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Title	Different responses of herbivore abundance to plant genotypic diversity depending on herbivore host specificity
Author(s)	Nakamura, Masahiro; Shan, Bingpin; Takafumi, Hino et al.
Citation	Trees - Structure and Function https://doi.org/10.1007/s00468-024-02521-w
Issue Date	2024-05-15
Doc URL	https://hdl.handle.net/2115/94833
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Type	journal article
File Information	genotypic_diversity_nakamura et al.pdf



**Title: Different responses of herbivore abundance to plant genotypic diversity
depending on herbivore host specificity**

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Abstract

Biological control is an important ecosystem service mediated through plant diversity. Responses of herbivore abundance to plant genotypic diversity may depend on host specificity, especially the host-feeding type. We conducted a common-garden experiment by manipulating plot-level genotypic diversity (number of source populations per plot) of Erman's birch (*Betula ermanii*) to understand how birch genotypic diversity affected the abundance of three herbivore feeding types (chewers, galls, and suckers). Specifically, we investigated whether the effects of plant genotypic diversity on herbivore abundance were additive or non-additive. Furthermore, we examined this mechanism as a possible change in plant phenotype (plant productivity and/or leaf traits) and/or herbivore foraging behavior (associational resistance with neighboring plant individuals). We found that genotypic diversity had a significant positive effect on condensed tannin concentration; however, it did not affect plant growth. There was no effect of increasing birch genotypic diversity on the density of chewers (generalists) and chewing herbivory, whereas the density of galls and suckers (specialists) decreased significantly. A negative non-additive effect was observed with regard to gall density and a negative additive effect on sucker density was observed. Gall density was not related to the increase in condensed tannins affected by birch genotypic diversity. This implies that associational resistance is more likely to explain the negative non-additive effects on gall density. Our study shows that the loss of birch genotypic diversity due to shrinking population sizes will strongly affect specialist herbivores in the boreal forests of Hokkaido.

Key Words: additive effects, associational resistance, common-garden experiment, non-additive effects, resource concentration hypothesis

Key Message: The response of herbivore abundance to birch genotypic diversity varies

54 depending on the host specificity of the herbivores. Specialist, but not generalist,
55 herbivores were affected by birch genotypic diversity.

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INTRODUCTION

The general loss of biodiversity is particularly severe in forest ecosystems (Hassan et al. 2005). Several experimental studies on multitrophic interactions have examined the effects of plant species diversity (Andow 1991; Knops et al. 1999; Koricheva et al. 2000; Haddad et al. 2001; Otway et al. 2005; Scherber et al. 2010). A meta-analysis of these studies showed that mixed forests were less vulnerable to herbivorous insects than monospecific forests (Jactel and Brockerhoff 2007). This type of biological control is an important service mediated through plant diversity (Moreira et al. 2016, Wan et al. 2022). Plants vary in their resistance and susceptibility to herbivorous insects, depending on their genotype (Maddox and Root 1987; Fritz and Price 1988). Similar to species diversity, plant genotypic diversity can also shape the community structure of herbivorous insects (Crutsinger et al. 2006; Hughes et al. 2008; Barton et al. 2015; Koricheva and Hayes 2018). Human activities resulting in land-use change and habitat fragmentation have dramatic impacts on species and genotypic diversity (Ellstrand and Elam 1993; Vitousek et al. 1997; Miura et al. 2017). It is thus important to elucidate the effects of plant genotypic diversity on multitrophic interactions; however, to date, the mechanisms underlying these effects are not comprehensively understood.

In general, additive effects are defined as arising from the independent effects of multiple factors, while non-additive effects are defined as arising from interactive effects among multiple factors. The effects of plant genotypic diversity on herbivore abundance may occur through additive (i.e., the sum of average effects of genotypes) or non-additive mechanisms (i.e., synergistic or antagonistic effects of multiple genotypes) (Schweitzer et al. 2005; Hughes et al. 2008; Utsumi et al. 2011; Hambäck et al. 2014). Additive effects (e.g., the resource concentration [RC] hypothesis) occur when plant genotypes experience the same herbivore abundance in single-genotype as in mixed-genotype plots (Root 1973); thus, the responses of herbivore abundance in mixed-genotype plots can be predicted based on those observed in single-genotype plots.

85 The RC hypothesis supports this mechanism and predicts that mixed-genotype plots
 86 should be diluted in terms of the abundance of preferred genotypes for specialist
 87 herbivores. In contrast, generalists are less sensitive to such dilution effects (Root 1973).
 88 Conversely, non-additive effects occur when the responses in diverse plant communities
 89 cannot be predicted from those in the single-genotype plots. Regarding herbivore
 90 abundance, non-additive effects in diverse plant communities arise because of changes
 91 in plant phenotypes and/or herbivore foraging behavior. **Changes in plant phenotypes**
 92 **affect plant productivity and defense mechanisms (leaf traits) (Barton and Bowers 2006;**
 93 **Bailey 2012; Genung et al. 2012).** The following mechanisms have been proposed to
 94 **explain changes in plant phenotypes. First, diverse plant communities are frequently**
 95 **more productive than simple plant communities, possibly because of sampling effects,**
 96 **niche partitioning, and more efficient resource use (Schwartz et al. 2000; Tilman et al.**
 97 **2001; Cardinale et al. 2002; Hooper et al. 2005; Cardinale et al. 2006; Cardinale et al.**
 98 **2007).** The increased plant productivity in diverse plant communities may result in more
 99 **food resources, thereby increasing generalist herbivore abundance (“more individual”**
 100 **(MI) hypothesis) (Srivastava and Lawton 1998).** Second, the increased plant
 101 **productivity in diverse plant communities may lead to reduced investment in plant**
 102 **defense via growth–defense trade-offs (Mraja et al. 2011; Moreira et al. 2014; McArt**
 103 **and Thaler 2013).** This decrease in plant defenses via growth–defense trade-offs may
 104 **promote an increase in generalist and specialist herbivore abundance.** By contrast,
 105 **changes in herbivore foraging behavior** may be based on associational resistance, which
 106 has frequently been attributed to visual and chemical masking of neighboring individual
 107 plants (Tahvanaian and Root 1972; Barbosa et al. 2009; Utsumi et al. 2011). This
 108 complexity makes it difficult for specialists to locate and move among host plants
 109 (Tahvanaian and Root 1972; Andow 1991). The above explanations of additive and
 110 non-additive mechanisms suggest that the response of herbivore abundance to changes
 111 in plant genotypic diversity may depend on herbivore host specificity (i.e., generalists or

specialists) (Castagneyrol et al. 2012; Barton et al. 2015).

The effects of plant genotypic and species diversity may differ between specialist and generalist herbivores. A meta-analysis examining the effects of plant genotypic diversity on herbivores found that damage caused by generalist herbivores was reduced at higher plant genotypic diversity, whereas damage caused by specialist herbivores was not for crops (Koricheva and Hayes 2018); however, several meta-analyses on the effects of plant species diversity have shown that the adverse effects of increased tree species diversity on herbivores is stronger in specialist herbivores than in generalists (Jactel and Brockerhoff 2007; Castagneyrol et al. 2014; Kambach et al. 2016; Jactel et al. 2021). This discrepancy may be due to differences in the mechanisms of plant genotypic and species diversity effects on herbivores. Even if genotypes of the same plant species are mixed, the overall density of specialist herbivores may not differ between stands with uniform and mixed genotypes because the concentration of host plants is the same in stands with uniform genotypes (Peacock and Herrick 2000; Utsumi et al. 2011). In contrast, for generalist herbivores, sequential feeding on different genotypes may reduce the resource utilization rate and decrease herbivory (McArt and Thaler 2013); however, this theory is based on only few studies (reviewed by Koricheva and Hayes 2018) and further research is required to fully understand how plant genotypic diversity affects specialist and generalist herbivores.

To examine how plant genotypic diversity affects insect community composition, we conducted a common-garden experiment in which the plot-level genotypic diversity (the number of source populations per plot) of Erman's birch (*Betula ermanii*), an important tree species in the boreal forests of Hokkaido (Japan), was manipulated.

Feeding type can be closely related to host specificity, as most chewers are polyphagous herbivores (generalists), whereas most galls and suckers are monophagous (specialists) (Moritsu et al. 1983; Yukawa and Masuda 1996; Barton et al. 2015). Because the host specificity of herbivores plays an important role in the response of herbivore abundance

to plant diversity (Castagneyrol et al. 2012; Barton et al. 2015; Jactel et al. 2021), different feeding types of herbivorous insects (i.e., chewers, galls, and suckers) were evaluated separately. Considering that plant genotypic diversity may have additive or non-additive effects on herbivore abundance, we addressed the following questions: 1) How does the genotypic diversity (changes in the number of source populations) of the birch affect **herbivore abundance** (the density of each feeding type and the herbivory of chewers (the area of damage (%) by chewers))? 2) How does the genotypic diversity of the birch affect **plant phenotypes** (plant growth and/or leaf traits)? 3) **How does the birch source population affect both plant phenotypes and herbivore abundance in the single-genotype plots?** 4) Are birch genotypic-diversity effects additive or non-additive **on both plant phenotypes and herbivore abundance?** 5) If birch genotypic diversity has non-additive effects on **herbivore abundance**, which leaf traits are **mechanistically** responsible for variations in density and herbivory?

MATERIAL AND METHODS

Common-garden experiment

A common-garden experiment was established in early May 2016 at Nayoro (44°19' N, 142°27' E), Hokkaido (the northern island of Japan). The average annual mean temperature in Nayoro is 5.5 °C, and the average annual precipitation is 969.2 mm. The common-garden experiment was manipulated by changing the plot-level genotypic diversity (the number of source populations per plot) of Erman's birch (*B. ermanii*). This common tree-line species is widely distributed from the plains to the mountains of Hokkaido (Ito 1987). Increasing the number of source populations of a plant species increases plot-level genotypic diversity (Crawford and Rudgers 2013), thus we collected saplings (approximately 30 cm tall) from six distinct source populations of *B. ermanii* in the Nakagawa Experimental Forest of Hokkaido University (19,364 ha, 44°85' N, 142°15' E; Table S1, Fig. S1) in September 2014. The distance between each of the source populations was at least 1 km to preclude gene flow. These saplings were temporarily planted in the common-garden of the Nakagawa Experimental Forest and then planted in the common-garden experiment at Nayoro in early May 2016, which was before the initiation of leaf flushing of these saplings. 95% of planted saplings was successfully established. Sixty-four 1 × 1 m plots were established, with 48 single-population plots (8 plots per source population), 8 three-population plots, and 8 six-population plots. We randomly selected source populations to establish three- and six-population plots. Each population was equally represented in all mixed plots. A block-design experiment was chosen to prevent the effects of spatial heterogeneity in the microenvironments. Eight blocks (eight replicates) were established with each block containing six single-population plots from each source population, one three-population plot, and one six-population plot (Fig 1c). The plots were randomly placed within each block. Each plot contained nine individual saplings (Fig 1a) at natural densities and was separated from neighboring plots by 2 m to prevent neighbor

effects (Fig 1b). Encroaching weeds were removed every month, manually and/or using machines.

Measurements

To assess effects of the source population and genotypic diversity of the birch on the density of each feeding type of herbivorous insects, three current-year shoots were randomly selected from each sapling, and the numbers of individuals of each herbivorous insect feeding type (chewers, galls, and suckers) were counted in late July 2016. Approximately 4% of the observed saplings had fewer than three current-year shoots for herbivore counts. Smaller-sized individuals (sapling stage) of a plant species are more susceptible to herbivore damage than larger-sized individuals (mature stage) because smaller ones are less resistant/tolerant to herbivore damage than larger ones (Boege & Marquis 2005). Thus, it is important that measurements are taken at the sapling stage, just after the initiation of the experiment. In the common-garden experiment, the dominant chewers were larvae of lepidoptera species, such as *Monema flavescens*, *Erannis golda*, and *Malacosoma neustria*, which are polyphagous on broadleaf trees. The dominant sucker was an aphid, *Callipterinella calliptera*, which is monophagous on birch (Moritsu 1983). The dominant galler was an unidentified gall midge of the family Cecidomyiidae which is essentially monophagous (Usuba 1982). Leaves with the galls fall to the soil in autumn and overwinter in pupae. Adults hatch from the galls just before leaf flushing in spring and lay eggs near the buds. As there is a secondary forest of Japanese white birch about 100 m away from the common-garden experiment, it is assumed that most of these herbivorous insects (chewers, galls, and suckers) gather from the secondary forest. Feeding types can be closely related to host specificity: chewers are generalist herbivores, as most chewers are polyphagous herbivores, whereas most galls and suckers are monophagous (specialists) (Moritsu 1983; Yukawa and Masuda 1996; Barton et al. 2015). Thus, classifying species into

ecologically and evolutionarily relevant feeding types (Simberloff and Dayan 1991) allows comparisons and generalizations that cannot be made using taxonomic groups alone (Landsberg and Smith 1992; Andrew and Hughes 2005). To determine the effects of the source population and genotypic diversity of the birch on chewing herbivory, the area of damage (%) by chewers (chewing herbivory) was evaluated in late July 2016, using the following six classes: 0 (0% = 0), 1 (1%–10%), 2 (11%–25%), 3 (26%–50%), 4 (51%–75%), and 5 (76%–100%) (Nakamura et al. 2014). The median of each group was used for the statistical analysis of chewing herbivory (i.e., 0%, 5%, 18%, 38%, 63%, and 88%, respectively).

The basal diameter and height of each sapling were measured twice (late June and early September) to evaluate the effects of source population and birch genotypic diversity on plant growth. To evaluate the effects of the source population and genotypic diversity of the birch on leaf traits (plant defenses), leaf mass per area (LMA), nitrogen (N) and carbon (C) content, the C/N ratio, and concentrations of condensed tannins and total phenolics of leaves were measured in early September 2016. We randomly selected three individual saplings from each single-population plot and one sapling of each source population from each mixed-population (three- and six-population) plot, and we collected two to three fully expanded leaves from the top of each sapling. Ten leaf disks (6 mm radius) were punched from the leaves of each sapling before they were oven-dried at 40 °C for at least 7 days. Phenolics are pyrolytic chemicals that do not decompose at 40 °C (Julkunen-Tiitto 1985). Mean LMA values were calculated. Leaves were ground into a fine powder using an analytical mill for chemical analysis. N and C content was measured using a C/N analyzer (NC-900, Sumika Chemical Analysis Service, Japan). C/N ratios were calculated as indicators of C-based chemical defense (Bryant et al. 1983). Total phenolics concentration was measured using the Folin-Ciocalteu method with gallic acid as the standard solution, and condensed tannin concentration was measured using the Butanol-HCl method with

Cyanidin chloride as the standard solution (Julkunen-Titto 1985). These leaf traits may be responsible for variations in the density of each feeding type (chewers, galls, and suckers) and/or chewing herbivory (Strong et al. 1984, Torres-Gurrola et al. 2011, Mitchell et al. 2016, Singh et al. 2021).

Statistical analyses

All statistical analyses were performed using R 4.31 software. Generalized linear mixed models (GLMMs) were fitted using the R package lme4 (Package 1.1-35.1) to determine whether birch genotypic diversity (the number of source populations per plot) affected plant phenotypes (sapling growth (30-day increments in height and basal diameter) and leaf traits (N content, C/N ratio, condensed tannins, total phenolics, and LMA)) and herbivore abundance (chewing herbivory and the density of each feeding type (chewers, galls, and suckers)); individual saplings were considered replicates, the effect of birch genotypic diversity was a fixed effect, and the block a random effect. GLMMs with a gamma distribution (log-link function) were used for the growth of saplings, leaf traits, and chewing herbivory because their distributions did not satisfy the assumption of normality (Shapiro-Wilk test, $P < 0.01$, each), and these values were positive. Because plant growth and chewing herbivory rate included zero and negative values, which are precluded from testing with a gamma family model using the GLMM function in R, a very small value (e^{-10}) was added to the data before the analysis. GLMMs with a Poisson distribution (log-link function) were used for the density of each feeding type, and the number of surveyed shoots was included as an offset variable to adjust the predicted values according to the number of the current-shoot surveyed in the models. A likelihood-ratio χ^2 test was performed using the R package car (Package 3.1-2) to determine whether the genotypic diversity of the birch affected the response variables in the models. When birch genotypic diversity was found to have a significant effect, the Wald test was used to compare single-population plot with each

262 mixed-population (three- or six-population) plot.

263 GLMMs were fitted using the R package lme4 to determine whether the birch
 264 source population affected plant phenotypes and herbivore abundance in the
 265 single-genotype plots. In the analyses, individual saplings were considered replicates,
 266 the birch source population was a fixed effect, and the block was included as a random
 267 effect. Data on plant growth, leaf traits, and chewing herbivory rates were analyzed
 268 using GLMMs with a gamma distribution (log-link function). The density of each
 269 herbivorous insect feeding type was analyzed using a GLMM with a Poisson
 270 distribution (log-link function), and the number of surveyed shoots was included as an
 271 offset variable to adjust the predicted values according to the number of the
 272 current-shoot surveyed. The likelihood-ratio χ^2 test of the R package car was used to
 273 determine whether the birch source population significantly affected the response
 274 variables. When a significant effect of the birch source population was determined,
 275 Tukey's all-pair comparisons were conducted post-hoc using the glht function of the R
 276 package multcomp (Package 1.4-25) for multiple comparisons among birch source
 277 populations. There were no galls and suckers on lots of saplings; thus, to stabilize the
 278 convergence, a count of 1 was added to each datapoint before the analysis.

279 We performed more in-depth analyses using the statistical approach described
 280 by Johnson et al. (2006) to determine whether the effects of birch genotypic diversity on
 281 plant phenotypes and herbivore abundance were additive (i.e., the sum of the average
 282 effects of genotypes) or non-additive (i.e., indicating a synergistic or antagonistic effect
 283 of multiple genotypes). A paired *t*-test was used to compare the expected and observed
 284 values for each mixed-population plot. The expected values for the mixed-population
 285 plot were calculated by summing the mean values of the single-population plots for
 286 each source population. The observed values were the values in each mixed-population
 287 (three- or six-population) plot. Individual plots were considered replicates.

288 When birch genotypic diversity had non-additive effects on herbivore

abundance, GLMMs were used, including all leaf traits (C/N ratio, N content, condensed tannin, total phenolics, and LMA) as fixed effects and the block as a random effect to examine which leaf traits **mechanistically** explained the variation in the density of each feeding type and/or chewing herbivory. Before performing the GLMM analysis, we examined all leaf traits for which the variance inflation factor (VIF) value exceeded 10, because VIF provides a measure of the extent to which the variance of an estimated regression coefficient is increased by multicollinearity. None of the leaf traits had a high VIF (> 10). Model selection was based on an information theory approach using Akaike's information criterion (AIC; Burnham and Anderson, 2002). AIC values and Akaike weights were computed for all alternatives to select the best-fitting model. According to the principle of parsimony, when alternative models had similar AIC values ($\Delta AIC < 2$), the model with fewer parameters was selected (Burnham and Anderson 2002).

RESULTS

Effects of birch source population

Birch source populations had no effect on any of the plant growth characteristics, including height ($X^2 = 6.53$, $P = 0.26$, Fig. S2a) and basal diameter ($X^2 = 4.06$, $P = 0.54$, Fig. S2b) of the saplings. Although there was no difference in N content and C/N ratios for leaf traits among birch source populations (N content: $X^2 = 9.78$, $P = 0.08$, Fig. S3a; CN ratios: $X^2 = 4.82$, $P = 0.44$, Fig. S3b), the birch source population had significant effects on the concentrations of condensed tannins and total phenols and on LMA (condensed tannins: $X^2 = 14.74$, $P < 0.05$, Fig. S3c; total phenols: $X^2 = 31.89$, $P < 0.01$, Fig. S3d; LMA: $X^2 = 11.50$, $P = 0.04$, Fig. S3e).

The responses of herbivore abundance to birch source populations differed depending on the feeding type (host specificity). Birch source populations did not significantly affect the density of chewers (generalists; $X^2 = 2.76$, $P = 0.73$, Fig. 2a) or chewing herbivory (area of damage by chewers; $X^2 = 2.24$, $P = 0.82$; Fig. 3) but significantly affected the densities of galls and suckers (specialists; galls: $X^2 = 95.50$, $P < 0.01$, Fig. 2b; suckers: $X^2 = 78.77$, $P < 0.01$, Fig. 2c).

Effects of birch genotypic diversity

Birch genotypic diversity had no effect on any plant growth characteristic (height: $X^2 = 0.35$, $P = 0.84$; Table 1; basal diameter: $X^2 = 0.10$, $P = 0.95$). By contrast, birch genotypic diversity had a significant effect only on the concentration of condensed tannins ($X^2 = 18.56$, $P < 0.01$, Table 1), which was significantly higher in the mixed-population (three- and six-population) plots than in the single-population plots ($P < 0.01$). None of the other leaf traits (C/N ratio, LMA, N content, total phenolics concentration) were significantly affected.

The response of herbivore abundance to birch genotypic diversity varied depending on feeding type. Thus, birch genotypic diversity did not significantly affect

the density of chewers ($X^2 = 3.13$, $P = 0.21$, Fig. 4a, Table 2) or chewing herbivory (area of damage by chewers; $X^2 = 0.69$, $P = 0.70$, Fig. 5). In contrast, birch genotypic diversity had a significant effect on the densities of galls and suckers (both $P < 0.01$; Table 2). The densities of galls and suckers in the mixed-population (three- and six-population) plots were significantly lower than those in the single-population plots (galls: $P < 0.01$, suckers: $P < 0.05$; Fig. 4b and c).

Additive versus non-additive mechanisms

The observed concentration of condensed tannins was significantly higher than the expected concentration in the three- and six-population plots ($t = 2.07$, $P = 0.01$; Fig. S4a; six-population plots, $t = 2.70$, $P < 0.01$; and Fig. S4b), suggesting that the genotypic diversity of the birch had a positive non-additive effect on the condensed tannin concentrations.

In the three- and six-population plots, the observed gall density was significantly lower than the expected gall density (three-population plots, $t = -2.26$, $P = 0.04$, Fig. 6a; six-population plots, $t = -2.60$, $P = 0.02$, Fig. 6b). By contrast, for sucker density, there was no significant difference between the observed and expected values at either the three- or the six-population plots (three-population plots, $t = -1.00$, $P = 0.33$, Fig. 6c; six-population plots, $t = -1.14$, $P = 0.27$, Fig. 6d). These results suggest that the genotypic diversity of the birch had a negative non-additive effect on gall density and an additive effect on sucker density.

Leaf traits explaining the abundance of each feeding type affected by non-additive effect

Regarding the variation in gall density, which was significantly affected by a non-additive effect, the model that included N content and C/N ratio had the smallest AIC; however, the ΔAIC s among the best four models were small ($\Delta AIC < 2$; Table 3).

359 According to the parsimony principle, the model that included the N content was the
360 best fit.
361

DISCUSSION

Our study provides insights into the various responses of different feeding types of herbivorous insects to birch genotypic diversity and birch source populations. Birch genotypic diversity did not affect plant growth, and among the leaf traits, it had a significant effect only on condensed tannin concentrations. Although there was no effect on the density of chewers (generalists) or chewing herbivory, the density of galls and suckers (specialists) decreased significantly with increasing birch genotypic diversity. This suggests that specialist, but not generalist, herbivores were affected by birch genotypic diversity; therefore, the response of herbivore abundance to birch genotypic diversity varies depending on the host specificity of the herbivores (i.e., generalist or specialist).

Mechanism of the effects of birch genotypic diversity on plant growth and leaf traits

Empirical and theoretical studies on plant species diversity have attributed the mechanisms of increased plant productivity with greater plant species diversity to a higher probability of including a highly productive species (i.e., a sampling effect) (Schwartz et al. 2000; Cardinale et al. 2006) and to reduced competition in a mixture of species due to niche partitioning (i.e., complementarity effects) (Tilman et al. 2001; Hooper et al. 2005; Cardinale et al. 2007) or facilitation among interacting species (Cardinale et al. 2002). Similarly, plant genotypic diversity has been reported to enhance primary plant production (Crutsinger et al. 2006; Kotowska et al. 2010; Cook-Patton et al. 2011; Schöb et al. 2015). Crutsinger et al. (2006) found that facilitation among genotypes likely contributed to an increase in productivity with increasing genotypic diversity of a dominant plant, *Solidago altissima*. In contrast, our study showed that birch genotypic diversity did not affect plant growth (height or basal diameter of the saplings). This lack of an effect may be due to the early-stage growth of

the plants in our experiment. Previous studies on plant species diversity that focused on the interactive effects of plant diversity and plant ontogenetic stages found a gradual increase in the magnitude of the effects of plant diversity with increasing duration of the experiment because of greater species complementarity (Cardinale et al. 2007; Moreira et al. 2017). The birch saplings used in our study may not have been large enough to overlap and compete for resources (e.g., soil nutrients and light intensity). Thus, the effect of birch genotypic diversity on plant growth may occur during the later stages of plant growth.

Several studies have suggested that growth–defense trade-offs are a possible mechanism underlying the negative effects of plant genotypic diversity on plant defenses (Mraja et al. 2011; McArt and Thaler 2013; Moreira et al. 2014). McArt and Thaler (2013) revealed a trade-off in which the increased productivity of the common evening primrose *Oenothera biennis* under high genotypic diversity resulted in reduced resistance to an exotic leaf herbivore, the Japanese beetle *Popillia japonica*. However, our study showed that the only leaf trait (in terms of plant defense) affected by birch genotypic diversity was condensed tannin concentration, which was significantly higher in the mixed-population than in the single-population plots; birch genotypic diversity had no effect on plant growth. The increased defense ability of plants in the presence of mixed-genotype diversity cannot be explained by a growth–defense trade-off mechanism. Similarly, Moreira et al. (2014) reported that the plant genotypic diversity of big-leaf mahogany *Swietenia macrophylla* enhanced stem (but not leaf) defenses but did not affect growth. Our experiment also showed that the genotypic diversity of the birch had a positive non-additive effect on condensed tannin concentrations. This suggests that an unknown mechanism other than the growth–defense trade-off may facilitate plant defense among birch populations. Knowledge of the mechanisms by which plant genotypic diversity affects plant defense is still limited, and further research is needed (Moreira et al. 2016).

Mechanism of the effects of birch genotypic diversity on the abundance of each herbivore feeding type

Several experimental studies have shown that plant genotypic diversity influences the total abundance and richness of herbivores (e.g., Crutsinger et al. 2006; Johnson et al. 2006). However, in our study, specialists (galls and suckers), but not generalists (chewers), were affected by the genotypic diversity of the birch. Thus, the response of herbivore abundance to birch genotypic diversity varies depending on the host specificity of the herbivores (generalist or specialist).

As birch source populations significantly affected the densities of galls and suckers (specialists), birch genotypic diversity may contribute to shaping the densities of these specialist herbivores, similar to species diversity. The effects of plant genotypic diversity on specialist herbivore abundance may be additive or non-additive (Schweitzer et al. 2005; Hughes et al. 2008; Utsumi et al. 2011; Hambäck et al. 2014). The RC hypothesis predicts additive effects, in which dilution alone is sufficient to decrease the abundance of specialist herbivores in mixed-genotype plots compared with single-genotype plots (Root 1973). Thus, the responses of herbivore abundance in mixed-genotype plots can be predicted based on those observed in single-genotype plots. In our experiments, sucker density decreased significantly with increasing birch genotypic diversity, although there were no significant differences between the expected and observed sucker densities in the mixed-population (three- and six-population) plots. These results suggest an additive effect of genotypic diversity owing to the dilution of the abundance of the preferred source populations for sucker density, which is consistent with the RC hypothesis.

In contrast to sucker density, the density of galls (specialists) significantly decreased with increasing birch genotypic diversity, and the observed gall density was significantly lower than the expected density in the mixed-population (three- and

six-population) plots. These results suggested a negative, non-additive effect of genotypic diversity on gall density. Non-additive effects in diverse plant communities arise because of possible changes in plant phenotypes (plant productivity and/or plant defenses) and/or herbivore foraging behavior (associational resistance with neighboring plant individuals). The MI hypothesis and the growth–defense trade-off together predict non-additive effects via changes in plant phenotype (plant productivity and/or defenses), resulting in an increase in herbivore abundance (Srivastava and Lawton 1998 Mraja et al. 2011; Moreira et al. 2014; McArt and Thaler 2013). Our experiment showed that birch genotypic diversity did not affect plant growth (plant productivity) but did increase the condensed tannin concentrations (plant defense). The best-fitting models of gall density did not include condensed tannins, thus ruling out changes in plant phenotype as a potential cause of decreased gall density. Furthermore, birch genotypic diversity decreased rather than increased the gall density. Thus, our results regarding gall density do not support the prediction made by the combination of the MI hypothesis and the growth–defense trade-off. Kostenko et al. (2017) reported similar results, namely that plant diversity (species diversity) altered the defenses of their focus plant (*Jacobaea vulgaris*), but the effects on the arthropod community were not mediated by this change in plant defenses. Alternatively, non-additive effects may be due to changes in foraging behavior, including associational resistance caused by visual and chemical masking of neighboring plants (Tahvanaian and Root 1972; Barbosa et al. 2009; Utsumi et al. 2011). Although foraging behavior was not investigated in this study, the masking of neighboring plant individuals during oviposition by the herbivorous insects may have led to a reduction in gall density in mixed-population plots. The negative, non-additive effects of birch genotypic diversity on gall density may be explained by this type of associational resistance.

In conclusion, our findings clearly demonstrate that the response of herbivore abundance to birch genotypic diversity varies depending on the host specificity of the

herbivores (i.e., generalists or specialists). Thus, specialist herbivores (galls and suckers) were affected by birch genotypic diversity, whereas generalist herbivores (chewers) were not. Accordingly, an accurate assessment of the impact of genotypic diversity on herbivore damage requires that the different feeding types of herbivorous insects be evaluated separately (Barton et al. 2015). The response of specialist herbivores to birch genotypic diversity in our experiment was similar to the response to plant species diversity reported in a previous meta-analysis by Koricheva and Hayes (2018), who noted that the concentration of host plants in mixed-genotype stands was the same as that in uniform-genotype stands. However, our study showed that birch source populations significantly affected the densities of galls and suckers (specialist herbivores), suggesting that the concentration of host plants in mixed-population plots for these specialist herbivores was not the same as the concentration of host plants in single-population plots. Our study suggests that the loss of birch genotypic diversity is an important biological control service for specialist herbivores in the boreal forests of Hokkaido. Given that specialist herbivores (gallers and suckers) have been shown to negatively affect plant fitness (Sacchi et al. 1988; Watanabe et al. 2018; Martini et al. 2021), future studies on the loss of birch genotypic diversity, such as due to shrinking population sizes (e.g., deforestation or forest fragmentation), should pay more attention to the possibility of further reductions in birch population size in combination with specialist herbivore attacks.

AUTHOR CONTRIBUTIONS

The idea for the study was conceived by MN. The methodology was designed by MN and BS. Data was collected by MN, BS, CT. Data analysis and interpretation was performed by MN, CT, and HT. MN, CT, and HT led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Northern Forestry Research and Development Office of Hokkaido University for their support. This work was supported by Grants from Japan Society for the Promotion of Science (No. 26450188 to MN and 20K06138 to HT). We would like to thank Editage (www.editage.jp) for English language editing.

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Figure captions

Figure 1 Schematic design of common-garden experiment in this study: (a) each plot (1 x 1m) contained nine individual saplings of Erman's birch; (b) each block contained six single-population plots from each source population (A, B, C, D, E and F), one three-population plot and one six-population plot; (c) 8 blocks (eight replicates) were set up in the common-garden experiment.

Figure 2 Density of each feeding type of herbivorous insects ((a) chewers, (b) galls, and (c) suckers) among source populations. Values are means $\pm$ SE. Tukey's all-pair comparisons (post hoc) test was used for multiple comparisons of densities among the birch source populations. Different letters indicate significant differences ($P < 0.05$).

Figure 3 Herbivory by chewers (the area of damage (%) by chewers) among birch source populations. Values are means $\pm$ SE.

Figure 4 Density of each feeding type of herbivorous insects ((a) chewers, (b) galls, and (c) suckers) among single-, three-, and six-population plots. Values are means $\pm$ SE. The Wald test was used to compare the densities between single-population plot and each mixed-population (three- or six-population) plot. Asterisks indicate significant differences (* $P < 0.05$).

Figure 5 Herbivory by chewers (the area of damage (%) by chewers) among single-, three-, and six-population plots. Values are means $\pm$ SE.

Figure 6 Observed and expected values of (a) gall density in three-population plots, (b) gall density in six-population plots, (c) sucker density in three-population plots, and (d) sucker density in six-population plots. Observed values from densities in mixed-birch population (three- and six-population) plots and expected values calculated from densities in single-birch population plots. Values are means $\pm$ SE. Asterisks indicate significant differences (* $P < 0.05$).

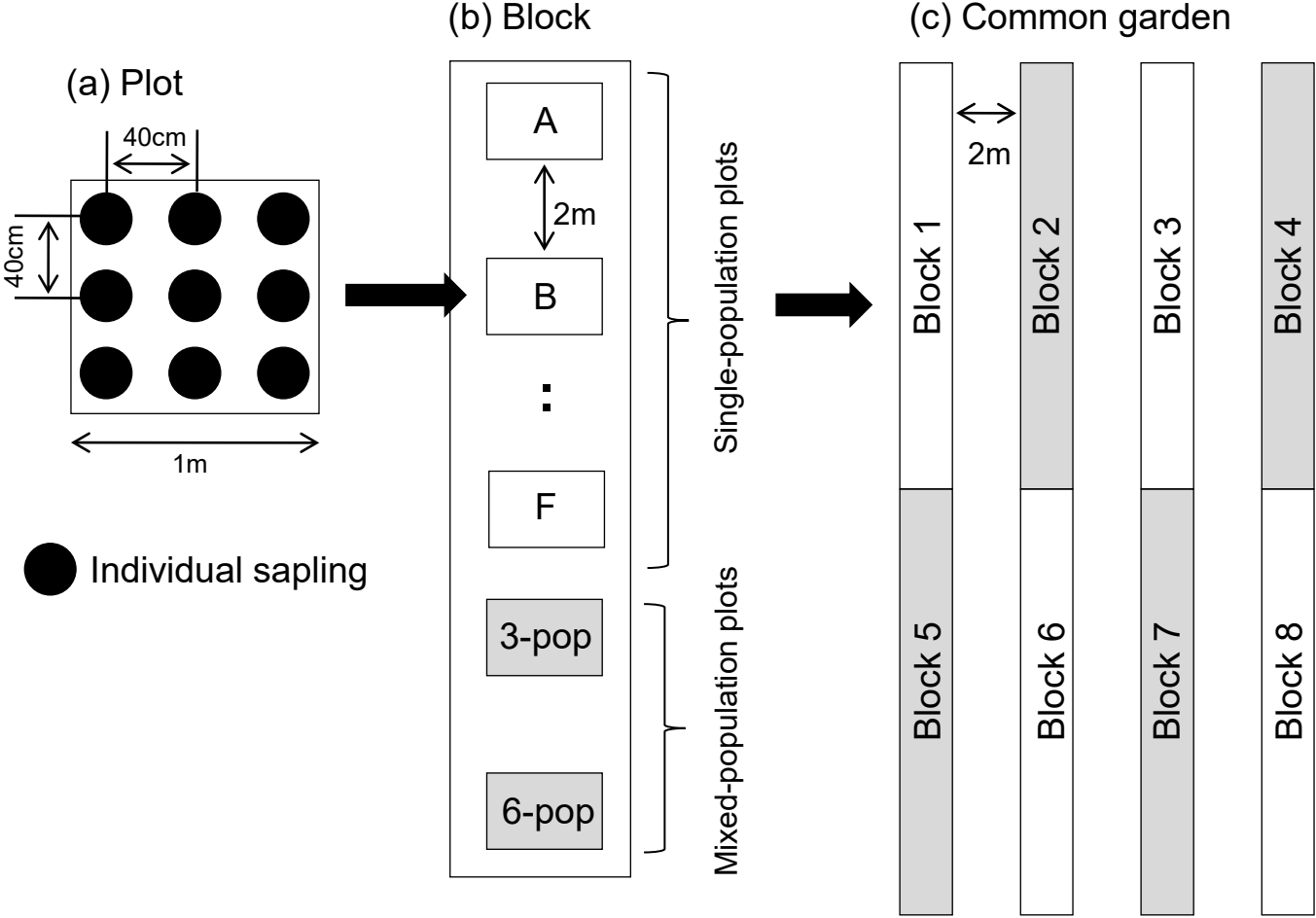
Supplemental figure captions

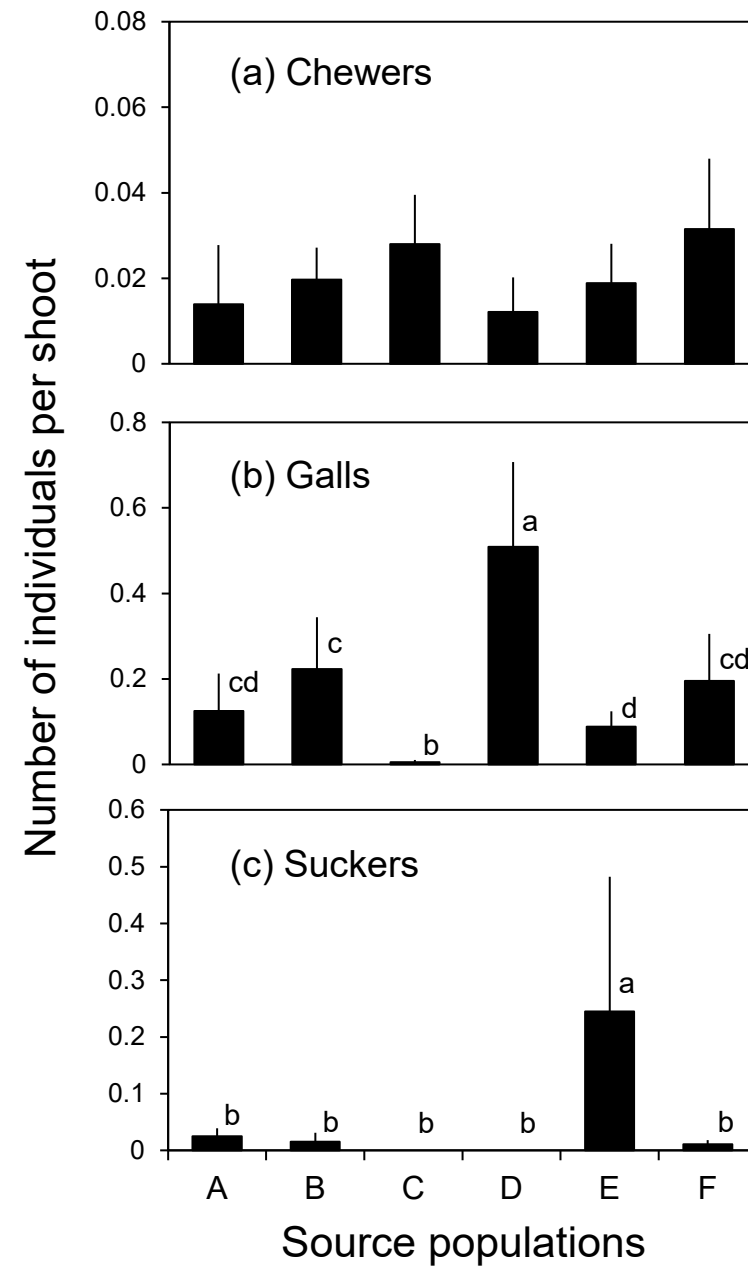
Figure S1 Location of Nakagawa Experimental Forest in Japan and six distinct source populations of the Erman's birch (*Betula ermanii*) in the forest. The distance between each of the source populations was at least 1 km to preclude gene flow.

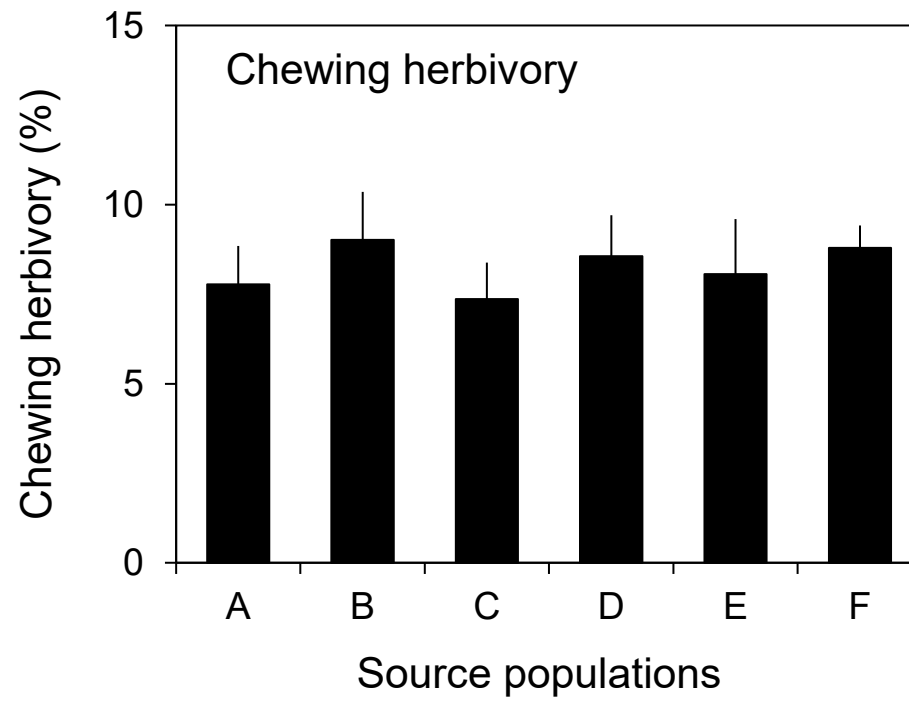
Figure S2 Plant growth ((a) sapling height growth, (b) sapling basal diameter growth) among birch source populations. Values are mean $\pm$ SE.

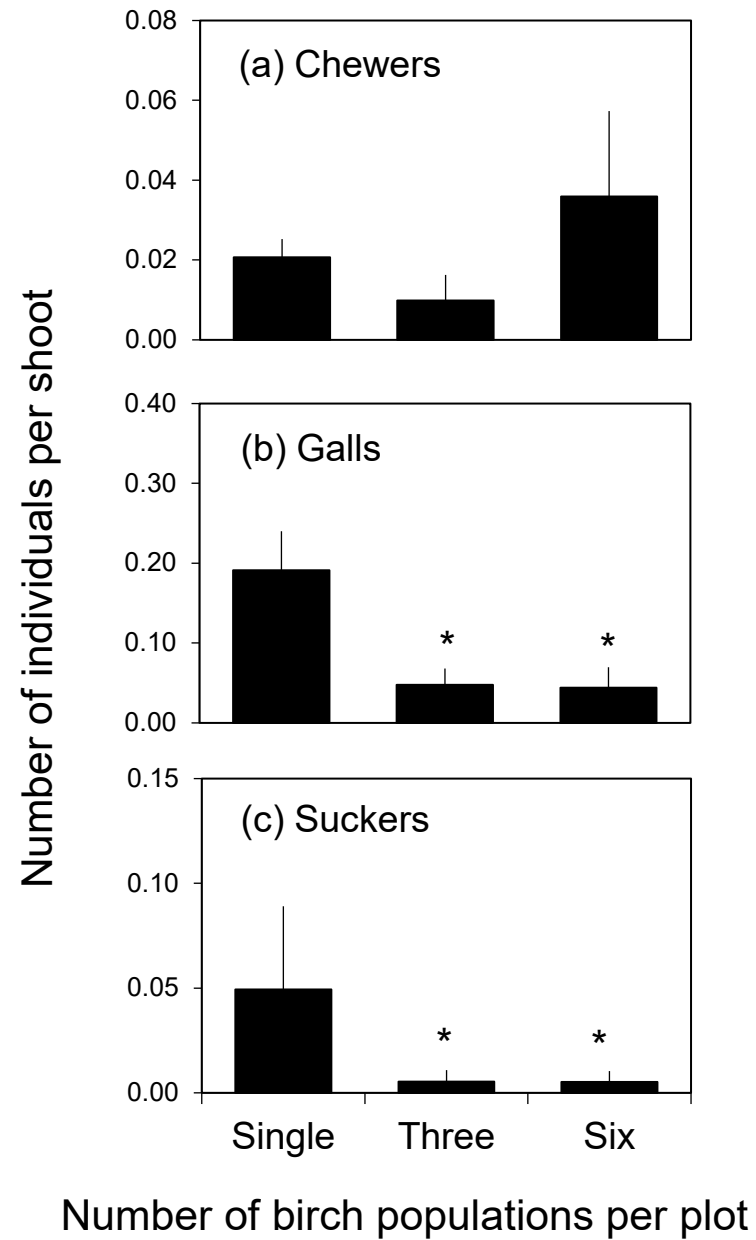
Figure S3 Leaf traits (nitrogen content, C/N ratio, condensed tannin, total phenolics, and LMA) among birch source populations. Values are mean $\pm$ SE. Tukey's all-pair comparisons (Post hoc test) was used for multiple comparison among source populations. Different alphabets indicate significant differences ($P < 0.05$). Birch source populations significantly affected concentrations of condensed tannins, total phenols, and LMA, but no significant differences were found for LMA and condensed tannins in the Post hoc test.

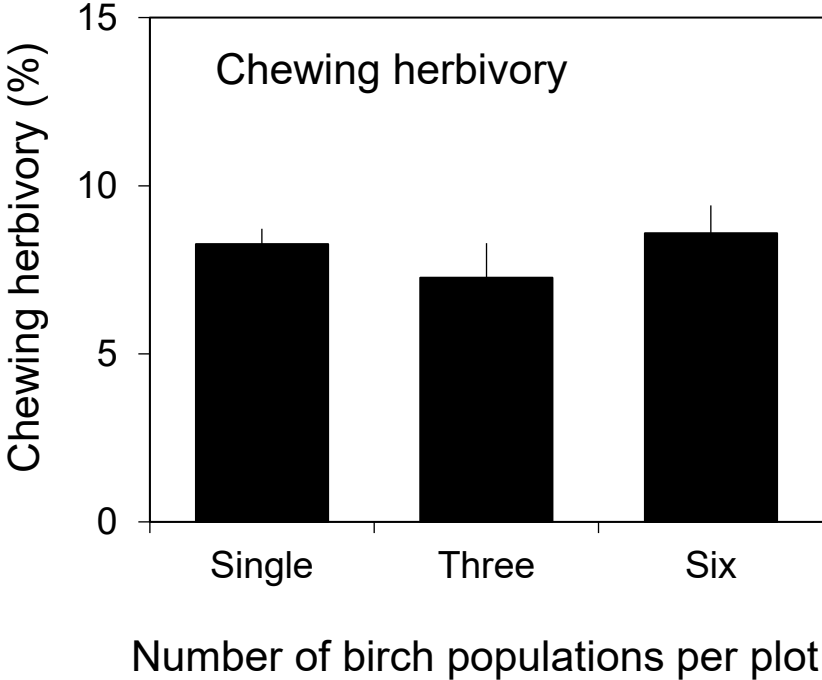
Figure S4 Observed and expected values of (a) condensed tannin concentration in three-population plots and (b) condensed tannin concentration in six-population plots. Observed values from concentrations in mixed-birch population (three- and six-population) plots and expected values calculated by concentrations in single-birch population plots. Values are mean $\pm$ SE. Asterisks indicate significant differences (* $P < 0.05$).

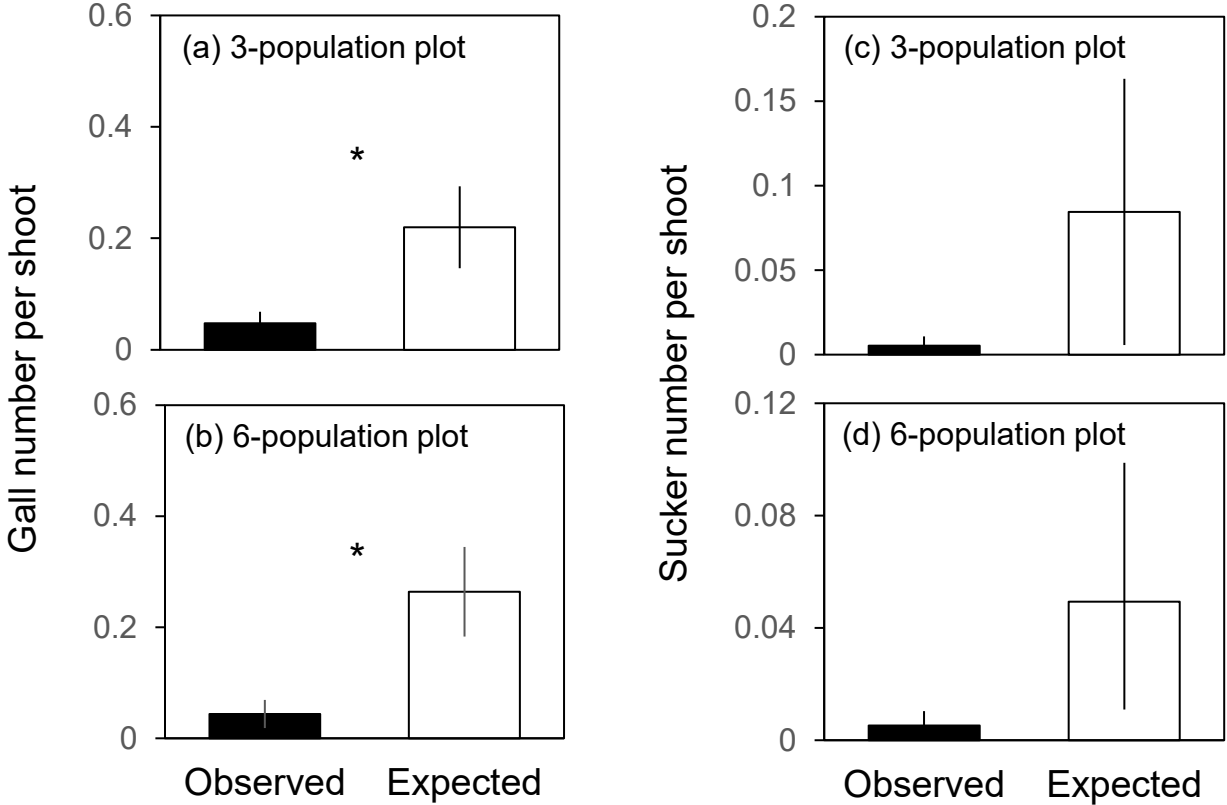


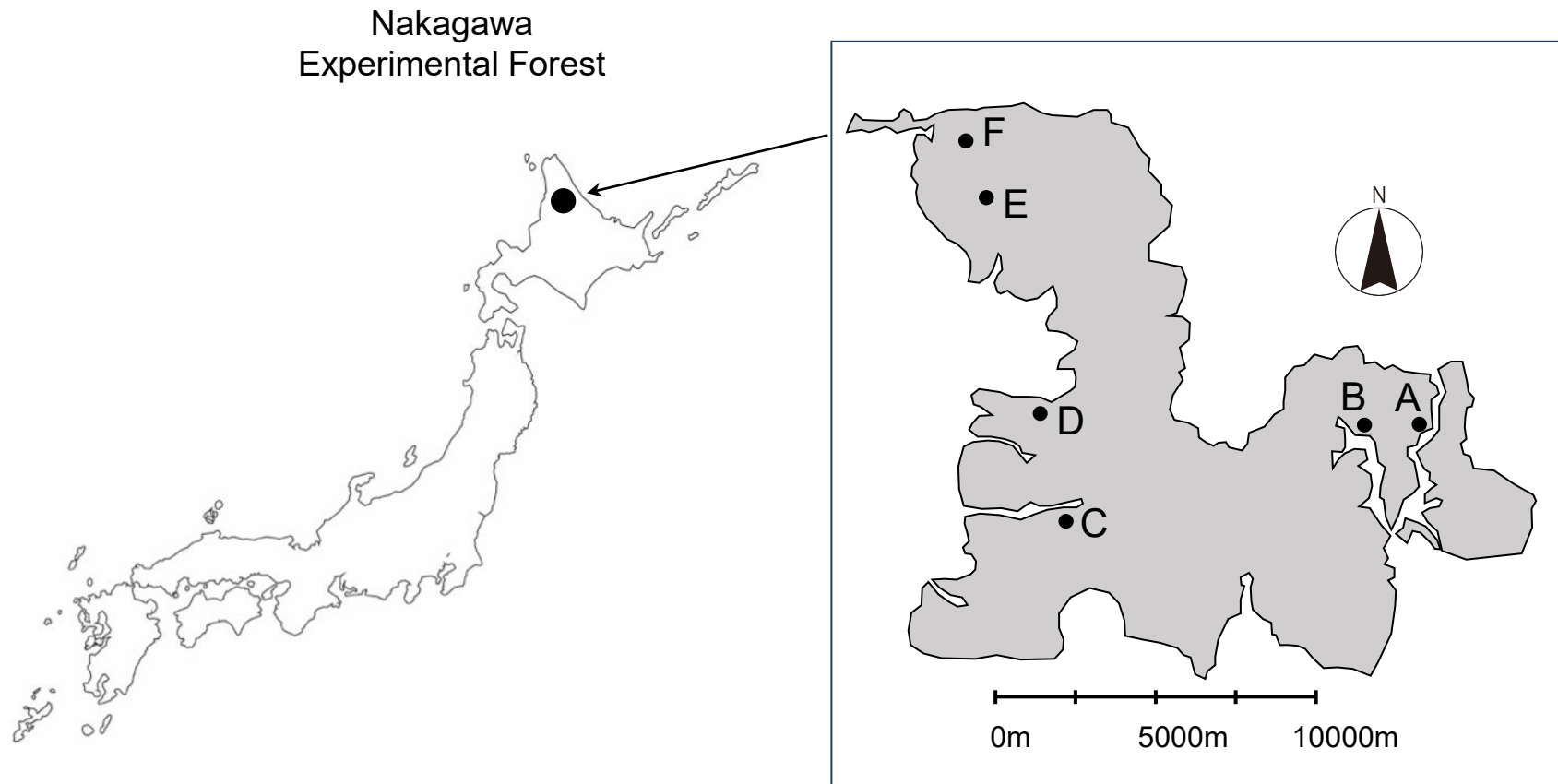


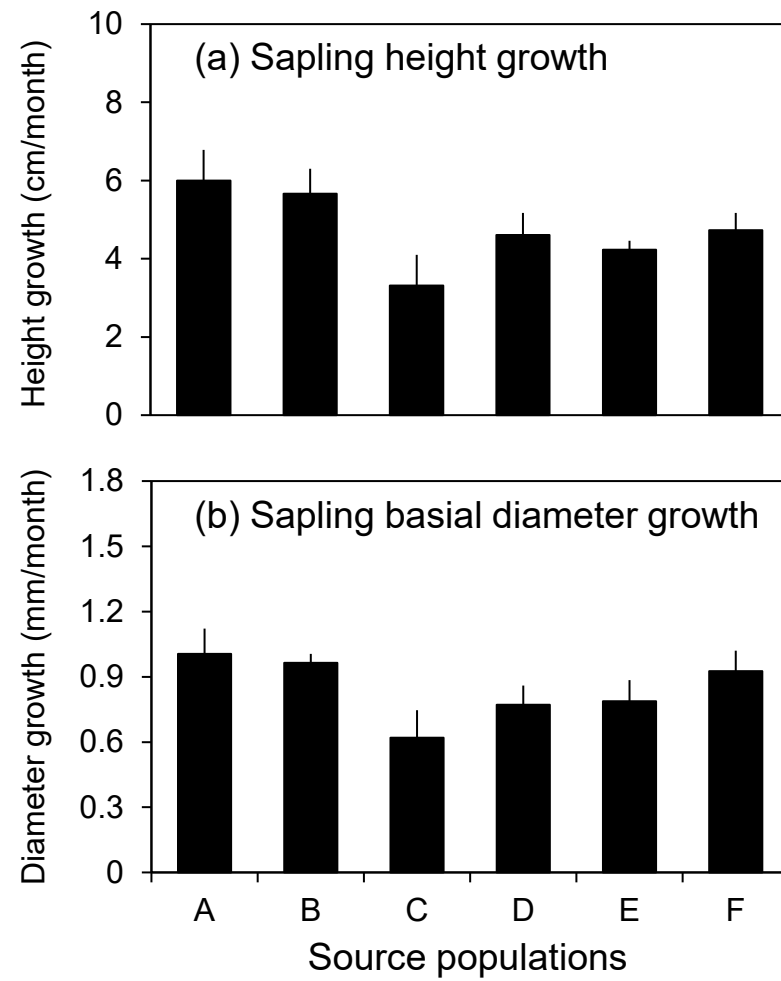


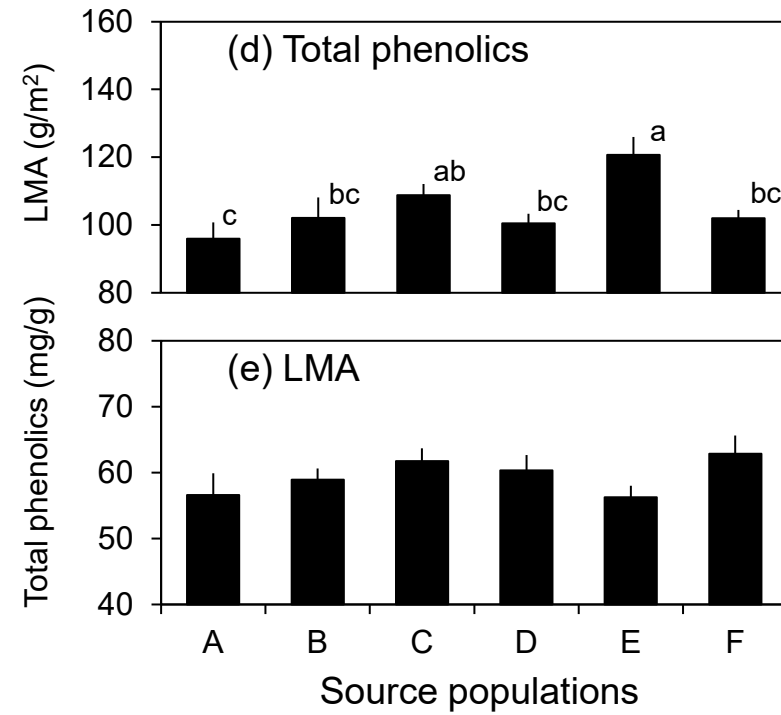
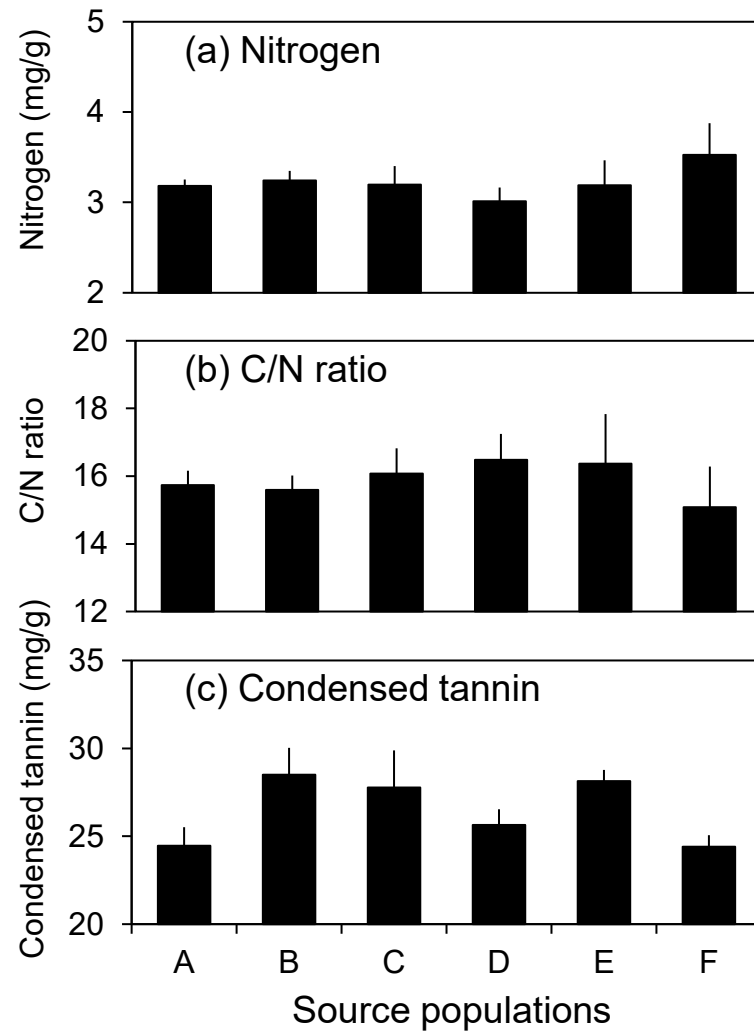












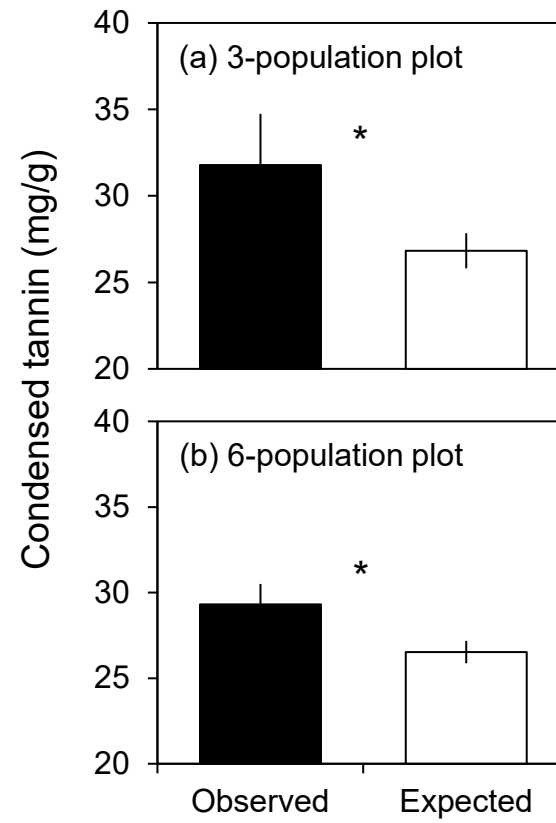


Table 1. Plant growth and leaf traits among single-, three-, and six-population plots. Values are mean + SE.

Wald test was used for comparison of these characteristics that are significantly affected by birch genotypic diversity between single-population plot with each mixed-population (three- or six-population) plot. Asterisk shows a significant difference (* $P < 0.05$).

Characteristics		Number of birch population per plot			Chi-square	<i>P</i> -value
		Single	Three	Six		
(a) Plant growth	Sapling height growth (cm/month)	0.85 (0.04)	0.85 (0.13)	0.82 (0.11)	0.35	0.84
	Sapling basal diameter growth (mm/month)	4.72 (0.28)	4.18 (0.68)	4.16 (0.78)	0.10	0.95
(b) Leaf traits	Nitrogen (mg/g)	32.24 (0.57)	32.08 (1.67)	33.72 (1.13)	2.89	0.24
	C/N ratio	15.89 (0.26)	16.06 (0.62)	15.10 (0.41)	5.34	0.07
	Condensed tannin (mg/g)	26.49 (0.50)	31.79 (1.90)*	29.26 (0.89)*	18.56	< 0.01
	Total phenolics (mg/g)	104.98 (1.60)	112.55 (4.44)	110.82 (2.69)	5.54	0.06
	LMA (g/m ²)	59.44 (0.76)	59.30 (2.24)	58.33 (1.57)	0.66	0.72

Table 2. Summary of likelihood-ratio chis-square test for effect of genotypic diversity (the number of source populations per plot) on density of each feeding type of herbivore insects ((a) chewers, (b) galls, and (c) suckers).

Feeding type	Df	Chi-square	<i>P</i> -value
(a) Chewers	2	3.11	0.21
(b) Galls	2	32.03	< 0.01
(c) Suckers	2	9.37	< 0.01

Table 3 Summary of the model selection procedure for the variation in densities of gall.

The most likely multivariate models ($\Delta AIC < 2$) are shown.

Log-likelihood value (LL), difference of the AIC between the current and the smallest model (ΔAIC), and Akaike weight (w_i) are shown.

Models	LL	ΔAIC	w_i
Gall			
CN + N	-234.110	0.0	0.181
N	-235.480	0.8	0.125
CN + LMA + N	-233.483	0.8	0.125
LMA + N	-235.014	1.8	0.073

Table S1. Geographic information (latitude, longitude, and altitude) for each source population.

Source population	Latitude	Longitude	Altitude (m)
A	44°80.0449N	142°26.0334E	142.7
B	44°79.8490N	142°24.6284E	154.1
C	44°77.2904N	142°10.7198E	163.4
D	44°80.4408N	142°10.3832E	144.4
E	44°87.8427N	142°08.8545E	252.8
F	44°88.9348N	142°08.4231E	217.3