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1 **Salmon carcasses influence genetic linkages between forests and streams**

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28 **Abstract**

29 Biodiversity at many scales (functional group, species, genetic) can result in emergent
30 ecological patterns. Here we explore the influence of tree genotypic variation and diversity on in-
31 stream ecosystem processes and aquatic communities. We test whether genetically diverse
32 inputs of leaf litter interact with a keystone organism, anadromous salmon, to influence in-
33 stream ecosystem function. We used reach-level manipulation of salmon carcasses and leaf
34 litter bags to examine how nutrient inputs interact with genetic variation in leaf litter
35 decomposition. Genotypic variation in black cottonwood (*Populus balsamifera* ssp. *trichocarpa*)
36 significantly influenced leaf litter chemistry, litter mass loss, and fungal biomass, but these
37 variables were only weakly influenced by salmon carcass presence or a genotype*salmon (G x
38 E) interaction. Mixtures of genotypes tended to demonstrate antagonistic effects (slower than
39 expected decomposition) in the absence of salmon, but synergistic effects (faster than expected
40 decomposition) when salmon were present. Our findings suggest that the influence of plant
41 genotypic variation in linking aquatic and terrestrial ecosystems may be altered, and in some
42 cases intensified in the presence of a keystone vertebrate species.

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45 **Keywords:** biodiversity-ecosystem function, genes-to-ecosystems, aquatic-terrestrial
46 interaction, litter mixtures

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51 **Introduction**

52 Over the last decade, a body of research has shown that genetic variation in several foundation
53 species can influence associated communities and ecosystem function, and these influences
54 can be as important as the influences of species-level diversity (Whitham et al. 2006; Hughes et
55 al. 2008; Bailey et al. 2009). A subset of this research shows significant intraspecific (within
56 species) variation in litter chemistry across a suite of plant species (including *Populus*
57 [cottonwood, aspen], *Quercus* [oak], and *Betula* [birch]) and subsequent litter decomposition
58 and nutrient cycling in both terrestrial and aquatic systems (Schweitzer et al. 2004; Madritch et
59 al. 2006; LeRoy et al. 2007; Silfver et al. 2007). The majority of previous genes-to-ecosystems
60 research has been conducted in common garden environments, but several recent studies have
61 taken a broader scope to examine genetic variation across environmental gradients. It is crucial
62 to move genes-to-ecosystems research outside the realm of common gardens because this will
63 help to place genetic variation within the context of broader environmental variation.

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64 Understanding genetic by environment (G x E) interactions is important because they
65 may elucidate situations in which the influence of genetic variation is either attenuated or
66 amplified by environmental factors. For example, in previous studies, G x E interactions were
67 rare for sea grass communities (Tomas et al. 2011), but relatively more common for terrestrial
68 insect communities (Johnson and Agrawal 2005; Tack et al. 2010; Rowntree et al. 2010;
69 Genung et al. 2012; Tétard-Jones et al. 2013). In the case of influences on ecosystem
70 functions, clear G x E interactions have been shown in both terrestrial (Madritch et al. 2006; He
71 et al. 2012) and aquatic (LeRoy et al. 2012) leaf litter decomposition studies and a soil nitrogen
72 transformation study (Pregitzer et al. 2013). In most cases, the environmental factor examined
73 is abiotic (such as nutrient enrichment), but occasionally the factor is biotic, such as herbivory
74 (Schweitzer et al. 2005b) or the presence of another genotype (Genung et al. 2012).

75 Emergent biodiversity patterns have been shown for both species mixtures and
76 genotype mixtures of litter. Diversity in detritus research represents a subset of the broader

77 biodiversity-ecosystem function literature (as reviewed by Gessner et al. 2010; Swan and
78 Kominoski 2012) and shows that litter mixtures have three possible effects on the
79 decomposition process: 1) additive effects where litter mixtures decompose at rates expected
80 based on each litter type in isolation 2) non-additive, synergistic effects where mixtures
81 decompose faster than expected, and 3) non-additive, antagonistic effects where mixtures
82 decompose slower than expected (Lecerf et al. 2011). Several recent papers have also shown
83 that mixtures of genotypes within species can influence decomposition and carbon cycling. As
84 with species diversity studies, these few results show that litter genotype mixtures can exhibit
85 significantly faster litter decomposition than expected (Schweitzer et al. 2005a), significantly
86 slower decomposition than expected (Madritch et al. 2006), or no difference between observed
87 and expected mass loss (Madritch et al. 2006; LeRoy et al. 2007).

88 Variation in environmental contexts may help explain the unpredictable additive and non-
89 additive results observed in past diversity studies; however, very few studies have manipulated
90 litter diversity and environmental variables together to test the consistency of emergent effects.
91 Rosemond et al. (2010) manipulated litter species diversity in the presence and absence of
92 elevated in-stream nutrients and showed that litter mixtures decomposed even faster in mixture
93 when also exposed to high in-stream nutrient concentrations. In a similar study, Bretherton et al.
94 (2011) examined litter species mixtures in the presence and absence of salmon carcasses (a
95 natural source of in-stream nutrients and organic matter) and also showed more synergistic
96 responses to litter mixing when salmon carcasses were present. This study further explores
97 these relationships, but goes to a finer level to ask how genotypic diversity effects may be
98 altered by nutrient enrichment via salmon carcass inputs.

99 Because anadromous salmon represent a major ecosystem-level influence on streams
100 and forests throughout the northern hemisphere (Gende et al. 2002), salmon presence is an
101 ideal biotic environmental factor to examine in this context. The presence of salmon carcasses
102 tends to increase in-stream algal and microbial productivity (Fisher-Wold and Hershey 1999)

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103 and alters the structure of macroinvertebrate communities (Wipfli et al. 1998). Although much is
104 known about inputs of both leaf litter and salmon carcasses to streams separately, only a few
105 studies have examined their interactions and the results have been mixed. For example, salmon
106 presence accelerated litter decomposition for relatively labile species like *Acer* (maple; Yanai
107 and Kochi 2005), *Salix* (willow; Kohler et al. 2008), mixed *Alnus* (alder) + *Acer* litters (Claeson et
108 al. 2006), and mixed *Alnus* + *Populus* (cottonwood) and *Alnus* + *Acer* litters (Bretherton et al.
109 2011), but slowed decomposition for both *Alnus* and *Acer* litters in isolation (Zhang et al. 2003),
110 and mixtures of *Populus* + *Acer* and *Populus* + *Alnus* litters (Bretherton et al. 2011).

111 Here we expand on previous research demonstrating genes-to-ecosystems linkages
112 across aquatic-terrestrial boundaries by examining genotypic variation in litter chemistry for
113 *Populus balsamifera* L. ssp. *trichocarpa* Torr. and A. Gray ex Hook (black cottonwood, hereafter
114 *Populus trichocarpa*; this study includes Nisqually-1, the first tree to be genomically sequenced;
115 Tuskan et al. 2006). We first examine how genetic variation influences leaf litter chemistry, then
116 we examine the influence of genetic variation and genotype mixing effects on leaf litter
117 decomposition as it interacts with an important biotic environmental factor, the presence of
118 anadromous salmon carcasses. We hypothesised that: 1) genetic variation in *P. trichocarpa*
119 would lead to differences in litter chemistry, 2) this genetic variation, as well as the presence of
120 salmon carcasses, and their interaction (genotype * salmon) would influence leaf litter mass loss
121 at all harvest dates, fungal biomass accumulation, and aquatic macroinvertebrate community
122 metrics; 3) leaf litter mass loss would be related to the suite of litter chemicals measured; 4)
123 genotypic mixing would accelerate mass loss and fungal biomass accumulation; and 5) salmon
124 carcasses, by providing nutrients and organic matter, would interact with genotype mixtures to
125 increase synergisms in mass loss, increase synergistic responses of fungal biomass accrual,
126 and alter macroinvertebrate communities.

127 Materials and Methods

128 *Study site*

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129 This study was conducted between 15 January and 4 April 2009 in McKenna Creek (46.93498
130 N 122.56394 W, elevation 107 m), a tributary of the Nisqually River, WA, USA. The stream
131 reaches were relatively low gradient channels with sandy substrate and ranged in width from 1
132 to 3 m. Although salmon spawning was not occurring in this location during our study period
133 (peak spawning is from September to November), salmon carcasses are seasonally present at
134 this location as detrital inputs earlier in the fall and the carcasses left by late-November
135 spawners are not likely to be fully degraded until mid-February. Access to this site was granted
136 by the Nisqually Land Trust and no further permits were required for this study location.
137 Throughout the study period, average stream temperatures ranged from 5.3 - 5.7°C, average
138 pH ranged from 6.3 - 7.2, and average dissolved oxygen ranged from 8.9 - 10.2 mg L^{-1}. The
139 riparian zone was dominated by *P. trichocarpa*, *Alnus rubra* Bong (red alder), *Acer*
140 *macrophyllum* Pursh (bigleaf maple), *Thuja plicata* Donn ex D. Don (western redcedar), and
141 *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir). Average annual precipitation at the site was
142 approximately 129 cm and average max and min temperatures were 25 and 0° C, respectively.

143 *Litter collection*

144 Leaf litter was collected at the Puyallup Research and Extension Center of Washington State
145 University's (WSU) R.L. Goss Research Farm in a 38-year old common garden of *P.*
146 *trichocarpa*. To establish the common garden, branches were collected randomly from naturally
147 existing stands across OR, WA and BC in 1976 and planted at this one location to isolate
148 genetic differences among genotypes (**Fig. 1**). Branches of full-grown, individual genotypes
149 were wrapped in mesh to collect genotype-specific litter. Naturally abscised litter was collected
150 weekly from 20 October to 21 November 2008 for two replicate clones of each of six *P.*
151 *trichocarpa* genotypes (**Fig. 1**; Chilliwack 61-154 [C], Arlington 88-596 [A], Snoqualmie 5-52 [S],
152 Nisqually-1 [N], Longview 9-91 [L], and Hoh 95-876 [H]). Genotypes dropped their leaves
153 throughout this period, but their leaf fall timing differed. We needed to wait until we had collected

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154 enough leaf litter from all genotypes before we could implement the study. Access to this site
155 was granted by WSU and no further permits were required for litter collection.

156 *Study Design*

157 Air-dried leaf litter was weighed into 2 g (± 0.05 g) quantities and experimentally placed into 1
158 cm-mesh litterbags (23 x 28 cm) for each of the 6 genotypes in isolation. Sixteen replicate
159 litterbags were created per genotype and for each of 3 harvest dates, yielding a total of N = 288
160 litterbags. Additionally, a suite of litter mixtures was created to address both the influence of
161 genotype richness and mixture compositional influences on litter mass loss. Five different
162 genotype mixtures included: one equal-weight mixture of all six genotypes (6-genotype), and
163 four equal-weight mixtures of 2 genotypes each (C+L, N+L, N+C, N+S). Genotype pairs were
164 chosen to represent a gradient in geographic distances among genotypes (see **Table S1**).
165 Sixteen replicate litterbags were created per mixture treatment for each of 3 harvest dates,
166 yielding an additional N = 240 litterbags.

167 The sixteen replicate litterbags for each single genotype and mixture treatment were
168 placed at 16 different locations in McKenna Creek using another common garden design. The
169 study was designed so that one replicate litterbag of each treatment and each intended harvest
170 date were placed along 2.5 m pieces of rebar similar to a blocked design with all 33 litterbags
171 randomly placed along each rebar. In an ideal situation, 8 blocks would have been randomly
172 treated with salmon carcasses and 8 would have been control blocks, but the inherent lack of
173 independence between up- and downstream locations in a riverine environment made a fully
174 randomized block design impossible. Instead, eight replicate "blocks" were placed perpendicular
175 to streamflow in downstream salmon manipulation reaches and eight more were placed directly
176 upstream in no-salmon control reaches. Blocks were separated by roughly 10 - 20 m to maintain
177 independence among experimental units and to place rebar lengths in similar environmental
178 conditions. We placed rebar lengths in depositional pools at similar depths (average depths
179 were 31 and 33 cm for salmon and no salmon rebars, respectively). On each rebar length,

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180 litterbags were randomly attached with colored cable ties to facilitate removal from the stream
181 on the appropriate harvest date (14, 28 or 78 d). We based collection dates on past studies that
182 showed only 25% mass remaining after 78 d (Bretherton et al. 2011).

183 Frozen whole carcasses (1 - 2 carcasses, approx 1 - 2 kg) of Chinook salmon
184 (*Oncorhynchus tshawytscha*; average length: 70 cm; average width: 17 cm) were wrapped in
185 wire mesh and attached along the top edge of the rebar lengths in downstream salmon reaches
186 to retain the carcasses directly upstream of litterbags, *sensu* Bretherton et al. (2011). Whole
187 carcasses were used instead of exact masses of salmon tissue to better simulate natural
188 spawning influences, but this may have resulted in unmeasured variation in salmon nutrient
189 loadings among blocks. Control blocks were identical and included the litterbags attached to the
190 rebar and the mesh envelope, except these envelopes did not enclose salmon carcasses. A
191 previous study used sand bags as no-salmon controls, but found significant microbial
192 colonization of the sand and bag (Bretherton et al. 2011) and so in this study, nothing was used
193 in place of the carcasses, which may have resulted in slightly altered flow environments in
194 control blocks. By 78 d the salmon carcasses were reduced to bones and small pieces of
195 amorphous tissue, and leaf litter bags contained between 0.0 and 0.97 g of AFDM (ash-free dry
196 mass) leaf material (representing 100% and 50% mass loss, respectively).

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197 *Litter chemistry*

198 Subsamples of air-dried litter were prepared for litter chemical analysis by grinding to pass a
199 0.42 mm-mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Initial litter % N
200 and % C were determined using elemental analysis (Carlo Erba NC2100 Elemental Analyzer).

201 A modified version of EPA Method 365.3 was used for litter % P analysis. Approximately
202 500 mg of dry leaf litter powder was combusted in a muffle furnace at 550ºC for 3 h, to which a
203 10 mL solution of dilute *aqua regia* was added. Diluted extracts were analysed using the
204 spectrophotometric ascorbic acid – phosphomolybdate method using a diode-array
205 spectrophotometer (Hewlett-Packard 8453, Germany). Proximate cellulose and lignin

206 percentages were determined using a gravimetric method modified by Gessner (2005). Values
207 for acid detergent cellulose (ADC) and acid detergent lignin (ADL) were converted to
208 percentages based on the initial dry weight of each sample. We analysed condensed tannins
209 using a modified butanol-HCl method (Porter et al. 1986; LeRoy et al. 2007). Tannin standard
210 for *P. trichocarpa* was prepared by exhaustive extraction using the methods of Hagerman and
211 Butler (1989). All standards and samples were analysed for absorbance at a wavelength of 550
212 nm using a diode-array spectrophotometer.

213 *Leaf litter mass loss*

214 Leaf litter bags collected from the stream were placed in polyethylene zipper bags and
215 transported to the lab for processing. Leaves were gently rinsed of sediment and
216 macroinvertebrates and 10 leaf punches (11 mm dia) were taken from leaf laminae in each litter
217 bag for ergosterol analysis (see below). The remaining leaf material was dried at 70°C for 72 h,
218 weighed, ground using a Wiley mill to pass a 0.42-mm mesh screen and subsampled (0.250 g)
219 for combustion in a muffle furnace at 550° C for 3 h to determine ash-free dry mass (AFDM)
220 remaining fraction.

221 *Fungal biomass*

222 Ergosterol concentrations were used to estimate fungal biomass from leaf discs via gas
223 chromatography-mass spectroscopy (GC-MS). Leaf discs were extracted in 5 mL of methanol
224 then spiked with 50 μL of 7-Dehydrocholesterol and 10 mL of 15% KOH/methanol. Vials were
225 incubated in a drying oven at 80º C for 90 min, cooled and then ergosterol was partitioned into a
226 pentane solution (1:3, DI water:pentane). After the extracts were evaporated to dryness under
227 nitrogen gas and slight warming, a derivatization reagent (15 μL of neat pyridine and 50 μL of
228 (N)- Bis(trimethylsilyl)trifluoroacetamide) was added to each sample vial. Vials were vortexed for
229 5 s, dried in an oven at 60ºC for 30 min, then cooled and 500 μL of toluene was added prior to
230 analysis by GC-MS (Agilent 7890A/5975C). Ergosterol concentrations were converted to fungal

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231 biomass (mg g^{-1} leaf) assuming an ergosterol concentration of 5.5 μg mg^{-1} of mycelial dry mass
232 (Gessner and Chauvet 1993).

233 *Macroinvertebrate analyses*

234 Aquatic macroinvertebrates were separated from leaf matter and preserved in 70% ethanol for
235 identification. All macroinvertebrates from the 28 d harvest were identified to the lowest
236 taxonomic level possible using Merritt et al. (2008) and dissecting microscopy. Aquatic
237 macroinvertebrates were collected under a Washington State Department of Fish and Wildlife
238 Scientific Collection Permit granted to the Evergreen State College. This study complied with all
239 relevant regulations, and no protected species were sampled.

240 *Statistical Analyses*

241 In order to examine genotypic differences in initial leaf litter chemistry (%), we transformed
242 percent data using the arcsine-square root transformation (this transformation normalizes the
243 distributions of percent data) and used analysis of variance (ANOVA) to compare differences
244 among the six *P. trichocarpa* genotypes. Significant ANOVAs were followed by Tukey's Honest
245 Significant Difference (HSD) posthoc tests. All data analyzed using parametric ANOVA met the
246 assumptions of normality and homogeneity of variances and tests were run in JMP Pro (11.0,
247 SAS Institute Inc., Cary, NC, 1989-2015) with an alpha = 0.05. All figures show back-
248 transformed means ± 1 standard error (SE).

249 Decomposition rates were compared using a general linear model (PROC GLM in SAS
250 8.01, SAS Institute, Inc. 1999-2000) with fixed effects and Type I Sums of Squares to determine
251 significant treatment effects, *sensu* Kominoski et al. (2007). Time was treated as a continuous
252 variable in the model, and each term (time, litter genotype presence or absence, salmon
253 carcass presence or absence, and diversity terms – genotype richness and composition) was
254 added sequentially to the model. The two diversity terms were composed of genotype richness
255 (one-, two-, or six-genotype litter mixtures) and genotype composition (C+L, N+C, N+L, N+S, 6-
256 geno) and allowed for separation of these two diversity effects.

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257 Linear relationships between decomposition rates and litter chemicals were determined
258 using multiple linear regression in JMP. Decomposition rate constants (*k*) were determined for
259 each leaf litter treatment with and without salmon by regressing the natural log of AFDM against
260 day (Jenny et al. 1949).
261 Two-way ANOVAs with tree genotype, salmon treatment, and genotype*salmon (G x E)
262 interaction as main factors were used to explore patterns in mass loss at individual harvest
263 dates, fungal biomass and simple invertebrate metrics (taxa richness, evenness, diversity, and
264 total abundance). Tukey's HSD post hoc tests were used to determine differences among all
265 pairwise treatments. In addition, mass loss and fungal biomass at individual harvest dates were
266 compared across genotype richness levels (1, 2 and 6 genotypes) using ANOVA.
267 To test for non-additivity of leaf litter mass loss in these same mixtures, Chi-square tests
268 were used to compare observed mass loss values in mixtures to expected values based on the
269 mass loss of each genotype in isolation at each harvest date. Additive responses were those
270 that did not vary from expectation while synergistic responses showed significantly higher mass
271 loss than expected and antagonistic responses showed significantly lower mass loss than
272 expected. Multiple comparisons required a Bonferroni-adjusted alpha to be set at a = 0.0016.
273 Macroinvertebrate data from litter bags were analysed using multivariate community
274 analysis methods. Non-metric multidimensional scaling (NMS) ordination and multi-response
275 permutation procedures (MRPP) were performed in PC-ORD (4.34, MJM software 1995-2005).
276 Relativization to taxa maximum was used to reduce the effects of hyper-abundant taxa. Two-
277 dimensional ordinations were chosen as the least-stressful representation of this complex
278 multidimensional dataset based on scree-plot evaluations. MRPP was used to determine
279 significant differences in macroinvertebrate assemblages among different leaf litter and salmon
280 treatments. Indicator Species Analysis was used to determine species-specific associations with
281 litter or salmon treatments (Dufrene and Legendre 1997).

282 **Results**

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283 *Litter chemistry*

284 Leaf litter chemistry differed among *P. trichocarpa* genotypes for all litter chemicals measured:
285 % N, % P, C:N, cellulose, lignin and condensed tannins (**Fig. 2**). Genotype accounted for large
286 percentages of the variation in litter chemistry variables, ranging from explaining 30% of the
287 variation in cellulose to 96.7% of the variation in N (see R^2 values in **Fig. 2**).

288 *Leaf litter decomposition*

289 Because leaf litter decomposition can be analysed with respect to both the overall rate of
290 decomposition through time, as well as the actual mass loss at individual harvest dates, we
291 present data on both the rate of decomposition (k day^{-1}) and mass loss (%). Multiple factors
292 significantly explained leaf litter decomposition rates overall, including mixed-genotype litter
293 richness ($p = 0.0318$) and individual litter genotype presence/absence for the genotypes Hoh (p
294 $= 0.0038$) and Nisqually ($p = 0.0200$, **Table 1**; Overall model: $F_{(65,327)} = 19.6$, $p < 0.0001$). We
295 originally hypothesised that salmon carcasses, by providing a pulse of nutrients, would increase
296 litter decomposition for all genotypes; however, this was not the case (**Table 1**; $p = 0.6947$).
297 Consistently for both salmon and control treatments through time, the genotype from the Hoh
298 River (H; Olympic Peninsula, WA) showed slower decomposition, and Nisqually (N; Nisqually
299 River, WA) showed accelerated decomposition. Both of these genotypes showed a significant
300 presence/absence effect on mixed litter decomposition rates (**Table 1**). Contrary to our
301 predictions, however, salmon carcasses did not significantly affect overall decomposition rates,
302 and instead interacted with genotypes and genotype mixtures differentially (see below).

303 Multiple linear regression models revealed that when salmon were absent, % P and %
304 cellulose significantly negatively influenced decomposition rates ($F_{(2,36)} = 10.31$, $p = 0.0003$), but
305 in the presence of salmon carcasses, % condensed tannins and % lignin negatively influenced
306 decomposition ($F_{(2,18)} = 17.36$, $p < 0.0001$). Decomposition rates for single genotypes of *P.*
307 *trichocarpa* litter ranged from 0.0084 (± 0.00124) for Longview litter in the presence of salmon to
308 0.0120 (± 0.00075) for Arlington litter in the absence of salmon. Decomposition rates for

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309 genotype mixtures were slightly more variable and ranged from 0.0076 (± 0.0009) for the
310 Nisqually x Longview mixture in the absence of salmon to 0.0125 (± 0.0004) for the Nisqually x
311 Snoqualmie mixture in the presence of salmon (**Table S2**). All exponential regressions used to
312 determine decomposition rates were significant at $p < 0.05$.

313 *Leaf litter mass loss*

314 Two-way ANOVAs for mass loss at individual harvest dates showed a significant
315 genotype effect, but no salmon effect, or genotype*salmon (G x E) interaction (**Table 2**). *Post*
316 *hoc* tests revealed that when salmon carcasses were present, genotypic effects on
317 decomposition were weaker (**Fig. 3a vs 3b**). Litter from different genotypes lost mass at
318 significantly different rates in the absence of salmon on both days 14 and 28 (**Fig. 3a** and **3e**,
319 respectively); however, in the presence of salmon carcasses, no significant differences in mass
320 loss among genotypes were detected until day 28 (**Fig. 3f**), and all genotype effects were
321 weaker in the presence of salmon. By day 78, genotype effects disappeared in both treatments
322 as remaining litter and salmon biomass were both low (**Fig. 3i and 3j**).

323 Although genotypic variation influenced leaf litter mass loss, genotype richness in
324 mixtures was a weak predictor of mass loss. In most cases, leaf litter bags with 2 or 6
325 genotypes did not lose mass faster or slower on average than single genotype litter bags (**Fig.**
326 **3c,d,g,k,l**), regardless of the presence of salmon. In only one case, mass loss on day 28 for leaf
327 litter mixtures in the presence of salmon carcasses, was there a significant increase in mass
328 loss for the 6-genotype mixture compared to single genotypes (**Fig. 3h**); however, looking more
329 closely at different mixtures of genotypes reveals further non-additive patterns. The direction
330 (both synergistic and antagonistic) and magnitude of mass loss effects depended on
331 compositional effects (which genotypes were present), as well as the presence of salmon (**Fig.**
332 **4, Table S3**). In the absence of salmon, antagonistic responses (significantly less mass lost
333 than expected; symbols fall below the 1:1 line) were more common and were found for the 6-
334 genotype mixture on day 14, and most mixtures on day 78 (**Fig. 4a**). In contrast, significant

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335 synergistic responses (significantly more mass lost than expected; symbols fall above the 1:1
336 line) occurred for the C+L mixture on day 28 and the N+C mixture on day 78 (**Fig. 4a**). In the
337 presence of salmon, synergistic responses were more common and were seen for the N+C
338 mixture on day 14, the 6-genotype mixture on day 28 and most mixtures on day 78 (**Fig. 4b**). In
339 contrast, only one antagonistic response was shown in the presence of salmon for the N+C
340 mixture on day 28 (**Fig. 4b**). The mean deviation in mass loss from expectation (observed –
341 expected) in the absence of salmon shows a significant antagonistic response, while the mean
342 deviation for mixtures in the presence of salmon shows a significant synergistic response (**Fig.
343 4c**).

344 *Fungal Biomass*

345 Aquatic fungal biomass at day 28 was most strongly influenced by litter genotype (**Table 2**).
346 Qualitatively, the difference appears stronger in the absence of salmon carcasses (**Fig. 5**), but
347 there was no significant salmon effect or G x E effect. Litter genotype mixing led to an
348 overwhelmingly synergistic response in fungal biomass with significantly more fungal biomass
349 on leaf litter mixtures than expected based on single genotype litterbags. This result was
350 observed for all treatments except for the 6-genotype mixture in the absence of salmon and
351 N+C mixture in the presence of salmon (**Fig. 5c**). Additionally, fungal biomass in the salmon-
352 treated reach showed even stronger synergistic responses to litter mixing than control reaches,
353 though both mixture effects were significantly synergistic (**Fig. 5d**). Finally, fungal biomass was
354 significantly influenced by genotype richness in both the absence and the presence of salmon
355 carcasses ($F_{(2,38)}$ = 3.4230, p = 0.0430; $F_{(2,37)}$ = 4.5609, p = 0.0170, respectively). Tukey's HSD
356 post hoc tests reveal that fungal biomass on single genotypes is significantly lower than that
357 found on 2-genotype mixtures in both experimental treatments, but the 6-genotype mixture did
358 not differ from either single of 2-genotype mixtures.

359 *Macroinvertebrates*

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360 Macroinvertebrate communities were not significantly different among litter genotypes when
361 compared using multi-response permutation procedure (MRPP) analysis; however, communities
362 differed between the salmon and no-salmon treatments (MRPP A = 0.035, $p < 0.0001$). Salmon
363 carcass presence significantly reduced macroinvertebrate taxa diversity as shown using
364 Shannon's and Simpson's Diversity Index values (**Table 2**). Overall macroinvertebrate taxa
365 abundance, richness and evenness did not significantly differ between salmon carcass and no-
366 salmon reaches. Invertebrate taxa abundance was significantly correlated with fungal biomass
367 in the no-salmon control reach ($R^2 = 0.1216$, $F_{(1,39)} = 5.4$, $p = 0.0254$) but not in the salmon
368 reach ($R^2 = 0.0056$, $F_{(1,37)} = 0.21$, $p = 0.6510$).

369 Several macroinvertebrate taxa were significant Indicator Species for either the no-
370 salmon control or the salmon carcass treatments. Members of the Trichopteran genus
371 *Lepidostoma* (p = 0.0010), and the Dipteran families Tipulidae (p = 0.0050) and Simuliidae (p =
372 0.0100) indicated for the no-salmon control treatments, while members of the Plecopteran
373 genus *Malenka* (p = 0.0480), the Trichopteran genus *Onocosmoecus* (p = 0.0010), the Dipteran
374 family Psychodidae (p = 0.0130) and the Mollusc family Physidae (p = 0.0020) indicated for the
375 salmon carcass treatment.

376 **Discussion**

377 As predicted, genetically distinct clones of *P. trichocarpa* differed in leaf litter chemistry, mass
378 loss at mid-stages of decay, and fungal biomass accrual. Contrary to our predictions, salmon
379 carcasses did not stimulate decomposition overall, which was somewhat unexpected because
380 water chemistry downstream of salmon carcasses can be elevated in ammonium (Claeson et al.
381 2006), and nutrient enrichment has been shown to stimulate leaf litter decomposition by 50% in
382 a large, recent meta-analysis (Ferreira et al. 2014). Instead, in this study, salmon carcass
383 presence altered the non-additive responses of genotype mixtures, stimulated fungal biomass,
384 and altered macroinvertebrate community structure. Although salmon influenced several in-
385 stream variables, we cannot clearly show any genotype by environment interactions with

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386 salmon carcasses using this experimental approach. Previous research in an aspen (*Populus*
387 *tremuloides*) system found evidence for G x E interactions between leaf litter and nutrient
388 additions (LeRoy et al. 2012), but there are some key methodological differences between these
389 two studies. First, the nutrient addition in LeRoy et al. (2012) took place while the aspen were
390 growing, and leaf litter decomposition for nutrient-enriched trees was compared to
391 decomposition for control trees. It is possible that the form of nutrient addition or the location of
392 the addition (in the forest versus in the stream) may alter G x E responses. Second, because of
393 key experimental design issues, this previous study also involved better randomization of
394 nutrient-enriched and control litterbags. The results we provide here suffer the same issues as
395 in-stream nutrient addition studies in terms of the complications of upstream versus downstream
396 treatments which may have confounded treatment effects with environmental effects.

397 In addition, this current study may underestimate the influence of both litter genetic
398 variation and salmon carcasses on the detrital food web based on the timing of the study. The
399 need to wait to collect litter from all genotypes and then prepare hundreds of litter bags meant
400 the study was placed in the stream 1-2 months later than peak litter fall and fewer shredding
401 and salmon-adapted invertebrates may have been present at this later time. Future research
402 should work to prepare and deploy both litter bags and salmon carcasses earlier to better match
403 the experimental inputs with natural allochthonous inputs.

404 Genotype mixture effects on mass loss were often non-additive, but highly idiosyncratic
405 and dependent on the composition of the genotype mixture and the environmental context in
406 which the litter decomposed (in the presence or absence of salmon). Similar patterns have been
407 shown in previous studies exploring mixtures of litter species (Lecerf et al. 2011), and so this
408 was not unexpected. In litter mixtures, the presence of salmon did not completely overwhelm
409 diversity effects, which was seen in a recent example using a long-term nutrient enrichment
410 experiment (Rosemond et al. 2010), but had the interesting effect of altering non-additive
411 outcomes. Several mixtures switched from additive mass loss in the absence of salmon to

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412 synergistic (faster than expected) mass loss in the presence of salmon. Additionally, several
413 litter mixtures that showed antagonistic (slower than expected) mass loss in the absence of
414 salmon switched to additive or synergistic mass loss in the presence of salmon carcasses.
415 These switches are not isolated to this study, but two other recent papers show a similar switch
416 when litter mixtures were exposed to a nutrient source in the stream environment (Rosemond et
417 al. 2010; Bretherton et al. 2011; **Fig. 6**). The pattern of slightly to very antagonistic mixture
418 effects for control treatments compared to synergistic effects for elevated nutrient treatments
419 (through fertilizer or salmon additions) is largely consistent across habitats, leaf species, and
420 nutrient environments, and should be explored in future studies. Further research could provide
421 evidence for a more broadly applicable Nutrient-Diversity Synergism Hypothesis (**Fig. 6**), but
422 this pattern would need to be further tested under other conditions and in other systems,
423 especially in light of a very recent study which showed synergistic litter effects in streams with
424 low nutrients compared to eutrophic systems (Lima-Fernandes et al. 2015). In this case, since
425 an environmental gradient in eutrophication was used instead of a nutrient manipulation, it is
426 possible that the eutrophic streams were otherwise degraded or had different shredder of
427 microbial communities that may have influenced mass loss in the opposite direction.

428 It is possible that idiosyncratic litter mixing responses may be due to environmental
429 variation across our study reaches. Since microhabitats were chosen to be as similar as
430 possible among both salmon and no-salmon control blocks, environmental differences were not
431 explicitly measured as covariates. In this study, any environmental variation would have resulted
432 in error in the leaf litter mass loss data, making it more difficult to see differences among
433 genotypes or mixtures. The fact that there are evident differences in mass loss among
434 intraspecific leaf litter treatments at mid-stages of decay shows that environmental variation was
435 not large enough to swamp these patterns. This does not suggest that environmental variation
436 was not present or important, just that genotypic variation was more important at mid-stages of
437 decay. At early and late stages of decay, it is possible that unmeasured environmental variables

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438 were relatively more important because neither genotype nor salmon carcass presence explain
439 much variation in mass loss at these time periods.

440 In contrast to the idiosyncratic mass loss effects in mixtures discussed above, genotype
441 mixture effects on fungal biomass accrual were consistently synergistic across treatments, and
442 more so in the presence of salmon carcasses. These results are relatively novel. One previous
443 study examined aquatic fungal biomass across many different genotypes of two *Populus*
444 species and their hybrids and found significant differences in fungal biomass accumulation on
445 genotypes within species (LeRoy et al. 2007), but work with mixtures of genotypes and
446 environmental interactions is new in this study, and should be further explored.

447 While litter genotype was a driver of patterns in litter mass loss and aquatic fungal
448 biomass, stream invertebrates were more sensitive to salmon carcass presence. Specifically,
449 the presence of salmon carcasses had a negative influence on the richness, evenness, and
450 diversity of the macroinvertebrates that colonized leaf surfaces. It is likely that the carcasses
451 provided more appealing substrate for a variety of macroinvertebrates and had the influence of
452 drawing certain components of the community away from litter bags, similar to the findings of
453 Zhang et al. (2003). Although there were no differences in overall macroinvertebrate
454 abundances on litter bags in no-salmon control and salmon carcass treatments, there was an
455 overall shift in the community structure found in litter bags from these two treatments. We found
456 no evidence of macroinvertebrate discrimination among *P. trichocarpa* genotypes or litter
457 mixtures, which is supported by previous studies comparing genotypes within species of
458 *Populus fremontii* and *Populus angustifolia* (LeRoy et al. 2007) and species mixtures in the
459 presence and absence of salmon (Bretherton et al. 2011). The timing of leaf fall in the Pacific
460 Northwest is generally October through November and the timing of fall salmon runs in these
461 rivers is generally August through November. The need to collect and prepare leaf litter and
462 salmon carcasses prior to the study required us to wait until just after these major natural inputs
463 and may have influenced in-stream invertebrate responses to both of these detrital inputs.

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464 We demonstrate that genotypically distinct leaf litters and salmon carcasses interact in
465 streams to influence leaf litter mass loss and fungal biomass growing on leaf surfaces, but only
466 carcass presence influenced aquatic macroinvertebrates. Thus while ecosystem function may
467 be sensitive to the interaction of G x E effects, stream invertebrates (often considered
468 bioindicators of stream health) are more sensitive to major biotic environmental factors like
469 salmon carcass presence than to the complex interactions between salmon, tree genetics, and
470 carbon-cycling through decomposition.

471 The study of salmon and leaf litter interactions is not simply a pairing of major energy
472 players in stream ecosystems. Salmon-derived nitrogen provides a limiting nutrient for trees and
473 vascular plants growing in riparian zones in the northern hemisphere and significantly fertilizes
474 riparian forests and increases above-ground net primary productivity (Helfield and Naiman
475 2001; Reimchen et al. 2003). The interaction between salmon carcasses and riparian trees
476 could result in a feedback to litter quality and potentially further influence litter dynamics
477 (Madritch et al. 2009; LeRoy et al. 2012), especially in systems with healthy salmon runs. For
478 example, Morris and Stanford (2011) found that salmon carcasses significantly enriched riparian
479 plants and lowered C:N ratios across the entire 2.5 km floodplain of the Kol River on the
480 Kamchatka Peninsula, Russian Far East. Further research into the plasticity of litter phenotypes
481 when exposed to salmon carcass additions could provide more insight into these interactions.

482 Our results highlight the importance of asking questions across the boundaries of
483 emerging fields of research, such as genes-to-ecosystems (Whitham et al. 2008), biodiversity-
484 ecosystem function (Lecerf and Richardson 2010; Cook-Patton et al. 2011; Lecerf et al. 2011),
485 and terrestrial-aquatic interactions (Richardson et al. 2010). More specifically, effects of
486 genotypic variation on ecosystem function can be dependent on biotic and abiotic environmental
487 contexts (Madritch et al. 2009; LeRoy et al. 2012; Pregitzer et al. 2013). Using leaf litter and
488 salmon carcass interactions highlights two major allochthonous energy inputs to streams and
489 riparian forests of the northern hemisphere and widens our understanding of terrestrial-aquatic

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490 interactions. The presence of salmon carcasses resulted in more frequent synergistic responses
491 in mixtures both for litter mass loss and fungal biomass. Understanding the circumstances
492 under which genetic variation may influence ecosystem function is a crucial area of research
493 since a variety of factors influence genetic diversity in both foundation and keystone species.
494 For example, range shifts under future climate conditions are likely to influence genetic variation
495 in foundation tree species (Excoffier et al. 2009). Both genetic variation in keystone species and
496 the overall influence of these organisms on ecosystem functions are likely reduced in situations
497 where the species (like anadromous salmon) have been extirpated or greatly reduced in most
498 natural riparian systems (only 6 to 7% of historic Pacific Northwest populations persist; Gresh et
499 al. 2000).

500 In this paper, salmon carcass presence altered the way in which leaves from genetically
501 diverse trees interacted with one another in mixtures, both through litter mass loss and fungal
502 decomposers, as well as altered the litter-dwelling aquatic macroinvertebrate community. It is in
503 these ways, and not in clear overall or interactive ways, that a major detrital input of nutrients
504 and organic matter influences the brown food web in this stream system. Thus, a genetic
505 perspective on ecosystem function becomes more important as riparian systems witness the
506 loss of key ecological players like anadromous salmon. The highly complex nature of these
507 systems warrants further investigation into the extended community and ecosystem effects of
508 plant genotypic variation across gradients of both abiotic and biotic environmental variation and
509 predictions for these systems as they experience large scale changes (Kominoski et al. 2013).
510

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682 **Table 1. ANOVA results for mass loss analysis.** ANOVA results for a general linear model
683 describing the effects of incubation time (Time), leaf genotype presence (Snoqualmie,
684 Longview, Chilliwack, Arlington, Hoh, and Nisqually genotypes), salmon carcass presence
685 (Salmon), genotype richness (Richness), and genotype composition (Composition) within leaf
686 litter bags as well as interactions among these factors on leaf litter decomposition. This model
687 uses fixed effects and Type I sums of squares. Significant effects are denoted in bold and with
688 asterisks (*).

Source	DF	Type I SS	Mean Square	F Value	p-value
Time	**2**	**64.8993**	**32.4496**	**600.68**	**<0.0001***
Snoqualmie	1	0.0184	0.0184	0.34	0.5600
Longview	1	0.0180	0.0180	0.33	0.5646
Chilliwack	1	0.0697	0.0697	1.29	0.2569
Arlington	1	0.0004	0.0004	0.01	0.9295
Hoh	**1**	**0.4578**	**0.4578**	**8.47**	**0.0038***
Nisqually	**1**	**0.2951**	**0.2951**	**5.46**	**0.0200***
Salmon	1	0.0083	0.0083	0.15	0.6947
Richness	**1**	**0.2511**	**0.2511**	**4.65**	**0.0318***
Composition	3	0.0792	0.0264	0.49	0.6903
Snoqualmie*Salmon	1	0.0090	0.0090	0.17	0.6828
Longview*Salmon	1	0.0169	0.0169	0.31	0.5767
Chilliwack*Salmon	1	0.0327	0.0327	0.61	0.4371
Arlington*Salmon	1	0.1240	0.124	2.30	0.1307
Hoh*Salmon	1	0.0421	0.0421	0.78	0.3781
Nisqually*Salmon	1	0.0900	0.0900	1.67	0.1977
Salmon*Richness	1	0.0059	0.0059	0.11	0.7418
Salmon*Composition	3	0.0647	0.0216	0.40	0.7537
Time*Snoqualmie	2	0.0072	0.0036	0.07	0.9354
Time*Longview	2	0.1133	0.0567	1.05	0.3515
Time*Chilliwack	**2**	**0.3880**	**0.1940**	**3.59**	**0.0287***
Time*Arlington	2	0.0861	0.0431	0.80	0.4514
Time*Hoh	2	0.0397	0.0199	0.37	0.6926
Time*Nisqually	2	0.2800	0.1400	2.59	0.0764
Time*Salmon	2	0.1362	0.0681	1.26	0.2848
Time*Richness	2	0.0432	0.0216	0.40	0.6705
Time*Composition	6	0.4610	0.0768	1.42	0.2053
Time*Snoqualmie*Salmon	2	0.1252	0.0626	1.16	0.3152
Time*Longview*Salmon	2	0.0372	0.0186	0.34	0.7090
Time*Chilliwack*Salmon	2	0.0861	0.0431	0.80	0.4514
Time*Arlington*Salmon	2	0.0081	0.0040	0.07	0.9279
Time*Hoh*Salmon	2	0.1970	0.0985	1.82	0.1632
Time*Nisqually*Salmon	2	0.0579	0.0289	0.54	0.5858

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692 **Table 2: Genotype, environment and G x E interactions**. Two-way ANOVA results showing
693 effects (p-values) of genotype, environment and G x E interactions on leaf litter decay and
694 consumers. Specific responses are shown for mass loss at days 14, 28 and 78, fungal biomass
695 at day 28, and the abundance, richness, evenness, Shannon's diversity index and Simpson's
696 diversity index values of aquatic macroinvertebrates at day 28. Significant effects are denoted in
697 bold and with asterisks (*).

Response Variable		Genotype	Salmon	G x E	R^2	*F*	*df*	*P*
Leaf litter decay								
	Mass loss (14 d)	0.2466	0.1764	0.2065	0.2132	1.55	11, 74	0.1357
	Mass loss (28 d)	**<0.0001***	0.3540	0.9264	0.3427	3.32	11, 81	**0.0010***
	Mass loss (78 d)	0.3248	0.7391	0.9725	0.0982	0.61	11, 73	0.8103
Consumers								
	Fungal biomass	**0.0095***	0.1926	0.2114	0.4115	2.35	11, 48	**0.0256***
	Invert Abundance	0.2907	0.1108	0.2577	0.3159	1.55	11, 48	0.1543
	Invert Richness	0.5466	0.6298	0.9640	0.1261	0.49	11, 48	0.9003
	Invert Evenness	0.6897	0.1003	0.4398	0.1368	1.01	11, 48	0.4477
	Shannon's Index	0.9486	**0.0002***	0.4490	0.2385	1.99	11, 48	**0.0420***
	Simpson's Index	0.8129	**0.0012***	0.4894	0.2098	1.69	11, 48	0.0936

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700 **Figure Legends**

701 **Figure 1: Map of study locations.** Map showing the collection locations for each *Populus*
702 *trichocarpa* genotype (●), the location of the common garden site at the Puyallup Research and
703 Extension Center (WSU, Puyallup, WA) used for litter collection (▲) and the location of the
704 stream (McKenna Creek; ▲) used for decomposition experiments. Map created in Arc-GIS 10.0
705 by Dylan G. Fischer.

706 **Figure 2. Intra-specific variation in black cottonwood litter chemistry.** Initial litter chemistry
707 differed significantly among genotypes of *Populus trichocarpa* leaf litter: a) % nitrogen, b) %
708 phosphorus, c) C:N ratio, d) % acid-detergent cellulose, e) % acid-detergent lignin, and f) %
709 condensed tannins. Bars represent means ± 1 SE, and lower case letters denote significant
710 differences among genotypes.

711 **Figure 3. Mass loss through time for individual genotypes and genotype mixtures.**
712 Percent mass loss for 6 genotypes of *Populus trichocarpa* leaf litter in isolation and compared to
713 mixtures of 2 and 6 genotypes after 14 d (a, b, c, d) 28 d (e, f, g, h), and 78 d (i, j, k, l) in the
714 stream. Gray bars and gray open circles represent control litter bags and black bars and black
715 open circles represent litter bags exposed to salmon carcasses. Bars represent means ± 1 SE,
716 and lower case letters denote significant differences among genotypes. Open circles represent
717 mass loss observations for genotype mixtures at different levels of genotype richness, and
718 horizontal dashed lines represent treatment means.

719 **Figure 4. Observed versus expected mass loss for genotype mixtures in the presence**
720 **and absence of salmon carcasses.** Observed % mass loss for all genotype mixture
721 treatments plotted as a function of the expected % mass loss based on average values for each
722 genotype in isolation: a) no-salmon controls, b) salmon carcass treatments, and c) overall non-
723 additive treatment effects (± 1 SE). Leaf litter treatments included the following mixtures: 6-
724 genotype mixture (6-geno), Chilliwack + Longview (C+L), Nisqually + Chilliwack (N+C),
725 Nisqually + Longview (N+L), and Nisqually + Snoqualmie (N+S). Diagonal lines represent 1:1

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726 equilibrium, and ellipses contain all means from each harvest date. Asterisks denote significant
727 non-additive responses in mixture (at Bonferroni-corrected alpha = 0.0016); asterisks above the
728 1:1 line show synergistic mass loss, asterisks below the line show antagonistic mass loss.
729 **Figure 5. Aquatic fungal biomass on individual genotypes and genotype mixtures**
730 **comparing mixtures in the presence and absence of salmon carcasses.** Mean aquatic
731 fungal biomass (mg g^{-1} leaf) on leaves of each *Populus trichocarpa* genotype in: a) no-salmon
732 control litter bags, and b) salmon carcass treatment litter bags. Gray bars represent means for
733 control litter bags and black bars represent means for litter bags exposed to salmon carcasses
734 (± 1 SE); c) Observed fungal biomass for each genotype mixture (y-axis) compared to expected
735 values of fungal biomass based on average values for each genotype in isolation (x-axis). Leaf
736 litter treatments included the following mixtures: 6-genotype mixture (6-geno), Chilliwack +
737 Longview (C+L), Nisqually + Chilliwack (N+C), Nisqually + Longview (N+L), and Nisqually +
738 Snoqualmie (N+S). Gray symbols represent control litter bags and black symbols represent litter
739 bags exposed to salmon carcasses. Non-significant additive responses denoted with "ns," all
740 other treatments were significantly non-additive (synergistic) at Bonferroni-corrected alpha =
741 0.005. Diagonal line represents 1:1 equilibrium; and d) overall non-additive treatment effects (±
742 1 SE) on fungal biomass.
743 **Figure 6**. **Patterns in synergistic and antagonistic mass loss across studies**. Additive
744 mass loss, synergistic mass loss (more mass lost than expected) and antagonistic mass loss
745 (less mass lost than expected) patterns from three studies comparing mixed leaf litter mass loss
746 in control and nutrient-enriched environments. Rosemond et al. (2010) used dripped fertilizer as
747 the nutrient enrichment and examined effects on species mixtures and two studies used salmon
748 carcasses as nutrient enrichment, Bretherton et al. (2011) examined effects on species mixtures
749 and the this study examines effects on genotype mixtures. Values represent mean non-additive
750 responses ± 1 SE, and may represent patterns that support a Nutrient-Diversity Synergism
751 Hypothesis.

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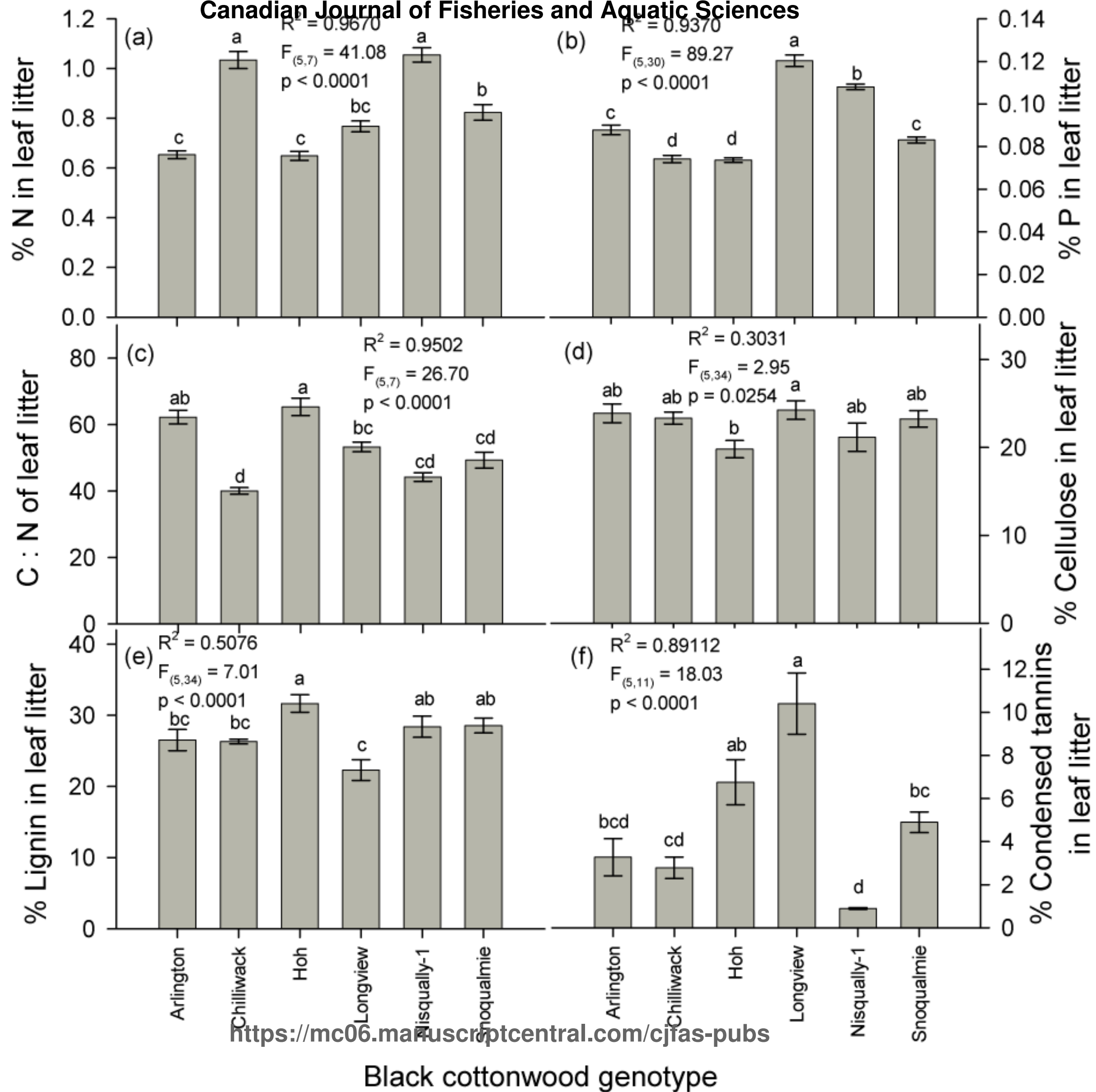
Chilliwack
Abbotsford
Surrey
Richmond
CANADA
UNITED STATES
Gulf of Alaska
Duncan
Bellingham
Saanich
Victoria
Mount Vernon
Oak Harbor
Arlington
Everett
Hoh
Seattle
Snoqualmie
Kent
Tacoma
Common Garden
Olympia
Nisqually
Aberdeen
McKenna Creek
N
Astoria
Longview
Longview
Legend
Genotype Origin Location
McKenna Creek Study Site
Common Garden Location
The Dalles
Hillsboro
Portland
Gresham
Newberg
0 15 30 60 90 120
Kilometers
D.G. Fischer 2013

Canadian Journal of Fisheries and Aquatic Sciences



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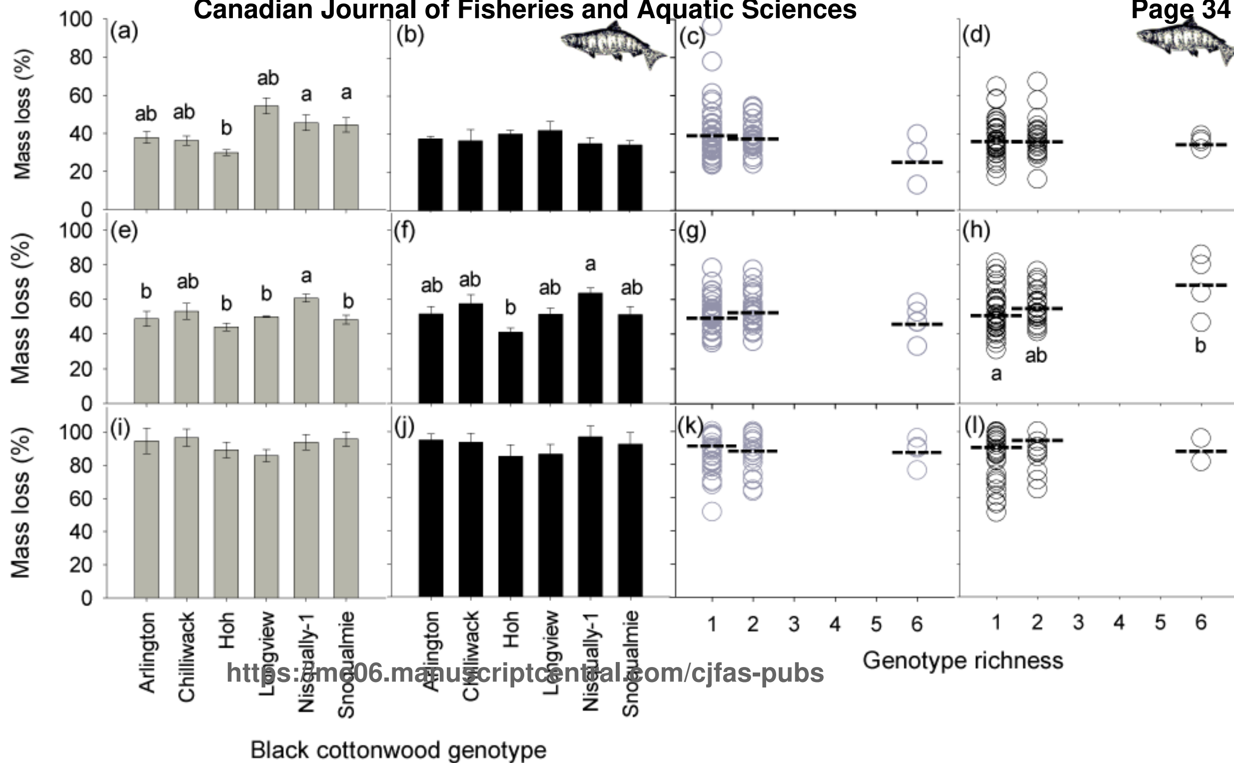
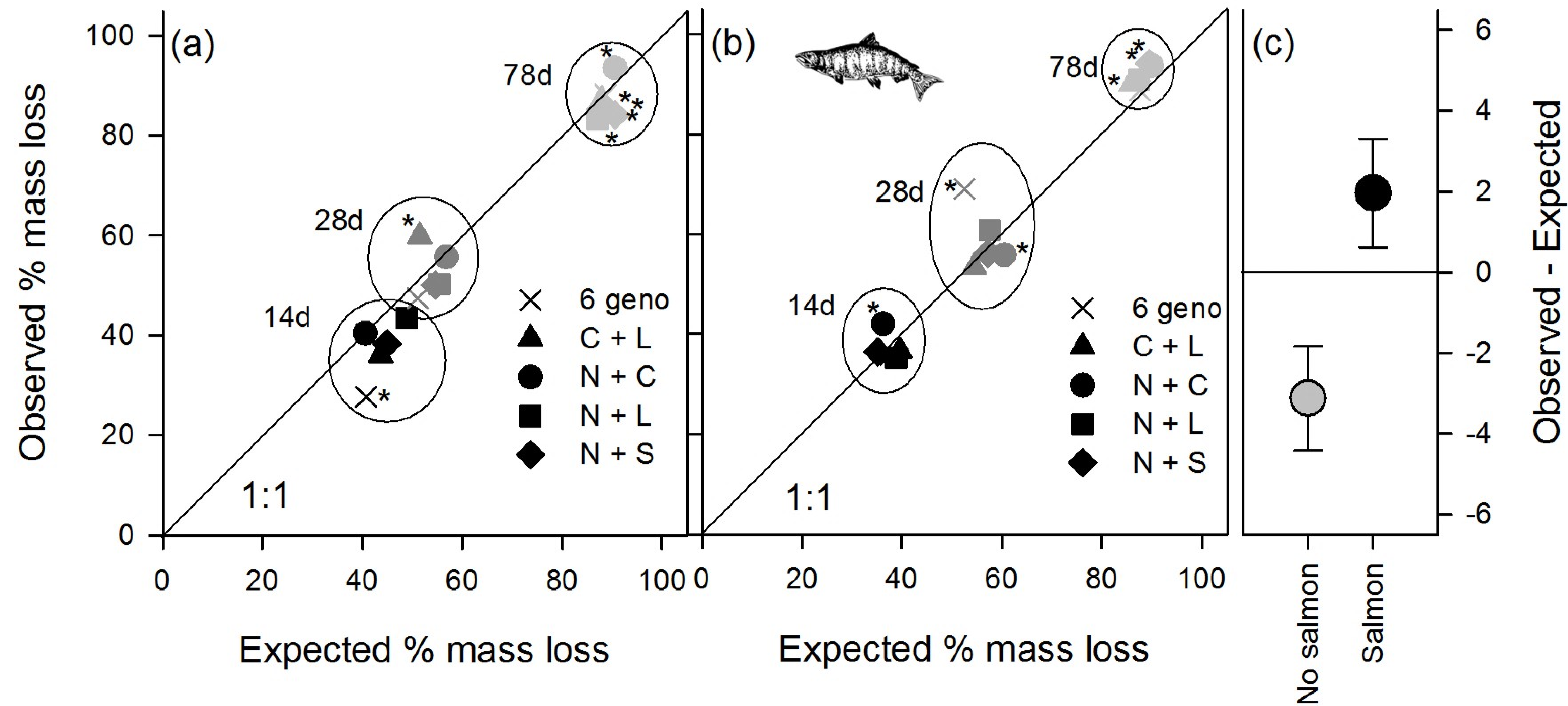
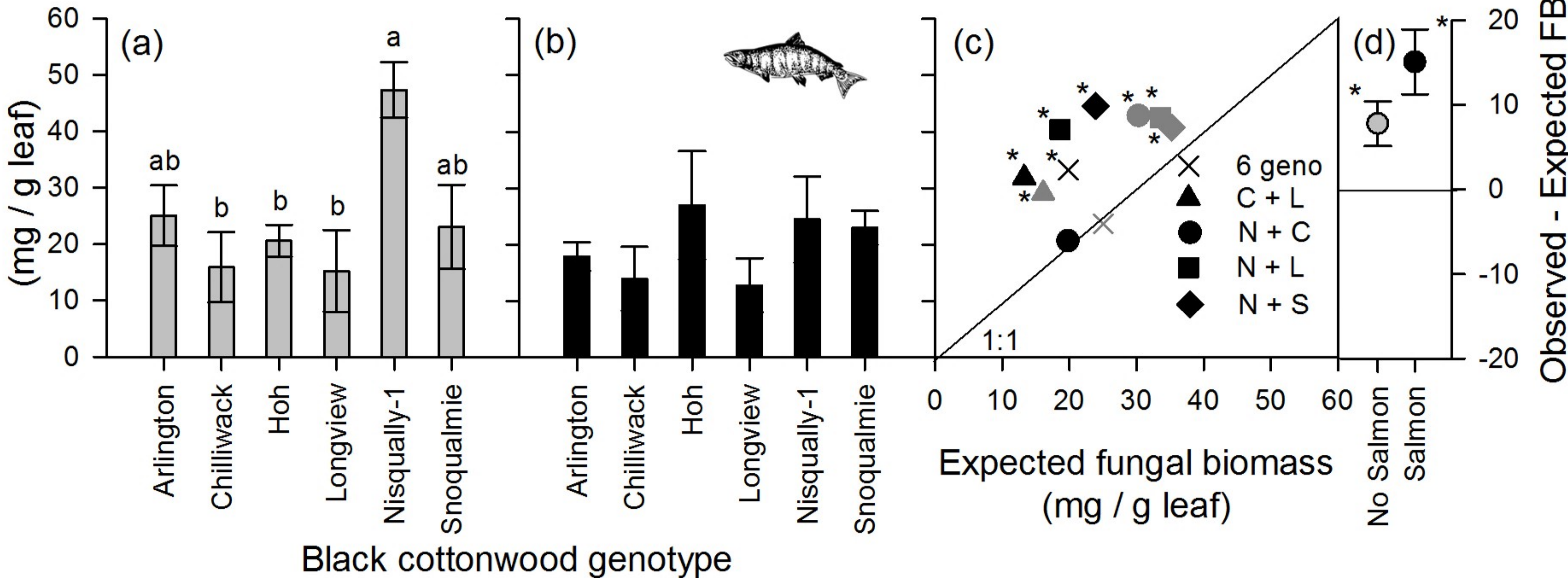
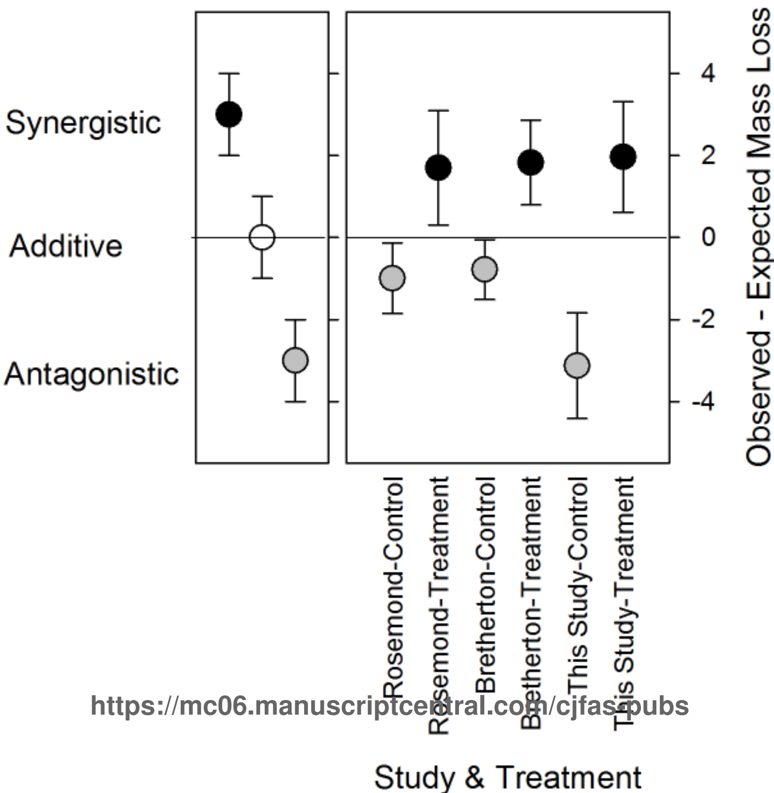


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
Mass loss (%)
ab
b
a
Arlington
Chilliwack
Hoh
Longview
Nisqually-1
Snoqualmie
Black cottonwood genotype
Genotype richness


(a)
(b)
(c)
Observed % mass loss
Expected % mass loss
Expected % mass loss
Observed - Expected
78d
28d
14d
1:1
6 geno
C + L
N + C
N + L
N + S
No salmon
Salmon
0
20
40
60
80
100
6
4
2
0
-2
-4
-6


(a)
(b)
(c)
(d)
Observed fungal biomass (FB)
(mg / g leaf)
0
10
20
30
40
50
60
ab
b
b
b
a
ab
Arlington
Chilliwack
Hoh
Longview
Nisqually-1
Snoqualmie
Black cottonwood genotype
6 geno
C + L
N + C
N + L
N + S
1:1
Expected fungal biomass
(mg / g leaf)
No Salmon
Salmon
Observed - Expected FB
-20
-10
0
10
20


Synergistic
Additive
Antagonistic
Observed - Expected Mass Loss
4
2
0
-2
-4
Rosemond-Control
Rosemond-Treatment
Bretherton-Control
Bretherton-Treatment
This Study-Control
This Study-Treatment
Study & Treatment