*399*	*14152*		

Reciprocal Transplant
Environment: 3, High Light – High Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*13081*	*4360*	*7.97*	*3*	*11868*	*3956*	*7.03*
Community	*1*	*282*	*282*	*0*	*1*	*434*	*434*	*0*
Ecology		*1*	*1*	*0*		*9*	*9*	*0*
Evolution		*314*	*314*	*1.51*		*320*	*320*	*1.54*
Eco x Evo		*-33*	*-33*	*0*		*105*	*105*	*0.26*
Environment	*1*	*112*	*112*	*0*	*1*	*81*	*81*	*0*
Spe x Com	*3*	*4155*	*1419*	*10.35*	*3*	*2921*	*967*	*6.76*
Spe x Env	*3*	*2084*	*695*	*5.05*	*3*	*1409*	*470*	*3.30*
Com x Env	*1*	*20*	*20*	*0*	*1*	*51*	*51*	*0*
Spe x Com x Env	*3*	*142*	*47*	*0.30*	*3*	*588*	*196*	*1.35*
Error	*384*	*2175*	*6*	*5.66*	*384*	*2519*	*7*	*6.56*
Total	*399*	*22051*			*399*	*19871*		

Reciprocal Transplant
Environment: 4, Medium Light – Low Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*10920*	*3640*	*7.32*	*3*	*9606*	*3202*	*6.27*
Community	*1*	*64*	*64*	*0*	*1*	*82*	*82*	*0.06*
Ecology		*12*	*12*	*0.04*		*86*	*86*	*0.41*
Evolution		*25*	*25*	*0.10*		*14*	*14*	*0.05*
Eco x Evo		*28*	*28*	*0.11*		*-18*	*-18*	*0.02*
Environment	*1*	*22*	*22*	*0*	*1*	*29*	*29*	*0*
Spe x Com	*3*	*491*	*279*	*2.23*	*3*	*58*	*6*	*0.02*
Spe x Env	*3*	*199*	*66*	*0.51*	*3*	*204*	*68*	*0.51*
Com x Env	*1*	*25*	*25*	*0*	*1*	*13*	*13*	*0*
Spe x Com x Env	*3*	*140*	*47*	*0.35*	*3*	*56*	*19*	*0.12*
Error	*384*	*1331*	*3*	*3.47*	*384*	*1165*	*3*	*3.03*
Total	*399*	*13193*			*399*	*11212*		

Reciprocal Transplant
Environment: 6, Medium Light – High Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*16118*	*5373*	*11.24*	*3*	*12124*	*4041*	*8.17*
Community	*1*	*150*	*150*	*0.02*	*1*	*219*	*219*	*0.13*
Ecology		*223*	*223*	*1.09*		*428*	*428*	*2.11*
Evolution		*25*	*25*	*0.10*		*81*	*81*	*0.38*
Eco x Evo		*-98*	*-98*	*0*		*-289*	*-289*	*0.15*
Environment	*1*	*8*	*8*	*0*	*1*	*13*	*13*	*0*
Spe x Com	*3*	*405*	*176*	*1.46*	*3*	*280*	*31*	*0.23*
Spe x Env	*3*	*320*	*107*	*0.86*	*3*	*152*	*51*	*0.38*
Com x Env	*1*	*17*	*17*	*0*	*1*	*13*	*13*	*0*
Spe x Com x Env	*3*	*197*	*66*	*0.51*	*3*	*180*	*60*	*0.45*
Error	*384*	*1642*	*4*	*4.28*	*384*	*1462*	*4*	*3.81*
Total	*399*	*18856*			*399*	*14444*		

Reciprocal Transplant
Environment: 7, Low Light – Low Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*13499*	*4500*	*8.70*	*3*	*11836*	*3945*	*7.16*
Community	*1*	*9*	*9*	*0*	*1*	*81*	*81*	*0.00*
Ecology		*50*	*50*	*0.21*		*77*	*77*	*0.35*
Evolution		*44*	*44*	*0.18*		*42*	*42*	*0.18*
Eco x Evo		*-85*	*-85*	*0*		*-38*	*-38*	*0*
Environment	*1*	*8*	*8*	*0*	*1*	*25*	*25*	*0*
Spe x Com	*3*	*263*	*142*	*1.02*	*3*	*432*	*196*	*1.39*
Spe x Env	*3*	*398*	*133*	*0.99*	*3*	*181*	*60*	*0.40*
Com x Env	*1*	*61*	*61*	*0*	*1*	*53*	*53*	*0*
Spe x Com x Env	*3*	*540*	*180*	*1.36*	*3*	*272*	*91*	*0.62*
Error	*384*	*1759*	*5*	*4.58*	*384*	*2019*	*5*	*5.26*
Total	*399*	*16537*			*399*	*14900*		

Reciprocal Transplant
Environment: 8, Low Light – Medium Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*12156*	*4052*	*7.67*	*3*	*14921*	*4974*	*8.79*
Community	*1*	*11*	*11*	*0*	*1*	*84*	*84*	*0*
Ecology		*103*	*103*	*0.48*		*44*	*44*	*0.15*
Evolution		*94*	*94*	*0.44*		*119*	*119*	*0.52*
Eco x Evo		*-185*	*-185*	*0*		*-79*	*-79*	*0*
Environment	*1*	*21*	*21*	*0*	*1*	*72*	*72*	*0*
Spe x Com	*3*	*585*	*145*	*1.07*	*3*	*814*	*454*	*3.17*
Spe x Env	*3*	*222*	*74*	*0.53*	*3*	*674*	*225*	*1.52*
Com x Env	*1*	*44*	*44*	*0*	*1*	*177*	*177*	*0*
Spe x Com x Env	*3*	*232*	*77*	*0.55*	*3*	*849*	*283*	*1.93*
Error	*384*	*1725*	*4*	*4.49*	*384*	*4013*	*10*	*10.45*
Total	*399*	*14996*			*399*	*21604*		

Reciprocal Transplant
Environment: 9, Low Light – High Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*15036*	*5012*	*9.01*	*3*	*12712*	*4237*	*7.44*
Community	*1*	*27*	*27*	*0*	*1*	*129*	*129*	*0*
Ecology		*46*	*46*	*0.20*		*247*	*247*	*1.20*
Evolution		*121*	*121*	*0.57*		*48*	*48*	*0.21*
Eco x Evo		*-140*	*-140*	*0*		*-167*	*-167*	*0*
Environment	*1*	*38*	*38*	*0*	*1*	*14*	*14*	*0*
Spe x Com	*3*	*781*	*278*	*1.98*	*3*	*475*	*421*	*2.94*
Spe x Env	*3*	*213*	*71*	*0.47*	*3*	*153*	*51*	*0.33*
Com x Env	*1*	*14*	*14*	*0*	*1*	*100*	*100*	*0*
Spe x Com x Env	*3*	*227*	*76*	*0.50*	*3*	*582*	*194*	*1.33*
Error	*384*	*2182*	*6*	*5.68*	*384*	*1566*	*4*	*4.08*
Total	*399*	*18518*			*399*	*15731*		

***In situ* time series**
Environment: 1, High Light – Low Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species*	*2*	*506*	*253*	*0.45*	*2*	*586*	*293*	*0.52*
Community	*1*	*18*	*18*	*0*	*1*	*35*	*35*	*0*
Ecology		*7*	*7*	*0.02*		*3*	*3*	*0*
Evolution		*29*	*29*	*0.13*		*50*	*50*	*0.23*
Eco x Evo		*-18*	*-18*	*0*		*-18*	*-18*	*0*
Environment	*1*	*65*	*65*	*0*	*1*	*163*	*163*	*0.13*
Spe x Com	*3*	*321*	*211*	*1.51*	*3*	*765*	*656*	*4.72*
Spe x Env	*3*	*194*	*97*	*0.68*	*3*	*113*	*57*	*0.39*
Com x Env	*1*	*62*	*62*	*0*	*1*	*61*	*61*	*0*
Spe x Com x Env	*3*	*144*	*72*	*0.50*	*3*	*395*	*198*	*1.41*
Error	*384*	*978*	*3*	*2.52*	*384*	*857*	*2*	*2.21*
Total	*399*	*2289*			*399*	*2976*		

**Local extinction of Wc, hence 2 df for Species*

***In situ* time series**
Environment: 2, High Light – Medium Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*1168*	*389*	*0.78*	*3*	*357*	*119*	*0.21*
Community	*1*	*7*	*7*	*0*	*1*	*20*	*20*	*0*
Ecology		*46*	*46*	*0.22*		*3*	*3*	*0.00*
Evolution		*75*	*75*	*0.37*		*16*	*16*	*0.07*
Eco x Evo		*-114*	*-114*	*0*		*1*	*1*	*0.00*
Environment	*1*	*6*	*6*	*0*	*1*	*24*	*24*	*0*
Spe x Com	*3*	*265*	*88*	*0.71*	*3*	*878*	*254*	*1.84*
Spe x Env	*3*	*94*	*31*	*0.25*	*3*	*136*	*45*	*0.32*
Com x Env	*1*	*18*	*18*	*0.02*	*1*	*19*	*19*	*0*
Spe x Com x Env	*3*	*27*	*9*	*0.07*	*3*	*327*	*109*	*0.78*
Error	*384*	*242*	*1*	*0.63*	*384*	*392*	*1*	*1.02*
Total	*399*	*1827*			*399*	*2154*		

***In situ* time series**
Environment: 3, High Light – High Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	3	*1242*	*414*	*0.76*	*3*	*2172*	*724*	*1.29*
Community	1	*27*	*27*	*0*	*1*	*22*	*22*	*0.00*
Ecology		*6*	*6*	*0.02*		*1*	*1*	*0*
Evolution		*49*	*49*	*0.24*		*16*	*16*	*0.07*
Eco x Evo		*-28*	*-28*	*0*		*5*	*5*	*0*
Environment	1	*6*	*6*	*0*	*1*	*6*	*6*	*0*
Spe x Com	3	*514*	*177*	*1.29*	*3*	*59*	*2*	*0.01*
Spe x Env	3	*54*	*18*	*0.12*	*3*	*76*	*25*	*0.17*
Com x Env	1	*13*	*13*	*0*	*1*	*23*	*23*	*0*
Spe x Com x Env	3	*108*	*36*	*0.25*	*3*	*316*	*105*	*0.74*
Error	384	*482*	*1*	*1.25*	*384*	*445*	*1*	*1.16*
Total	399	*2446*			*399*	*3119*		

***In situ* time series**
Environment: 4, Medium Light – Low Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species*	*3*	*4009*	*2004*	*4.06*	*3*	*3454*	*1727*	*3.49*
Community	*1*	*47*	*47*	*0*	*1*	*84*	*84*	*0*
Ecology		*20*	*20*	*0.08*		*18*	*18*	*0.06*
Evolution		*104*	*104*	*0.50*		*127*	*127*	*0.61*
Eco x Evo		*-77*	*-77*	*0*		*-61*	*-61*	*0*
Environment	*1*	*37*	*37*	*0*	*1*	*246*	*246*	*0.23*
Spe x Com	*3*	*369*	*327*	*2.64*	*3*	*425*	*195*	*1.56*
Spe x Env	*3*	*119*	*59*	*0.45*	*3*	*153*	*76*	*0.58*
Com x Env	*1*	*51*	*51*	*0.02*	*1*	*29*	*29*	*0*
Spe x Com x Env	*3*	*90*	*45*	*0.33*	*3*	*113*	*57*	*0.42*
Error	*384*	*1449*	*4*	*3.73*	*384*	*1674*	*4*	*4.31*
Total	*399*	*6170*			*399*	*6177*		

***In situ* time series**
Environment: 6, Medium Light – High Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*3627*	*1209*	*2.53*	*3*	*4997*	*1666*	*3.36*
Community	*1*	*9*	*9*	*0*	*1*	*11*	*11*	*0*
Ecology		*215*	*215*	*1.06*		*395*	*395*	*1.96*
Evolution		*235*	*235*	*1.16*		*387*	*387*	*1.92*
Eco x Evo		*-441*	*-441*	*0*		*-772*	*-772*	*0*
Environment	*1*	*41*	*41*	*0.02*	*1*	*21*	*21*	*0*
Spe x Com	*3*	*596*	*150*	*1.25*	*3*	*592*	*395*	*3.19*
Spe x Env	*3*	*62*	*21*	*0.16*	*3*	*132*	*44*	*0.34*
Com x Env	*1*	*6*	*6*	*0*	*1*	*9*	*9*	*0*
Spe x Com x Env	*3*	*134*	*45*	*0.36*	*3*	*101*	*34*	*0.25*
Error	*384*	*562*	*1*	*1.46*	*384*	*794*	*2*	*2.07*
Total	*399*	*5036*			*399*	*6656*		

***In situ* time series**
Environment: 7, Low Light – Low Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*4813*	*1604*	*3.10*	*3*	*7088*	*2363*	*4.57*
Community	*1*	*4*	*4*	*0*	*1*	*7*	*7*	*0*
Ecology		*56*	*56*	*0.26*		*72*	*72*	*0.34*
Evolution		*57*	*57*	*0.27*		*64*	*64*	*0.30*
Eco x Evo		*-108*	*-108*	*0*		*-129*	*-129*	*0*
Environment	*1*	*16*	*16*	*0*	*1*	*8*	*8*	*0*
Spe x Com	*3*	*641*	*156*	*1.21*	*3*	*661*	*102*	*0.77*
Spe x Env	*3*	*122*	*41*	*0.30*	*3*	*142*	*47*	*0.34*
Com x Env	*1*	*10*	*10*	*0*	*1*	*10*	*10*	*0*
Spe x Com x Env	*3*	*163*	*54*	*0.41*	*3*	*260*	*87*	*0.65*
Error	*384*	*695*	*2*	*1.81*	*384*	*1181*	*3*	*3.08*
Total	*399*	*6465*			*399*	*9358*		

***In situ* time series**
Environment: 8, Low Light – Medium Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*7615*	*2538*	*4.80*	*3*	*6871*	*2290*	*4.05*
Community	*1*	*151*	*151*	*0*	*1*	*9*	*9*	*0*
Ecology		*116*	*116*	*0.53*		*63*	*63*	*0.29*
Evolution		*513*	*513*	*2.52*		*51*	*51*	*0.23*
Eco x Evo		*-479*	*-479*	*0*		*-105*	*-105*	*0*
Environment	*1*	*22*	*22*	*0*	*1*	*17*	*17*	*0*
Spe x Com	*3*	*2117*	*791*	*5.94*	*3*	*514*	*48*	*0.32*
Spe x Env	*3*	*634*	*211*	*1.55*	*3*	*141*	*47*	*0.31*
Com x Env	*1*	*75*	*75*	*0*	*1*	*9*	*9*	*0*
Spe x Com x Env	*3*	*525*	*175*	*1.27*	*3*	*123*	*41*	*0.27*
Error	*384*	*2753*	*7*	*7.17*	*384*	*1334*	*3*	*3.47*
Total	*399*	*13892*			*399*	*9018*		

***In situ* time series**
Environment: 9, Low Light – High Nutrients

	Replicate mesocosm: 1				Replicate mesocosm: 2			
Source	**df**	**SS**	**MS**	**Variance Component**	**df**	**SS**	**MS**	**Variance Component**
Species	*3*	*4506*	*1502*	*2.70*	*3*	*7719*	*2573*	*4.52*
Community	*1*	*15*	*15*	*0*	*1*	*25*	*25*	*0*
Ecology		*119*	*119*	*0.58*		*190*	*190*	*0.93*
Evolution		*80*	*80*	*0.38*		*110*	*110*	*0.53*
Eco x Evo		*-184*	*-184*	*0*		*-275*	*-275*	*0*
Environment	*1*	*23*	*23*	*0*	*1*	*21*	*21*	*0*
Spe x Com	*3*	*606*	*202*	*1.44*	*3*	*785*	*145*	*0.98*
Spe x Env	*3*	*346*	*115*	*0.82*	*3*	*178*	*59*	*0.40*
Com x Env	*1*	*18*	*18*	*0*	*1*	*17*	*17*	*0*
Spe x Com x Env	*3*	*75*	*25*	*0.17*	*3*	*209*	*70*	*0.47*
Error	*384*	*629*	*2*	*1.64*	*384*	*1149*	*3*	*2.99*
Total	*399*	*6218*			*399*	*10102*		