Observing adaptation of duckweeds to their local microbiome depends on local pondwater

Ava Rose[1a], Anna M. O'Brien[1]*

1. University of New Hampshire, Department of Molecular, Cellular, and Biomedical Sciences

a. ava.rose@unh.edu

*Corresponding author

Anna O'Brien, anna.obrien@unh.edu

46 College Rd, Durham, NH, USA

Acknowledgments: The authors wish to acknowledge funding support by the National Science Foundation BIO-MCB (Award #2300059), NHAES (New Hampshire Agricultural Experiment Station) and USDA Hatch project number NH 00724, collections at sites managed by the UNH Office of Woodlands and Natural Areas and the Town of Durham, and valuable discussion with other O'Brien Lab members (Alex Trott, Admas Berisso, Ciana Lazu, Alyssa Daigle, Ethan Morgan).

Competing interests: The authors declare they have no known competing interests.

Author contributions: AR and AMO contributed to the conception and design of the experiment. AR led the acquisition of data. AR provided the initial manuscript draft and data analysis, and all authors contributed to revising the draft and data analysis, and approved the final version to be published.

Data Accessibility:

Data and scripts are available at https://github.com/amob/Ladpt-Water-Microbe-MS-analysis

ABSTRACT

Populations can locally adapt to both the biotic and abiotic factors of an environment, and observing adaptation to biotic factors can sometimes depend on the abiotic conditions, and vice versa. One important aspect of the biotic environment is the microbiome: interactions between microbiomes and their hosts are critical for host fitness and trait expression. Hosts may adapt to their local microbiomes (or vice versa), and hosts may depend on microbiomes for adaptation to the local environment. Using *Lemna minor* (duckweed) as a model organism, we examined differences in host fitness when grown in local and nonlocal microbiomes and in local and nonlocal water. We experimentally recombined duckweeds, microbes, and water from 4 different ponds around Durham, New Hampshire in well-plate microcosms in a growth chamber. Duckweed, microbe, and water source all affected microbial and duckweed growth, as well as duckweed traits. However, we observed only very weak local adaptation between duckweeds and microbes from their source site, and only very weak local adaptation of duckweeds to water from their source site that together affected frond final area positively, and microbial growth negatively.

Keywords: local adaptation, genotype environment interaction, duckweed, microbiome

INTRODUCTION

Populations often evolve in ways that increase their fitness in their local habitat, while experiencing a decay in fitness in other habitats due to drift or tradeoffs (Kawecki & Ebert, 2004; Hereford 2009; Blanquart et al., 2013). Biotic factors can influence local adaptation, but whether they consistently do so has remained a debate. Meta-analyses have reached opposing conclusions: that leaving biotic interactions intact strengthens the signal of local adaptation, and that biotic interactions do not alter signals of local adaptation (Briscoe-Runquist et al., 2020; Hargreaves et al., 2020).

For macroorganisms, one important local biotic habitat may be the microbiome: the community of microorganisms that live on, inside, or near larger host organisms (Berg et al., 2020). Host-associated microbiomes alter fitness and trait expression in a wide variety of host species (e.g., Turnbaugh et al., 2008; Kaiko & Stappenbeck, 2014; Gould et al., 2018). For example, microbes influence the timing of plant phenological events ranging from germination to fruiting pervasively across studied plants, regardless of microbe type, the nature of the interaction, and other factors (Lu et al., 2018; O'Brien et al., 2021). Indeed, effects of plant-microbiome interactions hold particular interest for us, and we hope that engineering microbial treatments matched to plants could increase crop yields and overall productivity in agriculture (Qiu et al., 2019).

Some of the trait and fitness effects of microbiomes on plants may derive from local adaptation. Plants appear generally adapted to some types of locally-associated microbes (Rúa et al., 2016), and microbes, in turn, can adapt to their locally-associated plants (Batstone et al., 2020). However, local adaptation between plants and associated microbes or microbiomes can be

conditional on the environment experienced, on the match of tested microbes to the full natural microbiome, and even on the environmental source of the plants and microbes (Johnson et al., 2010; O'Brien & Laurich et al., 2024; O'Brien & Sawers et al., 2024).

The common pond plant *Lemna minor*, hereafter "duckweed," has recently gained popularity as a model organism for host-microbiome interaction experiments (Ishizawa & Kuroda et al., 2020; Ishizawa & Tada et al., 2020; O'Brien et al., 2020, Anneberg et al., 2023; Jewell et al., 2023, Wei & Tan, 2023). Indeed, duckweed has been used as a model for plant biology since the 1950s (Acosta et al., 2021), largely because it's rapid doubling time and small size make it easy to manipulate and ideal for experiments (Zhang et al., 2010), and it's recent popularity has resulted in specialized experimental platforms and analyses (Mejbel & Simons 2018; Cox Jr. et al., 2022; O'Brien et al., 2022; Kose et al., 2023; Subbaraman et al., 2024).

Here, we used *Lemna minor* and its associated microbiome as a model to quantify abiotic and biotic local adaptation by collecting duckweeds, water, and microbiomes from four sites and factorially recombining all materials in a "common garden" in the lab. We hypothesized that 1) duckweeds would have higher fitness when grown in both local biotic (microbiome) and local abiotic (water source) contexts than when grown in local contexts for only the biotic or abiotic factors (i.e. local microbiome but nonlocal water), or when grown in entirely nonlocal contexts (neither the microbiome nor the water from the home site). We also hypothesized 2) that inoculated microbiomes would reach higher cell densities when grown in local biotic and abiotic conditions (duckweeds and water from their home pond) than those grown in partially or entirely nonlocal contexts.

MATERIALS AND METHODS

Collection Sites, Samples, and Storage

Duckweeds, microbes, and water were collected from four locations across Durham, New Hampshire: Mill (M), Upper Mill (UM), Woodman (W), and the Durham Reservoir (DR, Table S1). Two pairs of sites were within the same watersheds: Mill and Upper Mill are in the Oyster River Watershed, and Durham Reservoir and Woodman are in the Pettee Brook Watershed.

Duckweed samples were collected from each of the four sites during the spring of 2022, and we used a single frond from each site to found a clonally propagated line. We expect these lines to be essentially isogenic, because duckweeds have low segregating diversity (Ho, 2018), and our lines were never observed to flower in our lab conditions. We created axenic cultures from these isogenic lines following an existing protocol (Laurich & Carlson, 2023). Briefly, duckweeds were agitated by vortexing for ~5 minutes, placed in 1% bleach for 1 to 1.5 minutes, and then rinsed in sterile DI water three times. The first DI water rinse was 45 seconds long, and the second two were 5-10 minutes. The plants were transferred to sterile 0.5x Krazčič's media until fronds grew. A few fronds were then placed in Krazčič's media enriched with yeast and mannitol to check for failure of the procedure (microbial growth), and to rapidly amplify any successful axenic duckweed cultures. Lines were grown in this media, in natural light conditions on a windowsill, refreshing media when growth slowed, until they were needed for experimentation.

Microbes were collected from each of the four sites between September-October 2023. Fresh gloves were rinsed with 70% ethanol and worn for the entire collection process. All tubes used for collection were sterile, and were rinsed 3 times in the source water prior to microbiome collection. Three 45 mL tubes of water with live local microbiomes were collected at each site.

We centrifuged the collected water for 15 minutes at 4,000 rpm and 23°C. The supernatant was poured off, and the pellets were homogenized and resuspended in 2mL of ~25% sterile glycerol, then cryopreserved at -80°C until needed. Before experimentation, inocula were resuscitated from the cyropreserved microbiomes by unfreezing and diluting each microbiome with 2 mL of sterile DI water. To create control treatments (sterile mock inocula with an identical glycerol concentration to live inocula), sterile 25% glycerol was diluted by half in sterile DI water.

Water samples were collected from each of the four sites from September-October 2023. Three 40 mL samples of water were collected at each site. We placed the samples directly into a -20°C freezer until the experiment set-up. Before experimentation, we removed excess dirt and debris using gravity filtration and paper filters. We then filter-sterilized water samples by passing through a 0.2 µm filter.

Experimental Design

To evaluate the impacts of local and nonlocal microbiomes and water on duckweed fitness, four axenic isogenic duckweed lines, four filter-sterilized water sources, and four revived microbiomes or a no microbiomes control were factorially recombined (80 treatments, Figure 1) in a randomized-block, microcosm experiment with 12 replicates in 10, flat-bottom 96-well plates. Each block contained one replicate of each of the 80 treatments, and contained 4 sub-blocks, each randomly assigned to one duckweed line. Within each sub-block, the field-collected water, cryo-preserved microbes (or no microbes treatment) were completely randomized.

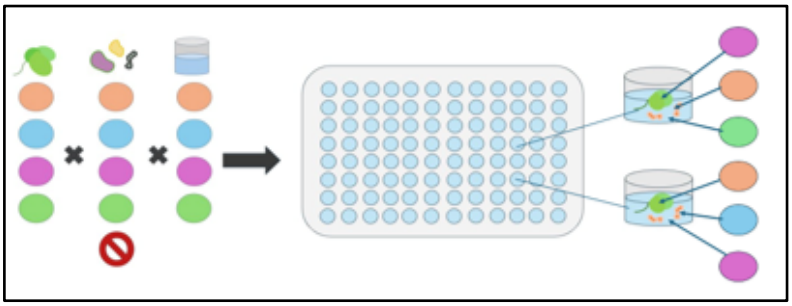

Figure 1: Experimental design for this project, depicting collection sites and the flow of work. Duckweeds, water, and microbes were collected from the four sites (Table S1). These sites are depicted by the green, orange, blue, and purple dots (Durham Reservoir, Woodman, Mill, and Upper Mill, respectively). Randomized crosses accounting for all combinations of duckweeds, water, and microbes (and a no-microbe treatment) were made in 96-well plates.

We added each treatment to the plates following the randomized block design in a biosafety cabinet (REDISHIP Purifier Logic+ Class II A2, Labconco). We first added 205 uL of the sterilized field-collected water treatments, and then added single attached units of sterile duckweed fronds approximately the same in total surface area (ranging from 1-4 individual fronds). The plates were next sealed with Breath Easier seals (Diversified Biotech) to prevent cross-contamination when inoculating microbiome treatments. We restored microbiomes to their field-collected concentrations by adding 9.1 µL of microbial inocula (or of sterile mock inocula for the control, no microbes treatment) to the 205 µL of pond water already in the wells. The inocula treatments (live or mock), were added to each well by punching through the Breath Easier seal with a pipette tip. The plates were then sealed with BreathEasy seals (Diversified Biotech) and gently covered with the plate's lid to slow evaporation in the growth chamber.

Once assembled, the experimental plates were placed in a Percival® model AR-75L3 growth chamber for 12 days. Lights and temperature cycled daily, with 16 hours at 350 µmoles/m^2/s and 23°C, followed by 8 hours of darkness at 18°C. The plates were placed on a stage inside the growth chamber that was positioned over an imaging system (4 cameras connected to Raspberry Pis, as in Kose et al., 2023). Images of the plates were taken at the end of the experiment (Day 12), and analyzed with DuckPlate software to measure the surface area of

duckweed fronds within each microcosm well (Kose et al., 2023). Frond area increases as the number of individuals increases and as individual quality (size) increases, and is therefore a commonly used fitness metric in duckweeds (O'Brien et al., 2020). After day 12, 200 µL of the remaining water was measured for optical density at 600 and 420 nm (OD_{600} and OD_{420}) with a BioTek Cytation 5 plate reader. Optical density at 600 nm correlates with cell density in duckweed microbiomes (O'Brien et al., 2020).

Data analysis

To test whether duckweed fitness was highest when duckweeds were paired with both water and microbes from their home site, and whether total microbial growth was highest when microbiomes were paired with both duckweeds and water from their home site, we used linear models. First, the 80 unique treatments were simplified into a matched treatment score based on whether, and if so how many, biological materials came from the same site, polarized by either the duckweed source or the microbe source. When polarizing by the duckweed source, the scores were based on whether the source site for the other materials was the same as the duckweed for: neither the water nor the microbiome (0), either the water or the microbiome (1), or both the water and the microbiome (2). When polarizing by the microbe source, the scores were based on whether the source site for the other materials was the same as the microbiome for: neither the water nor the duckweed (0), either the water or the duckweed (1), or both the water and the duckweed (2).

Statistical analysis was performed using linear models in R (R Core Team, 2021), with package MCMCglmm (Hadfield, 2010). Models were fit for the following equation for each response variable and each polarization (by duckweed or microbe source): $y \sim \alpha + \beta_T T +$

$N_D(0) + N_M(0) + N_W(0)$, where α is the intercept, T refers to the matched treatment score, and each $N(0)$ parameter is a random effect for duckweed, microbe, or water source (D, M, W, respectively). Random effects for each treatment material's source are included as strong effects of material source (e.g. high and low quality environmental matrix, maternal effects, etc.) can interfere with correct interpretation of tests for local adaptation (Blanquart et al., 2013). We also quantified these potential effects by fitting each of duckweed, microbe, and water source as the only effects in separate models for each response variable ($y \sim duckweed$, $y \sim microbe$, and $y \sim water$).

Various studies have found that sites within the same watershed share some commonality in their microbiomes, or other biotic communities (URycki et al., 2022; Comte et al., 2018). Because of this, the microbiomes or duckweed genotypes sampled from the same watershed may be more similar to each other than those from different watersheds. We therefore also fit models including a matched score for how many treatments matched the duckweed or microbe home watershed, again depending on which was used to polarize the match score. We then re-fit all models including this parameter: $y \sim \alpha + \beta_T T + \beta_{T_W} T_w + N_D(0) + N_M(0) + N_W(0)$, where T_w refers to the watershed treatment score.

We further tested the main effect of inoculating with any microbe as opposed to uninoculated (no microbes) treatments for each measured response variable. To force microbially inoculated treatments to be treated the same by the model, we dropped the random effect of source microbes, using model: $y \sim \alpha + \beta_I I + N_D(0) + N_W(0)$, where I indicates the presence of any microbes inoculated onto duckweeds in microcosm wells. Finally, we fit models estimating main effects of duckweed, water, and microbe source singly, each in turn, leaving the other two treatment variables as random effects. E.g. for plant effects this model was $y \sim \alpha +$

$\beta_D D + N_M(0) + N_W(0)$. For all tested models, we report p-values to evaluate whether fitted effects are biologically significant (pMCMC values), and we report means and standard errors to visualize results.

If microbiomes shift the values of some response variables differently from others, they would also be expected to alter the correlations among response variables. Most of our measured response variables were weakly positively correlated to each other experiment-wide (rho = 0.16 to 0.36), except for the two optical density measures, which were very strongly positively correlated (rho = 0.96). For one optical density measure (600 nm), frond greenness, and frond area, we tested if pairwise response correlations (3) were differed in microcosms filled with different water sources ($y \sim \beta_W : x + N_D(0) + N_M(0) + N_W(0)$, for each unique x and y pair of response variables). Similarly, we also tested if pairwise correlations between response variables differed when microcosms were inoculated with different microbiomes (β_M instead of β_W), or when different duckweed genotypes were added to microcosms (β_P instead of β_W).

RESULTS

Duckweed frond area, greenness, and both optical density measures varied substantially across the 80 unique treatments (Figure 2). There were significant differences at $p < 0.05$ for one or more duckweed, water or microbiome sources for most response variables, except that greenness did not differ across duckweed or water source, and neither optical density measure differed across microbiome source (Figure S1). This highlighted the importance of including duckweed, water, and microbiome source site as random effects in other models. Compared to microcosms that were not inoculated with microbiomes, microcosms that were inoculated with microbiomes from any source had higher optical cell density (i.e. because uninoculated wells are

not expected to have any, both $p < 0.05$), had duckweeds with greater frond greenness ($p < 0.05$), and duckweeds that grew marginally faster than uninoculated duckweeds ($p < 0.1$, see Figure S2).

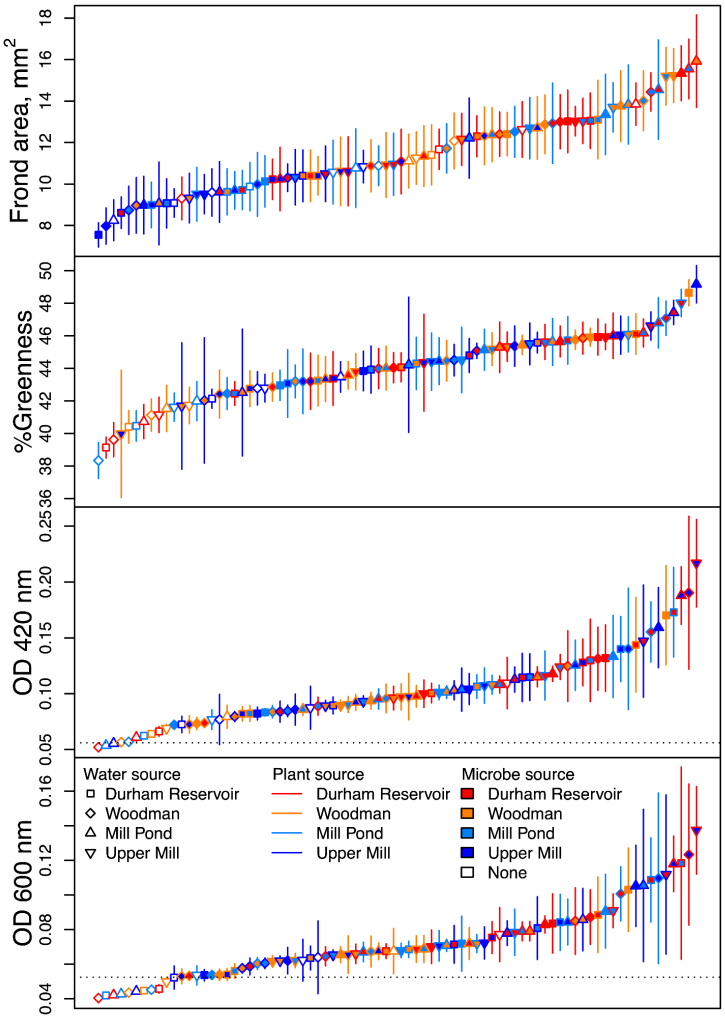

Figure 2: All treatment means for all response variables: duckweed frond area, duckweed frond percent green in pixel RGB values in images, and optical density at 420 nm and 600 nm in media. Points represent the average on day 14 of the experiment for each treatment category, while bars represent the standard error. N=12 for all except for optical density measures from three treatments, where N=11 due to NA values.

Only some of the variation across treatments was predicted by our tests for local adaptation. When treatments were increasingly drawn from the same source site as duckweeds (from 0, 1, or 2 of two additional treatments: microbes and water), microcosms had marginally

more duckweed final area ($p < 0.1$), marginally less microbial growth (optical density at 600 nm, $p < 0.1$), marginally lower optical density at 420 nm ($p < 0.1$), and non-significantly greener duckweed fronds (Figure 3). When treatments were increasingly drawn from the same source site as the microbes (from 0, 1, or 2 of two additional treatments: duckweeds and water), no significant trends in the response variables were detected. However, microcosms trended towards more duckweed final area and greener duckweed fronds when all source sites were the same as the microbe source (all n.s., Figure 3, no trends for optical density).

Our criteria for local adaptation were therefore met only for microbial and duckweed final area, and were only marginally significant. The observed pattern of local adaptation effects suggests that local adaptation between duckweeds and microbes from their source site, and local adaptation of duckweeds to water from their source site together affect frond final area positively, and microbial growth negatively. Adding a numeric score for the number of source materials sharing a watershed did not substantially alter trends, though it did increase the significance of the frond area result, and decrease the significance of the optical density at 420 nm and microbial growth results (Figure S3, watersheds were Pettee Brook for Durham Reservoir and Woodman, and Oyster River for Upper Mill and Mill Pond).

Despite effects of duckweed source, microbiome source, and water source on most response measures, only the correlations between optical density (600 nm) and frond greenness varied across these treatments. The positive correlation between frond greenness and the natural log of optical density at 600 nm was stronger when microcosms were inoculated with microbiomes from Durham Reservoir or Upper Mill than inoculated with microbiomes from the other sites. Frond greenness was also more strongly correlated to the natural log of optical

density at 600 nm when plants from Durham Reservoir or Mill Pond were added, or when microcosms received water from Durham Reservoir (Figure 4).

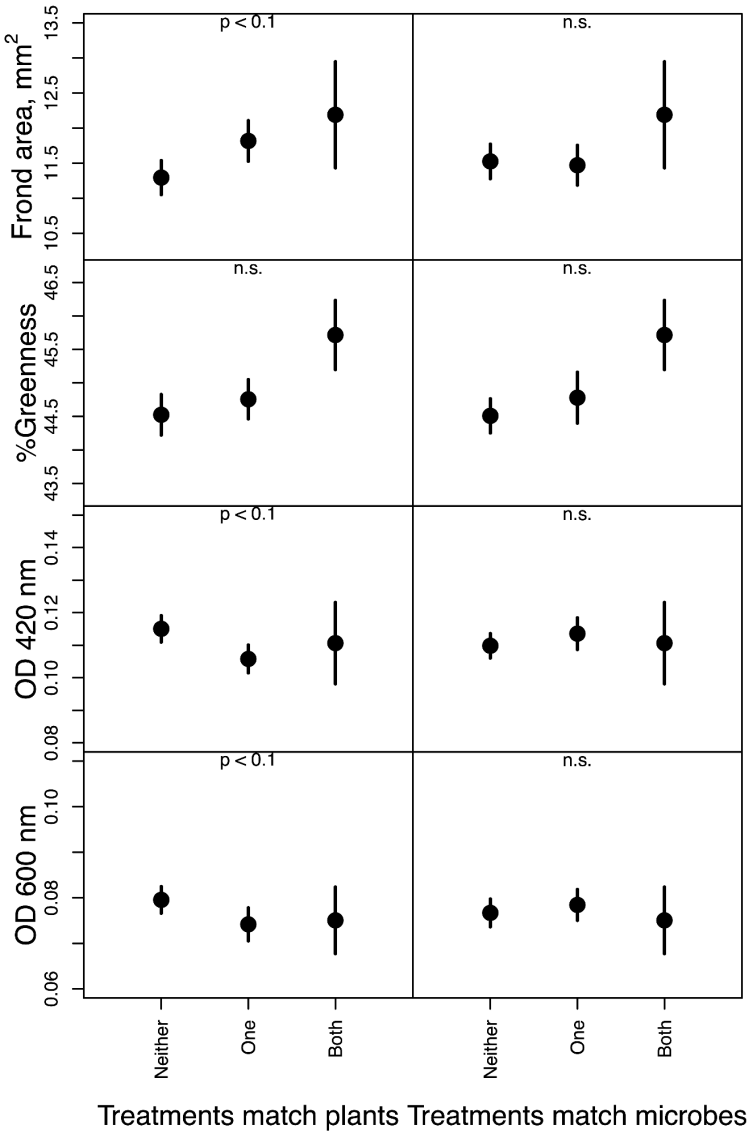

Figure 3: Duckweed frond area, duckweed frond percent green in pixel RGB values in images, and optical density at 420 nm and 600 nm in media. Points represent the average on day 14 of the experiment for each treatment category, while bars represent the standard error. Plots on the left show whether response variables change as other treatments increasingly matched the plant source site, while plots on the left show whether response variables changed as other treatments increasingly matched the microbe source site. "*" indicates that the p-value of the increasing match (slope, β_T) was different from 0 with p <0.05. Optical density measures are proxies for microbial cell density: optical density at 420 nm is expected to be affected by microbes with photosynthetic pigments and potentially fragmented host cells, while optical density at 600 nm is not. N = 432 for neither the water nor microbe source matches the plant source, and neither the

water nor plant source matches the microbe source. N = 288 for either the water or microbe source matches the plant source, and either the water or plant source matches the microbe source. N = 48 for when all three sources match, and is the same data between the left and right columns.

Correlations among response variables were generally very similar across treatments. Positive correlations between frond area and greenness remained significantly positive across all microbe, duckweed, and water source treatments (Figure 4). Positive correlations between frond area and the natural log of optical density at 600 nm were not significant in certain treatments (microbes from Durham Reservoir, duckweeds from Mill Pond, and water from Upper Mill, Figure 4). Positive correlations between frond greenness and the natural log of optical density at 600 nm were similarly insignificant in certain treatments (microbes from Upper Mill, duckweeds from Woodman, and water from Woodman or Upper Mill), but were also significantly different across microbe source, and were stronger in microbes from Durham Reservoir or Upper Mill than in microbes from Mill Pond (Figure 4).

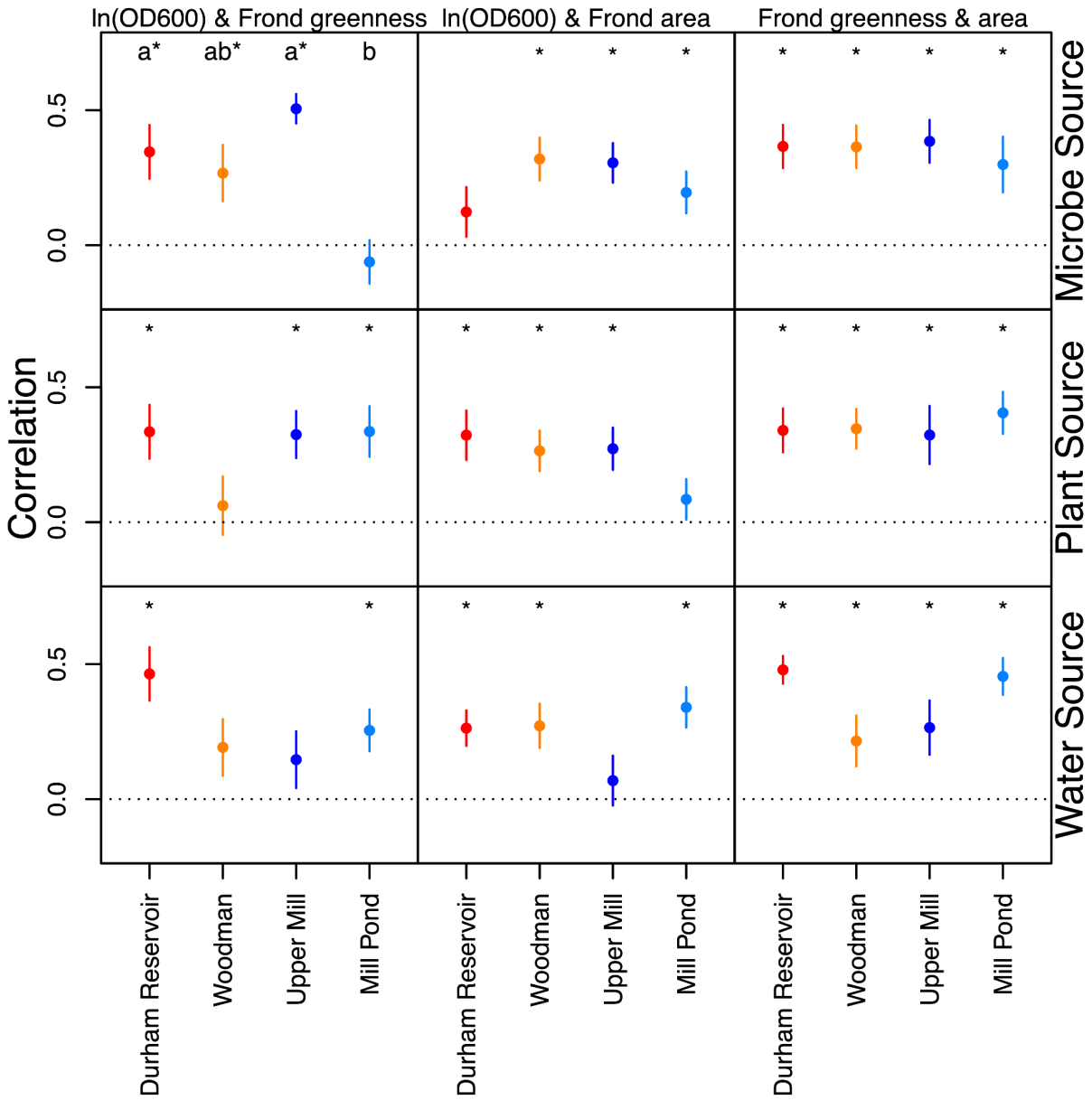

Figure 4: Correlations between three of the four measured response variables (the natural log of optical density at 600 nm, frond greenness, and frond area), averaged by source site for microbes, plants, and water separately. Correlations were calculated within each treatment (inoculated treatments only, 64 treatments, 12 datapoints each), then averaged for microbial, plant and water treatments separately (points, bars are plus or minus one standard error of the mean). "*" indicates that mean was significantly different from zero in linear model results, and letters (if any) indicate significant differences in correlations between measured response variables across treatments.

REFERENCES

Acosta, K., Appenroth, K. J., Borisjuk, L., Edelman, M., Heinig, U., Jansen, M. A., ... & Lam, E. (2021). Return of the Lemnaceae: Duckweed as a model plant system in the genomics and postgenomics era. *The Plant Cell*, *33*(10), 3207-3234.

Anneberg, T. J., Turcotte, M. M., & Ashman, T. L. (2023). Plant neopolyploidy and genetic background differentiate the microbiome of duckweed across a variety of natural freshwater sources. Molecular Ecology, 32(21), 5849-5863.

Batstone, R. T., O'Brien, A. M., Harrison, T. L., & Frederickson, M. E. (2020). Experimental evolution makes microbes more cooperative with their local host genotype. *Science*, *370*(6515), 476-478.

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., ... & Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*, 1-22.

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology letters*, *16*(9), 1195-1205.

Briscoe Runquist, R. D., Gorton, A. J., Yoder, J. B., Deacon, N. J., Grossman, J. J., Kothari, S., ... & Moeller, D. A. (2020). Context dependence of local adaptation to abiotic and biotic environments: a quantitative and qualitative synthesis. *The American Naturalist*, *195*(3), 412-431.

Clark, R. L., Connors, B. M., Stevenson, D. M., Hromada, S. E., Hamilton, J. J., Amador-Noguez, D., & Venturelli, O. S. (2021). Design of synthetic human gut microbiome assembly and butyrate production. *Nature communications*, *12*(1), 3254.

Comte, J., Culley, A. I., Lovejoy, C., & Vincent, W. F. (2018). Microbial connectivity and sorting in a High Arctic watershed. *The ISME journal*, *12*(12), 2988-3000.

Cox Jr, K. L., Manchego, J., Meyers, B. C., Czymmek, K. J., & Harkess, A. (2022). Automated imaging of duckweed growth and development. *Plant Direct*, *6*(9), e439.

Gould, A. L., Zhang, V., Lamberti, L., Jones, E. W., Obadia, B., Korasidis, N., ... & Ludington, W. B. (2018). Microbiome interactions shape host fitness. *Proceedings of the National Academy of Sciences*, *115*(51), E11951-E11960.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of statistical software*, *33*, 1-22. URL https://www.jstatsoft.org/v33/i02/ .

Hargreaves, A. L., Germain, R. M., Bontrager, M., Persi, J., & Angert, A. L. (2020). Local adaptation to biotic interactions: a meta-analysis across latitudes. *The American Naturalist*, *195*(3), 395-411.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist*, *173*(5), 579-588.

Ho, K. H. E. (2018). *The effects of asexuality and selfing on genetic diversity, the efficacy of selection and species persistence*. University of Toronto (Canada).

Ishizawa, H., Tada, M., Kuroda, M., Inoue, D., Futamata, H., & Ike, M. (2020). Synthetic bacterial community of duckweed: a simple and stable system to study plant-microbe interactions. Microbes and environments, 35(4), ME20112.

Ishizawa, H., Kuroda, M., Inoue, D., Morikawa, M., & Ike, M. (2020). Community dynamics of duckweed-associated bacteria upon inoculation of plant growth-promoting bacteria. FEMS microbiology ecology, 96(7), fiaa101.

Jacquiod, S., Spor, A., Wei, S., Munkager, V., Bru, D., Sørensen, S. J., ... & Blouin, M. (2022). Artificial selection of stable rhizosphere microbiota leads to heritable plant phenotype changes. *Ecology Letters*, *25*(1), 189-201.

Jewell, M. D., Van Moorsel, S. J., & Bell, G. (2023). Presence of microbiome decreases fitness and modifies phenotype in the aquatic plant Lemna minor. *AoB Plants*, *15*(4), plad026.

Johnson, N. C., Wilson, G. W., Bowker, M. A., Wilson, J. A., & Miller, R. M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences*, *107*(5), 2093-2098.

Kaiko, G. E., & Stappenbeck, T. S. (2014). Host–microbe interactions shaping the gastrointestinal environment. *Trends in immunology*, *35*(11), 538-548.

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology letters*, *7*(12), 1225-1241.

Kose, T., Lins, T. F., Wang, J., O'Brien, A. M., Sinton, D., & Frederickson, M. E. (2023). Accelerated high-throughput imaging and phenotyping system for small organisms. *PLoS One*, *18*(7), e0287739.

Krazčič, B., Slekovec-Golob, M., & Nemec, J. (1995). Promotion of flowering by Mn-EDDHA in the photoperiodically neutral plant Spirodela polyrrhiza (L.) Schleiden. *Journal of Plant Physiology, 147*, 397–400.

Lam, K. N., Alexander, M., & Turnbaugh, P. J. (2019). Precision medicine goes microscopic: engineering the microbiome to improve drug outcomes. *Cell host & microbe*, *26*(1), 22-34.

Laurich, J., & Carlson, C. (2023). Lemnaceae (Duckweed) culturing protocol.

Leducq, J. B., Charron, G., Samani, P., Dubé, A. K., Sylvester, K., James, B., ... & Landry, C. R. (2014). Local climatic adaptation in a widespread microorganism. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1777), 20132472.

Lu, T., Ke, M., Lavoie, M., Jin, Y., Fan, X., Zhang, Z., ... & Zhu, Y. G. (2018). Rhizosphere microorganisms can influence the timing of plant flowering. *Microbiome*, *6*, 1-12.

McGinnis, M. V. (1999). Making the watershed connection. *Policy Studies Journal*, *27*(3), 497.

McKay, S. K., Cooper, A. R., Diebel, M. W., Elkins, D., Oldford, G., Roghair, C., & Wieferich, D. (2017). Informing watershed connectivity barrier prioritization decisions: a synthesis. *River research and Applications*, *33*(6), 847-862.

Mejbel, H. S., & Simons, A. M. (2018). Aberrant clones: Birth order generates life history diversity in Greater Duckweed, Spirodela polyrhiza. *Ecology and Evolution*, *8*(4), 2021-2031.

Mondot, S., De Wouters, T., Doré, J., & Lepage, P. (2013). The human gut microbiome and its dysfunctions. *Digestive Diseases*, *31*(3-4), 278-285.

Mueller, U. G., & Linksvayer, T. A. (2022). Microbiome breeding: conceptual and practical issues. *Trends in microbiology*, *30*(10), 997-1011.

Najafi, S., Dragovich, D., Heckmann, T., & Sadeghi, S. H. (2021). Sediment connectivity concepts and approaches. *Catena*, *196*, 104880.

O'Brien, A. M., Yu, Z. H., Luo, D. Y., Laurich, J., Passeport, E., & Frederickson, M. E. (2020). Resilience to multiple stressors in an aquatic plant and its microbiome. *American Journal of Botany*, *107*(2), 273-285.

O’Brien, A. M., Sawers, R. J., Gasca-Pineda, J., Baxter, I., Eguiarte, L. E., Ross-Ibarra, J., & Strauss, S. Y. (2024). Teosinte populations exhibit weak local adaptation to their

rhizosphere biota despite strong effects of biota source on teosinte fitness and traits. *Evolution*, *78*(12), 1991-2005.

O'Brien, A. M., Ginnan, N. A., Rebolleda-Gómez, M., & Wagner, M. R. (2021). Microbial effects on plant phenology and fitness. *American Journal of Botany*, *108*(10), 1824-1837.

O'Brien, A. M., Laurich, J. R., & Frederickson, M. E. (2024). Evolutionary consequences of microbiomes for hosts: impacts on host fitness, traits, and heritability. *Evolution, 78*(2), 237-252.

O'Brien, A. M., Lins, T. F., Yang, Y., Frederickson, M. E., Sinton, D., & Rochman, C. M. (2022). Microplastics shift impacts of climate change on a plant-microbe mutualism: Temperature, CO2, and tire wear particles. *Environmental Research*, *203*, 111727.

Qiu, Z., Egidi, E., Liu, H., Kaur, S., & Singh, B. K. (2019). New frontiers in agriculture productivity: optimized microbial inoculants and in situ microbiome engineering. *Biotechnology advances*, *37*(6), 107371.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rúa, M. A., Antoninka, A., Antunes, P. M., Chaudhary, V. B., Gehring, C., Lamit, L. J., …& Hoeksema, J. D. (2016). Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. *BMC Evolutionary Biology*, *16*, 1-15.

Schupack, D. A., Mars, R. A., Voelker, D. H., Abeykoon, J. P., & Kashyap, P. C. (2022). The promise of the gut microbiome as part of individualized treatment strategies. *Nature reviews Gastroenterology & hepatology*, *19*(1), 7-25.

Subbaraman, B., de Lange, O., Ferguson, S., & Peek, N. (2024). The Duckbot: A system for automated imaging and manipulation of duckweed. *Plos one*, *19*(1), e0296717.

Trivedi, P., Batista, B. D., Bazany, K. E., & Singh, B. K. (2022). Plant–microbiome interactions under a changing world: responses, consequences and perspectives. *New Phytologist*, *234*(6), 1951-1959.

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell host & microbe*, *3*(4), 213-223.

URycki, D. R., Bassiouni, M., Good, S. P., Crump, B. C., & Li, B. (2022). The streamwater microbiome encodes hydrologic data across scales. *Science of The Total Environment*, *849*, 157911.

Wei, N., & Tan, J. (2023). Environment and host genetics influence the biogeography of plant microbiome structure. *Microbial Ecology*, *86*(4), 2858-2868.

Zhang, Y., Hu, Y., Yang, B., Ma, F., Lu, P., Li, L., … & Chen, S. (2010). Duckweed (Lemna minor) as a model plant system for the study of human microbial pathogenesis. *PLoS One, 5*(10), e13527.

SUPPLEMENTARY MATERIALS:

Watershed	Site name	Latitude	Longitude
Pettee Brook	Durham Reservoir	43.1476	-70.9441
Pettee Brook	Woodman Road	43.1374	70.9192
Oyster River	Mill Pond	43.1308	-70.9215
Oyster River	Upper Mill	43.1225	-70.9253

Table S1: Geographic coordinates for sampled sites, and their respective watersheds.

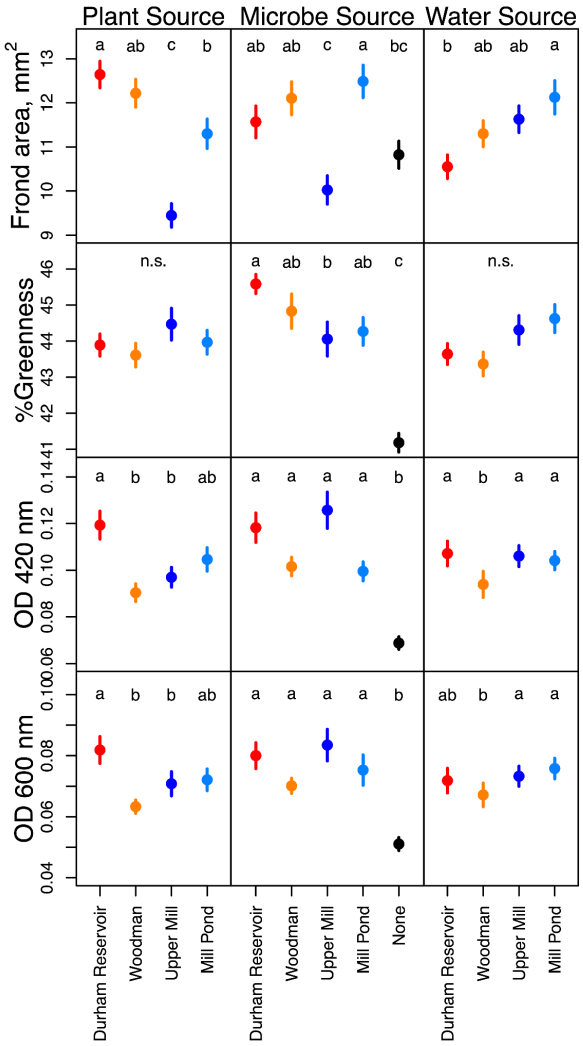

Figure S1: Means for each treatment, averaged across the others for all response variables: duckweed frond area, duckweed frond percent green in pixel RGB values in images, and optical density at 420 nm and 600 nm in media. Points represent the average on day 14 of the experiment for each treatment category, while bars represent the standard error. Each plant and water treatment has N=240; each microbe treatment has N = 192, except for optical density measures, where there were 3 NA values.

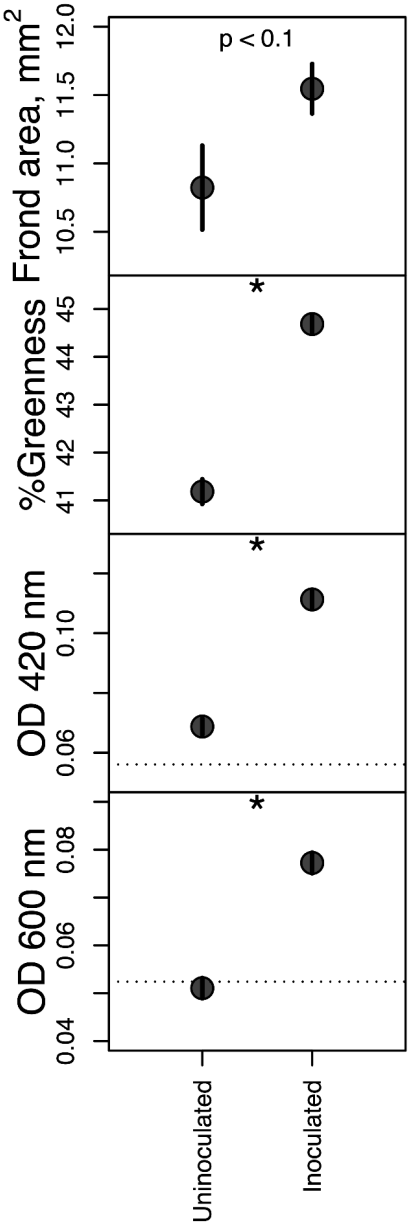

Figure S2: Duckweed frond area, duckweed frond percent green in pixel RGB values in images, and optical density at 420 nm and 600 nm in media, separated by whether microcosms were inoculated with microbes from any source, or received no microbes (uninoculated). Points represent the average on day 14 of the experiment for each treatment category, while bars represent the standard error. "*" indicates that the p-value of inoculation effect (slope, β_1) was different from 0 with $p < 0.05$. Optical density measures are proxies for microbial cell density: optical density at 420 nm is expected to be affected by microbes with photosynthetic pigments, and/or fragmented duckweed cells, while optical density at 600 nm is not. N = 768 for inoculated microcosms, and N = 192 for uninoculated microcosms.

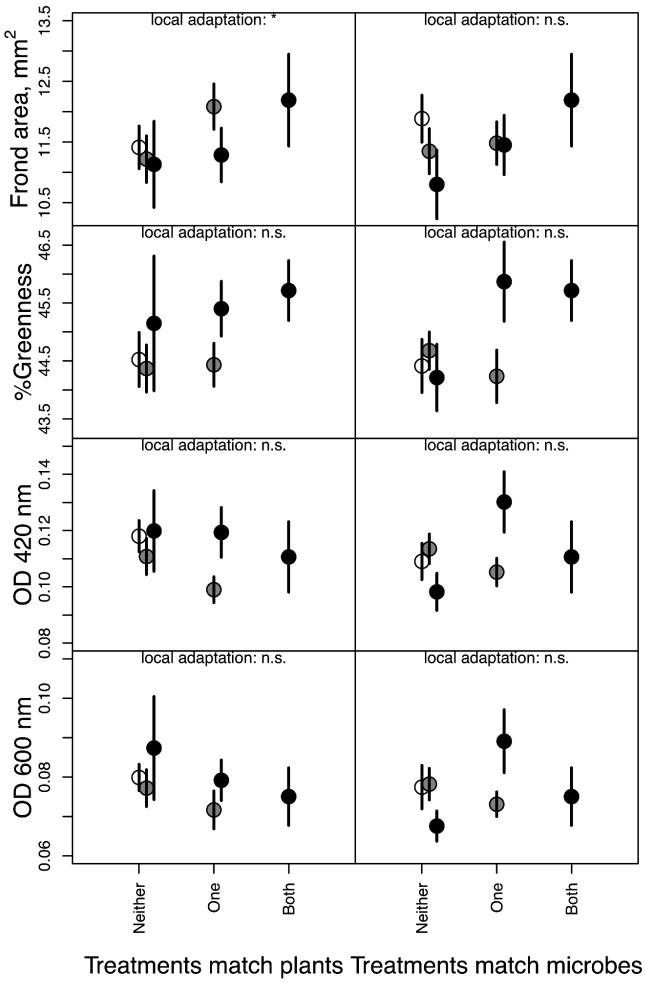

Figure S2: Data as in Figure 3, except that means and standard errors are further split by whether the watershed of the focal treatment (duckweeds on the left, microbes on the right) matches the watershed of neither of the other treatments (unfilled circles), one of the other treatments (grey circles), or all of the other treatments (filled circles). "*" indicates that the p-value of the increasing match (slope, β_T) was different from 0 with p <0.05. Effects of watershed match were never significant (β_{T_W}). Of the N = 432 for neither of the other two sources matches the focal treatment's source, 192 and 48 had one or two treatments' source sites in the same watershed as the focal treatment's source, respectively. Of the N = 288 for when one of the other treatmnt's source sites matches the focal treatment source, 96 had both of these sites from the same watershed as the focal treatment's source. N = 48 for when all three sources match, and is the same data between the left and right columns, and in Figure 3.