

1 The Gut-Associated Microbiome of the Eastern Spruce Budworm Does Not Influence Larval
2 Growth or Survival

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9 Running Head: Spruce Budworm Gut Microbiome does not Affect Growth

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20 **ABSTRACT**

21 Microbial communities have been shown to play an important role for host health in mammals,
22 especially humans. It is thought that microbes could play an equally important role in other
23 animal hosts such as insects. A growing body of evidence seems to support this, however most of
24 the research effort in understanding host-microbe interactions in insects has been focused on a
25 few well-studied groups such as bees and termites. We studied the effects of the gut-associated
26 microbial community on the growth of the eastern spruce budworm *Choristoneura fumiferana*,
27 an economically important lepidopteran forest pest in eastern Canada and the northeastern
28 United States. Contrary to our expectations, although antibiotics influenced spruce budworm
29 microbial community structure, the gut microbial community of spruce budworm larvae did not
30 influence host growth or survival. Our results agree with the hypothesis that lepidopteran larvae
31 lack resident microbial communities and are not nutritionally dependent on bacterial symbionts.
32 However, while most bacteria originating on foliage appear to be transient through the gut and
33 could not be linked with host growth, some bacteria may thrive better in the *C. fumiferana* gut.

34 **IMPORTANCE**

35 The importance of bacterial symbiosis has been clearly shown in humans. This has led to a
36 number of studies looking for similar levels of dependence on microbial communities for host
37 health in other organisms. The eastern spruce budworm (*Choristoneura fumiferana*) is an
38 economically important lepidopteran forest pest that feeds on the hard to digest needles of
39 balsam fir and spruce trees making it an ideal candidate for studying host-microbe interactions.
40 We found that disturbance of the gut microbiome with antibiotic treatment did not significantly
41 affect the growth of *C. fumiferana* larvae and the majority of bacteria in the *C. fumiferana* gut
42 were more abundant in diet samples. Our findings generally support the recent hypothesis that

43 lepidopteran larvae lack resident microbiota, however we also show that some rare taxa increase
44 in relative abundance in the gut suggesting weak selective pressures are at play in the larval *C.*
45 *fumiferana* gut.

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47 **INTRODUCTION**

48 The eastern spruce budworm (*Choristoneura fumiferana*) is a forest pest native to the north
49 eastern United States and eastern Canada that undergoes epidemic population outbreaks every
50 30-40 years. During these population outbreaks, lasting for approximately ten years, millions of
51 hectares of balsam fir (*Abies balsamea*) and spruce (*Picea spp.*) trees are defoliated (1–4).

52 Consequently spruce budworm has significant effects on forest productivity (5) is an
53 economically important defoliator in coniferous forests (6) and. While the role of factors such as
54 landscape context, parasitoids and other predators, and forest management practices have been
55 hypothesized to play a role in governing spruce budworm health and population dynamics (7, 8),
56 much less is known about the importance of the microbial life associated with spruce budworm
57 for their growth, population dynamics, and outbreak status.

58 Insects are associated with diverse communities of microorganisms including bacteria and fungi.

59 The collective set of microbial genomes associated with a host, the microbiome, has a much
60 greater functional diversity than the eukaryotic host genome (9). One of the most important
61 functions of the insect gut microbiome is its potential to aid in digestion by breaking down
62 compounds the host cannot digest (10, 11). Thus the microbiome can act as an extension of the
63 host gut. This is particularly important when considering the spruce budworm because it feeds on

64 conifer needles that are acidic, tough to digest and contain defensive compounds such as
65 terpenes.

66 Drawing generalizations about the extent to which insect microbiota are important to their hosts
67 is difficult due in large part to the morphological, physiological, and behavioral variation among
68 insects. Different physiology, life histories, and feeding strategies can all influence how
69 microbes interact with their host (12). There is evidence to support the assumption that gut
70 microbial symbiosis is important in a number of insects (12–19). There is a body of evidence
71 showing that termites depend on specialized bacteria or flagellates to allow them to digest
72 cellulose (11, 12, 17), and various species of bees benefit greatly from their associated
73 microbiota (13, 15, 18, 19). Bee-associated microbes have been shown to contribute to immune
74 function as well as to aid in nutrition through mediating the digestion of pectin (13, 19).

75 The lepidopteran microbiome has been described as a very simple microbial community
76 compared to other insects (20–27). One reason that lepidopteran gut microbial communities tend
77 to be simpler than other insects is that the lepidopteran larval gut is a simple tube without any
78 specialized structure for microbial cultivation as is seen in termite guts (12). Another unique
79 aspect of the lepidopteran larval gut is that unlike most insect midguts which are acidic and range
80 in pH between 4-7, lepidopteran midguts are highly alkaline ranging from pH 8-12 (12, 20, 23,
81 25). The alkaline nature of the spruce budworm gut could provide some advantage in digesting
82 acidic conifer needles.

83 The objectives of this study were to determine if the gut microbiota associated with spruce
84 budworm larvae, resident or otherwise, influence larval growth rates and survival, and to
85 determine if the eastern spruce budworm has a resident gut microbiome by comparing the
86 community composition of foliage, spruce budworm guts, and frass community assemblages that

87 is distinct from the microbial assemblages associated with foliage. We also sought to quantify
88 the effects that antibiotics would have on spruce budworm gut microbial diversity and
89 community composition.

90 Given the challenges associated with a diet of conifer needles, we hypothesized that the eastern
91 spruce budworm has a resident gut microbiome that contributes to larval growth and survival.
92 Thus, we further hypothesized that the disturbance of microbial communities with antibiotics
93 will negatively influence spruce budworm larval growth and survival. We also hypothesized that
94 the use of antibiotics will both reduce diversity of the microbial communities associated with
95 diet, guts, and frass, and would significantly alter the composition of the spruce budworm gut
96 microbial community in a way that would negatively influence larval growth and survival.

97 **RESULTS**

98 **Effects of diet and antibiotics on larval survival**

99 Diet did not have a significant effect on eastern spruce budworm larval survival rates (logistic
100 regression; $z = -0.897$, $p = 0.3695$), however antibiotic treatment tended to favor survival (logistic
101 regression; $z = -1.810$, $p = 0.0702$) (Fig S1). Because the synthetic diet was designed to be optimal
102 for spruce budworm growth and survival a second logistic regression was performed only on
103 larvae that fed on foliage and we found that there was no longer a trend of antibiotic treatment on
104 larval survival (logistic regression; $z = 0.110$, $p = 0.913$). The trend of antibiotic treatment favoring
105 survival seems to be driven by the difference between larvae feeding on spruce (30% survival)
106 that was not treated with antibiotics and larvae that fed on synthetic diet (60% survival) that
107 contained antibiotics. Larvae feeding on spruce and fir treated with antibiotics both had 50%
108 survival and larvae feeding on fir without antibiotics had 52.5% survival.

109 **Effects of diet and antibiotics on larval growth rate**

110 Antibiotic treatment and time significantly affected the weight of spruce budworm larvae (Table
111 1, Table S3). In the model, the estimates of time as a main effect represent larval growth rate.
112 The overall differences in growth rate observed between spruce budworm larvae in different
113 experimental groups were due to the antibiotic treatment, however this result must be interpreted
114 carefully because it appears to be largely driven by an interaction between diet and antibiotic
115 treatment rather than a consistent effect of antibiotics on growth rate. Individuals feeding on fir
116 treated with antibiotics grew less than those feeding on antibiotic treated spruce foliage ($-0.020 \pm$
117 0.005 (mean \pm SE); $p < 0.0001$), and larvae feeding on untreated fir foliage grew less than those
118 feeding on untreated spruce ($-0.017 \pm .006$; $p = 0.032$, Fig. 1, Table 1, Table S4). Growth rates of
119 larvae feeding on antibiotic treated and untreated foliage of the same type (i.e fir or spruce) did
120 not differ. Therefore, differences in growth rate observed among different groups of larvae are
121 due to differences in diet more so than any disturbance in the microbial community caused by
122 antibiotic treatment.

123 The growth rate of spruce budworm larvae was not strongly correlated with the microbial
124 community structure associated with foliage (Redundancy analysis (RDA); Fig. S2) or the
125 community structure in guts (Fig. S3). The influence of microbial community structure on the
126 growth rate of spruce budworm larvae was greater in foliage communities (19.01 % constrained
127 variance explained) than in gut communities (9.85 %). These data suggest that although the gut
128 microbial community is not well correlated with larval growth, there is some initial benefit from
129 bacteria as they pass through the gut even if they do not colonize the gut tissue. Foliage samples
130 collected at the first time point were used to determine the influence of foliage-associated

131 microbial communities because the community structure of foliage did not significantly differ
132 between collection times (Procrustes test; sum of squares = 0.88, $p=0.57$).

133 **Differences in the gut community of fast and slow growing larvae**

134 We compared the gut-associated communities of the fastest and slowest growing larvae (upper
135 and lower quartile of growth rates respectively) in each treatment group as an additional way to
136 determine if gut community structure impacts spruce budworm larval growth. Gut communities
137 of larvae did not differ between fast and slow growers regardless of diet, antibiotic treatment, or
138 distance measure used (PERMANOVA on unweighted UniFrac; antibiotic treated fir $F=1.89$,
139 $R^2=0.32$, $p=0.10$; untreated fir $F=0.74$, $R^2=0.15$, $p=0.86$; antibiotic spruce $F=1.07$, $R^2=0.26$,
140 $p=0.2$; untreated spruce $F=2.17$, $R^2=0.35$, $p=0.10$).

141 **Effects of antibiotic treatment on microbial communities**

142 Foliage that was treated with antibiotics had slightly lower diversity (Shannon diversity = $1.8 \pm$
143 0.12) than untreated foliage (2.20 ± 0.12) (ANOVA; $F=5.834$ $p=0.019$) however the difference
144 was not significant according to post hoc tests (Tukey's honestly significant difference test
145 (TukeyHSD); $p = 0.051$, Fig. 2A). Larvae feeding on synthetic diet that contained antibiotics,
146 had lower microbial diversity in their guts (0.52 ± 0.13) than larvae feeding on foliage treated
147 with antibiotics (1.52 ± 0.13) or untreated foliage (1.47 ± 0.13) (ANOVA; $F=20.5$, $p<0.001$:
148 TukeyHSD; $p<0.001$, Fig. 2B). The Shannon diversity of microbial communities sampled from
149 spruce budworm frass did not differ among any of the treatments (Fig. 2C).

150 The composition of diet-associated microbial communities (fir foliage, spruce foliage, and
151 synthetic diet) was different between antibiotic treatments (PERMANOVA on unweighted
152 UniFrac; $F=1.43$, $R^2=0.022$, $p=0.0027$) regardless of the ecological distance measure used (Fig.

153 S4). When foliage samples (not including synthetic diet) were compared, antibiotic treatment
154 only had an effect on foliage-associated community composition when UniFrac distances were
155 weighted by relative abundance (Fig. S4D-C). Antibiotic treatment only affected gut microbial
156 community structure when synthetic diet-fed larvae were excluded and the comparisons were
157 made with weighted UniFrac distances (PERMANOVA on weighted UniFrac; $F=2.40$, $R^2=$
158 0.060 , $p=0.025$, Fig 3C-D). There was no effect of antibiotic treatment on microbial community
159 composition associated with the frass of larvae from any treatment group.

160 Antibiotic treatment impacted the microbial community structure associated with the diets fed to
161 larvae in each treatment. Spruce budworm gut-associated communities, however, were only
162 affected by antibiotic treatment when distance measures were weighted by the relative
163 abundance of OTUs. In addition, because the microbial diversity of spruce budworm gut-
164 microbial communities was the same between larvae fed on treated and untreated foliage, this
165 suggests that antibiotic treatment affected the relative abundance of OTUs in gut communities
166 but not overall community membership.

167 **Does spruce budworm have a resident microbiome?**

168 To test if spruce budworm has a resident gut microbiota, we compared the associated microbial
169 communities among diets (foliage and artificial diet), guts, and spruce budworm frass, both at a
170 community-wide scale and at the scale of individual OTUs. Microbial diversity was significantly
171 different among sample types (ANOVA; $F=23.68$, $p<0.001$, Fig. 2). Unsurprisingly microbial
172 diversity was lower in guts (1.16 ± 0.09 (mean \pm SE)) than in foliage samples (1.74 ± 0.1)
173 (TukeyHSD; $p<0.001$). Interestingly microbial diversity was also lower in guts than in frass
174 samples (1.76 ± 0.06) (TukeyHSD; $p<0.001$). There was no significant difference in microbial
175 diversity between frass and foliage communities (TukeyHSD; $p=0.986$, Fig. 2).

176 The structure of microbial communities associated with diets (including artificial diet), guts, and
177 frass were significantly different from each other based on the phylogenetic relatedness of OTUs
178 among the communities weighted by their relative abundances (PERMANOVA on weighted
179 UniFrac distance; $F=4.50$, $R^2=0.034$, $p<0.001$). Because these communities are different, we
180 analyzed the effects of each sample type separately using both unweighted and weighted
181 UniFrac. Overall diet-associated communities were different from each other regardless of
182 distance measure used (PERMANOVA on unweighted UniFrac; $F=1.66$, $R^2=0.052$, $p<0.001$,
183 Fig. S4). The largest difference among diet-associated communities in terms of composition was
184 between foliage communities and the communities in the synthetic diet (Fig. S4). Differences
185 between spruce- and fir-associated communities were also evident (PERMANOVA on
186 unweighted UniFrac; $F=1.27$, $R^2=0.0255$, $p=0.031$), however the difference between spruce- and
187 fir- associated communities was not evident when OTUs were weighted by relative abundance
188 which suggests that the abundant OTUs are shared between diet types and the difference between
189 spruce and fir foliage is driven by rare taxa.

190 Gut-associated communities follow a similar trend but differences are less pronounced than
191 observed in the foliage- and synthetic diet-associated communities. In most of the comparisons
192 of diet-associated communities, diet type (tree species or artificial diet) was identified as being a
193 significant factor driving community structure. Gut community structure on the other hand was
194 only affected by diet type when larvae that fed on synthetic diet were included in the analysis
195 (PERMANOVA on unweighted UniFrac; $F=12.97$, $R^2=0.31$, $p<0.001$, Fig. 3) regardless of
196 distance measure. There was no difference in gut-associated community composition between
197 spruce and fir foliage fed larvae (Fig. 3). This indicates that antibiotics affect the relative
198 abundances of gut microbiota in the spruce budworm gut, but differences between host foliage

199 do not. Across all gut samples the five most abundant OTUs were identified as: *Enterococcus* sp.
200 (39.7 % relative abundance), *Shewanella* sp. (13.6%), *Shewanella* sp. (8.8%), a member of the
201 family *Halomonadaceae* (7.4%), and *Halomonas* sp. (7.4%) (Fig. 4).

202 Frass communities of spruce budworm larvae only differed in structure between diets when
203 larvae fed on synthetic diet were included in the analysis (PERMANOVA on weighted UniFrac;
204 $F=2.052$, $R^2=0.0493$ $p=0.0031$), there was no difference between frass-associated communities
205 of foliage fed larvae. There was no difference among any of the treatments in frass based on
206 unweighted UniFrac distances, a purely phylogenetic distance measure (Fig. S5). Frass-
207 associated microbial communities in general were more similar among different diet types and
208 treatments than either the guts or diet samples.

209 Generally, our results show that the type of diet (spruce foliage, fir foliage, or synthetic diet) and
210 antibiotic treatment had a significant effect on the communities associated with the diets fed to
211 larvae from each treatment. When we analyzed the gut-associated bacterial communities we
212 were still able to detect effects on community structure attributed to diet type but that difference
213 was driven entirely by the difference in community structure between synthetic diet and fresh
214 foliage. When the guts of larvae that were fed synthetic diet were removed from the analysis, the
215 only difference that we detected between communities was due to antibiotic treatment when
216 weighted UniFrac was used as the distance measure. Because the only difference that we
217 observed in community structure was calculated with a metric weighted by abundance, this
218 suggests that antibiotic treatment applied to the different diets had some effect on spruce
219 budworm microbiota relative abundances but not on overall community membership.

220 **Are gut-associated bacteria more abundant in foliage or the gut?**

221 We compared the relative abundances of individual OTUs between foliage, guts, and frass
222 samples to identify OTUs that were differentially abundant between samples using ANCOM
223 (Table S5). Identifying differentially abundant OTUs was done to further determine if the spruce
224 budworm gut has a resident microbiome. Among all diets and treatments, ANCOM detected 9
225 differentially abundant OTUs between gut and foliage communities (Fig. 5). Each OTU
226 identified as being differentially abundant was more abundant in foliage-associated communities.
227 Within the group of larvae feeding on antibiotic treated fir however, there was one differentially
228 abundant OTU between the gut (denovo8018: *Halomonas sp.*, relative abundance in gut: 0.0003
229 ± 0.0001) and foliage communities (relative abundance in foliage 0.0 ± 0.0) that was more
230 abundant in the gut. *Halomonas sp.* accounts for less than 1% of the gut associated bacteria in
231 larvae feeding on antibiotic treated fir however (Fig. S6). We also detected three differentially
232 abundant OTUs between gut and frass communities two of which were more abundant in frass
233 while the third, denovo4154: *Mollicutes*, was more abundant in guts (Fig S7). Finally, no OTUs
234 were identified as being differentially abundant between fast and slow growing larvae.

235 **DISCUSSION**

236 Our results provide evidence to support the idea that microbial symbionts are not critical to
237 lepidopteran nutrition\survival and that lepidopteran larvae lack a resident microbiota (28, 29).
238 Although antibiotics influenced spruce budworm microbial community structure, the gut
239 microbial community of spruce budworm larvae did not influence host growth. Contrary to other
240 studies suggesting that microbial communities of lepidopteran larvae lack resident microbes (28,
241 29), we present evidence suggesting that a small number of bacterial taxa are able to persist and
242 increase in relative abundance in the spruce budworm gut such as *Halomonas sp.* and a member

243 of *Molicutes*. These microbes do not appear to be necessary for the growth or survival of the
244 spruce budworm, and their functions in the gut remain unclear.

245 **Influence of the gut microbial community on larval health**

246 One of our hypotheses was that the use of antibiotics would reduce the bacterial diversity and
247 alter community composition in the gut of spruce budworm and that those differences in the
248 microbial community would result in a reduction of spruce budworm larval growth. Although
249 there are some differences in spruce budworm larval gut microbial communities due to diet
250 choice and antibiotic treatment, none of the observed changes in gut microbiota associated with
251 antibiotic treatment resulted in any significant change in larval survival or growth under
252 laboratory conditions.

253 Taken together with similar results from a growth experiment where the microbiome of
254 *Manduca sexta* was eliminated via antibiotic treatment and no change in growth was detected
255 (28), our findings suggest that the eastern spruce budworm, and perhaps many other lepidopteran
256 species, are not nutritionally dependent on a microbial symbiosis. One possible explanation for
257 this could be the bulk feeding strategy utilized by spruce budworm and many other herbivorous
258 lepidopteran species. It is possible that because spruce budworm larvae consume so much food
259 during their development it is not as imperative to efficiently extract nutrients from their diet.
260 Another possible explanation could be that the alkalinity of the spruce budworm guts allows
261 them to extract nutrients or tolerate the secondary compounds associated with conifer foliage.
262 Along with previous work showing that carnivorous and herbivorous larval microbiomes did not
263 differ significantly in composition (29), this suggests that lepidopteran larvae may not select for
264 gut bacteria based on their nutritional needs, providing further evidence lepidopteran larvae do
265 not rely on microbial symbiosis to extract the necessary nutrients from food.

266 **Is the spruce budworm gut microbiome resident or transient?**

267 Although antibiotic treatment did not significantly affect the growth of spruce budworm, our
268 results shed some light on an ongoing discussion in the literature about the nature of gut
269 microbiota in lepidopteran larvae. Our second hypothesis was that as microbes are passed
270 through the spruce budworm gut via feeding, the physio-chemical environment of the gut would
271 select for bacteria that could thrive in that environment, but that the community would still
272 reflect the source community originating from the foliage diet. This pattern would provide
273 evidence that spruce budworm larvae possess a resident gut microbiome, however our results did
274 not support this hypothesis, and our findings provide further evidence that lepidopteran larvae
275 lack resident gut microbial communities. The main argument for this conclusion is that the larval
276 midgut community is composed of diet- and environmentally-derived microbes, and further
277 supported by evidence that lepidopteran larvae are not nutritionally dependent on microbial
278 associations.

279 While we provide evidence that generally supports the hypothesis that lepidopteran larvae lack a
280 resident microbiome, our data suggests that the microbial community associated with diet
281 changes as it travels through the spruce budworm gut. As microbes pass through the spruce
282 budworm gut, differences in the community structure attributed to diet and antibiotic treatment
283 present in foliage diminish. Thus weak selective pressures seem to be at play in the spruce
284 budworm gut, possibly due to the high pH in the lepidopteran larval gut. Looking at finer scales
285 to examine the responses of individual taxa, we found that spruce budworm larvae host a few
286 bacteria that increase in relative abundance while in the spruce budworm gut relative to the
287 foliage diet such as *Halomonas sp.* and a member of the class *Mollicutes*. This supports the
288 hypothesis that the dominant taxa in the spruce budworm gut are food-derived transient

289 microbes, but that rare foliage-associated microbes are able to persist in the spruce budworm gut.
290 The functional role of these rare gut-associated taxa has yet to be fully elucidated. While it is
291 clear that spruce budworm is not nutritionally dependent on its microbiota, it is possible that
292 other aspects of spruce budworm health could be influenced by these rare taxa that are able to
293 colonize the gut. More studies will be necessary to determine the influence of the microbiome on
294 spruce budworm reproductive fitness, fecundity, and parasitism rates for example through field
295 studies with wild populations of spruce budworm.

296 Although we observed weak selective pressures on the relative abundance of diet-derived
297 bacteria as they passed through the spruce budworm gut, we were unable to determine whether
298 these bacteria were dormant or active. If for example taxa that are active on foliage become
299 dormant or are otherwise unable to colonize the gut but do not die, it is possible that their intact
300 cells could survive the passage through the gut and still be detectable through DNA sequencing.
301 Future studies that measure bacterial activity and function directly in the gut will be required to
302 address this question.

303 Microbial communities in fecal material or frass are often presumed to provide a surrogate
304 measure of the microbial communities present in the gut. In our study, microbial community
305 structure in frass differed only between artificial diet versus all other treatments, in contrast with
306 the results for gut and foliage associated communities. Due to logistical constraints, frass was
307 allowed to accumulate over time before being collected, and it is possible that any differences in
308 gut microbial community structure in the gut were obscured by microbial growth in frass after
309 excretion. Because we observed weak selective pressures on the microbial community in the gut,
310 it is likely that the observed phenomenon in the frass-associated microbial community is a
311 combination of both selective pressures and methodological issues resulting in frass-associated

312 community structure being a less sensitive measure of variation in microbial community
313 structure in the gut than direct sampling of gut tissues. For this reason we suggest that the use of
314 frass to quantify insect gut microbiomes is not currently advised until a better method for
315 collecting frass can be developed.

316

317 **Conclusions**

318 Overall we observed that spruce budworm does not maintain a resident gut microbiome but that
319 there appears to be weak selective pressures on ingested bacteria in the spruce budworm gut. Our
320 findings did not support our hypothesis that alteration of the spruce budworm gut-associated
321 microbial community would result in a reduction of spruce budworm larval growth. We did
322 however provide evidence in support of the hypothesis that, although some rare microbial taxa
323 may be able to colonize the gut, the gut microbial community of lepidopteran larvae is largely
324 transient and unimportant for larval growth.

325 **MATERIALS AND METHODS**

326 **Insect rearing**

327 We acquired approximately 1,000 spruce budworm second instar larvae that had completed
328 diapause from the Insect Production Services at the Great Lakes Forestry Centre, (Sault Ste.
329 Marie, ON, Canada). Larvae were packaged between a sheet of parafilm and a sheet of cheese
330 cloth and stored at 4°C prior to the start of the experiment. Sections of the parafilm containing
331 approximately 30-40 larvae were cut using scissors sterilized for 5 seconds with 70% ethanol and
332 placed on cups of synthetic diet containing antibiotics in autoclaved magenta boxes. Larvae were
333 allowed to emerge from their hibernacula and feed on the common diet for one week. The

334 purpose of rearing larvae on a common diet for the first week was twofold: to ensure larvae were
335 large enough to successfully eat foliage, and so that all larvae started to feed on the same food to
336 control for variation in the starting microbiota among second instar larvae. Throughout the
337 experiment larvae were maintained at 24°C at 60% relative humidity under a 16h:8h light:dark
338 cycle.

339 After 1 week of feeding on the common diet, 200 larvae were randomly selected and split
340 equally among 5 treatments (n=40): artificial diet with antibiotics, black spruce (*Picea mariana*)
341 foliage treated with antibiotics, untreated spruce foliage, balsam fir (*Abies balsamea*) foliage
342 treated with antibiotics, and untreated balsam fir foliage. Each replicate consisted of an
343 individual larva in an autoclaved magenta box. Spruce foliage was collected from saplings
344 housed in the greenhouse at the Université du Québec à Montréal and stored at -20°C for
345 approximately 4 weeks. Fir foliage was collected from trees near Baie Comeau, Québec and
346 stored in sterile bags at -20°C for 4 weeks. In both cases we took care to use only foliage that had
347 fresh growth. Foliage was placed in a 2ml microcentrifuge tube filled with sterile water, or 50
348 µg/ml streptomycin for antibiotic treatments, and sealed with parafilm to reduce desiccation of
349 the cut foliage during the experiment. For antibiotic treatments, a 1500 ppm solution of methyl
350 paraben and a 50 µg/ml solution of streptomycin were each sprayed on the foliage every other
351 day. Untreated foliage were not manipulated other than placing the cut stem in a microcentrifuge
352 tube containing sterile water.

353 **Health assessment: measuring larval growth and survival**

354 Larval health was assessed every other day by measuring larval weight and calculating growth
355 rates. Overall survival was calculated as well. We chose larval weight as a measure of health
356 because it is often used as a measure of fitness in pupae and therefore can also be used as a

357 representation of overall health (28). We removed each larva from its magenta box using a fine
358 paintbrush, placed it on a sterile weigh boat, and recorded the mass. Re-application of antibiotics
359 on foliage occurred at this time via spray bottle. All work was done in an ethanol sterilized fume
360 hood. The paintbrush used to manipulate the larvae was sterilized for 5 seconds with 70%
361 ethanol between each replicate. Larvae that were dead at the time of weighing were discarded.

362 **Sample collection**

363 We collected sixth-instar larvae just prior to pupation, placed them in microcentrifuge tubes, and
364 left them at room temperature for 4 hours before freezing at -80°C. This was to allow for any
365 remaining food to pass through their guts, providing us with a more accurate approximation of
366 the true gut microbiota as opposed to microbes that simply pass through the gut along with the
367 food. Larval midguts were extracted from surviving individuals, using forceps and scissors
368 sterilized with 70% ethanol, by cutting the posterior and anterior ends of the individual off to
369 separate the midgut from the hindgut and foregut; remaining midgut was extracted from the larva
370 using the forceps. Extracted guts were placed directly in MoBio PowerSoil bead beating tubes
371 (Qiagen) and stored at -20°C until nucleic acid extraction.

372 We sampled frass and foliage samples twice during the experiment, once 7 days after exposure to
373 treatments and again after 14 days when larvae were also collected. Foliage was collected by
374 taking 5 needles with ethanol sterilized forceps, placed in microcentrifuge tubes, and
375 immediately frozen at -80°C. Frass was collected from the bottom of the magenta box, placed in
376 microcentrifuge tubes, and immediately frozen at -80°C. Samples were then assessed for
377 microbial community diversity and composition following DNA sequencing.

378 We sampled frass and foliage communities along with the gut microbiota. Using these three
379 communities to determine how the relative abundance of microbes changes from the source
380 (foliage) through an environmental filter (gut) and by comparing gut communities with frass
381 communities (or foliage communities) makes it possible to determine which taxa are able to
382 persist in the gut versus which taxa simply pass through the larval gut.

383 **DNA extraction and processing**

384 We extracted DNA from the midguts of all surviving larvae (n=96). In addition 10 individuals
385 were selected randomly from each of the 5 treatment groups to extract DNA from foliage or
386 synthetic diet (n=101) and frass (n=99), both collected at each of the two time points. All
387 genomic DNA from the guts, foliage, and frass was extracted using the MoBio PowerSoil DNA
388 extraction kit (Qiagen). We used a slightly altered protocol, as described below, in order to
389 increase DNA yields. Guts were homogenized by vortexing for 10 minutes in the provided
390 PowerSoil bead beating tubes and centrifuged at room temperature for 1 min at 10,000g. The
391 supernatant was transferred to a sterile 2 ml microcentrifuge tube and sonicated with the
392 Bioruptor UCD-200 sonicator (Diagenode) for 1 min on the low setting (160W at 20kHz) for 5
393 min. After sonication the DNA extraction proceeded as per the manufacturer's instructions.

394 Foliage and synthetic diet samples were placed in thick walled 2 ml tubes with three 2.3mm
395 diameter stainless steel beads (BioSpec Products, Bartlesville, OK, USA) and 250 µl of the
396 PowerSoil bead tube buffer. Diets were homogenized using a MiniBead Beadbeater-16 (BioSpec
397 Products, Bartlesville.) for 1.5 minutes. The remaining buffer from the bead beating tube was
398 added to the resulting homogenate, sonicated at the high setting (320W at 20kHz) for 2 minutes
399 and re-introduced to the bead beating tubes. Frass samples were sonicated for 2 minutes at the
400 high setting (320W at 20kHz) for 2 minutes with 250 µl of the bead beating buffer. Following

401 sonication, samples were transferred back to the bead beating tubes. For diet and frass samples,
402 after sonication the DNA extraction was performed following the manufacturer's instructions.
403 Following DNA extractions all samples were cleaned using the Zymo OneStep-96 PCR inhibitor
404 removal kit. Polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene from the
405 extracted DNA. We used the chloroplast excluding primers (799F and 1115R) (30) to target the
406 V5-V6 region of the 16S rRNA gene. Each primer also contained 1 of 20 unique bar codes and
407 an Illumina adaptor to allow sequences to bind to the flow cell of the MiSeq sequencer. PCR was
408 performed using 25 μ l reactions prepared with 1 μ l genomic DNA diluted 1:10 in molecular-
409 grade water, 5 μ l 5x HF buffer (Thermo Scientific), 0.5 μ l dNTP's (10 μ M each), 0.5 μ l forward
410 and reverse primer (10 μ M each), 0.75 μ l DMSO, 0.25 μ l Phusion HotStart II polymerase
411 (Thermo Scientific), and 16.5 μ l molecular-grade water. Each reaction began with 30 seconds of
412 denaturation at 98°C followed by 35 cycles of: 15s at 98°C, 30s at 64°C, 30s at 72°C, and a final
413 elongation step at 72°C for 10 minutes. Each PCR included a positive control and a negative
414 control that were verified using gel electrophoresis on an agarose gel prior to sequencing.
415 Amplicons were cleaned and normalized to 0.55 ng/ μ l using the Invitrogen SequelPrep
416 normalization plate kit. After normalization equal volumes of amplicon DNA per sample were
417 pooled and sequenced. In addition to sequencing experimental samples, positive and negative
418 controls from each PCR were sequenced.

419 **Amplicon sequencing**

420 We sequenced 16S rRNA gene amplicons using the Illumina MiSeq platform using V3
421 chemistry. After sequencing, we first trimmed Illumina adapters from our sequences using the
422 program BBduk version 35.76 ([https:// sourceforge.net/projects/bbmap](https://sourceforge.net/projects/bbmap)) and created paired end
423 sequences using PEAR version 0.9.5 (31). The resulting paired end sequences were

424 demultiplexed and passed through a quality control workflow using QIIME version 1.9.1 (32)
425 where chimeric sequences, sequences with more than two errors in the primer sequence, and
426 sequences with an average quality score lower than 25 were removed. Except where otherwise
427 noted, default settings were used for all bioinformatics analyses. In total 3,568,621 sequences
428 were obtained across all samples after the initial quality control steps and the removal of
429 chimeric sequences.

430 Sequences passing quality control parameters were binned into 8,593 operational taxonomic
431 units (OTU) based on a 97% sequence similarity using the uclust algorithm. The most abundant
432 sequence for each OTU was used as a representative sequence that was taxonomically identified
433 to the lowest possible level (50% consensus cutoff) using a BLAST search of the Greengenes
434 16S gene database (33). In addition an alignment of each OTU's representative sequence was
435 used to create a phylogeny using the FastTree 2 software (34). Positive controls were identified
436 as *E. coli* and were distinct from experimental samples as expected. Negative controls had few to
437 zero sequences and did not pass quality control steps. Following quality control, we analyzed
438 community composition, structure, and the effects of gut communities on spruce budworm
439 growth and survival using the statistical software R (35).

440 **Growth and survival analysis**

441 The effects of antibiotic treatment and diet (spruce or fir) on spruce budworm growth and
442 survival were tested using two separate models. A mixed-effects model implemented with the R
443 package nlme (36) was used to test for differences in larval growth. Larval weights were log
444 transformed and used as the response variable in the model. Time, antibiotic treatment, diet, and
445 their interactions were used as fixed effects, where time as a fixed effect is an estimate of growth
446 rate, and time nested within individual larvae was used as the random effect for the model.

447 Differences in larval survival were tested using a separate logistic regression with survival as a
448 binary response variable and antibiotic treatment, diet, and their interaction as main effects.

449 **Community analysis**

450 We tested for differences in community composition and diversity both among and within
451 sample types (foliage, guts, frass). When we made comparisons among sample types, data were
452 analyzed as a single dataset so we could ensure that each sample type had equal sampling depth
453 for comparisons. When comparing treatments within sample types we analyzed separate datasets
454 for each sample type that were rarefied separately. Sample types were rarefied separately to
455 ensure the maximum number of sequences could be used in our analysis, allowing for more
456 statistical power when testing within sample type differences.

457 Prior to our analysis we removed extremely rare OTUs (< 10 sequences) and samples that had
458 fewer than 500 total sequences. A total of 1,020 OTUs remained after removing rare OTUs.
459 When all sample types were analyzed together samples were rarefied to 1,000 sequences. When
460 analyzed separately, gut samples were rarefied to 2,500 sequences per sample while diet and
461 frass samples were rarefied to 1,000 sequences each. We calculated Shannon diversity based on
462 relative abundances of rarefied samples for each data set as a measure of diversity (37).

463 Community structure was explored using non-metric multidimensional scaling (NMDS) using
464 two complementary distance measures; unweighted UniFrac, and weighted UniFrac (38).

465 Permutational multivariate ANOVA (PERMANOVA) with 10,000 permutations was used to test
466 for differences in community structure among diets and between antibiotic treatments using the
467 unweighted UniFrac and weighted UniFrac distance measures. PERMANOVA was implemented
468 using the R package Vegan (39)

469 Gut communities were further analyzed to test for correlations between growth rate (calculated
470 from log transformed weights as the slope of a simple linear model for each larva) and gut
471 community structure using redundancy analysis (RDA) with growth rate, diet, and treatment as
472 environmental variables. Individual growth rates were used for redundancy analysis rather than
473 the estimate derived from the mixed effects model in order to incorporate the communities of
474 each sample separately rather than a single average community. Individual growth rates
475 calculated in this manner were comparable to the estimate derived from the mixed effects model.
476 Redundancy analysis was also performed on foliage communities collected at the first time point
477 to test for correlations between plant-associated microbial communities and larval growth. A
478 Procrustes analysis of NMDS ordination results calculated with weighted UniFrac was used to
479 test for differences in foliage-associated community structure between collection times.

480 In addition to using redundancy analysis to examine how growth rate and gut community
481 structure are related, the fastest and slowest growing larvae, defined as the larvae in the upper
482 and lower quartile of growth rates respectively, were selected for each experimental group and
483 we compared their gut community structure.

484 Finally to test for differences in relative abundance of individual taxa between groups we used a
485 differential expression analyses using the R package ANCOM (40), which tests for differences in
486 relative abundances of taxa between communities. Differentially abundant OTUs were identified
487 using the Kruskal-Wallis test with the less stringent multiple testing correction option provided
488 by ANCOM and an alpha of 0.05, based on samples rarefied to 1000 sequences per sample and a
489 pseudocount of 1 added to each abundance. We chose to use this method for calculating
490 differentially abundant OTUs because it has been shown to perform better than other methods

491 (41). A table summarizing all of the statistical tests used in this study and the hypotheses tested
492 by each can be found in the supplementary material (Table S2)

493 **Nucleotide sequence accession numbers**

494 16S rRNA amplicon sequence reads, including positive and negative controls, were submitted to
495 the NCBI sequence read archive under the SRA accession number SRP139053 which covers all
496 samples collected for this study between the accession numbers SRX3908565 and SRX3908861
497 (<https://www.ncbi.nlm.nih.gov/sra/SRP139053>).

498

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508

509 **REFERENCES**

510 1. Boulanger Y, Arseneault D. 2004. Spruce budworm outbreaks in eastern Quebec over the last 450
511 years. *Can J For Res* 34:1035–1043.

- 512 2. Royama AT, Mackinnon WE, Kettela EG, Carter NE, Hartling LK, Ecology S, May N. 2005. Analysis
513 of Spruce Bud Worm Outbreak Cycles in New Brunswick, Canada, Since 1952 86:1212–1224.
- 514 3. Sainte-Marie GB, Kneeshaw DD, MacLean D a., Hennigar CR. 2015. Estimating forest vulnerability to
515 the next spruce budworm outbreak: will past silvicultural efforts pay dividends? Can J For Res 45:314–
516 324.
- 517 4. Burton PJ, Svoboda M, Kneeshaw D, Gottschalk KW. 2015. Options for Promoting the Recovery and
518 Rehabilitation of Forests Affected by Severe Insect Outbreaks. Restor Boreal Temp For 495–517.
- 519 5. MacLean DA. 1984. Effects of spruce budworm outbreaks on the productivity and stability of balsam
520 fir forests. For Chron 60:273–279.
- 521 6. Fournier C, Baucé É, Dupontb A, Berthiaumea R. 2010. Wood losses and economical threshold of Btk
522 aerial spray operation against spruce budworm. Pest Manag Sci 66:319–324.
- 523 7. Robert L, Kneeshaw D, Sturtevant BR. 2012. Effects of forest management legacies on spruce
524 budworm (*Choristoneura fumiferana*) outbreaks. Can J Res 42:463–475.
- 525 8. Régnière J, Nealis VG. 2007. Ecological mechanisms of population change during outbreaks
526 of the spruce budworm. Ecol Entomol 32:461–477.
- 527 9. Lapierre P, Gogarten JP. 2009. Estimating the size of the bacterial pan-genome. Trends Genet 25:107–
528 110.
- 529 10. Feldhaar H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect hosts.
530 Ecol Entomol 36:533–543.
- 531 11. Douglas AE. 2009. The microbial dimension in insect nutritional ecology. Funct Ecol 23:38–47.
- 532 12. Engel P, Moran NA. 2013. The gut microbiota of insects - diversity in structure and function. FEMS
533 Microbiol Rev 37:699–735.

- 534 13. Kwong WK, Mancenido AL, Moran NA. 2017. Immune system stimulation by the native gut
535 microbiota of honey bees. *R Soc Open Sci* 4:170003.
- 536 14. De Souza DJ, Lenoir A, Kasuya MCM, Ribeiro MMR, Devers S, Couceiro JDC, Della Lucia TMC.
537 2013. Ectosymbionts and immunity in the leaf-cutting ant *Brain Behav Immun* 28:182-187.
- 538 15. Koch H, Schmid-Hempel P. 2011. Socially transmitted gut microbiota protect bumble bees against an
539 intestinal parasite. *Proc Natl Acad Sci USA* 108:19288-19292.
- 540 16. Prado SS, Hung KY, Daugherty MP, Almeida RPP. 2010. Indirect effects of temperature on stink bug
541 fitness, via maintenance of gut-associated symbionts. *Appl Environ Microbiol* 76:1261-1266. doi:
542 10.1128/AEM.02034-09
- 543 17. Rosengaus RB, Zecher CN, Schultheis KF, Brucker RM, Bordenstein SR. 2011. Disruption of the
544 termite gut microbiota and its prolonged consequences for fitness. *Appl Environ Microbiol* 77:4303-4312.
545 doi: 10.1128/AEM.01886-10.
- 546 18. Emery O, Schmidt K, Engel P. 2017. Immune system stimulation by the gut symbiont *Frischella*
547 *perrara* in the honey bee (*Apis mellifera*). *Mol Ecol* 26:2576–2590. <https://doi.org/10.1111/mec.14058>.
- 548 19. Engel P, Martinson VG, Moran NA. 2012. Functional diversity within the simple gut microbiota of
549 the honey bee. *Proc Natl Acad Sci USA* 109:11002-11007.
- 550 20. Broderick NA, Raffa KF, Goodman RM, Handelsman J. 2004. Census of the Bacterial Community of
551 the Gypsy Moth Larval Midgut by Using Culturing and Culture-Independent Methods. *Appl Environ*
552 *Microbiol* 70:293–300. doi: 10.1128/AEM.70.1.293-300.2004.
- 553 21. Mason CJ, Raffa KF. 2014. Acquisition and structuring of midgut bacterial communities in gypsy
554 moth (*Lepidoptera: Erebidae*) larvae. *Environ Entomol* 43:595–604.

- 555 22. Xiang H, Wei G-F, Jia S, Huang J, Miao X-X, Zhou Z, Zhao L-P, Huang Y-P. 2006. Microbial
556 communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa*
557 *armigera*). *Can J Microbiol* 52:1085–1092.
- 558 23. Tang X, Freitag D, Vogel H, Ping L, Shao Y, Cordero EA, Andersen G, Westermann M, Heckel DG,
559 Boland W. 2012. Complexity and variability of gut commensal microbiota in polyphagous lepidopteran
560 larvae. *PLoS One* 7:1–9.
- 561 24. Brinkmann N, Martens R, Tebbe CC. 2008. Origin and diversity of metabolically active gut bacteria
562 from laboratory-bred larvae of *Manduca sexta* (*Sphingidae*, *Lepidoptera*, *Insecta*). *Appl Environ*
563 *Microbiol* 74:7189–7196. doi: 10.1128/AEM.01464-08
- 564 25. Belda E, Pedrola L, Peretó J, Martínez-Blanch JF, Montagud A, Navarro E, Urchueguía J, Ramón D,
565 Moya A, Porcar M. 2011. Microbial diversity in the midguts of field and lab-reared populations of the
566 European Corn Borer *Ostrinia nubilalis*. *PLoS One* 6: e21751.
567 <https://doi.org/10.1371/journal.pone.0021751>.
- 568 26. Robinson CJ, Schloss P, Ramos Y, Raffa K, Handelsman J. 2010. Robustness of the bacterial
569 community in the cabbage white butterfly larval midgut. *Microb Ecol* 59:199–211.
- 570 27. Landry M, Comeau AM, Derome N, Cusson M, Levesque RC. 2015. Composition of the Spruce
571 Budworm (*Choristoneura fumiferana*) Midgut Microbiota as Affected by Rearing Conditions. *PLoS One*
572 10:e0144077. <https://doi.org/10.1371/journal.pone.0144077>
- 573 28. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SL, Fierer N. 2017. Caterpillars lack a resident gut
574 microbiome. *PNAS* 249:84–89.
- 575 29. Whitaker MRL, Salzman S, Sanders J, Kaltenpoth M, Pierce NE. 2016. Microbial communities of
576 lycaenid butterflies do not correlate with larval diet. *Front Microbiol* 7:1920.

- 577 30. Chelius MK, Triplett EW. 2001. The Diversity of Archaea and Bacteria in Association with the Roots
578 of *Zea mays* L. *Microb Ecol* 41:252–263.
- 579 31. Zhang J, Kobert K, Flouri T, Stamatakis A. 2014. PEAR: A fast and accurate Illumina Paired-End
580 reAd mergeR. *Bioinformatics* 30:614–620.
- 581 32. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG,
582 Goodrich JK, Gordon JI, Huttley G a, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone C a,
583 Mcdonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters W a, Widmann J,
584 Yatsunenko T, Zaneveld J, Knight R. 2010. Correspondence QIIME allows analysis of high- throughput
585 community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nat*
586 *Publ Gr* 7:335–336.
- 587 33. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P,
588 Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench
589 compatible with ARB. *Appl Environ Microbiol* 72:5069–5072. doi: 10.1128/AEM.03006-05
- 590 34. Price MN, Dehal PS, Arkin AP. 2010. FastTree 2 - Approximately maximum-likelihood trees for
591 large alignments. *PLoS One* 5:e9490. <https://doi.org/10.1371/journal.pone.0009490>
- 592 35. R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for
593 Statistical Computing. Vienna, Austria. <https://www.R-project.org/>.
- 594 36. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2017. nlme: Linear and Nonlinear Mixed
595 Effects Models. R package version 3.1-131.
- 596 37. Haegeman B, Hamelin J, Moriarty J, Neal P, Dushoff J, Weitz JS. 2013. Robust estimation of
597 microbial diversity in theory and in practice. *ISME J* 7:1092–1101.
- 598 38. Lozupone C, Knight R. 2005. UniFrac : a New Phylogenetic Method for Comparing Microbial
599 Communities. *Appl Environ Microbiol* 71:8228–8235. doi: 10.1128/AEM.71.12.8228-8235.2005

600 39. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlenn D, Minchin PR, O'Hara RB,
601 Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2017. vegan: Community Ecology
602 Package. R package version 2.4-3. <https://CRAN.R-project.org/package=vegan>

603 40. Mandal S, Treuren W Van, White RA, Eggesbø M, Peddada SD, Mandal S, Treuren W Van, White
604 RA, Eggesbø M. 2015. Analysis of composition of microbiomes: a novel method for studying microbial
605 composition. *Microbial ecology in health and disease* 26:2235.

606 41. Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-
607 Baeza Y, Birmingham A, Hyde ER, Knight R. 2017. Normalization and microbial differential abundance
608 strategies depend upon data characteristics. *Microbiome* 5:27.

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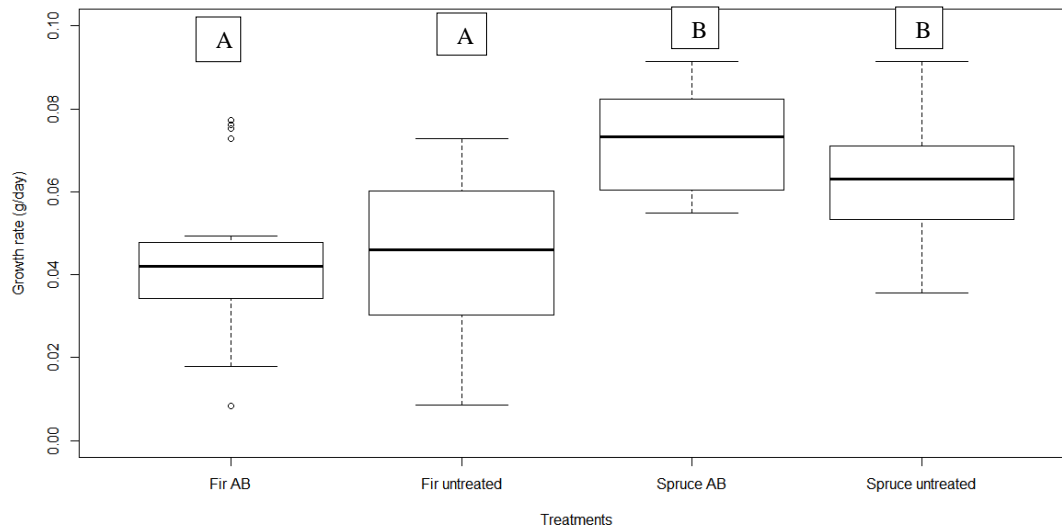
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620 **FIGURES AND TABLES**



621
622 Figure 1. Growth rate (grams/day, \pm S.E) of spruce budworm larvae among different diets
623 (spruce versus fir foliage) and antibiotic treatments (AB = antibiotic treated). Letters indicate
624 treatment combinations that differed significantly ($p < 0.05$) according to a Tukey's Honest
625 Significant Difference post-hoc test, based on a mixed model (see Methods section for details).

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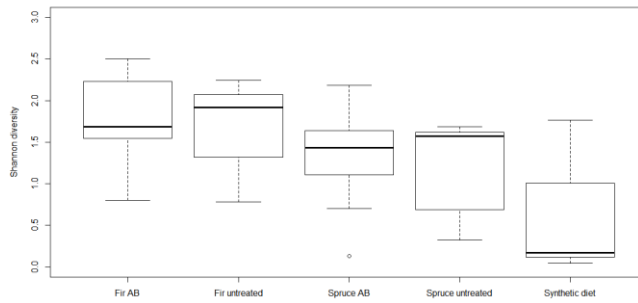
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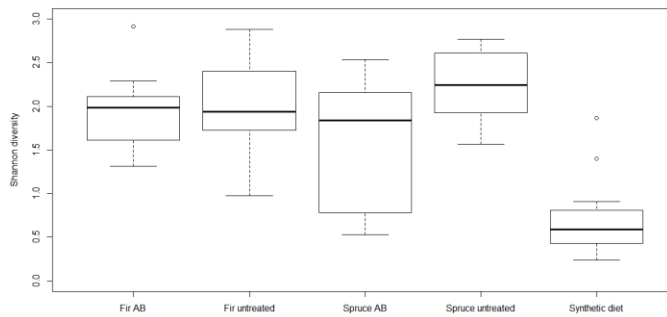
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632 A)



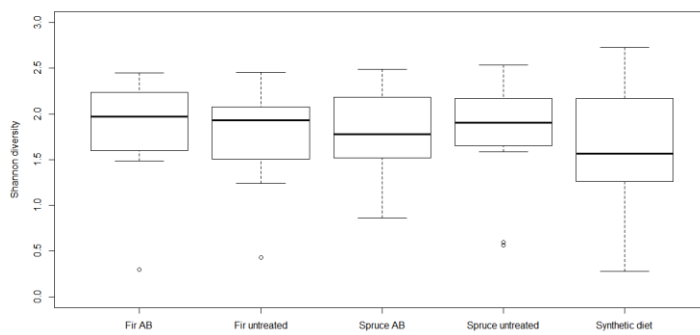
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634 B)



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636 C)



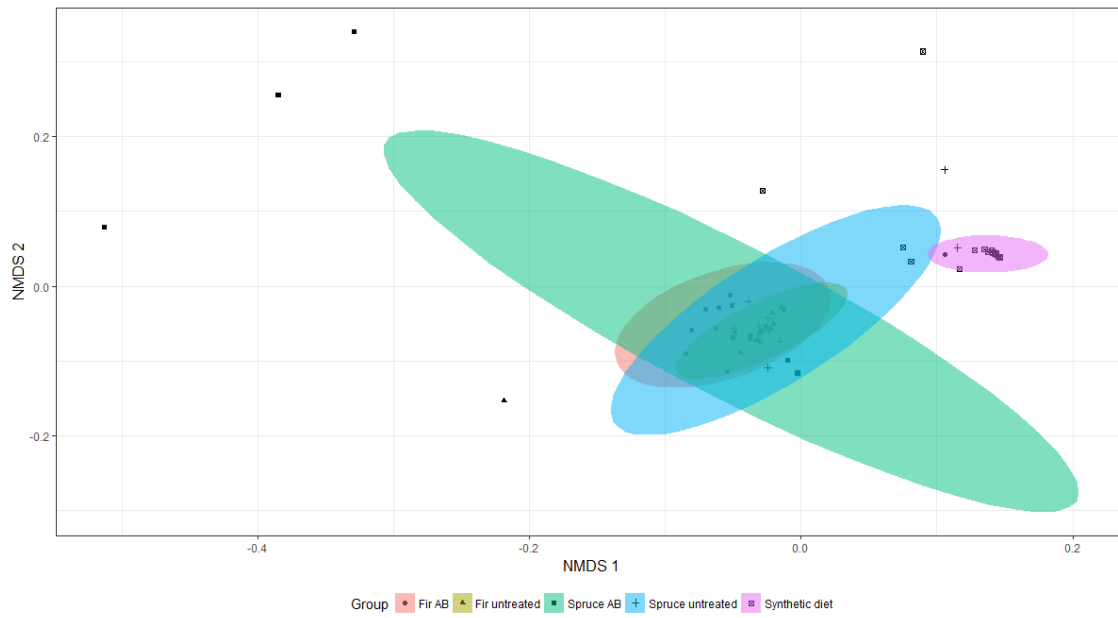
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638 Figure 2. Mean (\pm SE) Shannon diversity of (A) spruce budworm diets (B) guts and (C) frass
639 associated microbial communities among different diets (spruce versus fir foliage and synthetic
640 diet) and antibiotic treatments (AB= antibiotic treated).

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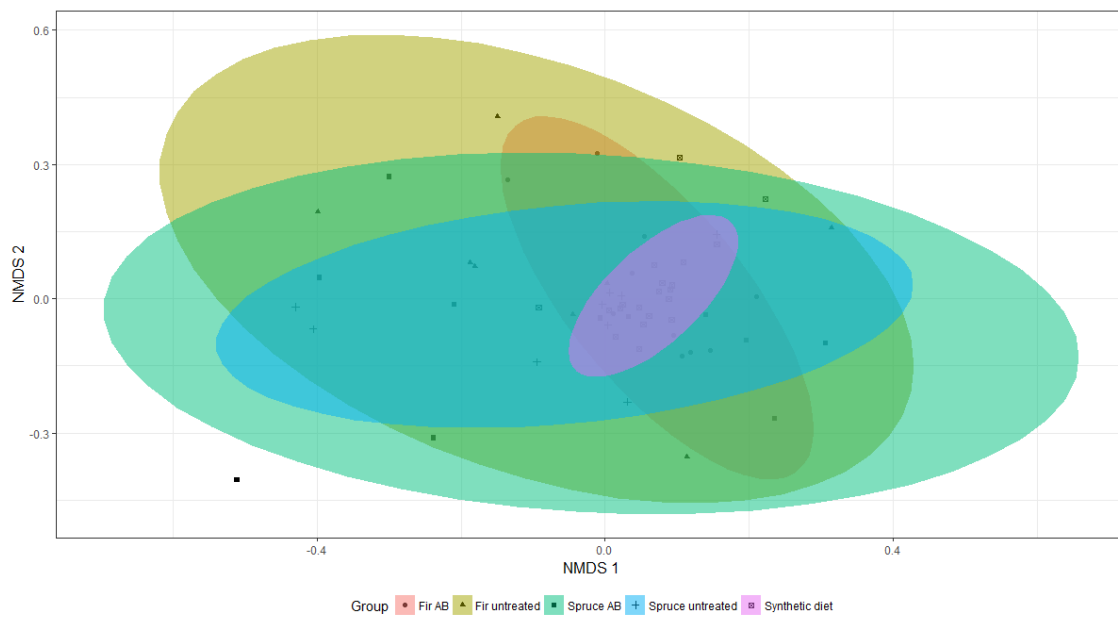
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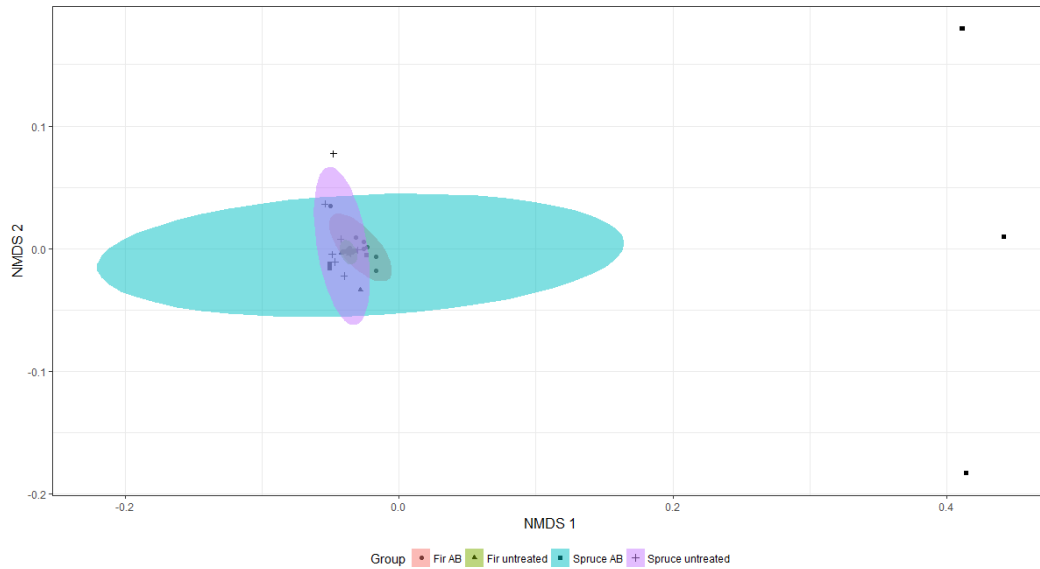
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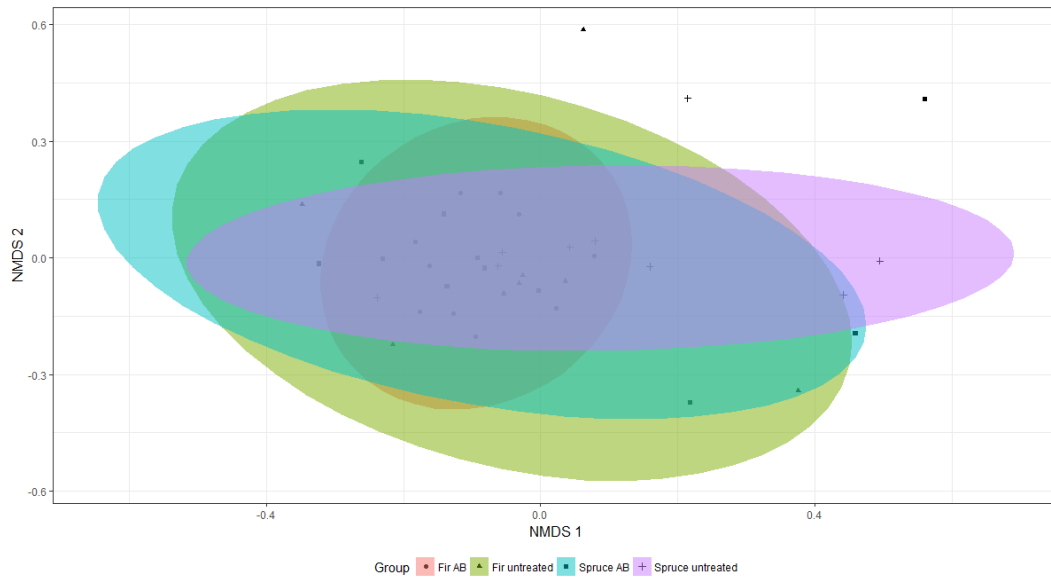
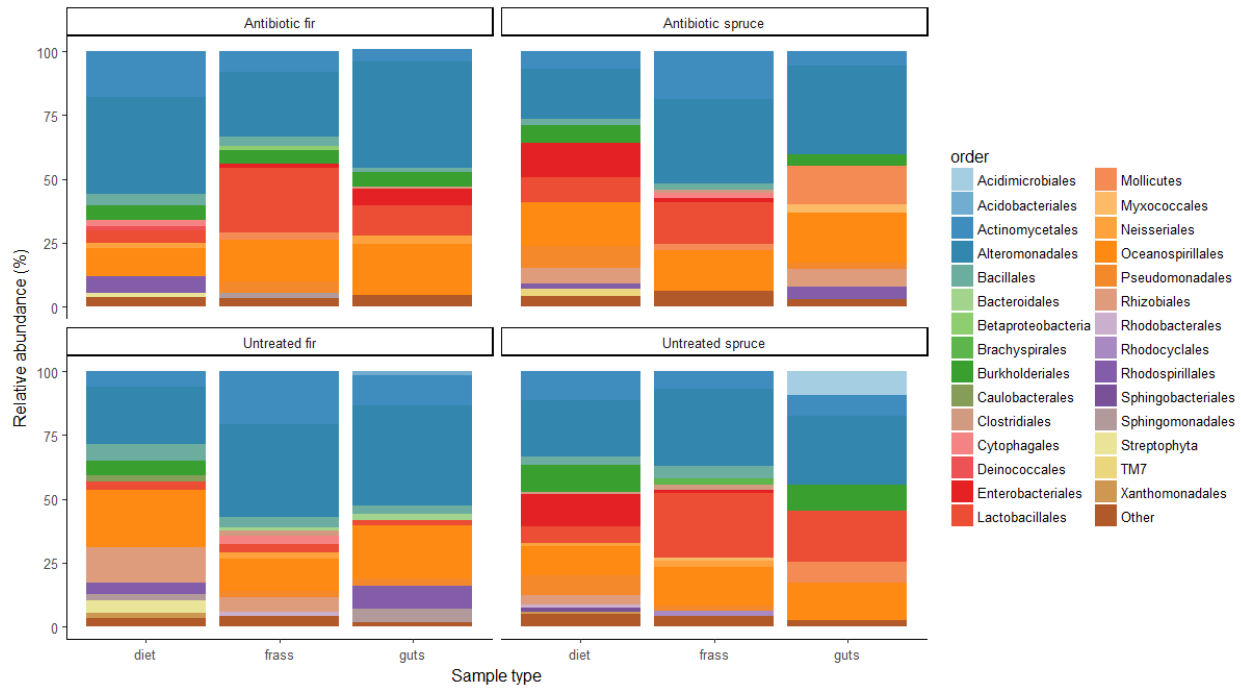


Figure 3. NMDS ordinations of gut-associated microbial communities based on weighted and unweighted UniFrac distances. (A: all guts weighted UniFrac stress =0.07, B: All guts UniFrac stress =0.21, C: guts of larvae feeding on foliage weighted UniFrac stress =0.07, D: guts of larvae feeding on foliage unweighted UniFrac stress =0.10). Ellipses represent 95% confidence

655 intervals around samples from different treatments (Fir.AB = antibiotic treated fir, Spruce.AB =
656 antibiotic spruce, Fir.untreated = untreated fir, and Spruce.untreated = untreated spruce).

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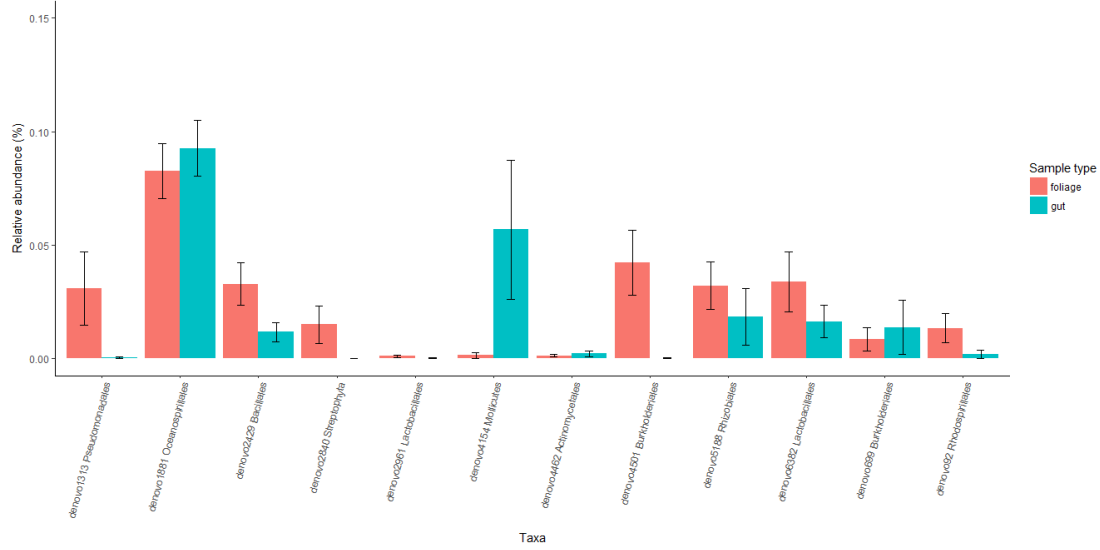
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659 Figure 4. Mean relative abundance (%) of bacterial taxa across sample types, i.e diet (either
660 spruce or fir foliage), budworm midguts, and budworm frass in each treatment group. Taxa were
661 identified to the level of order, however if taxa remained unassigned at the order level they were
662 labelled by taxonomic phylum or class.

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667 Figure 5. Mean relative abundance (\pm SE) of OTUs identified as being differentially abundant
668 between foliage- and gut-associated communities based on an ANCOM test (ANCOM; adjusted
669 $p < 0.05$). Blue bars represent gut samples and red bars represent foliage samples.

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678 Table 1. Growth rates of spruce budworm larvae feeding on spruce and fir needles with and
679 without antibiotics calculated as the estimate of time as fixed effect of a mixed effect model
680 comparing larval weights with time, antibiotic treatment, and diet and their interactions as fixed
681 factors and time nested within individual as random factors. In our model time as a fixed effect
682 represents the growth rate of larvae

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Diet	Treatment	Growth rate (g/day)	Standard error	Lower confidence limit	Upper confidence limit
Fir	AB	0.044	0.0039	0.036	0.052
Fir	None	0.045	0.0038	0.038	0.053
Spruce	AB	0.073	0.0038	0.065	0.081
Spruce	None	0.062	0.0049	0.053	0.072

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