# Presence of the microbiome decreases fitness and modifies phenotype in the aquatic plant Lemna minor

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**Abstract**

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 supresses 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.

## Presence of the microbiome decreases fitness and modifies phenotype in the aquatic plant *Lemna minor*

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**Abstract**

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 supresses 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.

*Keywords: growth rate, intraspecific variation, plant phenotype, root length, frond area*

## 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), and reduced herbivory (Hubbard et al. 2019). Examples include nitrogen-fixing arbuscular mycorrhizal fungi, whose mutualistic association with terrestrial plants often releases the host from severe nitrogen or phosphorus limitation (Smith and Read 2008). In addition, there are other important mutualistic plant-microbe interactions, namely plant growth-promoting bacteria (PGPB), which have the potential to increase the yield of agricultural plant crops and other applications (Glick 2012). However, the majority of reported plant–microbe interactions are negative (Bever 2003, Kulmatiski et al. 2008, Van der Putten et al. 2013), which is thought to be 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). 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 $20^{\text{th}}$ C, with the observation of an association with N-fixing bacteria (Bottomley 1920), and has accelerated in recent years (Ishizawa et al. 2017a, 2017b, 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

### *L. minor sampling and microbiome removal and reintroduction*

Eight natural rivers, ponds, and swamps supporting populations of*L. minor* were located within a 10km radius of Montreal, Canada (Table S1 & Fig. S1, Supplementary materials). 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.

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 Supplementary materials, Table S2). 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. S2, Supplementary materials). 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.

### *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, $20^0$C, 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 ($20^0$C, 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$r = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t}$ 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). 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) and for seven of the eight genotypes (Fig. 2a). 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 the Supplementary materials (Table S3).

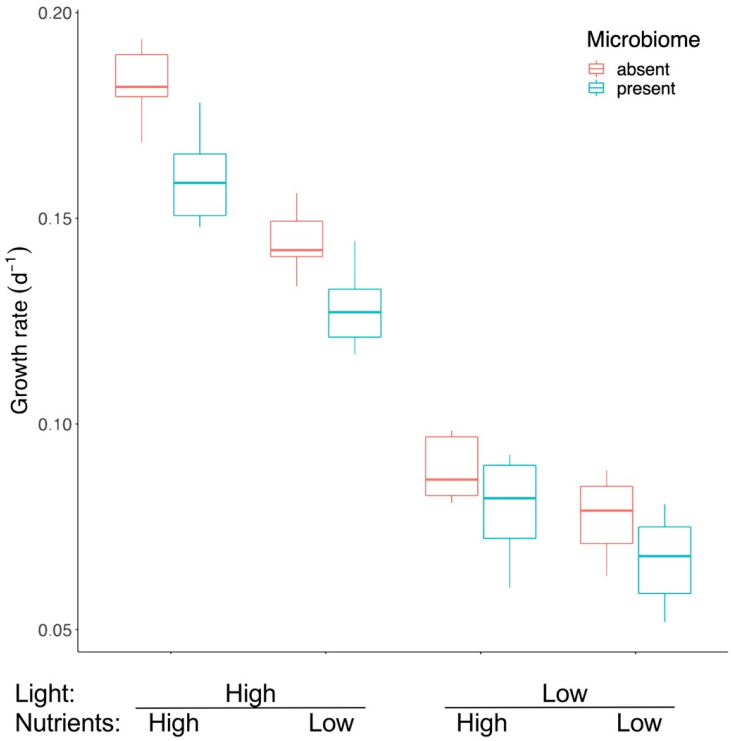



*Fig. 1. Population growth rate for Lemna minor assayed in four modified environmental* conditions*, with and without its natural microbiome. Each box and whisker represent the variation among 8 independent populations (3 replicate flasks were averaged for each of the 8* genotypes*). Boxes represent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

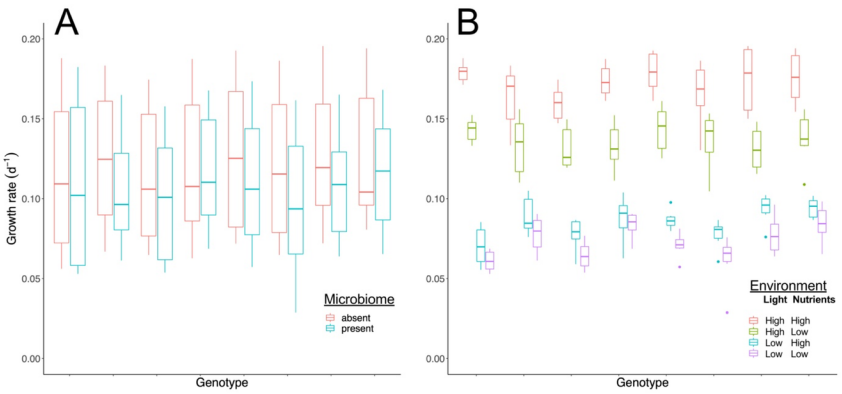
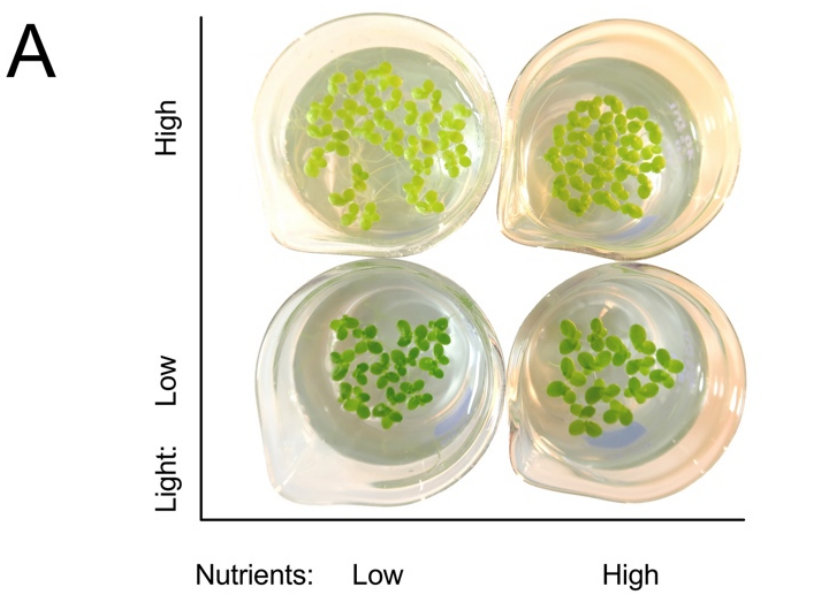



*Fig. 2. Growth rates for eight genotypes of Lemna minor, grown in four different environmental conditions, with and without their microbiomes. A) Variation is the result of 3 replicate assays in each of four environments all grouped together. B) Variation is the result of 3 replicate assays with and 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. 3c). 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. 3c).

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. 3a). 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 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. 3b), 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).



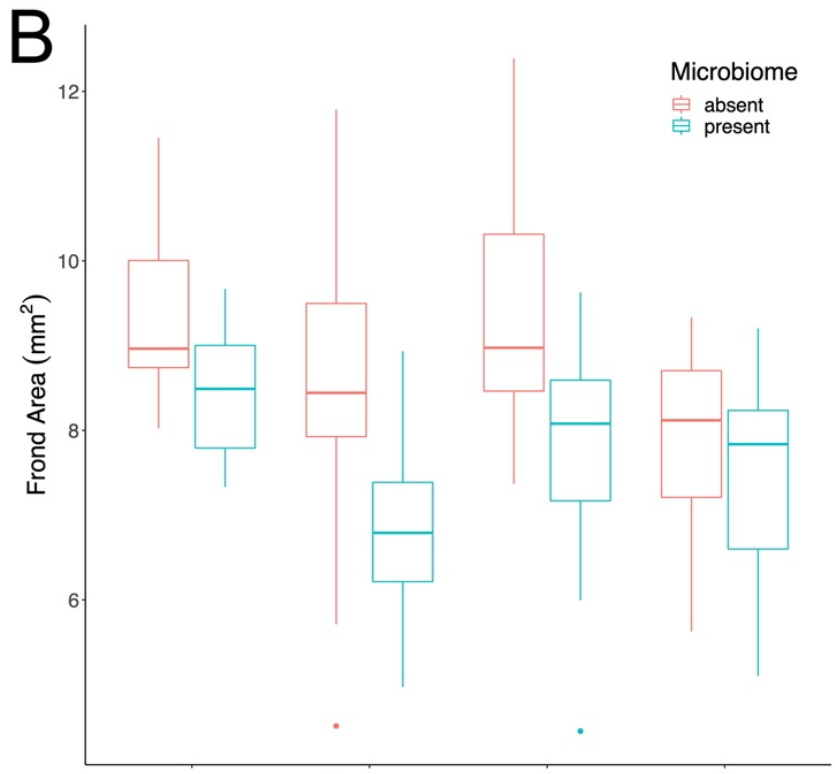




*Fig. 3. Phenotypic consequences of growth in four modified environmental conditions for Lemna minor, with and without its natural microbiome. A) Photo of a single genotype grown in four environmental* conditions *without the microbiome. B) Variation in frond area ($mm^2$) C) Variation in Root length (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.*

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. 4). 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. 4).

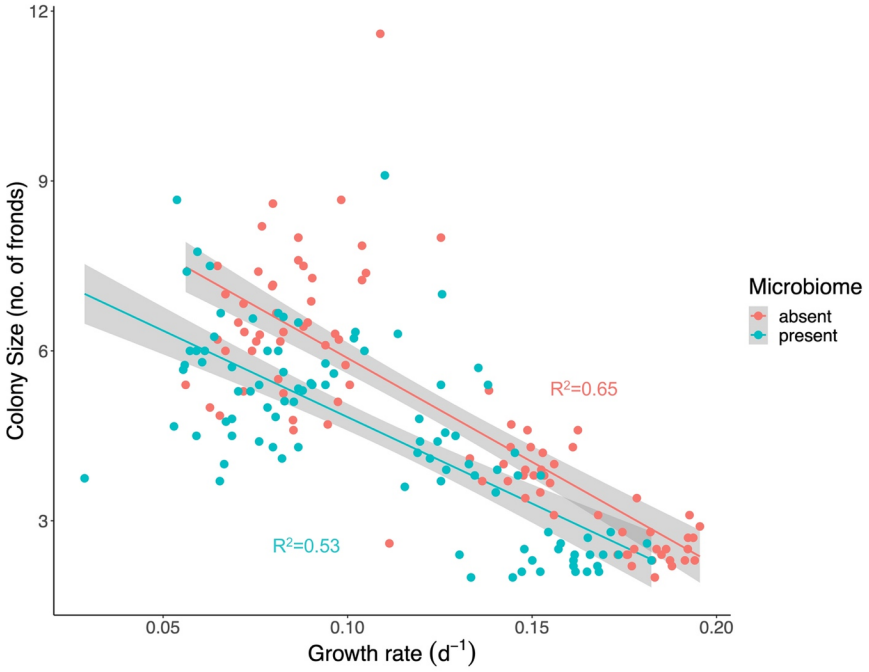



*Fig. 4. Lemna minor colony size as a function of its growth rate for populations with and without their microbiome. Each regression is the result of 96 points (8* genotypes *x 4 environments x 3 replicates). Shading around each regression line are 95% confidence intervals.*

*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.

*Presence of the microbiome consistently decreased growth rates and modified phenotypes of the host plants*

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. 2017a, 2017b, 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), and for seven of the eight genotypes (Fig. 2a). 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. 2017b).

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. 3). 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. 4). 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 supressed 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 (eg. O'Brien et al. 2020a, 2020b, Tan et al. 2021), is that the majority of these studies are explicitly looking to identify only these mutualistic associations. Due to its extremely rapid growth rate, among the fastest of all plants, *L. minor* research is often in the context of its 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 carefully chosen 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 specialisation 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 board functional groups and their interactions with the plant host.

*Light and nutrient treatments had strong effects on plant growth and phenotype*

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).

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. 3c). 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. 3b), 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. 3a), 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.

*Evidence of genetic variation in traits 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 supresses 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 microbiome clearly includes important symbionts including several nitrogen fixers, it is a mistake to discount the important pathogenic, parasitic, and competitive interactions, whose influence seem to override that of mutualists.

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**Authors' contributions:** The study was conceived together by MDJ and GB. MDJ and SVM performed the experiment. The manuscript was written by MDJ. SVM and GB contributed substantially to revisions.

**Competing interests:** The authors declare no competing interests.

**Data availability:** Raw data from which all figures were generated will be stored in the Dryad repository before publication of the article.

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**Supplementary Materials**

Table S1. 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 S2. 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 |

| | Low | Med | High |
|---|---|---|---|
| $CuSO_4$ 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. 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.

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 | $< 0.001$ |

| Source | Df | Sum Sq | Mean Sq | $F$ | $p$ |
|---|---|---|---|---|---|
| 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 | | | |

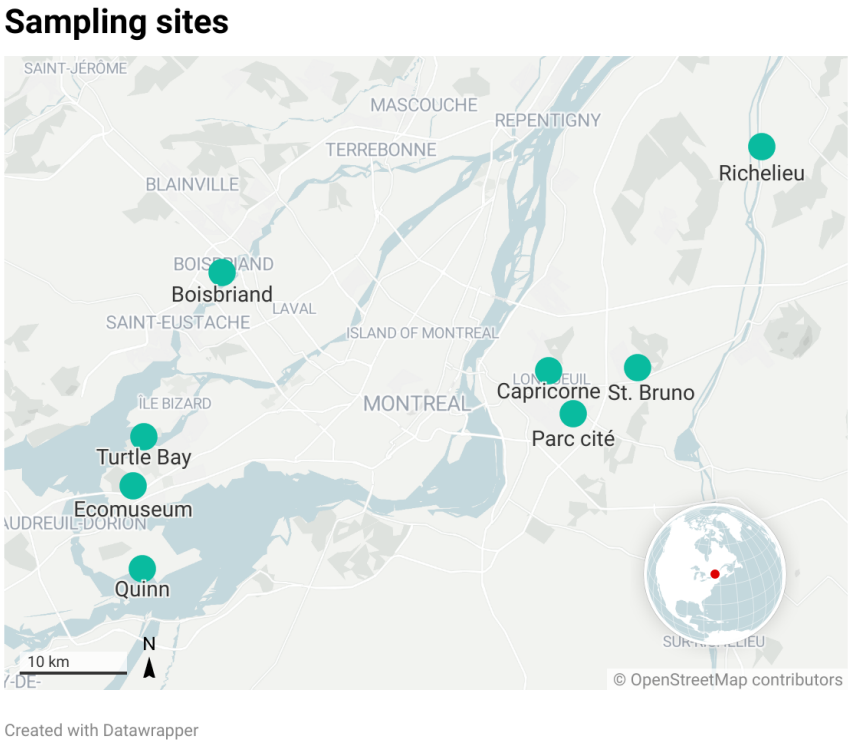


**Figure S1.** Map of the sampling sites around Montreal, Quebec, Canada. Created with Datawrapper.

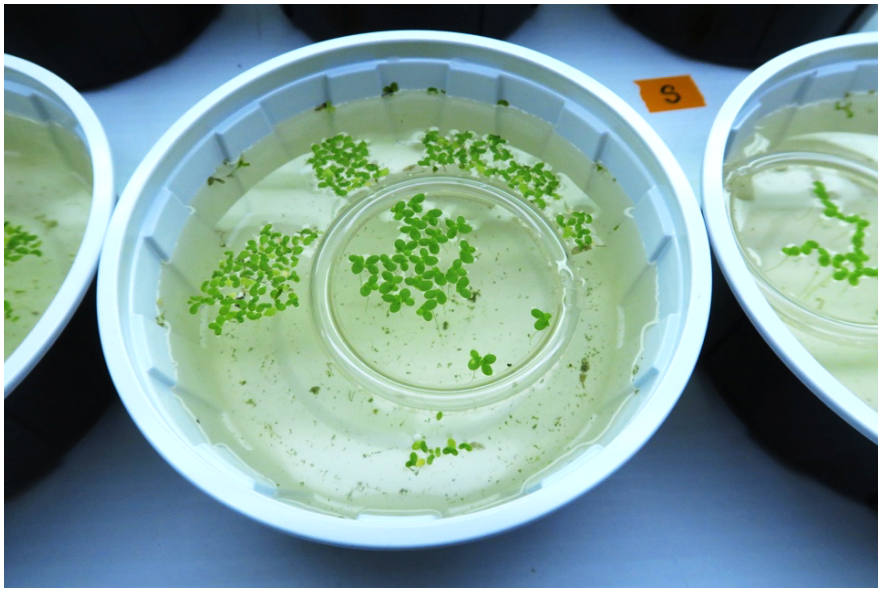


*Fig. S2. Reinoculation of the microbiome back to axenic Lemna minor fronds. The container, filled with natural pond water is filled with fronds from the same site, with their microbiome intact. The floating circular boom isolates the sterilised target fronds. Although the fronds are spatially separated 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 4 generations).*