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ORIGINAL RESEARCH

Intraspecific variation in leaf litter alters fitness metrics and the gut microbiome of consumers

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Abstract

Biodiversity can have cascading effects throughout ecosystems. While these effects are better understood at coarser taxonomic scales of biodiversity, there has been a resurgence in investigating how biodiversity within species may have cascading effects on communities and ecosystems. We investigate the broader trophic implications of intraspecific variation in the riparian tree, *Alnus rubra*, where immediately local or 'home' litter decomposes faster than 'away' litter in aquatic and terrestrial systems. With climate change shifting the distributions of plants across the globe, it is essential to understand how shifts in the intraspecific traits of leaf litter may have reverberating effects throughout ecosystems. Here, we find that intraspecific variation in leaf litter has fitness implications for invertebrate consumers, including the algivorous *Dicosmoecus* and detrivorous *Psychoglypha* caddisflies, which exhibited increased body size and muscle nitrogen content when incubated within in-situ river mesocosms supplied with local *A. rubra* litter. Litter source altered caddisfly gut microbiomes by increasing relative abundance of methanogens and methanotrophs among the non-local treatment group. Additionally, *Dicosmoecus* supplied with non-local litter may have shifted their diet towards a higher proportion of algae, as inferred from shifts in gut microbiome composition and isotopic ratios of muscle tissue. Overall, our study demonstrates that shifting distributions of plant genotypes across the globe may cause plant–microbe mismatches that will disrupt patterns of decomposition and may have consequences on the fitness and foraging behavior of consumers.

Keywords Cross-ecosystem subsidies · Trophic interactions · Microbiome · *Alnus* · Caddisflies

Introduction

Biodiversity at varying taxonomic scales can have broad ecosystem effects (Whitham et al. 2006). Emerging evidence suggest that even intraspecific variation within plants can have wide ranging implications on communities and ecosystems (Madritch and Hunter 2002; Schweitzer et al. 2005; Crutsinger et al. 2006). Leaf litter decomposition is a key ecosystem function, with broader implications on global carbon cycles (Cebrian 1999). Decomposition processes are regulated by litter quality, including composition of nutrients, secondary defense compounds, and structural components such as lignin (Klotzbücher et al. 2011; Mithöfer and Boland 2012; Jackrel and Morton 2018). While certain components of litter quality have wide-spread value to decomposers, such as high N-content, quality can be highly dependent on the availability of resources in an environment and the metabolic capacities of a decomposer community. A notable example of this context dependency in litter decomposition has become known as the home-field advantage (HFA) (Gholz et al. 2000).

In this phenomenon, which must be demonstrated using reciprocal transplant experiments of leaf litter, decomposer communities are able to breakdown locally derived or 'home' leaf litter more rapidly than non-local or 'away' litter. The HFA may be driven in part by host-associated microbes inhabiting senescent leaf litter as well as free-living microbes residing in soil and aquatic environments that receive leaf litter fall (Veen et al. 2019). The HFA appears widespread in forest ecosystems in Europe and the Americas (Ayres et al. 2009; Austin et al. 2014). In addition to studies that have documented accelerated decomposition of leaves

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originating from local versus non-local tree species, several studies have also demonstrated accelerated decomposition of litter from local tree genotypes (Lecerf and Chauvet 2008; Madritch and Lindroth 2011), including a sizable HFA among genotypes growing less than 1 km apart (Jackrel and Wootton 2014). Whether such spatial variation in litter quality at the intraspecific scale may have broader implications on consumer fitness is unknown. One study by Fenoy et al. (2021) investigated the effects of intraspecific leaf litter variation on the fitness of aquatic shredder macroinvertebrates, but found little evidence of adaptive phenotype plasticity. Understanding the wider implications of spatially variable decomposition rates, particularly accelerated decomposition of local litter, is particularly timely as the distributions of plant species and genotypes are shifting due to climate change, habitat destruction, and reforestation.

Here, we test for cascading trophic effects of intraspecific variation in leaf litter in freshwater streams of the Pacific Northwest. In this system, we have documented a consistent pattern of the HFA for local genotypes of red alder, *Alnus rubra*, using reciprocal transplant experiments (Jackrel and Wootton 2014; Jackrel et al. 2016, 2019). We therefore evaluated the implications of intraspecific variation in leaf litter on two species of consumers that occupy different trophic positions, including those feeding directly on leaf litter and those that may experience indirect effects of shifts in litter quality. Using in-river mesocosms, we tested larval *Dicosmoecus* caddisflies and larval *Psychoglypha* caddisflies as the predominant grazers and decomposers in the system. *Dicosmoecus* spp. typically graze river stone for periphyton and *Psychoglypha* spp. feed in packs of decaying leaf litter, however, both genera have been frequently observed consuming leaves of *A. rubra* in association with and independent of our experimental leaf pack studies. Caddisflies and other invertebrates are thought to be the predominant cause of leaf mass loss in river ecosystems, followed by fungi and bacteria (Hieber and Gessner 2002).

For each species, we evaluated effects of leaf origin on correlates of organismal fitness, including survival, growth, and nutrient composition of muscle tissue. Furthermore, because invertebrate decomposers, including caddisflies, are aided by microbiota that thrive in their digestive system, we probed the effects of intraspecific variation in leaf litter on caddisfly gut microbiomes. We specifically aimed to evaluate what metabolic pathways might be involved under local versus non-local leaf treatments, and how this in turn may translate into caddisfly fitness. The gut microbiomes of decomposers have been found to play key roles in processing litter in other systems. Recalcitrant structural compounds are often digested with the aid of bacteria residing in the digestive tract, such as methanogens that aid in cellulose breakdown in termites (Ohkuma et al. 1995). Further, variation in diet quality has been shown to affect the composition and function of gut microbial communities, such as shifts in methanogen communities and the quantity of methane emitted in both insects and ruminants (Zhou and Hernandez-Sanabria 2010; Gijzen et al. 1994, 1991). Additionally, microbes within the insect digestive system may assist in detoxification of plant secondary defenses, which according to our prior work, play a key role in driving the HFA patterns previously documented among *A. rubra* genotypes (Hammer and Bowers 2015; Jackrel et al. 2016).

Lastly, we hypothesized that access to local versus non-local leaf litter may shift trophic positions of our target taxa. For example, a shift in leaf litter origin may cause *Dicosmoecus* spp. to shift their diet from predominantly allochthonous to autochthonous primary productivity. We aimed to detect such shifts in diet via the isotopic signatures of animal tissue (Post 2002; Vander Zanden et al. 1997; Wootton 2012b). A second indication of such trophic shifts is a change in gut microbiome composition, such as an increased consumption of bacteria as an alternative food resource. Some aquatic invertebrates when challenged with low-resource environments shift to feeding directly on microbes, such as microbial biofilms on the surface of litter (Fredeen 1964; Grey and Deines 2005; Goedkoop and Johnson 1992).

Methods

Study sites

We deployed in-situ river mesocosms on the South Fork of the Pysht River (48.167°N, 124.157°W) in the Olympic Peninsula of Washington State, USA. We used four deployment sites that each had a riparian zone consisting of early successional forest dominated by *A. rubra* (see Table S1 for additional baseline information about each site). We identified five *A. rubra* individuals growing immediately upstream of each deployment site for a total of 20 trees in total, that would be used for obtaining leaves for the local treatment. We also identified 20 riparian *A. rubra* trees growing along the Sekiu River (48.165°N, 124.245°W), which also has a riparian zone dominated by *A. rubra* and is located 30 km W of the Pysht River. These trees on the Sekiu River were used to source all leaves for the non-local treatment. According to baseline measures of %C, %N, %P, carbon and nitrogen isotopic ratios, and carbon to nutrient ratios of 20 trees collected from across the Sekiu and Pysht Rivers in a prior 2012 study, leaf nutrients did not differ between rivers (analysis of variance models, $p > 0.10$ for all metrics; Jackrel et al. 2016). However, as we have previously reported in detail, the relative abundance of secondary metabolites in *A. rubra,* including ellagitannins and diarylheptanoids, varies significantly across rivers in the Olympic Peninsula (see Jackrel et al. 2016).

Experimental design

In July and August of 2016, we deployed 24 mesocosms that were each constructed from a plastic, lidded container with cut outs in each side lined with 0.64 cm plastic mesh to allow for water flow while retaining caddisflies within the enclosure. To construct a more natural riverbed, we weighed down the floor of each mesocosm with river stone sourced from the immediately adjacent river channel. We applied our leaf treatment to each of these mesocosms by deploying leaf packs of either local or non-local *A. rubra* genotypes. We constructed each leaf pack with ten leaves: two from each of the five local trees immediately upstream of the deployment site, or two from each of five trees on the Sekiu River for the non-local treatment. For these leaf packs, we hand-picked fresh, green leaves with little or no visible damage from herbivores or pathogens. Each river mesocosm received leaves from the same five *A. rubra* trees through the duration of the experiment. We added two leaf packs to each mesocosm and allowed leaves to incubate for seven days prior to adding caddisflies to the mesocosms, which ensured leaves at varying stages of decomposition were immediately available to caddisflies for feeding. We eliminated one mesocosm receiving the local leaf treatment from the study due to damage to the enclosure netting.

To assess the effects of leaf treatment on fitness of animals at varying trophic levels, we enclosed algivorous *Dicosmoecus* caddisflies and detritivorous *Psychoglypha* caddisflies. As juvenile caddisflies are challenging to identify definitively to species, we refer to each by the genus name. Our study likely included individuals of only one of the two species known to occur in Washington State, *D. gilvipes* or *D. atripes*, as co-occurrence of both species is rare. At our study sites, we frequently observed *Dicosmoecus* scraping periphyton from river rock, which is a foraging behavior more characteristic of *D. gilvipes* (Wiggins and Richardson 1982), however, we also frequently observed individuals clinging to the mesh bags of leaf packs and feeding within the mesh openings on the decaying leaves, which is more characteristic of *D. atripes* (Mihuc and Mihuc 1995). Although these individuals may have been scraping microbial biofilms off of decaying leaves, we inferred they were eating the leaf itself because beneath *Dicosmoecus* clinging to the bags were hexagonal-patterned sections of skeletonized leaves that matched the hexagonal mesh netting pattern, suggesting that the *Dicosmoecus*, which are too large to enter the bags, were removing leaf tissue accessible to them through the mesh openings.

At each deployment site, we collected baseline measurements of caddisflies, including body mass, isotopic content of muscle tissue, and composition of the microbiome. We collected 20 *Psychoglypha* spp. and 20 *Dicosmoecus* spp. from across the four sites (i.e., 5 per caddisfly genus per site). Caddisfly larvae were removed from their cases, blotted dry with paper towels and weighed to the nearest milligram. Muscle tissue was excised from caddisflies, oven dried and stored until elemental and isotopic analyses described below. After harvesting muscle tissue, the gut from each caddisfly was removed and stored at – 80 °C at Argonne National Laboratory until DNA extraction for 16S rRNA gut microbiome analysis.

To each mesocosm, we added caddisflies collected locally from each deployment site, including 6 *Dicosmoecus* and 6 *Psychoglypha*. Among the caddisflies we were able to collect at each of the deployment sites, we selected the largest available individuals to reduce likelihood of escape through the mesocosm mesh netting. Due to the hard outer cases of caddisflies, it was not feasible to accurately measure initial body length. Instead, initial and final length and width were measured of caddisfly cases, and at the end of the experiment, larvae were removed from their case and measured using calipers.

At the end of the 27-day enclosure period, recaptured individuals were sealed in individual WhirlPak bags filled with immediately adjacent river water and kept cool during transport to the lab. Recapture rates were recorded. Final body mass, width and length for caddisflies were recorded. Caddisfly case length and width were also recorded. Muscle tissue and the gut from each caddisfly was harvested and stored as described above.

We used the additional caddisflies collected at the same time and locations at the beginning of the experiment to confirm that caddisflies assigned to the two treatment groups did not differ. We found that *Dicosmoecus* case length was significantly predictive of body mass ($R^2 = 0.63$), and *Psychoglypha* case width was significantly predictive of body mass ($R^2 = 0.32$). We found no differences in the length or width of caddisfly cases between leaf treatments for either species at the beginning of the experiment or among the individuals recovered at the end of the experiment (all LMER not significant). Based on our calculation of initial body mass using case dimensions and regression equations, we detected no differences at the beginning of the experiment (LMER: $p = 0.28$) or at the end of the experiment (LMER: $p = 0.36$), indicating that each treatment group received caddisflies of similar initial body mass and that recovered individuals were not size biased. Therefore, while size bias from the beginning of the experiment remains a possibility because it is not possible to obtain an initial body mass without the caddisfly case, the above factors suggest this as unlikely.

In addition to the primary experiment, leaf packs were also deployed to confirm repeatability of the previously documented locally accelerated decomposition pattern in these rivers (Jackrel et al. 2019, 2016; Jackrel and Wootton 2014). We compared decomposition rates of leaf packs containing leaves from local and non-local genotypes of riparian *A.*

rubra. At each of the four sites along the Pysht River, we deployed two local and two non-local leaf packs in the river channel adjacent to the river mesocosms. Further, we used a subset of the leaf packs added to each river mesocosm to determine whether leaf origin influenced decomposition rate when caddisflies were enclosed within the mesocosms. One set of leaf packs that had been added to the mesocosms prior to adding the caddisflies were removed after 16 days to measure percent leaf mass remaining (i.e. days 1–7 of decomposition occurred in mesocosms without caddisflies, and days 8–16 of decomposition occurred in the presence of caddisflies).

Elemental and isotopic analyses

Oven dried muscle tissue collected from caddisflies was ground into a fine powder using a BioSpec Mini-BeadBeater. We packed ~ 1.25 mg of powdered tissue into 3.5 × 5 mm tin capsules. Samples were analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer for % N, %C, $^{15}N/^{14}N$, and $^{13}C/^{12}C$ at the University of California Davis Stable Isotope Facility. The reproducibility was ± 0.08‰ for ^{15}N and 0.16‰ for ^{13}C, with ten reference standards as isotopic controls.

Microbiome analyses

We extracted DNA from gut samples using PowerSoil DNA isolation kits (MoBio Laboratories, Carlsbad, CA, USA). We amplified the 254 base pair V4 region of extracted DNA using the Earth Microbiome Project universal primer 515F, 806 GoLay-barcoded reverse primers (Walters et al. 2016). We sequenced DNA fragments in a MiSeq 2- by 151-bp run at the Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory and analyzed our bacterial sequencing data using the QIIME pipeline (Caporaso et al. 2010, 2012). We classified operational taxonomic units (OTUs) from Illumina reads at the 97% similarity level using open reference-based clustering with uclust. We assigned a taxonomy using the RDP taxonomic assignment comparing the OTU sequences against the Greengenes database, version 13_8, and predicted metabolic function of the assigned communities with the functional annotation tool PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al. 2013).

Statistical analyses

To determine whether the leaf origin treatment significantly affected leaf decomposition and body composition metrics, we used linear mixed effects models with leaf origin as our fixed effect and our blocking factors as our random effects. Caddisfly genus was also used as a fixed effect for models assessing body composition. Outliers were removed as necessary to meet model assumptions of normality and homogeneity of variances. Frequency histograms and quantile–quantile plots of all linear mixed effects models are reported in Fig. S1.

Microbial diversity, richness, and evenness metrics were calculated using the phyloseq package in R (McMurdie and Holmes 2013; Team 2000). Microbial community composition differences between treatments were assessed with principal coordinates analyses using the non-phylogenetically based Bray–Curtis distance metric, the relative abundance-weighted phylogenetically-based UNIFRAC distance metric, and the unweighted UNIFRAC distance metric. Distance matrices were calculated using the vegdist function in vegan and the UniFrac function in phyloseq. Beta-dispersion tests in the vegan package were used to confirm homogeneity of dispersion among groups. Adonis tests with caddisfly genus and leaf treatment as fixed effects and an interaction between genus and leaf treatment, as well as pairwise post-hoc comparisons with false discovery rate corrections were completed using the adonis and pairwise.perm.manova functions, respectively in the RVAideMemoire package in *R*.

All microbial relative abundance data were analyzed using the EdgeR package in *R* using standard operating procedures (Robinson et al. 2009). Specifically, raw relative abundance counts were normalized using the calcNormFactors function and read counts for each OTU were fit with quasi-likelihood negative binomial generalized log-linear models using the glmQLFit function. Separate models were fit for *Dicosmoecus* and *Psychoglypha* caddisflies. Within the model for each caddisfly genus, model contrasts of the gut microbiome community were used to compare individuals enclosed in mesocosms receiving the local versus non-local leaf treatment. These contrasts generated log-fold change values of each bacterial OTU in individuals given the local versus non-local leaf treatment. To probe for any functional implications of taxonomic shifts in the gut microbiome, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and the KEGG Orthology (KO) database of molecular functions, which are represented in terms of functional orthologs. Specifically, we tested whether any KEGG metabolic pathways, and KO terms within these pathways, differed significant by leaf treatment group using the PICRUST phylogenetically-based inference tool. These inferences regarding metagenome functions were derived from bacterial taxa within short evolutionary distances, thus yielding more accurate predictions (average nearest sequences taxon index = 0.050) (Langille et al. 2013). To determine whether any of the pathways assigned to "Metabolism" under the KEGG hierarchy differed by leaf treatment, we used linear mixed effects models with

our blocking factors as random effects. Model results were corrected for multiple comparisons via Benjamini–Hochberg false discovery rate (Benjamini and Hochberg 1995). We then filtered our whole gene prediction table by those genes assigned to a specific metabolic pathway. For each of the ten metabolic pathways with the lowest corrected p-values for *Dicosmoecus* and *Psychoglypha*, we tested which genes within these pathways significantly differed by leaf treatment with the same type of linear mixed effect models.

Results

Documentation of intraspecific variation in leaf litter decomposition

Local leaves decomposed more than non-local *A. rubra* leaves when deployed within the main river channel ($p < 0.001$, $n = 15$, $\mu_{local} = 20.0\%$ leaf mass lost, $\mu_{non\text{-}local} = 3.2\%$) and when deployed within river mesocosms ($n = 22$, $\mu_{local} = 10.4\%$ leaf mass lost, $\mu_{non\text{-}local} = 6.3\%$) (Fig. 1). Without a full reciprocal transplant, this does not verify the HFA pattern that has been repeatedly documented in this system, however, these results are consistent with HFA patterns. Non-local leaf packs were greater in initial mass than local leaf packs, resulting in mesocosms assigned the non-local treatment to contain greater initial leaf mass than mesocosms assigned the local leaf pack treatment (Sekiu River leaf packs = 12.2 ± 0.328 SE, Pysht River leaf packs = 9.9 ± 0.332 SE, ANOVA $F_{1,20} = 8.4$, $p = 0.009$). However, additional leaf packs were added throughout the experiment so that leaves were never limited in quantity in any mesocosm.

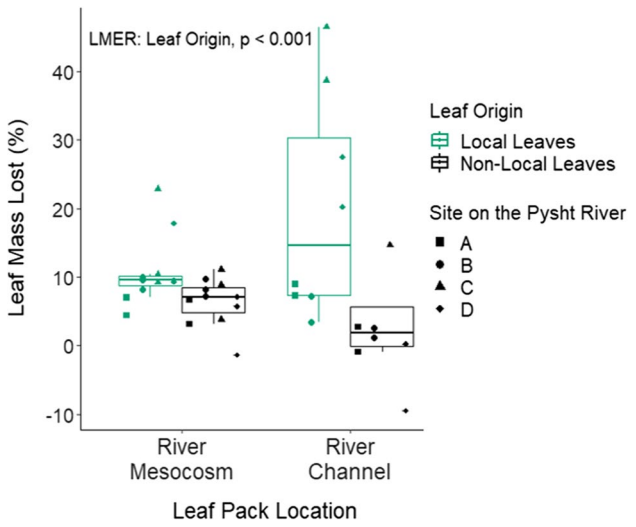


Fig. 1 Locally derived *A. rubra* leaves from the riparian zone of the Pysht River, WA decomposed more over a 16-day incubation period than non-locally derived *A. rubra* leaves from the riparian zone of the Sekiu River, WA. This pattern was evident both in leaf packs deployed in the main river channel ($n = 15$) and within enclosed in-situ river mesocosms containing caddisflies that were deployed at four sites along the Pysht River ($n = 22$). A linear mixed-effects model accounts for blocks nested within each deployment site as a random effects term, and leaf origin and leaf pack location (i.e. either the river channel or mesocosms) as fixed effects

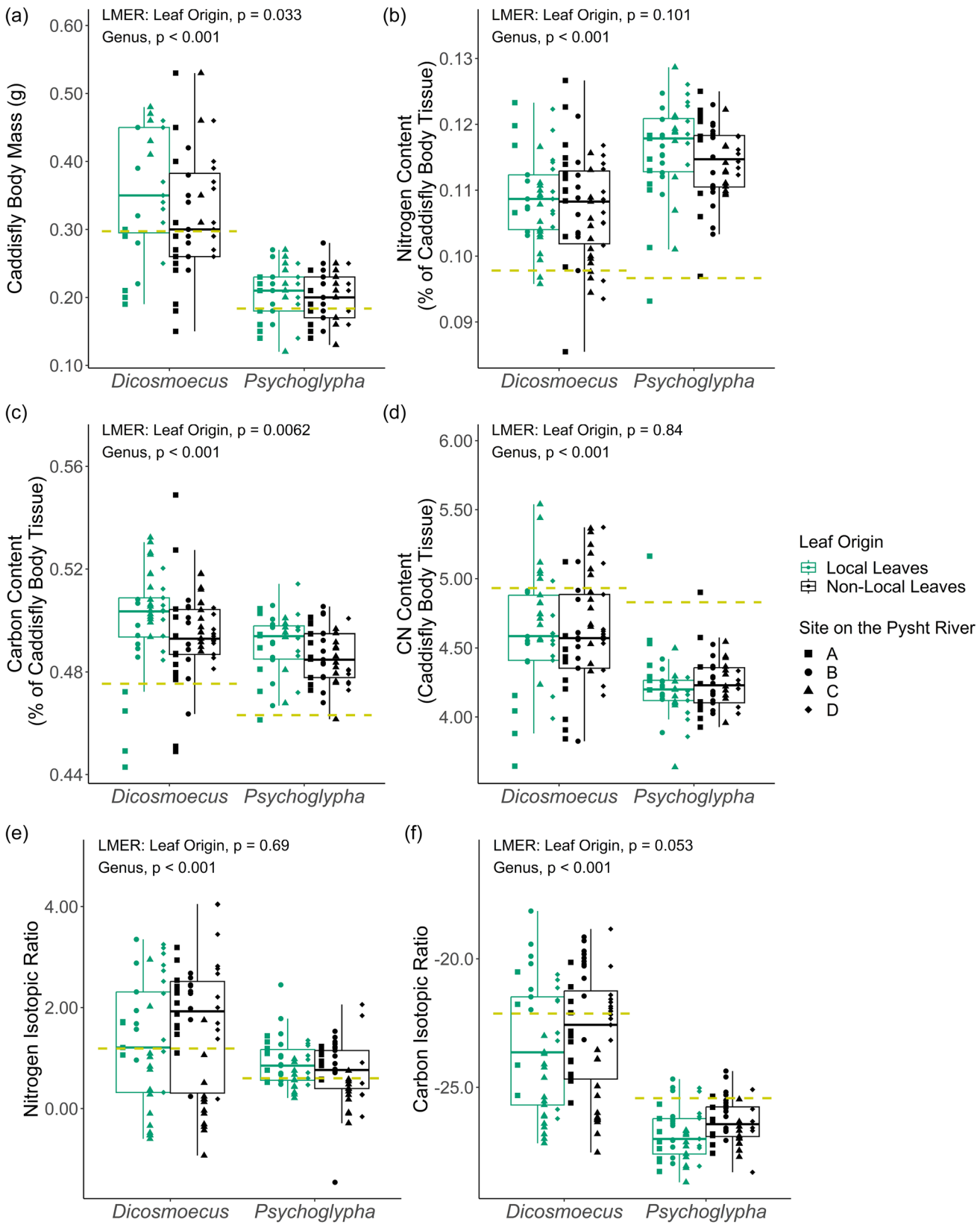
Intraspecific variation in leaf litter effects metric of consumer fitness

Caddisflies residing in mesocosms with the local leaf treatment attained greater body mass than caddisflies receiving the non-local leaf treatment (Fig. 2a, LMER: Leaf Origin $p = 0.033$, $n = 154$; *Dicosmoecus*, Local: 0.357 ± 0.018 SE g, Non-local: 0.321 ± 0.014 SE g; *Psychoglypha*, Local: 0.208 ± 0.006 SE g, Non-local 0.199 ± 0.005 SE g). Similar numbers of *Dicosmoecus* from each leaf treatment sealed their cases for metamorphosis (Local: $n = 11$, 29.7%, Non-local: $n = 13$, $n = 24.5\%$). These individuals were excluded from analyses because they would not have been feeding throughout the entirety of the experiment. Recapture rate of caddisflies also did not differ by leaf treatment (LMER: Leaf Origin $p = 0.22$).

Intraspecific variation in leaf litter effects elemental and isotopic composition of consumers

Percent Nitrogen tended to be higher among caddisflies in the Local rather than Non-local leaf litter treatment, particularly for *Psychoglypha* spp. (Fig. 2b, $\Delta = 1.92\%$, $p = 0.101$; Local litter: $\mu = 0.113 \pm 0.00785$; Non-local litter: $\mu = 0.110 \pm 0.00797$). Percent carbon also tended to be higher in caddisflies in the local rather than non-local leaf litter treatment (Fig. 2c, $\Delta = 1.02\%$, $p = 0.0062$; Local litter: $\mu = 0.495 \pm 0.0167$; Non-local litter: $\mu = 0.490 \pm 0.0148$). Due to this increase in both %N and %C among the local treatment, C:N was similar between the local and non-local leaf litter treatment (Fig. 2d, p = 0.84; Local litter: $\mu = 4.41 \pm 0.380$; Non-local litter: $\mu = 4.53 \pm 0.363$). N^{15}/N^{14} and C^{13}/C^{12} isotopic ratios did not statistically differ by treatment groups, but averaged higher among the non-local treatment group (Fig. 2e, N^{15}/N^{14}: $p = 0.69$; Local litter: $\mu = 1.116 \pm 0.920$; Non-local litter: $\mu = 1.231 \pm 1.128$. Figure 2f, C^{13}/C^{12}: p = 0.053; Local litter: $\mu = -25.228 \pm 2.516$; Non-local litter: -24.493 ± 2.513).

Body composition differed significantly between genera of caddisfly. Percent Nitrogen of muscle tissue differed between genera of caddisflies (Fig. 2b, $\Delta = 6.92\%$, $p < 0.001$; *Dicosmoecus*: $\mu = 0.108 \pm 0.00748$, $n = 87$; *Psychoglypha*: $\mu = 0.115 \pm 0.00658$, $n = 83$). Percent Carbon in the muscle tissue of caddisflies differed significantly between genera (Fig. 2c, $\Delta = 1.81\%$, $p < 0.001$; *Dicosmoecus*:


(a)
LMER: Leaf Origin, p = 0.033
Genus, p < 0.001
Caddisfly Body Mass (g)
0.60
0.50
0.40
0.30
0.20
0.10
Dicosmoecus
Psychoglypha
(b)
LMER: Leaf Origin, p = 0.101
Genus, p < 0.001
Nitrogen Content (% of Caddisfly Body Tissue)
0.13
0.12
0.11
0.10
0.09
Dicosmoecus
Psychoglypha
(c)
LMER: Leaf Origin, p = 0.0062
Genus, p < 0.001
Carbon Content (% of Caddisfly Body Tissue)
0.56
0.52
0.48
0.44
Dicosmoecus
Psychoglypha
(d)
LMER: Leaf Origin, p = 0.84
Genus, p < 0.001
CN Content (Caddisfly Body Tissue)
6.00
5.50
5.00
4.50
4.00
Dicosmoecus
Psychoglypha
Leaf Origin
Local Leaves
Non-Local Leaves
Site on the Pysht River
A
B
C
D
(e)
LMER: Leaf Origin, p = 0.69
Genus, p < 0.001
Nitrogen Isotopic Ratio
4.00
2.00
0.00
Dicosmoecus
Psychoglypha
(f)
LMER: Leaf Origin, p = 0.053
Genus, p < 0.001
Carbon Isotopic Ratio
-20.0
-25.0
Dicosmoecus
Psychoglypha

◂**Fig. 2** *Dicosmoecus* and *Psychoglypha* caddisflies that were supplied with locally derived *A. rubra* leaves within in-situ river mesocosms within the Pysht River, WA versus caddisflies supplied with non-locally derived *A. rubra* leaves from the riparian zone of the Sekiu River, WA, attained **A** greater body mass, and **B** tended to have a higher % N content and **C** higher % C content of their muscle tissue. Muscle tissue of caddisflies supplied with local versus non-local leaves tended to have similar **D** C:N, as well as **E** $^{15}N/^{14}N$ and **F** $^{13}C/^{12}C$ isotopic ratios. Linear mixed-effects models account for blocks nested within each deployment site as a random effects term, and leaf origin and caddisfly genus as fixed effects. Total sample size was 170 caddisflies. Yellow dashed lines indicate initial baseline measures of these metrics prior to the start of the mesocosm study (see Fig. S2 for illustrations of these data)

$\mu = 0.497 \pm 0.0180$; *Psychoglypha*: $\mu = 0.488 \pm 0.0113$). The C:N ratio, as well as the isotopic ratios of N^{15}/N^{14} and C^{13}/C^{12}, also all differed significantly between genera (Fig. 2d C:N $\Delta = 9.44\%$, *Dicosmoecus*: $\mu = 4.66 \pm 0.489$, *Psychoglypha*: $\mu = 4.22 \pm 0.213$; Fig. 2e, N^{15}/N^{14} $\Delta = 91.6\%$, *Dicosmoecus*: $\mu = 1.54 \pm 1.26$, *Psychoglypha*: $\mu = 0.806 \pm 0.540$; Fig. 2f, C^{13}/C^{12} $\Delta = 13.1\%$, *Dicosmoecus*: $\mu = -23.1 \pm 2.41$, *Psychoglypha*: $\mu = -26.6 \pm 0.944$; all p-values < 0.001). Initial conditions for each of these metrics are summarized in Fig. S2.

Grazers and decomposers have a core gut microbiome

To test for the occurrence of a core microbiome, we used individuals collected during our initial surveys that would not have experienced a shift in the microbiome due to experimental treatment. Both *Dicosmoecus* and *Psychoglypha* caddisflies had a core microbiome, in which $88.7\% \pm 1.1$ and $86.6\% \pm 3.7$ of bacterial sequences belonged to core OTUs, which were defined as those OTUs occurring in the majority of congeners analyzed. Caddisfly gut microbiomes were dominated in relative abundance by several taxonomic groups: the Enterobacteriaceae family of γ-Proteobacteria ($\mu = 41.1 \pm 2.6\%$ SE in *Dicosmoecus*, $52.7 \pm 2.2\%$ in *Psychoglypha*), the Clostridiales and Lactobacillales orders of the Firmicutes ($14.9 \pm 1.4\%$ in *Dicosmoecus*, $19.7\% \pm 1.4\%$ in *Psychoglypha*, and $6.4 \pm 0.8\%$ in *Dicosmoecus*, $9.5 \pm 0.8\%$ in *Psychoglypha*), and the Rhizobiales order of α-Proteobacteria ($8.2 \pm 0.9\%$ in *Dicosmoecus*, $1.4 \pm 0.2\%$ in *Psychoglypha*) (Table S2). In initial surveys of the two caddisfly genera prior to the mesocosm experiment, both genera were similar in Shannon's diversity and Pileou's evenness, but differed slightly in taxonomic richness (Fig. S3). The two genera differed in gut microbiome community composition using either the non-phylogenetic β-diversity metric Bray–Curtis, or the phylogenetic metrics of abundance-weighted UNIFRAC, and unweighted UNIFRAC metrics (Fig. 3, adonis pairwise post-hoc tests, $p < 0.01$).

Intraspecific variation in leaf litter alters the gut microbiome

Among individuals recaptured at the end of the mesocosm experiment, caddisflies given the non-local leaf treatment, particularly *Dicosmoecus*, exhibited significantly greater Shannon's diversity, Pileou's Evenness, and taxonomic richness (Fig. S4). Further, although caddisfly size was not predictive of diversity metrics in our initial pre-experiment survey (Fig. S3), larger caddisflies in the mesocosms tended to harbor gut microbiomes with lower diversity and evenness (Fig. S4). *Dicosmoecus* receiving the non-local leaf treatment harbored a significantly different microbiome compared to *Dicosmoecus* receiving the local leaf treatment (Fig. 3, $p < 0.01$ for adonis pairwise post-hoc test using each of the three distance metrics). *Psychoglypha* exhibited weaker differences by leaf treatment ($p = 0.042$, 0.079, and 0.14 for Bray–Curtis, abundance weighted UNIFRAC and unweighted UNIFRAC respectively).

In addition to community-wide metrics, caddisflies receiving the two leaf treatments were significantly over and under-represented in numerous taxa (Fig. 4, Table S3). Gut microbiomes of *Dicosmoecus* receiving the non-local leaf treatment were over-represented in certain OTUs of the Rhizobiales of the α-Proteobacteria, Lactobacillales and Clostridiales of the Firmicutes, and Enterobacteriales of the γ-Proteobacteria. Similarly, *Psychoglypha* receiving the non-local leaf treatment were over-represented in certain OTUs of the Clostridiales and Enterobacteriales, as well as Bacteroidetes. *Dicosmoecus* receiving the non-local treatment were under-represented by OTUs in Bifidobacterium of the Actinobacteria and Dysgonomonas spp. of the Bacteroidetes, while *Psychoglypha* were under-represented in a diversity of Firmicutes and Proteobacteria.

Among *Dicosmoecus*, predicted gene content in the methane metabolism pathway was significantly elevated in the non-local leaf treatment group (Table S4). Increased methane metabolism within the non-local treatment group of *Dicosmoecus* was supported by significantly increased relative abundances of methanotrophic taxonomic groups (Fig. 5). Note that methanotrophs did not appear in the analyses illustrated in Fig. 4 because methanotrophs were often absent entirely from the local leaf treatment group, whereas differential abundance analyses requires occurrence in both groups being compared. Additional pathways that differed by leaf treatment in *Dicosmoecus* were broad, but suggestive of pathways related to aromatic plant secondary metabolites, including pathways for certain categories of plant secondary metabolites (including terpenoids and polyketides), enzymes catalyzing the biosynthesis of aromatic amino acids (see K03856) that form the building blocks of secondary metabolites, and enzymes that may degrade aromatic rings (see K00074 and K00626) (Table S4). Further suggestive

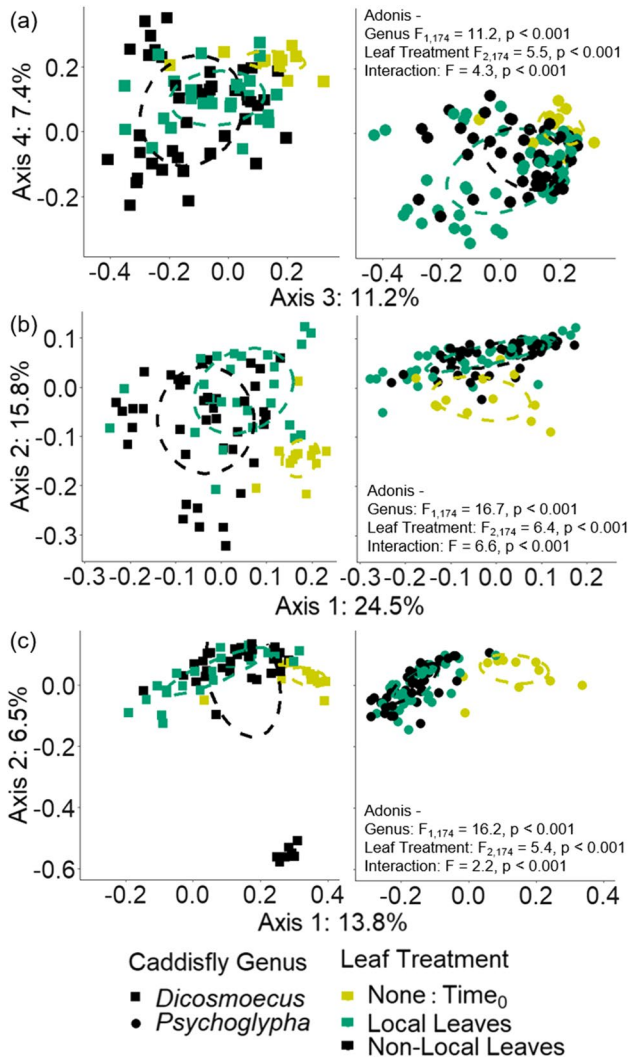


Fig. 3 Gut microbiome composition of caddisflies enclosed within in-situ river mesocosms deployed at the Pysht River. Axes list taxonomic differences in microbial community composition in terms of (**A**) Bray–Curtis distances, (**B**) abundance-weighted UNIFRAC distances, and (**C**) abundance-unweighted UNIFRAC distances from principal coordinate analyses. Significance of separation by caddisfly genus and leaf treatment were determined using analysis of variance on distance matrices (adonis), as well as adonis pairwise post-hoc tests, and visualized with 50% confidence ellipses. The assumption of homogeneity of dispersion for adonis tests were met, except for the abundance-unweighted UNIFRAC metric ($p=0.022$). *Dicosmoecus* ($n=78$) and *Psychoglypha* spp. ($n=97$) depicted in separate panels (left and right, respectively) to minimize overlapping data points

that *A. rubra* aromatic secondary metabolites may influence gut microbiome communities was the predicted higher proportion of the enzyme caffeoyl-CoA O-methyltransferase in *Dicosmoecus* given the non-local versus local leaf treatment. This enzyme is key to the biosynthesis of diarylheptanoids, a group of aromatic secondary metabolites known in *A. rubra* to be abundant, structurally diverse, and spatially variable. Among *Psychoglypha*, PICRUST results suggested that the gut microbiomes of individuals differed by leaf treatment in gene content corresponding to Vitamin B6 and lipopolysaccharide biosynthesis (Table S5).

Methanogens and methanotrophs were greater in relative abundance in *Dicosmoecus* given the non-local leaf treatment but still rare overall. The most abundant families and genera of methanotrophs were Beijerinckiaceae (average occurrence of most common OTU: average 0.45%, maximum 3.85%), Methylocystaceae (avg. 0.10%, max. 1.20%), and Crenothrix (avg. 0.022, max. 0.22%). The most abundant genera of methanogens were Methanobacterium (avg. 0.020%, max. 0.22%), Methanobrevibacter (avg. 0.0045%, max. 0.18%), Methanoregula (avg. 0.0086%, max. 0.11%), Methanosarcina (avg. 0.0055%, max. 0.091%), and Methanolinea (avg. 0.0054%, max. 0.091%).

One group of individuals within *Dicosmoecus* from the non-local leaf treatment group was notably divergent in their gut microbiome. This group consisted of 7 individuals used in the reported analyses, as well as 4 additional individuals that were removed due to their stone cases being closed. These 11 individuals had Shannon's Diversity, Pileou's Evenness and taxon richness notably higher than all others (Fig. S3). Their gut microbiome communities were distinct enough to form their own group in PCoA analyses (Fig. 3), and were particularly enriched in methanogenic and methanotrophic bacteria (Fig. 5). This group does not appear to be an artifact of the experiment design as these individuals occurred in 7 different mesocosms located across 3 of the 4 deployment sites.

Discussion

While accelerated decomposition of local leaf litter has been documented in numerous ecosystems, few studies have investigated the broader consequences of this phenomenon. Our study demonstrates that intraspecific variation in leaf litter has physiological consequences for invertebrate consumers. Using small streams that have previously been shown to exhibit a remarkably strong HFA pattern to individual tree genotypes, our results advance our understanding of the broader fitness consequences for the predominant macroinvertebrate consumers in this system. In addition to suppressed weight gain among these consumers receiving only non-local leaves, we observed a notable shift in their body condition and in their gut microbiomes.

Intraspecific variation in leaf litter effects correlates of fitness in consumers

Consumers inhabiting mesocosms with local leaves gained more mass over the course of the study and attained higher % N content, compared to those inhabiting mesocosms with

Fig. 4 Relative to caddisflies supplied with local *A. rubra* leaves, the gut microbiomes of caddisflies enclosed in in-situ river mesocosms supplied with non-local *A. rubra* leaves were significantly under and over represented in numerous taxa. Positive logFC values indicate taxa significantly over-represented among algivorous *Dicosmoecus* ($n=66$) or detrivorous *Psychoglypha* ($n=85$) supplied with the non-local leaf treatment relative to those supplied with the local leaf treatment. Negative logFC values indicate taxa significantly under-represented in the non-local treatment group. Differential abundances calculated in edgeR with significance reported at $p<0.05$ after false-discovery rate correction. Relative abundance in the over-represented group indicated by point diameter (see Table S3 for relative abundances in each group)

non-local leaves. These treatment effects on body condition may have resulted from (a) feeding directly on the local versus non-local leaves, (b) feeding on the microbes inhabiting these leaves, or (c) a dietary shift. For example, the physiology of the caddisflies themselves or their gut microbiomes as discussed further below, may have affected how efficiently caddisflies could convert local versus non-local leaves into muscle tissue. Alternatively, free-living microbes could have also affected caddisfly fitness. While all leaves enter the detrital pool with a host-associated microbial community, in soil systems, it has been found that local leaves generally acquire a community of free-living microbes more rapidly and harbor higher densities of microbes (Altaf and Rashid 2021). Whether this pattern holds in aquatic systems is not yet known, however, it has been found that most actively growing microbes inhabiting leaf litter in riverine systems colonized from the water column rather than entering the water in association with leaf litter (Hayer et al. 2022). Therefore, caddisflies supplied local leaves may have had the opportunity to consume a larger quantity of microbial

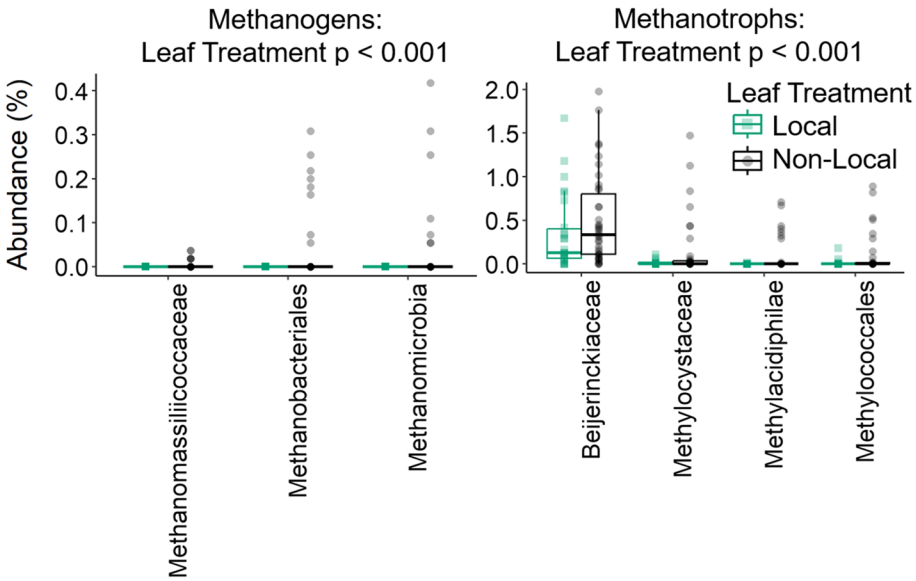


Fig. 5 *Dicosmoecus* caddisfly gut microbiomes contained greater relative abundances of methanogens and methanotrophs when individuals were given the treatment of non-local *A. rubra* leaves compared to local *A. rubra* leaves. Abundance values shown are sums per individual ($n = 64$) across all OTUs within each taxonomic group. Separate linear mixed effects models were ran for methanogens versus methanotrophs. Each model included fixed effects of Leaf Treatment and OTU, as well as a spatial blocking factor as a random effect

decomposers, which are generally richer in N and P relative to leaves (Enriquez et al. 1993). Lastly, these treatment effects for our dietary generalist, *Dicosmoecus*, could have ultimately been the result of a dietary shift between leaves and algae, as discussed further below.

Intraspecific variation in leaf litter effects gut microbiomes of consumers

Caddisflies feed on litter that is being actively decomposed by a rich array of microbes, and so undoubtedly consume sizable quantities of microbes. Therefore, it is important when interpreting shifts in the gut microbiome to first consider whether caddisflies even have a resident community of microbes in their gut. Notably, the bacterial taxa that we found to dominate in relative abundance in the caddisfly gut microbiome were indeed well-known associates with the gastrointestinal tracks of animals. Further, approximately 88% of bacterial sequences in each gut microbiome belonged to a conserved core microbiome that was common across most surveyed individuals. This is in stark contrast to a study that found a general lack of a resident microbiome in caterpillars, which are the terrestrial counterpart to larval caddisflies in aquatic systems (Hammer et al. 2017). Additionally, unlike in Hammer et al (2017), we found that the dominant taxa observed in association with the gut microbiome differed sharply from the dominant taxa associated with decomposing leaf litter, which we had previously documented in this system as the orders Burkholderiales, Sphingomonadales, and Myxococcales of the Proteobacteria, as well as the orders Cytophagales and Flavobacteriales of the Bacteroidetes (Jackrel et al. 2019). We infer therefore that the dominant orders of Enterobacterales and Clostridiales that we found in the gut were not transient taxa passing through the digestive system, but rather resident microbes. Resident microbes with more persistent associations with their host are more likely to confer fitness effects onto their hosts. A study of another stream macroinvertebrate detritivore, cranefly (Tipulidae), found that these larvae harbor diverse and dense communities of resident microbiomes within the lumen and directly attached to the gut wall (Klug and Kotarski 1980). Klug and Kotarski noted that Tipulidae larvae had an enlarged hindgut often referred to as a fermentation chamber, with physiological similarities to termites and cockroaches (1980). Aquatic invertebrates from marine systems have also been shown to harbor resident gut microbiota that differ markedly from their surrounding environments (Harris 1993). Future studies could more conclusively identify the role of caddisfly microbiomes by measuring caddisfly growth with and without their intact microbiome through the use of antibiotics. Such types of experiments typically show limited growth effects in insects that have mostly a transient microbiome, such as ants, and more significant growth effects for bees, beetles and true bugs thought to have persistent resident microbiomes (Hammer et al. 2017; Salem et al. 2013; Ceja-Navarro et al. 2015; Raymann et al. 2017).

A primary aim of evaluating the gut microbiome of caddisflies was to determine whether caddisfly endosymbionts may play a role in the degradation of aromatic secondary metabolites. Specifically, in the Pacific Northwest study system, we know that aromatic secondary metabolites are a key driver of locally accelerated decomposition patterns that have been repeatedly documented in these rivers. In *A. rubra*, two classes of secondary metabolites, the ellagitannins and diarylheptanoids, were found to be highly geographically structured across home and away sites. Using

leaf extracts of these secondary metabolites imbued into artificial diets, we observed a similar HFA pattern as observed with whole leaves (Jackrel et al. 2016). Further, we have started to resolve the free-living bacterial community associated with decomposition with indicators that home leaves were inhabited by bacterial communities with distinct functional capacities to degrade aromatic compounds (Jackrel et al. 2019). However, the breakdown of aromatic rings is fundamentally different in the presence versus absence of oxygen. Unlike free-living aerobic bacteria that use well-studied oxygenases, the anaerobes that we might expect to dominant a caddisfly's digestive system would use different enzymes (Fuchs et al. 2011; Boll et al. 2014). While we had previously observed significant differences in numerous KO terms involved in the degradation of aromatic compounds among communities of free-living bacteria that were decomposing home versus away *A. rubra* leaves, we did not see these terms significantly differ in relative abundance among the caddisfly microbiomes analyzed in this study. We also found no evidence that KO terms involved in the anaerobic degradation of aromatic compounds differed in predicted relative abundance by treatment. The only indication that *A. rubra* aromatic secondary metabolites may drive the structure of the caddisfly gut microbiome was that *Dicosmoecus* microbiomes differed by treatment in predicted relative abundance of K00588, or caffeoyl-CoA O-methyltransferase, which belongs to the "Stilbenoid, diarylheptanoid and gingerol biosynthesis" pathway (see Table S4).

Although we found limited evidence that the caddisfly microbiome plays a role in the degradation of aromatic compounds, we did observe two notable shifts in the gut microbiomes of *Dicosmoecus* by leaf treatment. *Dicosmoecus* supplied non-local leaves contained higher relative abundances of methanotrophs and methanogens. Endosymbiotic methanogens are most typically associated with challenging herbivorous diets, such as grass and wood (St-Pierre and Wright 2013). Although methanogens remained rare in *Dicosmoecus* given the non-local leaf treatment, low relative abundance is common among organisms noted for their endosymbiotic methanogens including termites and cows, where abundance rarely exceeds 4% of the total microbiome (Brauman et al. 2001; Wallace et al. 2015). Insects have been surveyed extensively for their ability to produce methane because despite small emissions per individual, due to their numerical abundance, insects could contribute sizably to atmospheric methane emissions. Although to our knowledge, methane production has not been measured in caddisflies, methanogens are known to occupy the gut microbiomes of other detritivorous insects including millipedes, termites, cockroaches, and beetle larvae (Hackstein and Stumm 1994). While an alternative explanation of methanotrophic bacteria occurring in the gut is direct consumption of these bacteria as a food resource, our isotopic results suggest this is unlikely the case. Aquatic macroinvertebrates have been found to compensate for poor diet by increasing consumption of methanotrophic bacteria, however such feeding shifts results in muscle tissue with a markedly depleted ^{13}C isotopic signature because methane and methanogens are exceptionally light relative to other energy sources at the base of aquatic food webs (Deines and Fink 2011). Instead, caddisflies supplied with the non-local litter treatment in our study tended to have greater ^{13}C content.

Intraspecific variation in leaf litter may cause dietary shifts in a consumer

Results from our study suggest the possibility that the non-local litter treatment may have caused *Dicosmoecus* caddisflies to shift their diet away from *A. rubra* litter and towards an algivorous diet. While algae typically contain lower nitrogen content than *A. rubra* litter, and so such a shift might result in reduced caddisfly growth rate, this shift may still be advantageous if caddisflies are maladapted to the secondary chemistry of non-local *A. rubra* leaves. The structural components and secondary defense compounds of terrestrial leaf litter can substantially reduce the bioavailability of this potential energy resource to consumers that lack the necessary enzymes to degrade the non-local signature of *A. rubra* secondary metabolites. Relatively labile freshwater eukaryotic algae, which typically produce few secondary defenses, may therefore be a viable alternative resource (Brett et al. 2017). We note several trends that are collectively suggestive of a diet shift, but some of these trends are not statistically significant, and therefore should be investigated further with a targeted study on dietary behavior and inclusion of larger sample sizes. Additionally, we do not have archived samples of all potential food sources from the time of our study to generate quantitative predictions from isotope mixing models. Prior work at the sites used in our study on the Pysht River indicate that algae have a less negative ^{13}C and positive ^{15}N, whereas *A. rubra* litter has a more negative ^{13}C and a negative ^{15}N (Wootton 2012a, 2012b). The directional shifts in the mean isotopic signatures of *Dicosmoecus* caddisflies, albeit non-significant, suggest this group may have incorporated more algae into their diets when supplied with non-local leaves. Considering the non-significant trends for the isotopic analyses, a larger scale study is needed to conclusively determine whether intraspecific variation in leaf litter may cause dietary shifts. Another potential line of evidence of this diet shift can be seen in the gut microbiome. We found significant increases in the relative abundance of five OTUs within the Rhizobiales among *Dicosmoecus* receiving the non-local leaf treatment. The persistent presence of these Rhizobiales in both the local and non-local leaf litter treatments (i.e. see Table S2), indicates a treatment effect on the differential growth rates of Rhizobiales within

the caddisfly gut. If instead, presence/absence of these taxa had been observed, this would have suggested the introduction of novel microbes by diet type. Our result is in line with studies of the foraging ecology of ants, which are thought to overcome the limited nitrogen content of their herbivorous diet by symbiotic associations with Rhizobiales (Stoll et al. 2007; Russell et al. 2009). Considering that *A. rubra* leaves are rich in nitrogen due to the tree's association with N-fixing bacterial symbionts (Benson and Dawson 2007), a shift to a more algivorous diet may have necessitated that *Dicosmoecus* receiving the non-local leaf treatment compensate for this diet by harboring microbiomes enriched in nitrogen-fixing bacteria.

There are however other factors that may explain the observed compositional shifts in caddisfly gut microbiomes. For example, our prior work has found that although *A. rubra* leaves contain many of the same chemical compounds across multiple riparian zones of the Olympic Peninsula, the relative abundance of these compounds is highly geographically structured (Jackrel et al. 2016). Likely as a consequence of this geographic variation in chemistry, local versus non-local *A. rubra* leaves harbor different bacterial communities on their leaves during decomposition (Jackrel et al. 2019). Therefore, the observed shifts in the relative abundance of leaf secondary metabolites and bacterial communities inhabiting the decomposing leaf surface, may also be causes of the observed shift in caddisfly gut microbial community composition.

Interestingly, we found limited treatment effects on the macroinvertebrate in our system that is more strongly associated with decaying leaf litter. *Psychoglypha* are typically found in high densities feeding on decaying leaves that have settled in slow moving pools, while in contrast, *Dicosmoecus* are most notably associated with grazing on periphyton. Although, we have also observed *Dicosmoecus* feeding on *A. rubra* leaf litter in natural leaf packs, as well as commonly on our experimental leaf packs. These feeding differences between genera were supported by our elemental and isotopic analysis of muscle tissue, as well as their gut microbiomes. Relative to *Dicosmoecus, Psychoglypha* had higher %N, lower ^{15}N, and fewer Rhizobiales in their gut microbiomes, which are each indicative of a diet comprised largely of nitrogen rich *A. rubra* leaf litter. Therefore, as more of an obligate detritivore, *Psychoglypha* may have been relatively unaffected by leaf treatment due to a specialization on the consumption of more recalcitrant leaf litter. A more generalist consumer such as *Dicosmoecus* could presumably shift to an alternative food resource when necessary. As such, *Psychoglypha* may have already undergone stronger selection for a broader capacity to consume *A. rubra* leaf litter regardless of quality, and so may have acquired a gut microbiome better able to consume leaf litter containing a broader range of secondary metabolites.

Limitations and conclusions

There are several limitations to our study. First, our measure of fitness differences by leaf treatment is dependent on no significant difference among individuals initially assigned to the local versus non-local leaf treatment group. By maintaining large sample sizes, randomly assigning individuals to mesocosms, and having observed no significant difference in proxies of body mass, such as caddisfly case length and case width, it is unlikely but possible that caddisflies assigned the local leaf treatment were initially significantly larger. Additionally, our inferences regarding the metabolic capacity of the gut microbiome is inherently limited by the use of our inference tool, PICRUST. While PICRUST predictions were drawn from closely related bacterial reference genomes, these predictions are not a substitute for directly observing gene abundance in a metagenomics sample or directly measuring a metabolic function. Further, several treatment effects may have been driven by increased variation in response rather than an overall shift in mean values. In particular, 11 individuals of *Dicosmoecus* assigned to the non-local treatment (7 with open cases, 4 with closed cases) demonstrated markedly distinct gut microbiomes. While linear mixed effects and adonis model assumptions, including homogeneity of variance, were still met for most metrics (but see Fig. 3C), we acknowledge this distinct subgroup that comprised 20% of the non-local *Dicosmoecus* individuals likely had a substantial effect on final results. Regardless, whether the non-local leaf treatment effected the variance versus mean values, this is an intriguing result that is likely to have real biological consequences on *Dicosmoecus* population biology. None of our metadata explained what may have caused this subgroup, which had individuals across three of our four experimental blocks. We cannot eliminate the possibility that this variance is caused by using two species that are known to occur in the state of Washington, because adult morphology is required to identify *Dicosmoecus* to species. However, considering that co-occurrence is rare and that these 11 individuals appeared only in the non-local treatment group and neither in the local treatment group or our Time_0 control individuals, the odds that this pattern is due to chance assignment of one species to only one treatment group and not the consequence of the experimental treatment appears exceedingly unlikely.

Overall, our results point to the broader cascading implications of intraspecific variation in leaf litter on consumer communities. Further research in aquatic and terrestrial systems should investigate the potential community and ecosystem implications of this phenomenon, particularly in the context of anthropogenic global changes that are shifting the historic ranges of plant genotypes and species. If global changes spur discordance between plants and their

consumers, nutrient and carbon cycles may be disrupted with cascading implications through an ecosystem.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-023-05435-5.

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Author contribution statement SLJ designed and carried out study, and completed all microbiome analyses. TYB completed nutrient and isotope sample preparation and data analyses. SLJ wrote the paper with contributions from TYB.

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Data availability All data will be deposited in Dryad.

Code availability All code will be made available through GitHub.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional guidelines for the care and use of animals were followed.

Consent to participate Not applicable.

Consent for publication Not applicable.

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Intraspecific variation in leaf litter alters fitness metrics and the gut microbiome of consumers.

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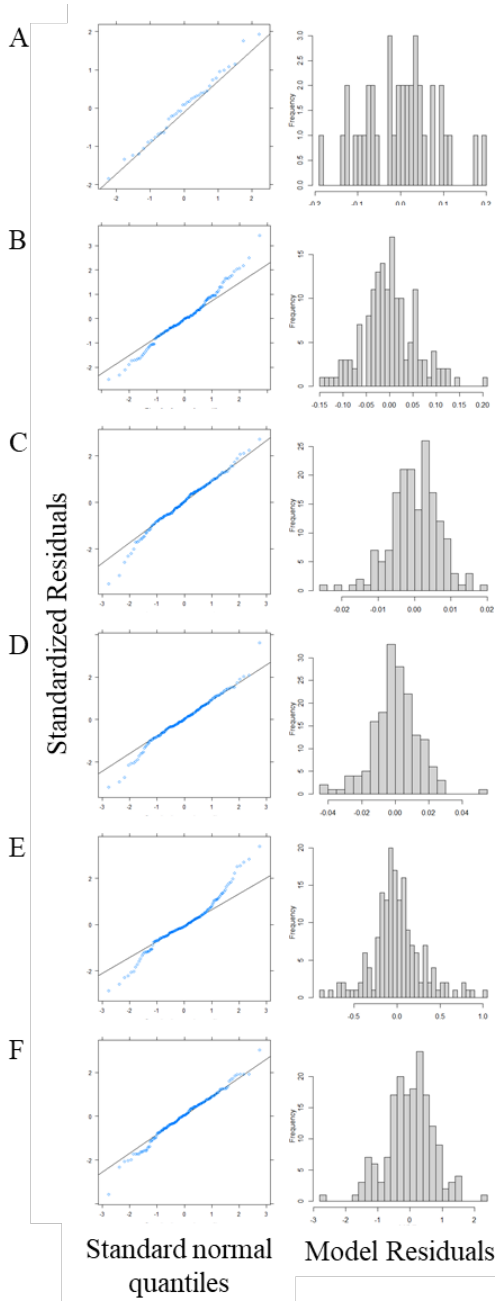
Abstract-
Biodiversity can have cascading effects throughout ecosystems. While these effects are better understood at coarser taxonomic scales of biodiversity, there has been a resurgence in investigating how biodiversity within species may have cascading effects on communities and ecosystems. We investigate the broader trophic implications of intraspecific variation in the riparian tree, *Alnus rubra*, where immediately local or 'home' litter decomposes faster than 'away' litter in aquatic and terrestrial systems. With climate change shifting the distributions of plants across the globe, it is essential to understand how shifts in the intraspecific traits of leaf litter may have reverberating effects throughout ecosystems. Here, we find that intraspecific variation in leaf litter has fitness implications for invertebrate consumers, including the algivorous *Dicosmoecus* and detrivorous *Psycoglypha* caddisflies, which exhibited increased body size and muscle nitrogen content when incubated within in-situ river mesocosms supplied with local *A. rubra* litter. Litter source altered caddisfly gut microbiomes by increasing relative abundance of methanogens and methanotrophs among the non-local treatment group. Additionally, *Dicosmoecus* supplied with non-local litter may have shifted their diet towards a higher proportion of algae, as inferred from shifts in gut microbiome composition and isotopic ratios of muscle tissue. Overall, our study demonstrates that shifting distributions of plant genotypes across the globe may cause plant-microbe mismatches that will disrupt patterns of decomposition and may have consequences on the fitness and foraging behavior of consumers.

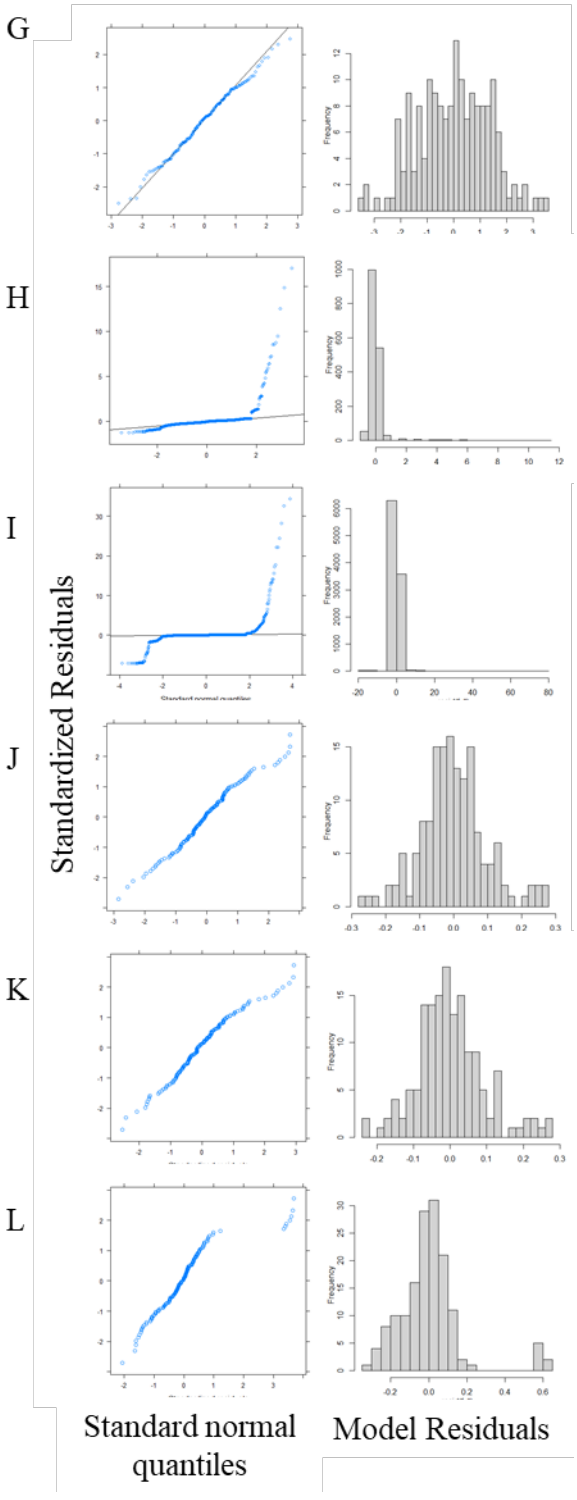
Supplementary Materials

Table S1. Temperature for four sites along the South Fork of the Pysht River were collected during the mesocosm study. Average daytime water temperatures were determined from 10-minute interval readings using HOBO data loggers where daytime water temperature values corresponded to a non-zero light measurement. Minimum and maximum water temperatures include daytime and nighttime measurements.

	ELK Downstream Site A	ELK Upstream Site B	E3000 Site C	W3000 Site D
Stream order	3	3	3	3
Latitude (48°N + °)	0.167	0.164	0.170	0.173
Longitude (124°W+ °)	0.157	0.156	0.159	0.169
Elevation	268	303	184	152
Average daytime water temperature (°C)	60.4	60.3	57.7	59
Minimum water temperature (°C)	55.2	54.7	53.8	53.5
Maximum water temperature (°C)	67.2	67.6	67.4	64.1

Fig. S1. Quantile-quantile plots (left-hand side) and frequency histograms (right-hand side) for all linear mixed effects model including A) leaf mass lost corresponding to results reported in Figure 1, B) caddisfly body mass, C) nitrogen content, D) carbon content, E) C:N content, F) nitrogen isotopic ratio, and G) carbon isotopic ratio for model results reported in Figure 2, H) methanogens and I) methanotrophs for model results reported in Figure 5, and J) Shannon's Diversity, K) Pileou's Evenness and L) Taxon Richness of gut microbiomes for model results reported in Figure S3.




G
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Standardized Residuals
Standard normal quantiles
Model Residuals

Figure S2. Survey of caddisflies for baseline metrics in advance of the experimental phase of this study. Initial metrics of caddisflies collected along the Pysht River include A) body mass, B) C:N ratio, C) nitrogen isotopic ratio, D) carbon isotopic ratio, E) percent nitrogen content and F) percent carbon content.

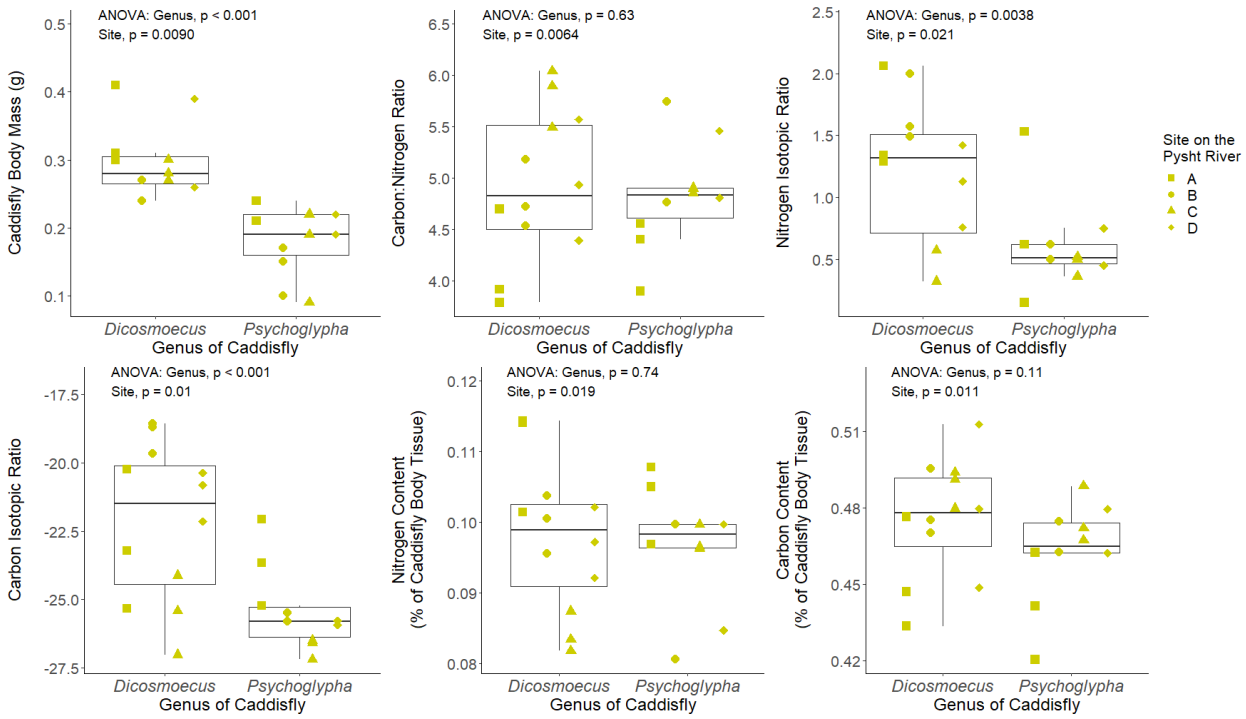

Figure S3. Two genera of caddisflies, the algivore *Dicosmoecus* and detritivore *Psychoglypha*, were similar in various diversity metrics in their gut microbiome prior to the start of the river mesocosm experiment. A) Taxonomic Richness was greater in *Dicosmoecus* but B) Shannon's Diversity and C) Shannon's Evenness did not differ by caddisfly genus. None of these metrics were significantly correlated with caddisfly body mass as determined with stepwise linear mixed effects modeling.

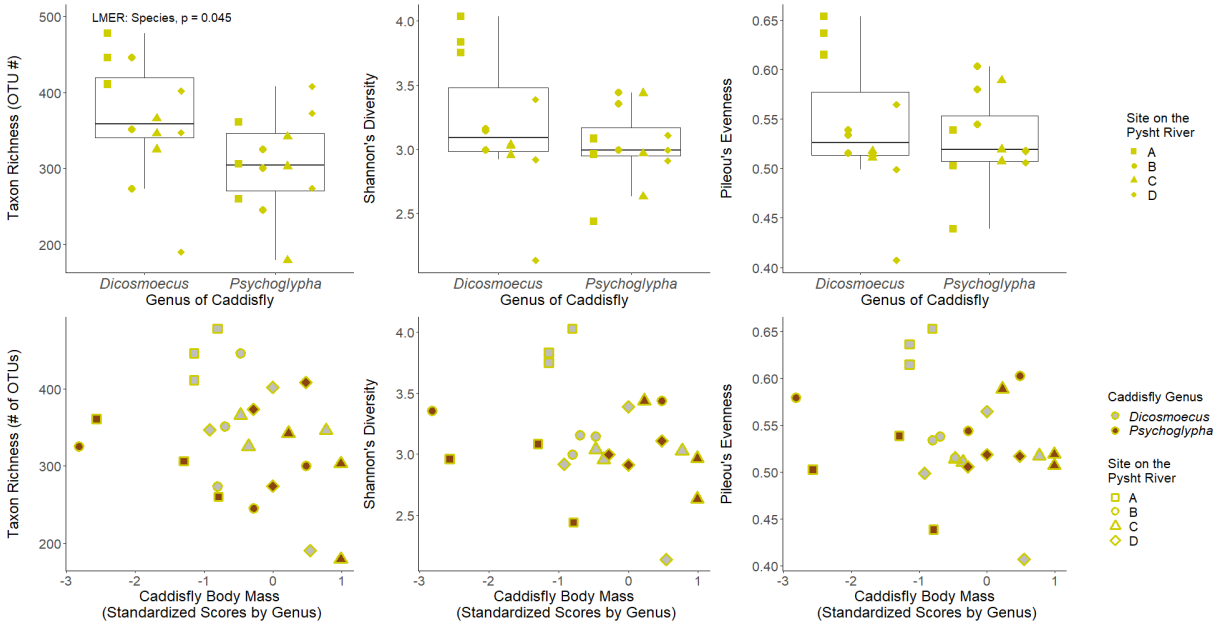

Figure S4. In the river mesocosm experiment, caddisfly genus and leaf treatment were significant predictors of gut microbiome diversity metrics, including A) Shannon's diversity, B) Pileou's evenness, and C) taxonomic richness. Linear-mixed effects models state which of these fixed effects and whether the interaction between fixed effects predict each diversity metric. Models included blocks nested within each deployment site as a random effects term. Independent variables are log transformed as needed to meet model assumption of homogeneity of variances, however transformation moderated but did not eliminate heterogeneity of variance for taxon richness (Levene's Test p = 0.007). Caddisfly body mass was predictive of D) Shannon's diversity, and E) Pileou's evenness, and F) taxonomic richness.

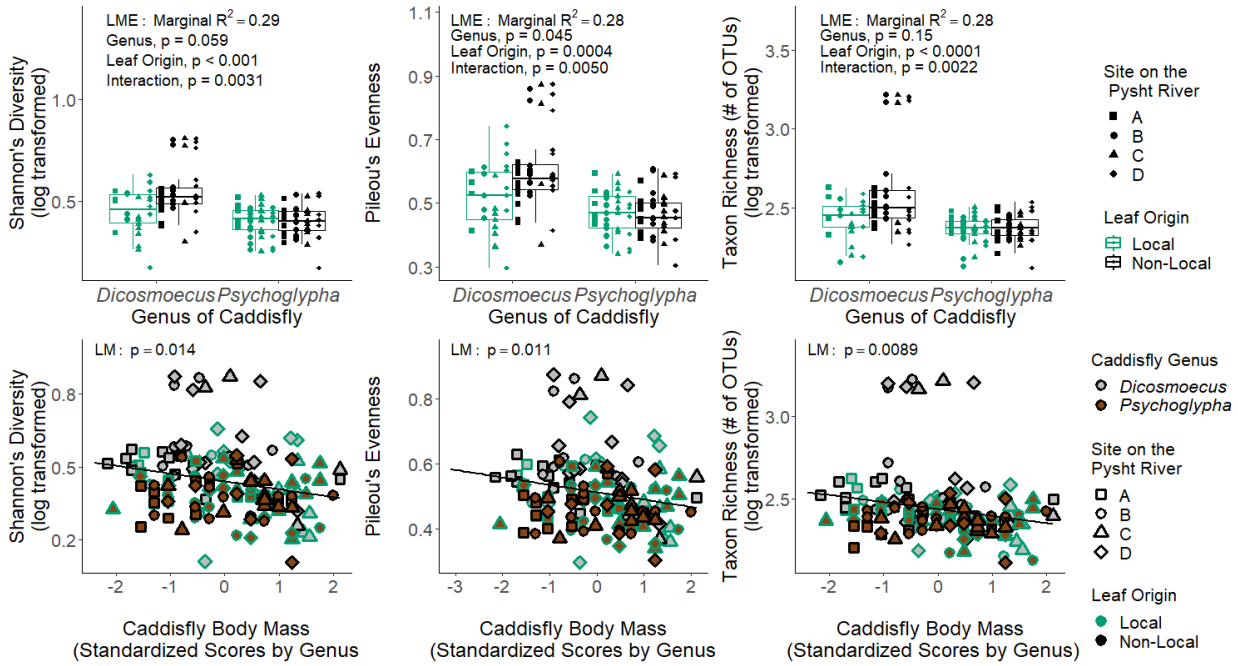

Table S2. Bacterial taxa most relatively abundant in the gut microbiomes of the algivorous *Dicosmoecus* and detrivorous *Psychoglypha* caddisfly. Caddisfly gut microbiomes were surveyed from four sites along the Pysht River, WA immediately prior to starting the mesocosm experiment at these sites (i.e. 'Initial' samples). Gut microbiomes were also surveyed after caddisflies were enclosed in mesocosms for a 27-day period with either locally-derived *A. rubra* leaves from the Pysht River, WA versus non-locally derived *A. rubra* leaves from the riparian zone of the Sekiu River, WA. Reported are those bacterial taxa comprising on average at least 1% of the population among the six treatment groups.

		Dicosmoecus						*Psycoglypha*					
		Initial (n = 12)		Local (n = 26)		Non-Local (n = 40)		Initial (n = 12)		Local (n = 41)		Non-Local (n = 44)	
#OTU	Taxonomy	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE
922761	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	14.97	5.86	20.39	3.67	9.85	1.88	26.68	5.26	17.81	2.79	16.29	2.15
1111294	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	20.66	5.27	8.75	2.57	6.62	1.77	7.36	2.85	10.70	2.69	20.22	3.36
837283	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae,Serratia spp.	0.37	0.07	15.46	3.23	8.53	1.80	0.20	0.06	11.38	2.17	12.15	1.86
N.R.OTU197	Alphaproteobacteria,Rhizobiales	10.77	1.49	0.19	0.07	0.32	0.07	0.36	0.20	0.01	0.003	0.01	0.002
368099	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella spp.	0.66	0.43	0.08	0.05	0.86	0.56	10.36	3.40	0.03	0.02	1.20	0.56
1111582	Firmicutes,Bacilli,Lactobacillales,Enterococcaceae,Enterococcus spp.	0.90	0.27	1.87	0.49	6.76	1.29	2.59	0.60	7.47	1.19	6.11	0.91
N.R.OTU426	Firmicutes,Clostridia,Clostridiales,Ruminococcaceae	0.02	0.01	0.15	0.06	0.24	0.09	6.76	1.72	1.81	0.47	1.74	0.37
585419	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella dispar	3.19	0.83	3.45	1.04	3.03	0.90	0.81	0.32	5.68	1.67	3.12	0.82
1084865	Firmicutes,Bacilli,Bacillales,Staphylococcaceae,Staphylococcus spp.	0.41	0.26	2.19	1.07	1.62	0.60	0.22	0.14	5.12	1.69	1.57	1.04
606927	Firmicutes,Clostridia,Clostridiales,Peptostreptococcaceae	4.41	3.05	0.26	0.16	2.90	1.15	0.20	0.13	2.11	0.82	0.66	0.21
N.R.OTU431	Alphaproteobacteria,Rhizobiales	3.66	0.75	0.02	0.01	0.04	0.01	0.33	0.20	0	0	0.002	0.002
583117	Bacteroidetes,Bacteroidia,Bacteroidales,Bacteroidaceae,Bacteroides spp.	0.002	0.002	0.003	0.003	0.001	0.001	0	0	0.04	0.02	3.44	1.63
N.R.OTU73	Firmicutes,Clostridia,Clostridiales,Lachnospiraceae	0.02	0.01	0.49	0.18	0.26	0.09	2.95	1.05	0.68	0.13	0.76	0.15
687940	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae,Trabulsiella spp.	0.05	0.02	0.13	0.06	0.05	0.01	0.07	0.01	2.55	1.04	1.30	1.17
828483	Firmicutes,Clostridia,Clostridiales,Clostridiaceae,Clostridium spp.	0.65	0.35	0.73	0.52	0.64	0.27	1.92	0.70	2.35	0.77	0.81	0.26
4403259	Actinobacteria,Coriobacteriia,Coriobacteriales,Coriobacteriaceae	0.003	0.002	0.05	0.03	0.03	0.01	2.16	0.59	0.20	0.04	0.19	0.04
744029	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	0.02	0.01	1.83	1.14	0.45	0.14	1.92	0.69	1.22	0.31	1.55	0.39
932696	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae,Serratia marcescens	0.01	0.01	0.13	0.13	1.87	1.06	0.003	0.002	0.04	0.02	0.67	0.35
N.R.OTU321	Tenericutes,Mollicutes,RsaHF231	0.07	0.03	1.87	0.69	1.55	0.25	0.02	0.01	0.004	0.002	0.01	0.01
818854	Alphaproteobacteria,Rhodobacterales,Rhodobacteraceae,Rhodobacter spp.	1.86	0.34	0.47	0.15	0.60	0.09	0.79	0.22	0.05	0.01	0.11	0.03
583656	Bacteroidetes,Bacteroidia,Bacteroidales,Bacteroidaceae,Bacteroides fragilis	0	0	0.001	0.001	0.001	0.001	0	0	1.70	1.16	0.01	0.01
N.R.OTU114	Tenericutes,Mollicutes,RsaHF231	0.12	0.04	1.61	0.41	1.70	0.24	0.23	0.09	0.03	0.01	0.03	0.01
505587	Firmicutes,Clostridia,Clostridiales,Tissierellaceae,Finegoldia spp.	0.02	0.01	0.22	0.08	1.68	0.72	0.29	0.16	0.12	0.04	0.13	0.07
1106733	Betaproteobacteria	0	0	0.05	0.03	0.03	0.02	1.65	1.64	0.02	0.01	0.004	0.002
746799	Alphaproteobacteria,Rhodobacterales,Rhodobacteraceae,Rhodobacter spp.	1.61	0.53	0.81	0.22	0.86	0.17	0.59	0.12	0.06	0.02	0.12	0.03
1117031	Planctomycetes,Planctomycetia,Pirellulales,Pirellulaceae,	1.57	0.29	0.09	0.03	0.12	0.03	0.53	0.28	0.01	0.01	0.03	0.01
528421	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae,Citrobacter spp.	1.34	0.56	0.19	0.12	0.32	0.12	0.18	0.06	0.75	0.46	1.52	0.66
N.R.OTU281	Fusobacteria,Fusobacteriia,Fusobacteriales,Leptotrichiaceae	0.002	0.002	0.02	0.01	0.03	0.02	1.28	0.57	1.40	0.40	1.52	0.36
523589	Firmicutes,Clostridia,Clostridiales,Clostridiaceae,Clostridium neonatale	0.05	0.05	0.55	0.40	0.05	0.03	0.003	0.002	1.51	0.77	0.55	0.22
N.R.OTU417	Betaproteobacteria,Burkholderiales,Comamonadaceae,	0.04	0.03	0.73	0.34	1.10	0.56	1.51	0.44	0.86	0.19	0.62	0.13
585435	Firmicutes,Bacilli,Lactobacillales,Carnobacteriaceae,Carnobacterium spp.	0.003	0.002	0.11	0.05	0.72	0.45	0.03	0.01	1.49	0.43	0.50	0.15
775567	Alphaproteobacteria,Rhizobiales	1.40	0.24	0.02	0.01	0.03	0.01	0.12	0.09	0.002	0.001	0.01	0.005
N.R.OTU254	Tenericutes,CK-1C4-19	0.11	0.05	0.72	0.17	1.38	0.33	0.25	0.10	0.11	0.03	0.08	0.02
559527	Actinobacteria,Bifidobacteriales,Bifidobacteriaceae,Bifidobacterium spp.	0	0	1.35	0.52	0.22	0.16	0	0	0.05	0.03	0.51	0.19
N.R.OTU211	Firmicutes,Clostridia,Clostridiales,Lachnospiraceae,Dorea formicigenerans	0	0	0.01	0.004	0.004	0.003	1.33	0.46	0.11	0.04	0.10	0.02
N.R.OTU328	Alphaproteobacteria,Rhizobiales	1.32	0.18	0.13	0.06	0.16	0.03	0.12	0.06	0.01	0.00	0.02	0.01
752012	Bacteroidetes,Bacteroidia,Bacteroidales,Porphyromonadaceae,Dysgonomonas spp.	0.005	0.003	0.92	0.49	1.32	0.63	0.26	0.23	0.61	0.29	0.87	0.34
4422456	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella parvula	1.25	0.69	0.01	0.01	0.26	0.14	0.04	0.02	0.005	0.002	0.002	0.001
737645	Alphaproteobacteria,Rhizobiales	1.23	0.39	0.01	0.01	0.03	0.01	0.01	0.01	0	0	0	0
1099914	Firmicutes,Clostridia,Clostridiales,Tissierellaceae,Peptoniphilus spp.	0.03	0.02	0.32	0.14	1.21	0.46	0.003	0.003	0.32	0.18	0.22	0.09
235024	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	0.005	0.002	0.88	0.55	1.17	0.43	0.005	0.003	0.35	0.14	0.10	0.03
568228	Firmicutes,Bacilli,Lactobacillales,Enterococcaceae,Vagococcus salmoninarum	0.003	0.002	0.13	0.03	0.19	0.06	0.30	0.18	1.13	0.30	0.51	0.17
830893	Alphaproteobacteria,Rhizobiales,Phyllobacteriaceae	0.35	0.08	0.81	0.24	1.05	0.25	0.84	0.23	0.13	0.04	0.11	0.03
N.R.OTU445	Betaproteobacteria,Neisseriales,Neisseriaceae,Vitreoscilla	0	0	0.02	0.01	0.03	0.01	1.04	1.03	0.01	0.005	0.02	0.01

Table S3. Bacterial taxa significantly differentially-represented in the gut microbiomes of caddisflies receiving the local versus non-local *A. rubra* leaf treatment. Significance based on quasi-likelihood F-tests using the glm approach in edgeR. Since edgeR works with raw count data, reported means (± SE) are from the rarefied dataset used in all preceding analyses.

					Initial		Local		Non-Local	
OTU #	Taxonomy	Caddisfly	FDR	logFC	μ	SE	μ	SE	μ	SE
515709	Alphaproteobacteria,Rhizobiales,Hyphomicrobiaceae,Rhodoplanes spp.	Dicosmoecus	1.2E-08	8.91	0.03	0.01	0.0003	0.0003	0.16	0.05
754628	Alphaproteobacteria,Rhizobiales,Methylocystaceae	Dicosmoecus	4.1E-08	8.57	0.04	0.01	0.0006	0.0004	0.12	0.04
812496	Acidobacteria,DA052,Ellin6513	Dicosmoecus	5.9E-08	7.43	0	0	0	0	0.04	0.01
562311	Alphaproteobacteria,Rhizobiales,Hyphomicrobiaceae,Rhodoplanes spp.	Dicosmoecus	4.0E-06	6.39	0.05	0.02	0.002	0.001	0.07	0.02
1096310	Verrucomicrobia,Pedosphaerae,Pedosphaerales,Ellin515	Dicosmoecus	6.3E-05	5.93	0	0	0.001	0.001	0.08	0.02
876519	Alphaproteobacteria,Rhizobiales,Hyphomicrobiaceae	Dicosmoecus	2.2E-07	5.75	0.04	0.01	0.0003	0.0003	0.02	0.01
932696	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae,Serratia marcescens	Dicosmoecus	0.001	5.02	0.01	0.002	0.11	0.10	1.45	0.82
505587	Firmicutes,Clostridia,Clostridiales,Tissierellaceae,Finegoldia spp.	Dicosmoecus	0.002	3.92	0.03	0.02	0.17	0.06	1.44	0.57
580625	Alphaproteobacteria,Rhizobiales,Bradyrhizobiaceae	Dicosmoecus	0.001	3.78	0.13	0.03	0.01	0.004	0.07	0.02
495396	Firmicutes,Clostridia,Clostridiales,Tissierellaceae,Anaerococcus spp.	Dicosmoecus	0.026	3.30	0.0008	0.0008	0.12	0.05	0.60	0.25
368099	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella spp.	Dicosmoecus	0.026	3.27	0.67	0.43	0.11	0.05	1.91	0.82
606927	Firmicutes,Clostridia,Clostridiales,Peptostreptococcaceae	Dicosmoecus	0.048	3.14	4.39	3.00	0.45	0.27	2.26	0.89
1099914	Firmicutes,Clostridia,Clostridiales,Tissierellaceae,Peptoniphilus spp.	Dicosmoecus	0.043	3.07	0.03	0.02	0.25	0.11	1.08	0.38
622288	Firmicutes,Bacilli,Lactobacillales,Enterococcaceae,Enterococcus spp.	Dicosmoecus	0.001	3.07	0.01	0.003	0.01	0.004	0.04	0.01
N.C.R.OTU60615	Alphaproteobacteria,Rhodobacterales,Rhodobacteraceae,Rhodobacter spp.	Dicosmoecus	0.008	3.07	0.02	0.01	0.01	0.004	0.06	0.04
535955	Firmicutes,Clostridia,Clostridiales,Peptostreptococcaceae	Dicosmoecus	0.030	2.94	0.10	0.07	0.01	0.01	0.04	0.02
667570	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	Dicosmoecus	0.009	2.92	0.31	0.09	0.16	0.03	0.78	0.38
940737	Betaproteobacteria,Rhodocyclales,Rhodocyclaceae	Dicosmoecus	0.030	2.90	0	0	0.02	0.01	0.07	0.05
N.C.R.OTU3483	Alphaproteobacteria,Rhizobiales,Beijerinckiaceae	Dicosmoecus	0.029	2.70	0.0008	0.0008	0.02	0.01	0.11	0.05
1062748	Actinobacteria,Actinobacteria,Actinomycetales,Mycobacteriaceae,Mycobacterium spp.	Dicosmoecus	0.037	2.21	0.05	0.02	0.01	0.002	0.02	0.004
810399	Firmicutes,Bacilli,Lactobacillales,Enterococcaceae,Enterococcus spp.	Dicosmoecus	0.026	2.15	0.08	0.05	0.01	0.003	0.04	0.01
1111582	Firmicutes,Bacilli,Lactobacillales,Enterococcaceae,Enterococcus spp.	Dicosmoecus	0.008	1.98	0.85	0.26	2.37	0.44	6.09	1.07
1109623	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	Dicosmoecus	0.041	-1.91	0.002	0.002	0.07	0.02	0.01	0.003
559527	Actinobacteria,Actinobacteria,Bifidobacteriales,Bifidobacteriaceae,Bifidobacterium spp.	Dicosmoecus	0.012	-2.67	0	0	1.65	0.44	0.18	0.12
128382	Firmicutes,Clostridia,Clostridiales,Veillonellaceae	Dicosmoecus	4.5E-04	-3.68	0.002	0.001	0.28	0.13	0.01	0.01
974121	Gammaproteobacteria,Pseudomonadales,Pseudomonadaceae,Pseudomonas spp.	Dicosmoecus	3.7E-05	-4.05	0.0008	0.0008	0.18	0.09	0.01	0.003
369429	Firmicutes,Clostridia,Clostridiales,Lachnospiraceae,Ruminococcus	Dicosmoecus	4.5E-04	-4.16	0	0	0.75	0.31	0.02	0.01
523589	Firmicutes,Clostridia,Clostridiales,Clostridiaceae,Clostridium neonatale	Dicosmoecus	4.5E-04	-4.20	0.05	0.05	1.21	0.74	0.04	0.02
532521	Firmicutes,Clostridia,Clostridiales,Peptostreptococcaceae,Peptostreptococcus anaerobius	Dicosmoecus	4.5E-05	-4.65	0	0	0.39	0.30	0.01	0.005
583117	Bacteroidetes,Bacteroidia,Bacteroidales,Bacteroidaceae,Bacteroides spp.	Psychoglypha	5.7E-06	7.24	0.0008	0.0008	0.04	0.02	3.46	1.64
124906	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Megasphaera spp.	Psychoglypha	1.4E-05	6.52	0	0	0.02	0.01	0.78	0.41
368099	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella spp.	Psychoglypha	4.5E-04	5.08	10.37	3.43	0.03	0.02	1.20	0.56
N.R.OTU259	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella spp.	Psychoglypha	1.9E-07	5.02	0.08	0.03	0	0	0.01	0.01
932696	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae,Serratia marcescens	Psychoglypha	0.001	4.22	0.005	0.003	0.04	0.02	0.69	0.36
559527	Actinobacteria,Actinobacteria,Bifidobacteriales,Bifidobacteriaceae,Bifidobacterium spp.	Psychoglypha	0.001	3.70	0.0008	0.0008	0.04	0.03	0.49	0.18
968954	Firmicutes,Bacilli,Lactobacillales,Streptococcaceae,Streptococcus	Psychoglypha	0.032	2.76	0	0	0.04	0.02	0.32	0.14
235024	Gammaproteobacteria,Enterobacteriales,Enterobacteriaceae	Psychoglypha	0.023	-2.20	0.002	0.002	0.37	0.14	0.10	0.03
10485	Deltaproteobacteria,Desulfovibrionales,Desulfovibrionaceae	Psychoglypha	0.020	-2.30	0.03	0.02	0.05	0.02	0.01	0.002
114821	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella parvula	Psychoglypha	0.014	-2.39	0.02	0.01	0.03	0.01	0.01	0.004
518743	Firmicutes,Clostridia,Clostridiales,Veillonellaceae,Veillonella parvula	Psychoglypha	0.020	-2.42	0.01	0.01	0.09	0.03	0.02	0.01
582691	Firmicutes,Clostridia,Clostridiales,Clostridiaceae	Psychoglypha	0.037	-2.52	0.0008	0.0008	0.25	0.12	0.07	0.03
628226	Firmicutes,Clostridia,Clostridiales,Clostridiaceae	Psychoglypha	0.003	-3.29	0.005	0.003	0.16	0.11	0.02	0.01
N.R.OTU182	Deltaproteobacteria,Desulfarculales,Desulfarculaceae	Psychoglypha	3.9E-04	-3.56	0	0	0.0002	0.0002	0.001	0.0006

Table S4. Based on gene predictions from 16S rRNA sequence data using the PICRUST tool, various metabolic pathways were differentially abundant in the gut microbiomes of *Dicosmoecus* caddisflies given the local versus non-local *A. rubra* leaf treatment. Top 10 pathways are shown, including 3 KO terms per pathway plus any additional terms with FDR p-values < 0.10.

KEGG Ontology Pathway	P-value	FDR-correct P-value
Polyketide sugar unit biosynthesis	**0.0003**	**0.035**
K00067: dTDP-4-dehydrorhamnose reductase	0.001	0.008
K01710: dTDP-glucose 4,6-dehydratase	0.003	0.015
K00973: glucose-1-phosphate thymidylyltransferase	0.005	0.032
K01790: dTDP-4-dehydrorhamnose 3,5-epimerase	0.011	0.068
Methane metabolism	**0.0004**	**0.052**
K11261: formylmethanofuran dehydrogenase subunit E	0.0003	0.043
K07812: trimethylamine-N-oxide reductase (cytochrome c)	0.001	0.102
K01834: cofactor dependent phosphoglycerate mutase	0.001	0.133
Amino acid metabolism	**0.0005**	**0.0698**
K00549: cobalamin-independent methionine synthase	0.0001	0.0439
K03856: 3-deoxy-7-phosphoheptulonate synthase	0.0001	0.0555
K00074: 3-hydroxybutyryl-CoA dehydrogenase	0.0002	0.0767
K07008: gamma-glutamyl hercynylcysteine S-oxide hydrolase	0.0002	0.0773
K01479: formiminoglutamase	0.0002	0.0855
K00609: aspartate carbamoyltransferase catalytic subunit	0.0002	0.0984
Terpenoid backbone biosynthesis	**0.001**	**0.095**
K00806: undecaprenyl diphosphate synthase	0.0004	0.013
K00919: 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	0.002	0.072
K00626: acetyl-CoA C-acetyltransferase	0.003	0.090
Amino sugar and nucleotide sugar metabolism	**0.001**	**0.102**
K00884: N-acetylglucosamine kinase	0.001	0.006
K00820: glutamine---fructose-6-phosphate transaminase	0.003	0.227
K01784: UDP-glucose 4-epimerase	0.003	0.235
Biosynthesis of vancomycin group antibiotics	**0.001**	**0.137**
K01710: dTDP-glucose 4,6-dehydratase	0.003	0.003
Synthesis and degradation of ketone bodies	**0.002**	**0.213**
K00626: acetyl-coA C-acetyltransferase	0.003	0.023
K01640: hydroxymethylglutaryl-CoA lyase	0.005	0.036
K01028: 3-oxoacid CoA-transferase subunit A	0.012	0.100
Selenocompound metabolism	**0.002**	**0.281**
K00549: 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	0.0001	0.002
K00384: thioredoxin reductase	0.001	0.015
K01874: methionyl-tRNA synthetase	0.011	0.243
Stilbenoid diarylheptanoid and gingerol biosynthesis	**0.002**	**0.282**
K00588: caffeoyl-CoA O-methyltransferase	0.003	0.003
Citrate cycle TCA cycle	**0.002**	**0.291**
K00161: pyruvate dehydrogenase E1 component alpha subunit	0.001	0.037
K00116: malate dehydrogenase	0.001	0.047
K01610: phosphoenolpyruvate carboxykinase	0.001	0.054
K00174: 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit alpha	0.002	0.071
K00382: dihydrolipoamide dehydrogenase	0.002	0.086
K00030: isocitrate dehydrogenase	0.002	0.097

Table S5. Based on gene predictions from 16S rRNA sequence data using the PICRUST tool, various metabolic pathways were differentially abundant in the gut microbiomes of *Psychoglypha* caddisflies given the local versus non-local *A. rubra* leaf treatment. Top 10 pathways area shown, including 3 KO terms per pathway plus any additional terms with FDR p-values < 0.10.

Pathway	P-value	FDR P-value
Vitamin B6 metabolism	**0.0001**	**0.008**
K00831: phosphoserine aminotransferase	0.0002	0.002
K00275: pyridoxamine 5'-phosphate oxidase	0.0002	0.002
K03474: pyridoxine 5-phosphate synthase	0.0002	0.003
K03473: erythronate-4-phosphate dehydrogenase	0.0003	0.003
K03472: D-erythrose 4-phosphate dehydrogenase	0.004	0.049
K00097: 4-hydroxythreonine-4-phosphate dehydrogenase	0.008	0.083
Lipopolysaccharide biosynthesis proteins	**0.0003**	**0.032**
K03269: UDP-2,3-diacylglucosamine hydrolase	0.0001	0.011
K10012: undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	0.001	0.070
K02560: lauroyl-Kdo2-lipid IVA myristoyltransferase	0.001	0.095
Nicotinate and nicotinamide metabolism	**0.001**	**0.068**
K00324: H+-translocating NAD(P) transhydrogenase subunit alpha	0.001	0.027
K00322: NAD(P) transhydrogenase	0.001	0.036
K08281: nicotinamidase/pyrazinamidase	0.001	0.040
K00325: H+-translocating NAD(P) transhydrogenase subunit beta	0.001	0.049
K01916: NAD+ synthase	0.003	0.084
Lipopolysaccharide biosynthesis	**0.001**	**0.086**
K03269: UDP-2,3-diacylglucosamine hydrolase	0.0001	0.005
K02560: lauroyl-Kdo2-lipid IVA myristoyltransferase	0.001	0.048
K00748: lipid-A-disaccharide synthase	0.002	0.097
Toluene degradation	**0.001**	**0.129**
K01061: carboxymethylenebutenolidase	0.131	1.000
K00055: aryl-alcohol dehydrogenase	0.150	1.000
K00141: benzaldehyde dehydrogenase	0.158	1.000
Glycolysis Gluconeogenesis	**0.001**	**0.141**
K01792: glucose-6-phosphate 1-epimerase	0.001	0.080
K00163: pyruvate dehydrogenase E1 component	0.001	0.085
K03841: fructose-1,6-bisphosphatase I	0.002	0.138
Glycerolipid metabolism	**0.001**	**0.148**
K00631: glycerol-3-phosphate O-acyltransferase	0.001	0.021
K03429: processive 1,2-diacylglycerol beta-glucosyltransferase	0.002	0.051
K00864: glycerol kinase	0.004	0.120
Glyoxylate and dicarboxylate metabolism	**0.001**	**0.149**
K01433: formyltetrahydrofolate deformylase	0.000	0.008
K00281: glycine dehydrogenase	0.001	0.068
K00124: formate dehydrogenase iron-sulfur subunit	0.003	0.245
Geraniol degradation	**0.001**	**0.164**
K00632: acetyl-CoA acyltransferase	0.006	0.012
K01640: hydroxymethylglutaryl-CoA lyase	0.038	0.076
Riboflavin metabolism	**0.002**	**0.243**
K14652: 3,4-dihydroxy 2-butanone 4-phosphate synthase	0.015	0.326
K05368: NAD(P)H-flavin reductase	0.021	0.471
K08096: GTP cyclohydrolase IIa	0.032	0.696