Genetic and functional variation of an *Abies mariesii* meta-population in a subalpine ecosystem

A DISSERTATION PRESENTED

BY

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TO

THE GRADUATE SCHOOL OF ENVIRONMENT AND INFORMATION SCIENCES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF ENVIRONMENT SCIENCE

IN THE SUBJECT OF

ECOLOGY

YOKOHAMA NATIONAL UNIVERSITY

YOKOHAMA, KANAGAWA

MARCH 2015

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Abstract

Within the high-elevational areas, mosaics of habitats are generally distributed at small spatial scales. Initiation of plant growth and reproduction is also said to be triggered different ways for species inhabiting contrasting habitats. Therefore, plants often appear in spatially discrete colonies. Due to their small population size, spatially discontinuous populations may face an increased threat of extinction under changing environments. Unlike ecosystems that features a diverse of species, high-elevational ecosystem are generally consisted of fewer species. Hence, even small and discrete population patches of dominant species (known as "foundation species") can be critically important. Foundation species are likely irreplaceable considering their role in providing necessary habitats for subordinate species. Meanwhile, critical ecosystem processes such as biomass production and biogeochemical cycling, are also considered to be closely correlated with the foundation species. Furthermore, habitat modifications (e.g. soil conditions) through foundation species can lead to possible feedbacks to above-ground plant communities, resulting indirect plant-plant interactions between foundation species and associated species. Study of how foundation species respond to changing environments not only improves the understandings of their persistence, but also gains ecological importance and insights of maintaining correlated ecosystem processes in high-elevational systems.

The studies in this dissertation were conducted in a local-scale subalpine

ecosystem (within about 3 km²) that includes forest habitats near the wind-blown mountain ridge and habitats in the nearby moorlands in central Japan. The topography and wind direction results in heavier snow accumulation in moorlands, compared to the forest habitat. Molecular tools and *in situ* field experiments were used to study the responses of *Abies mariesii*, a foundation coniferous species of many Japanese subalpine ecosystems, to heterogeneous and fragmented habitats.

Firstly, the genetic variation and functional variation of *A. mariesii* were quantitatively investigated. Though *A. mariesii* populations are spatially discrete and undergoing strong habitat heterogeneity, they are less likely to be genetically differentiated. Gene flow among the *A. mariesii* populations was biased toward the moorlands. Compared to the forest populations, the genetic diversity in the moorland populations was found to be significantly higher, suggesting that the moorlands could serve as sinks of genetic diversity for *A. mariesii*. Meanwhile, *A. mariesii* showed considerable physiological variation across habitats. Being able to adjust tree morphology in contrasting eco-habitats may strengthen the competitiveness of *A. mariesii*. This may also make a tangible contribution to the maintenance of populations in contrasting habitats.

Then, potential causes and consequences of within-species variation of *A. mariesii* were evaluated. Considering that one major source of a plant's physiological trait variation may be related to its nutrient use strategies, the nutrient use strategies of *A. mariesii* distributed across the two eco-habitats (i.e. the forest habitat and the moorland habitat) were therefore investigated. To do

so, the nitrogen (N)-resorption efficiency and the mean residence time (*MRT*) of needles as well as foliar N were quantified. In order to explore the consequences of intraspecific variation, an *in situ* litterbag transplant experiment was conducted to examine whether intraspecific variation of *A. mariesii* can cause impacts on litter decomposition, one of the key ecosystem processes. The results suggested that *A. mariesii* individuals in snowy moorlands were more conservative in using acquired nutrients, as they showed higher nitrogen resorption efficiency and tended to retain needles and foliar nitrogen longer than those in snowless forest habitat. This resulted in reduced nitrogen lost through needle senesce for *A. mariesii* in humid and harsh conditions, and led to the production of both nutrient-poor and nutrient-rich needle litter within this foundation species. Litter nutrient quality later became one crucial factor affecting the release of nitrogen back to this local-scale ecosystem. By investigating both the causes and consequences of intraspecific variations in nitrogen-related physiological traits of *A. mariesii*, important details could be added to our knowledge for the maintenance of foundation species and its contribution to the functioning of local ecosystems.

Based on the research of within-species patterns of *A. mariesii*, a conceptual framework has been proposed to bridging the gaps between ecological studies. This framework demonstrates how levels of organization in ecology, ranged from individual organisms and populations to ecosystems, can be linked and considered in a more systematic perspective through the focus of intraspecific patterns (i.e. individual- and population-level patterns). The framework also

highlights some pathways which have not received enough attentions in current ecological studies, or some novel pathways which may bare great significance in future. More attentions need to be paid on those critical but easy-to-be ignored novel pathways.

Contents

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The following authors contributed to **Chapter 3**: Saori Fujii, Kobayashi Makoto and Akira S. Mori.

Acknowledgments

First and foremost, I would like to extend my sincere gratitude to Akira S. Mori for accepting me as his Ph.D. student. I am deeply grateful of his guidance for my research. His consistent and illuminating instruction has inspired me, and allowed me to grow as a scientist. I want to thank Saori Fujii, Ishii Hiroshi, Yuji Isagi, Shingo Kaneko, Fumito Koike, Kobayashi Makoto, Makiko Mimura, Eri Mizumachi, Yukira Mochida, Taizo Nakamori, Aino Ota, Akiko Sakai and Saito Waka (in alphabetical order by last name) for their kind assistance and advices during my fieldwork, laboratory work and publication of papers. My gratitude also goes to Keiichi Ohno, who is my first Japanese supervisor, for his kind support and instruction while I was working for my master's degree. I thank Ryo Kitagawa, Hirofumi Kondo, Uryu Shinya, Shinichi Tatsumi (in alphabetical order by last name) and all the rest laboratory members who haven't been listed here for sharing casual and professional conversations with me every day. They made my research life in Japan an enjoyable memory, and their brilliant comments and suggestions greatly improved my research.

Lastly, I would like to express my deep appreciation to my family and friends. Words cannot express how grateful I am to my parents for their constant support. I would like to thank Yanhui Gu for his valuable advices and support since the day I decided to study abroad. I also want to thank my sincere friends Qiuyun Xu, Kaixuan Chen and Jie Lin for their company and encouragement all these years.

1

General Introduction

1.1 Ecosystems at high-elevations

Ecosystems at high-elevations are characterized by rough topography and heterogeneous environmental gradients (e.g. snow accumulation patterns and soil properties), which could strongly affect the growth and distribution of living organisms in these areas (Körner 2003). Snow, for example, can promote the growth of plants by protecting them from frost events early in the growing season (e.g. Mori and Komiyama 2008; Rixen et al. 2010) or promote germination rates (e.g. Shimono and Kudo 2005); heavy snowpack can at the same time cause mechanical pressures therefore limiting the growth of species (e.g. Kajimoto et al. 2002; Seki et al. 2005).

In some Japanese subalpine ecosystems, snow accumulation can be extremely

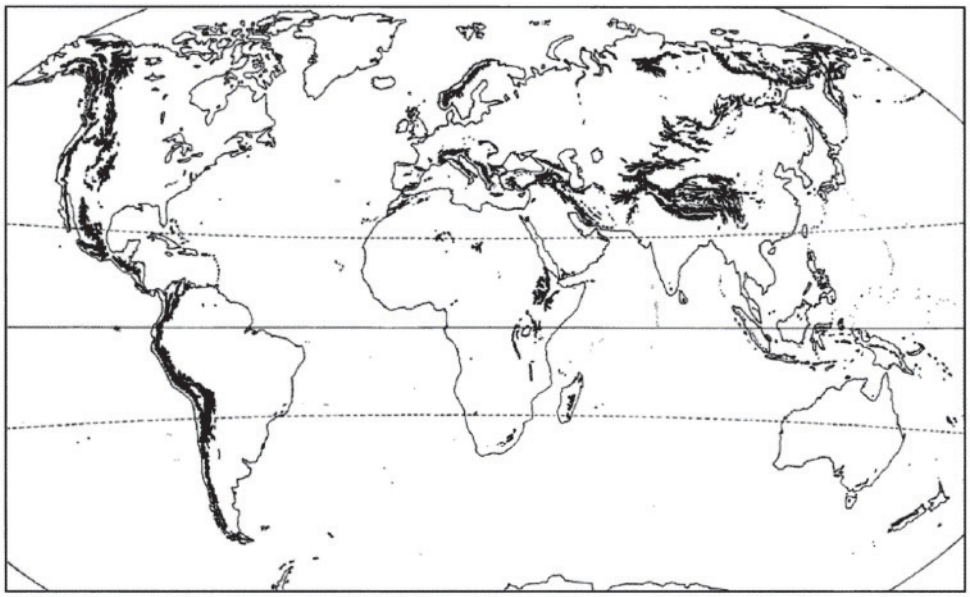

Figure 1.1.1: The global distribution of the high-elevational alpine life zone. (Figure from Körner 1995)

heavy in areas with gentle slopes (e.g. up to 4-6 m in subalpine moorlands; Fig. 1.1.2), resulting less diverse plant communities and infertile soils. On the contrary, less snow will accumulate in wind-blown mountain ridges, resulting in snowless habitats and usually earlier snowmelt compared to the heavily snowy sites. The unsynchronized snowmelt timing would further affect the duration of plant growth, and contribute to the shape of varied plant communities that have been associated with early- or late-snowmelt (Hülber et al. 2010; Körner 2003).

In addition to the snow, soil characteristics (e.g. physical properties and nutrient availability) can also become critical factors in determine species compositions and community structures. Soil moisture and physical soil properties such as grain size, water potential or bulk density are influenced by the micro-typography, forming a complex pattern or soil types, which seem to be a characteristics feature for soils at high-elevations (Holtmeier 2009; Körner 2003).

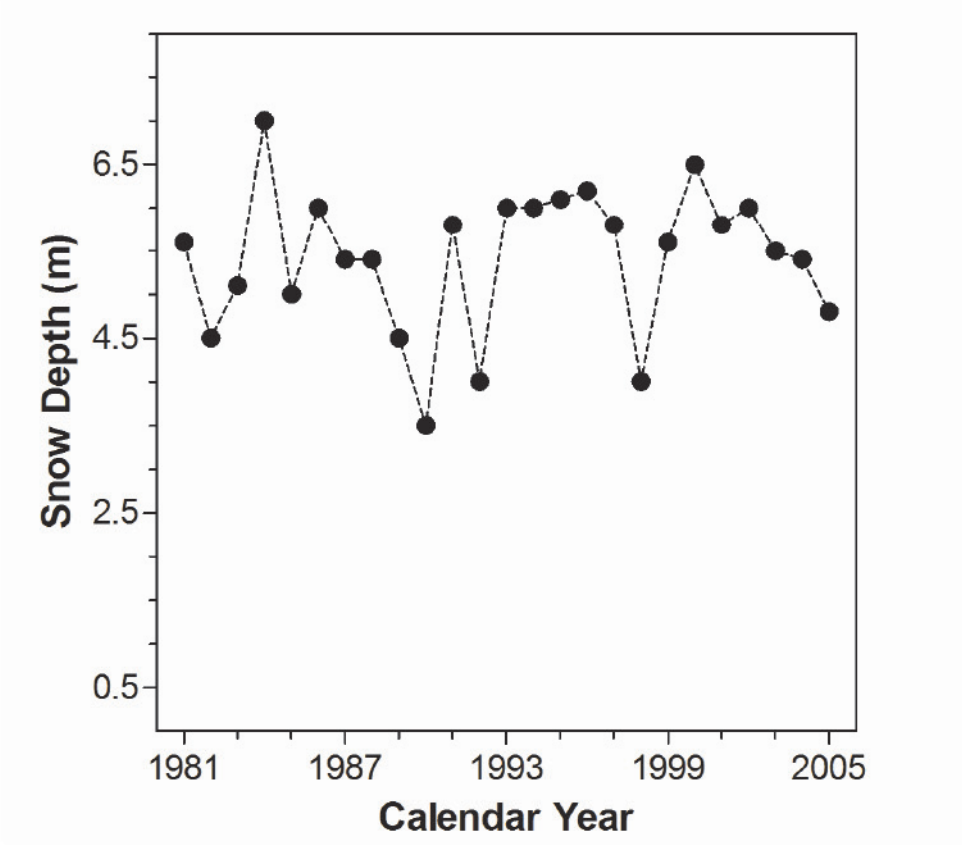

Figure 1.1.2: Snow depth in a subalpine moorland in Tateyama Mountain range in central Japan between 1981 and 2005. (Doi *unpublished data*)

1.2 The distribution of plants in high-elevational ecosystems

Within the high-elevational areas, mosaics of habitats are generally distributed at small spatial scales. Initiation of plant growth and reproduction is also said to be triggered different ways for species inhabiting contrasting habitats (e.g. snowless wind-blown ridges and heavily snowy snow-beds; Fig. 1.2.1). Therefore, plants often appear in spatially discrete colonies. Due to their small population size, spatially discontinuous populations may face an increased threat of extinction under changing environments. In some conditions, patchily distributed populations can together shape the meta-populations structures. As long as small patchy populations across landscape are connected by immigration, local populations as a whole (i.e. the meta-population) can still be maintained by reaching a certain level of equilibrium, which is determined by the rates of extinction and colonization of local populations, and by the quality of habitat patches (Lande 1987). Sites that are vacant right now may have been occupied at other times, and vice versa. Population patches may have acted as sources or sinks—they might have expanded, merged, or become extinct.

Besides the patchiness, fragmentation, and displacement of suitable habitats, the maintenance of gene flow among area patches is also a crucial characteristic of meta-populations. Gene flow is not only a function of dispersal, but also of the success of the migrants among populations that could involve a number of evolutionary processes. Insights into detailed gene flow patterns thus give the basis for understanding the migration among spatially structured populations, as well as understanding the population genetic structures in heterogeneous habitats (Kawecki and Ebert 2004). Currently available molecular approaches provide good opportunities to study how spatially discrete populations are structured and maintained. Also, these approaches have advanced the evaluation and prediction of population changes (e.g. local population extinction) in

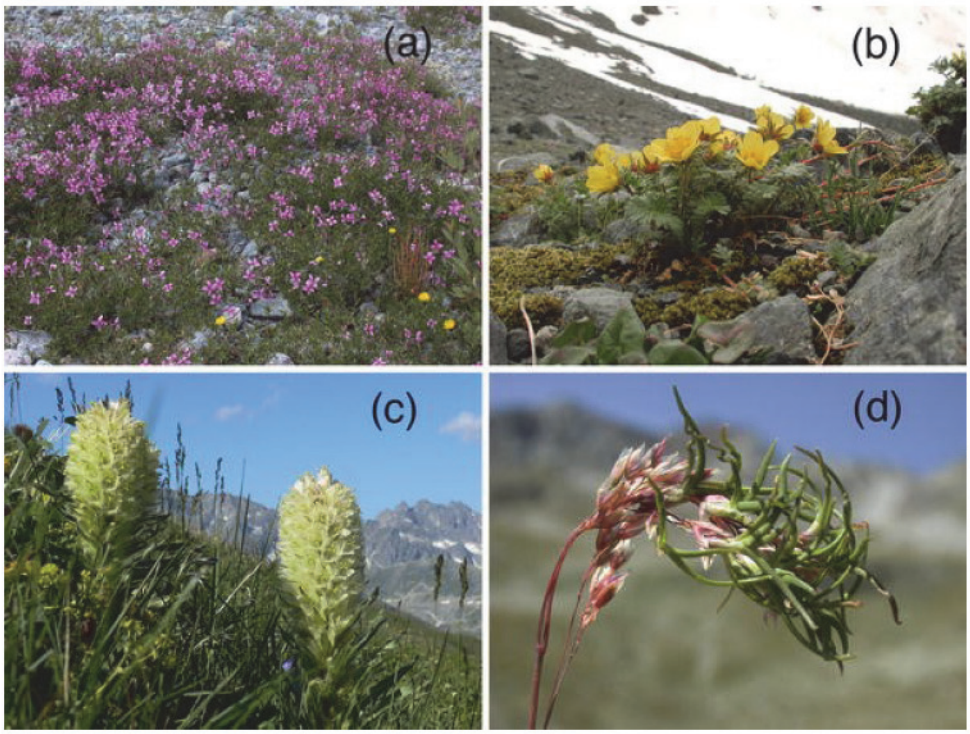

Figure 1.2.1: Patchily distributed habitats of three alpine plant species. (a) *Epilobium fleischeri*, (b) *Geum reptans*, (c) *Campanula thyrsoides* and (d) Poa alpine. (Photos from Stöcklin et al. 2009)

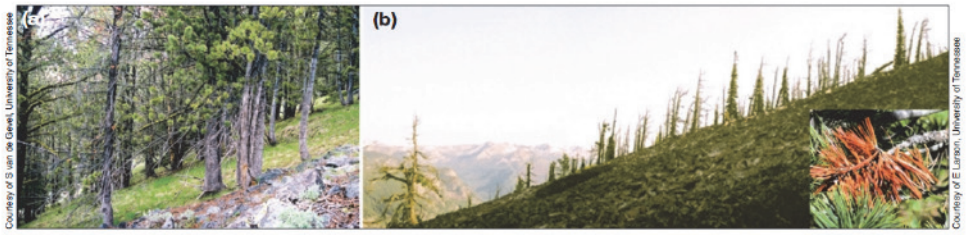

Figure 1.2.2: High-elevation stands of whitebark pine (*Pinus albicaulis*). This species is transformed from (a) healthy stands to (b) dead stands through the interaction of fire suppression, the introduced pathogen Cronartium ribicola that causes white pine blister rust (inset), and the native bark beetle Dendroctonus ponderosae. (Photos from Ellison et al. 2005)

high-elevational ecosystems in the future.

1.3 Foundation species and the maintenance of local systems at high-elevations

Unlike ecosystems that features a diverse of species, high-elevational ecosystem are generally consisted of fewer species. Hence, even small and discrete population patches of dominant species can be critically important to subordinate species in the ecosystem (i.e. foundation species, Ellison et al. 2005; Fig. 1.2.2). The population patches are likely irreplaceable considering their role in providing necessary habitats for arthropods or microbes (Ellison et al. 2005), and their contribution to the persistence of associated species (Callaway 2009).

Meanwhile, critical ecosystem processes such as biomass production and biogeochemical cycling, are also considered to be closely correlated with the foundation species in local systems (Al Hayek et al. 2014; Block et al. 2012; Ellison et al. 2005; Yu et al. 2010). Furthermore, habitat modifications (e.g. soil conditions) through foundation species can lead to possible feedbacks to above-ground plant communities, resulting indirect plant-plant interactions between foundation species and subordinate species (Hendriks et al. 2013; van de Voorde et al. 2011; Van der Putten 2003). Study of how foundation species respond to changing environments not only improves the understandings of their persistence, but also gains ecological importance and insights of maintaining correlated ecosystem processes in high-elevational systems.

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2

Genetic and functional variation of an *Abies mariesii* meta-population

2.1 Introduction

Species at high-elevations that are distributed across heterogeneous mountain environments are shaped by, but not limited to, variations in topographical position, soil properties, and snow accumulation patterns; therefore, they often appear in spatially discrete colonies (Harris 1984; Horikawa et al. 2008; Körner 2003; Segelbacher et al. 2003). Because spatially discontinuous populations may face an increased threat of extinction under changing environments, due to their small population size, an understanding of how these species persist in such discontinuous habitats is crucial for predicting future changes. The meta-population concept suggests that, as long as small patchy populations

across landscape are connected by immigration, local populations as a whole (i.e., the meta-population) can still be maintained by reaching a certain level of equilibrium, which is determined by the rates of extinction and colonization of local populations, and by the quality of habitat patches (Lande 1987; Levins 1970). Following this concept, a number of studies have underlined the importance of habitat quality or connectivity among habitat patches, arguing that large habitat patches or high connectivity among local populations could contribute to longer overall population persistence (Hanski 1998; Hanski and Ovaskainen 2000; Higgins and Lynch 2001; Ovaskainen and Hanski 2003).

High connectivity, however, which could contribute to the size of a meta-population, thus persistence, has an antagonistic effect when populations among heterogeneous environments are locally adapted. When some local populations have diverged and become locally adapted to specific environmental conditions, the niche of an individual would be much smaller than what we expect based on the species range alone. In this case, increasing the connectivity by increasing the movement of genes among populations (a.k.a. gene flow) may bring maladaptive genes into the local population and reduce the population's fitness (Bolnick and Nosil 2007; Kremer et al. 2012; Savolainen et al. 2007; Schiffers et al. 2013).

Genetically diverged populations, in some cases, can be shaped by small-scale habitat heterogeneity, for instance, in alpine herbs such as *Primula cuneifolia* (Hirao and Kudo 2008) and *Potentilla matsumurae* (Shimono et al. 2009) where differentiated flowering due to the heterogeneous snowmelt patterns acted as the predominant factor. Nevertheless, long-lived tree species are unlikely to see differentiation in locally discrete habitats due to their strong dispersal abilities (Hamrick 2004). Only rare cases (i.e., reproductive isolation in trees) have been mentioned, like the diverged flowering in *Eucalyptus globulus* (Foster et al. 2007). Reproductive isolation can be a major factor in shaping asymmetric dispersal among local populations (Cortés et al. 2014). Nevertheless, gene flow is not only a function of dispersal, but also of the success of the migrants among populations that could involve a number of evolutionary processes. Insights into detailed gene

flow patterns thus provide the basis for understanding the directions of migration among spatially structured populations, as well as understanding the population genetic structures in heterogeneous habitats (Kawecki and Ebert 2004).

We conducted this study in a local scale subalpine ecosystem (within about 2.8 km^2) that includes forest habitats near the wind-blown mountain ridge and habitats in the nearby moorlands (hereafter referred to as the two eco-habitats). The topography and wind direction results in heavier snow accumulation in moorlands, compared to forest habitats. *Abies mariesii*, a long-lived dominant coniferous tree, shows spatially discrete populations across the two eco-habitats. *Abies mariesii* populations in the forest habitat consist of large-sized individuals, while *A. mariesii* populations in the moorland habitats consist of mainly dwarfed individuals, with a patchy distribution. We first examined the genetic characteristics of *A. mariesii* populations in both eco-habitats, while considering the possibility of reproductive isolation driven by the heterogeneous snow accumulation. Second, we investigated the gene flow patterns among the forest populations and moorland populations, to investigate whether or not the gene flow among *A. mariesii* populations in the two eco-habitats is unrestricted (e.g., biased gene flow between the two eco-habitats). Third, since previous studies mention that *A. mariesii* presents apparent morphological differences at the local scale (Doi et al. 2007), we quantified the morphological variation of *A. mariesii* to further understand the maintenance of discrete *A. mariesii* populations.

2.2 Materials and methods

2.2.1 Study sites and fieldwork

We conducted this study in a subalpine ecosystem that includes areas from the upper slopes near the mountain ridge and relatively low moorland sites with gentle slopes (latitude: 36°33′ to 36°34′ and longitude: 137°32′ to 137°33′; elevation ranging from 1850 m to 2010 m) in the Tateyama Mountain Range of central Japan (Fig. 2.2.1). In central Honshu Island in Japan, mountainous areas

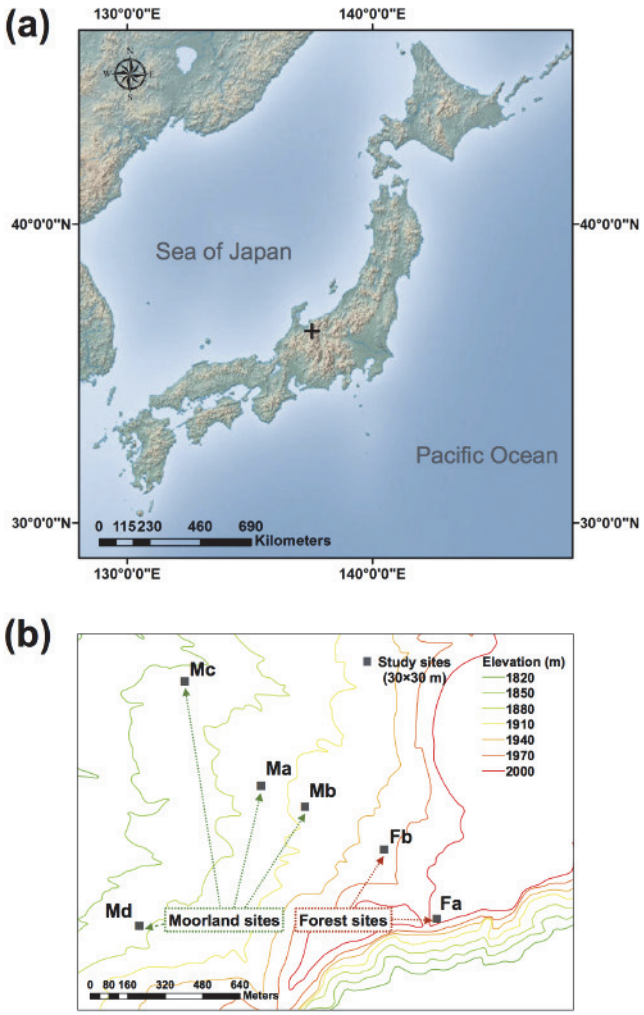

Figure 2.2.1: Study sites in Midagahara, Tateyama Mountain Range in central Japan. (a) The black cross indicates the location of the study sites within Tateyama Mountain Range. Land coloring is based on elevation and environment: highlands are colored in brown and lowlands are colored in green, and (b) a total of six study sites were established across two kinds of habitats at the local scale: sites Fa and Fb were established in a forested upper slope area and sites Ma, Mb, Mc, and Md were established in the nearby moorlands. Solid squares indicate the study sites. Contour lines are colored from green to red in order to show the differences in elevation, greener contour lines suggest lower elevations and redder contour lines suggest higher elevations.

near the Sea of Japan receive extremely heavy winter snow (Imanishi 1937). Consequently, deep snow accumulates in this subalpine ecosystem creating a long period of snow-cover (Fukai 1974). Snowfall generally begins in late-October and snow cover lasts until mid-June. In the past three decades, the maximum snow depth was approximately 4-6 m in the moorlands. Large *A. mariesii* trees dominate the major areas in the snowless and forested upper slope habitat, while others thrive in the adjacent moorlands, though individuals remain short. Most of the dwarfed moorland individuals are completely buried under heavy snow until snowmelt (Appendix 1), and are patchily distributed. These poorly drained moorlands support few woody species other than the dwarfed *A. mariesii* (Appendix 2).

We established a total of six 30 m × 30 m *A. mariesii*-dominated sites at a local scale across two habitats; forest-type sites (Fa and Fb), which were set on the upper slopes near the mountain ridge; and moorland-type sites (Ma, Mb, Mc and Md), which were set in the nearby moorlands. We arranged sites Fa, Fb, Ma, and Mb along elevational gradients, while sites Mc and Md were arranged at a similar elevation to site Ma but relatively distant from it (Fig. 2.2.1b). We arranged sites Mc and Md in this way trying to make our sampling design include both elevational and distance gradients. We collected current-year shoots from 30 mature *A. mariesii* individuals from each site of Fa, Fb, and Ma in 2008 for developing microsatellite markers; one sample from Fa was lost and excluded. We then harvested current-year shoots of 30 individuals per site from sites Mb, Mc, and Md in September 2012 to complete the genotyping. Harvested shoots were placed in paper bags to air dry. Then, samples were moved into plastic bags and stored in a freezer at −40°C prior to DNA extraction. In July and August 2013, we randomly selected 15 individuals from each study site for scaling their morphological traits. All shoots were collected from branches that were exposed to full sunlight, and tree height and diameter at breast height (DBH) were recorded at the sampling. According to Mori and Mizumachi (2009), shoots from the upper part of tree crowns and shoots from the lower part may show different properties. Therefore, we chose to harvest only shoots from the middle part of *A.*

mariesii crowns to minimize the individual tree-level morphological variation.

Finally, we observed the number of flowering individuals during the initial flowering stage within each of the 30 m × 30 m study sites. We walked through each site and counted flowering individuals twice: the first time we recorded the number of individuals that had set male flowers, and then we walked through again to record the number of individuals that had set female flowers.

2.2.2 Genotyping and genetic data analysis

We extracted DNA following a modified hexadecyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980) from all samples collected in 2008 and 2012 (179 individuals from six sites). In this study, we developed six microsatellite markers (Am003, Am247, Am351, Am364, Am418, and Am548) specifically for *A. mariesii* using an improved technique for isolating codominant microsatellite markers (Kaneko et al. 2008; Lian and Hogetsu 2002; Lian et al. 2006). Development process, DDBJ accession numbers, and primer sequences were described in detail in Appendix 3. We conducted polymerase chain reaction (PCR) using amplification mixtures and a thermal profile described in Appendix 3. We detected microsatellite fragment sizes using ABI Model 3100 Genetic Analyzer (Applied Biosystems, Weiderstadt, Germany) with GeneScan™ 500 LIZ® (Applied Biosystems) used as an internal size standard. We performed allele scoring using GENEMARKER® software (SoftGenetics, LLC, State College, PA, USA) and confirmed all allele calls manually.

We calculated standard genetic diversity parameters, namely, the mean number of alleles (A), mean observed heterozygosity (H_O), and mean expected heterozygosity (H_E) across all six loci for each *A. mariesii* population using GenAlEx 6.5 (Peakall and Smouse 2012). We corrected the mean number of alleles by rarefaction using HP-RARE 1.0 (Kalinowski 2005). In addition, we compared A, A_S, H_E, and H_O between the eco-habitats using linear mixed models with habitat as the fixed effect and site as a random effect. We then extracted the overall means of A, A_S, H_E, and H_O for each habitat from identical models

without intercepts to compare the mean genetic diversity at the habitat level (Cortés et al. 2014; Schielzeth 2010). We tested linkage disequilibrium across the entire dataset (179 samples) and within each of the six populations, and explored the population genetic structure by calculating pairwise F_{ST} following Weir and Cockerham (1984) in FSTAT 2.9.3.2 (Goudet 2001). We computed the relatedness coefficients across all pairs of individuals following Lynch and Ritland (1999) using COANCESTRY 1.0.1.2 (Wang 2011). While F_{ST} is a comparison among populations, relatedness coefficients are computed at the individual level and work as a measure of the inbreeding coefficient. We ran the Mantel test in R 3.0.2 (R Core Team 2013) with 99,999 permutations to test for correlations between the matrices of $F_{ST}/(1 - F_{ST})$ (Rousset 1997) and the geographic distance (three-dimensional distance calculated from latitude, longitude, and elevation), or relatedness (summarized for each site) and geographic distance. When comparing the correlation between relatedness and $F_{ST}/(1 - F_{ST})$, we used the partial Mantel test to account for geographic distance.

We used BOTTLENECK 1.2.02 (Piry et al. 1999) to investigate genetic bottlenecks in two methods. First, we used the Wilcoxon's sign rank test, which examines whether or not populations exhibit a greater level of heterozygosity than predicted in a population at mutation-drift equilibrium. Second, we used the mode-shift test, which is most appropriate for detecting population declines that have occurred more recently, specifically, over the last few dozen generations (Cornuet and Luikart 1996). Under the mode-shift test, an L-shaped distribution of alleles is expected in the absence of a bottleneck, whereas, a distribution with a shifted mode is expected in a population that has gone through a bottleneck. We performed 10,000 simulations using BOTTLENECK in six *A. mariesii* populations under the stepwise mutation model (SMM) and the two-phase model (TPM) with 95% single step mutations and a variance of 12, as recommended by Piry et al. (1999). *P*-values from the Wilcoxon's test were used as evidence for the occurrence of bottlenecks and were assessed for significance

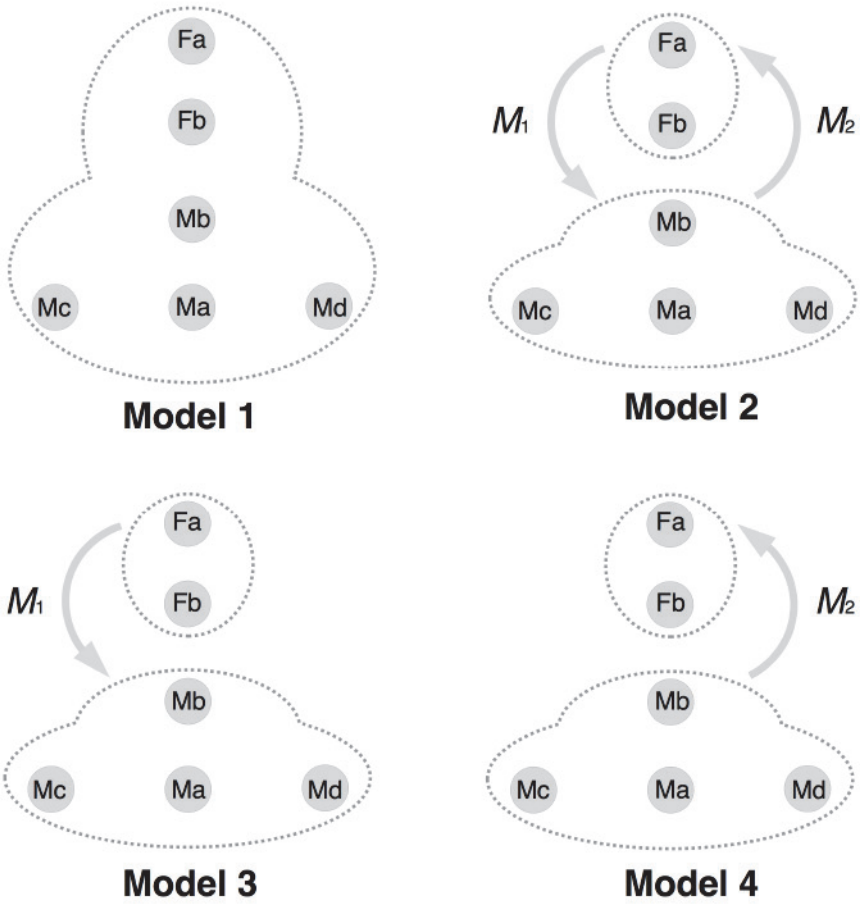

Figure 2.2.2: Models of different gene flow patterns simulated for *A. mariesii* across two habitats. Closed circles indicate study sites and dotted lines suggest re-labeled population groups used for model simulation. M_1 indicates the migration rate from forest populations to moorland populations, and M_2 indicates the migration rate from moorland populations to forest populations. Model 1 represents a population model that assumes a completely random mating pattern among *A. mariesii* individuals in six sites; model 2 is the panmixia model that assumes individuals from forest populations and individuals from moorland populations were separated into two population groups with a bidirectional gene flow between the two habitats; model 3 assumes the same population grouping as in model 2 but suggests a one-directional gene flow from the forest to the moorland populations; model 4 assumes the same population grouping as in model 2 and 3, but with the direction of gene flow going from moorland to forest populations.

Table 2.2.1: Estimates of average genetic diversity and results from BOTTLENECK for six *A. mariesii* populations

Population	Sample size	Summary of genetic diveristy				Bottleneck		
		A	A_S	H_O	H_E	TPM (Wilcoxon *P*-value)	SMM (Wilcoxon *P*-value)	Model-shift test
Fa	29	5.667	5.560	0.563	0.551	0.313	0.406	L-shape
Fb	30	6.500	6.410	0.514	0.557	0.078	0.313	L-shape
Ma	30	7.000	6.760	0.539	0.531	0.688	0.891	L-shape
Mb	30	7.833	7.570	0.594	0.565	0.953	0.984	L-shape
Mc	30	7.500	7.270	0.506	0.565	0.953	0.969	L-shape
Md	30	7.000	6.840	0.577	0.525	0.594	0.688	L-shape

A, mean number of alleles; A_S, mean number of alleles corrected by rarefaction; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity

at $\alpha = 0.05$.

We tested models of different population gene flow patterns with a Bayesian coalescent framework implemented in MIGRATE 3.2.6 (Beerli and Palczewski 2010) to exam gene flow asymmetry. We set up and verified four migration models (Models 1, 2, 3, and 4; Fig. 2.2.2). Initially, we ran exploratory runs to determine the required run length and priors to obtain good posterior distributions. The conditions chosen for the final simulations were under the default options in MIGRATE with the following exceptions: the Brownian motion mutation model was used for microsatellite data; uniformed theta (scaled population size) priors {min. = 0, max. = 100, delta = 25}; uniformed migration priors {min. = 0, max. = 200, delta = 50}; increment between sampled geneologies {200}; recorded steps {500,000}; and number of discard trees per chain (burn-in) {250,000}. We ran eight static heated chains {temperatures: 1.00, 1.17, 1.40, 1.75, 2.33, 3.50, 7.00, 1,000,000.00}. We obtained model ranks and probabilities based on the Log Bayes Factor (LBF), which was calculated from the Bezier log marginal likelihood (Bezier lmL) values summarized for individual models (Beerli and Palczewski 2010).

2.2.3 Measurement of tree architecture and morphology

We evaluated the Height-DBH relationship and estimated asymptotic maximal height (H_{Max}) using an exponential distribution described by Thomas (1996). We used the equation $H = H_{Max}[1 - e^{(-aDb)}]$ and nonlinear regression functions

in R 3.0.2 (R Core Team 2013) to estimate H_{Max}. H and D in the equation represent observed tree height and stem diameter, respectively. For harvested one-year shoots, we measured the length (L_{N_1}) and width (W_{N_1}) of needle clusters on each shoot. Next, we removed needles and recorded stem length and stem diameter. We flattened the removed needles and scanned them using a high-resolution scanner to obtain the projected needle area (PNA) and number of needles per shoot (N_{N_1}). We processed all scanned images using ImageJ 1.4.7 (U. S. National Institutes of Health, Bethesda, MD, USA). Finally, we oven dried the shoot stems and needles at 70°C for 72 hours to measure needle dry-matter content (M_{N_1}) and stem dry-matter content (M_{S_1}) (Pérez-Harguindeguy et al. 2013).

We calculated four parameters based on the measurements of shoots, stems, and needles to evaluate the following shoot- and needle-level morphological characters of A. mariesii individuals grown in the two eco-habitats: needle cover (NC) (Stenberg et al. 1995), needle mass ratio (NMR) (Mori and Takeda 2005), individual needle area (INA), and needle mass per unit area (NMA). We used NC to roughly evaluate the needle arrangement on shoots in relation to the light capture efficiency (Mori and Mizumachi 2009) and calculated it as $NC = PNA/PEA$, from which the projected shoot envelope area (PEA) can be obtained using the equation $PEA = 0.25 \times 3.14 \times L_{N_1} \times W_{N_1}$. We calculated NMR, which indicates the biomass allocation between photosynthetic and respiratory tissues within a shoot, using the following equation: $NMR = M_{N_1}/(M_{S_1} + M_{N_1})$. Finally, we defined INA as $INA = PNA/N_{N_1}$ and NMA using the equation: $NMA = M_{N_1}/PNA$. We then tested the differences in these four parameters across the six study sites using a one-way ANOVA, followed by a Tukey's HSD (honest significant difference) test of significance (R Core Team 2013).

2.3 Results

2.3.1 Genetic diversity, patterns of divergence and gene flow

The parameters for genetic diversity at the population level, using six developed microsatellite markers, are summarized in Table 2.2.1. Mean genetic diversity was significantly higher in the moorland habitats than in the forest habitats, in terms of allelic richness (A) (7.333 for moorland and 6.084 for forest, respectively; F = 9.839, P = 0.035) and number of alleles corrected by rarefaction (A_S) (7.110 for moorland and 5.985 for forest, respectively; F = 8.503, P = 0.043). No significant differences were found for mean observed heterozygosity H_O (0.554 for moorland and 0.539 for forest, respectively; F = 0.219, P = 0.664) and mean expected heterozygosity (H_E,) (0.547 for moorland and 0.554 for forest, respectively; F = 0.214, P = 0.668) between the two eco-habitats. After adjusting the significance levels for multiple comparisons, none of the loci showed significant linkage disequilibrium for all pairwise locus combinations.

Overall, F_{ST} was low among the six populations (overall F_{ST} = 0.0250, ranging from 0.0048 to 0.0471), and did not differ significantly between within-habitat (F_{ST} = 0.0245 ± 0.0041 S.E.) and among-habitat (F_{ST} = 0.0243 ± 0.0043 S.E.) comparisons (t = 0.041, P = 0.968). Pairwise individual relatedness was lower in moorland habitats than in forest habitats (average pairwise relatedness = –0.0046 ± 0.0019 S.E. for moorland and 0.0306 ± 0.0050 S.E. for forest, respectively; t = 6.6280, P < 0.0001). In addition, individuals within the same habitat (average pairwise relatedness = 0.0022 ± 0.0018 S.E.) were significantly more related than among-habitat (average pairwise relatedness = –0.0150 ± 0.0019 S.E.) individual pairs (t = –6.5655, P < 0.0001). The Mantel test revealed no significant correlations in pairwise comparisons of $F_{ST}/(1 - F_{ST})$ vs. geographic distance (Spearman's r = 0.300, P = 0.154; Fig. 2.3.1a), relatedness vs. geographic distance (Spearman's r = –0.204, P = 0.793; Fig. 2.3.1b), and relatedness vs. $F_{ST}/(1 - F_{ST})$ (Spearman's r = –0.646, P = 0.992; Fig. 2.3.1c).

Results from the bottleneck test showed no evidence for changes in population

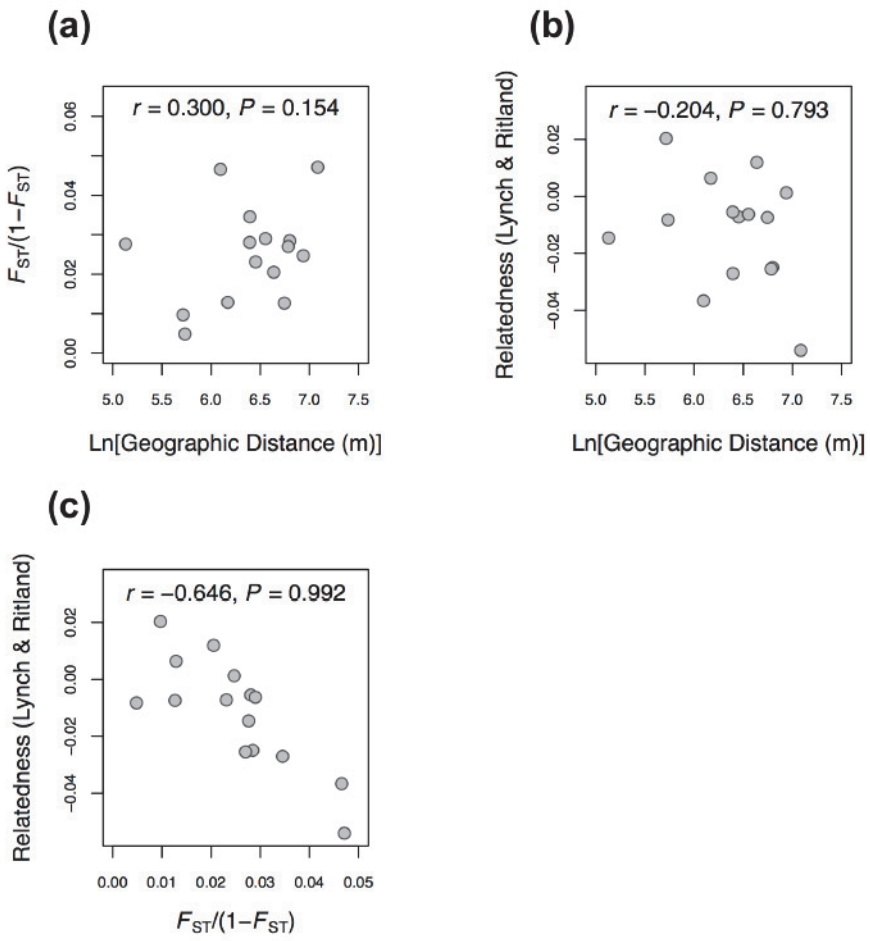

Figure 2.3.1: Correlations between genetic distance, relatedness, and geographic distance of *A. mariesii* populations in two eco-habitats. (a) Comparison of $F_{ST}/(1 - F_{ST})$ versus natural logarithm of the geographic distance, (b) comparison of relatedness coefficient according to Lynch and Ritland (1999) versus natural logarithm of the geographic distance, and (c) comparison of relatedness coefficient versus $F_{ST}/(1 - F_{ST})$.

size over either the long- or short-term. All six populations were detected with normal L-shaped distribution of alleles (Table 2.2.1).

Model comparisons of different gene flow patterns suggest that the model assuming that the populations in two eco-habitats were connected through one-directional gene flow from forest to moorland populations is the best model (Model 3 in Fig. 2.2.2) ($M_1 = 15.089$; Bezier lmL: –6338.73; LBF: 0; model rank: first; model probability: 1.00). Another model (Model 4), which also assumes one-directional gene flow between forest and moorland populations but with gene flow going from moorland to forest, was ranked second ($M_2 = 56.882$; Bezier lmL: –7009.97; LBF: –671.24; model rank: second; model probability: 3.04×10^{292}). Because the probability of this second-ranked model (Model 4) was extremely low, compared to Model 3, it is unlikely to be representative of the actual gene flow patterns among forest and moorland *A. mariesii* populations. Model 1 (the full migration model; model rank: third; model probability: 3.62×10^{691}) and Model 2 ($M_1 = 15.099$; $M_2 = 2.432$; model rank: fourth; model probability: 1.67×10^{2623}) were even less likely than Model 4.

2.3.2 Observed first flowering in spring

The flowering observation of *A. mariesii* individuals in the study sites showed that, despite a much heavier snow accumulation in moorlands, first flowering was only slightly delayed when compared with the forest populations in 2013. In the forest populations, the first and second *A. mariesii* individuals were found to have set the first male flowers on 28 June and on 2 July, with no other individuals found with blooming male flowers. On 2 July, two individuals with both male and female flowers were first observed in moorland sites, only 4 days (the observation interval), after the first flowering was observed in forest populations in 2013 (Appendix 4).

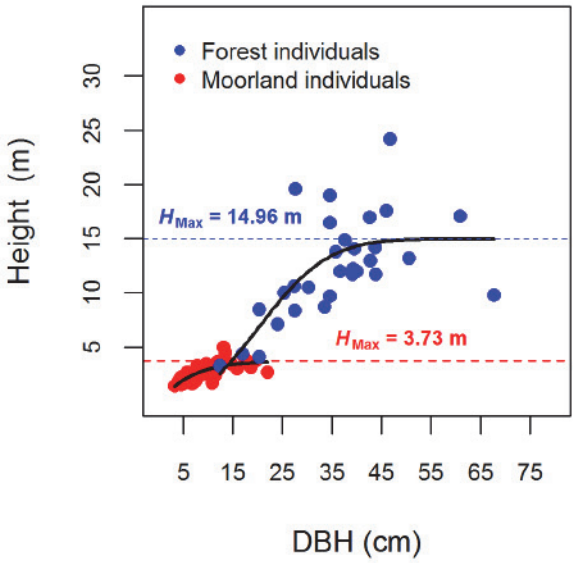

Figure 2.3.2: Height-DBH relationship and estimated asymptotic maximal height (H_{Max}) of *A. mariesii* individuals in two habitats. Blue dots indicate *A. mariesii* individuals from forest populations (n = 30); red dots indicate *A. mariesii* individuals from moorland populations (n = 60); dashed lines suggest the estimated asymptotic maximal height (H_{Max}). H_{Max} was estimated at 14.96 meters for forest individuals and at 3.73 meters for moorland individuals.

2.3.3 Tree architecture and shoot and needle morphology

Tree architecture is described by the Height-DBH relationship; and H_{Max} , which showed clear differences in the two eco-habitats (Fig. 2.3.2). The DBH of most forest individuals ranged from 20 cm to 60 cm, and was larger than the DBH of most moorland individuals, ranging from 5 cm to 20 cm. Estimated H_{Max} was 14.96 m for forest individuals and 3.73 m for moorland individuals. Meanwhile, forest individuals showed higher variation regarding the Height-DBH relationship (Fig. 2.3.2).

The analysis of shoot and needle morphology revealed that *A. mariesii* individuals exhibited additional morphological differences other than tree architecture. Individuals from the two eco-habitats had significantly distinct *INA* and *NMA* (Fig. 2.3.3c, d; as indicated by different letters above each box). *NMR* of most moorland individuals (in sites Ma, Mb, and Mc) was also significantly different from individuals distributed in sites Fa and Fb. Nevertheless, *NC* was not significantly different across all six study sites. No significant differences were found at the site level among either forest or moorland populations for most of the important parameters that are generally used to characterize the physical properties of plant leaves, such as *INA* and *NMA*, even though distinct differences were observed across habitats.

2.4 Discussion

Our results indicate that, though *A. mariesii* populations are spatially discrete and undergoing strong habitat heterogeneity, which has probably led to their morphological differentiation between habitats, they are not genetically differentiated. Gene flow was biased toward the moorlands, and the moorlands could be potential sinks of genetic diversity in the landscape.

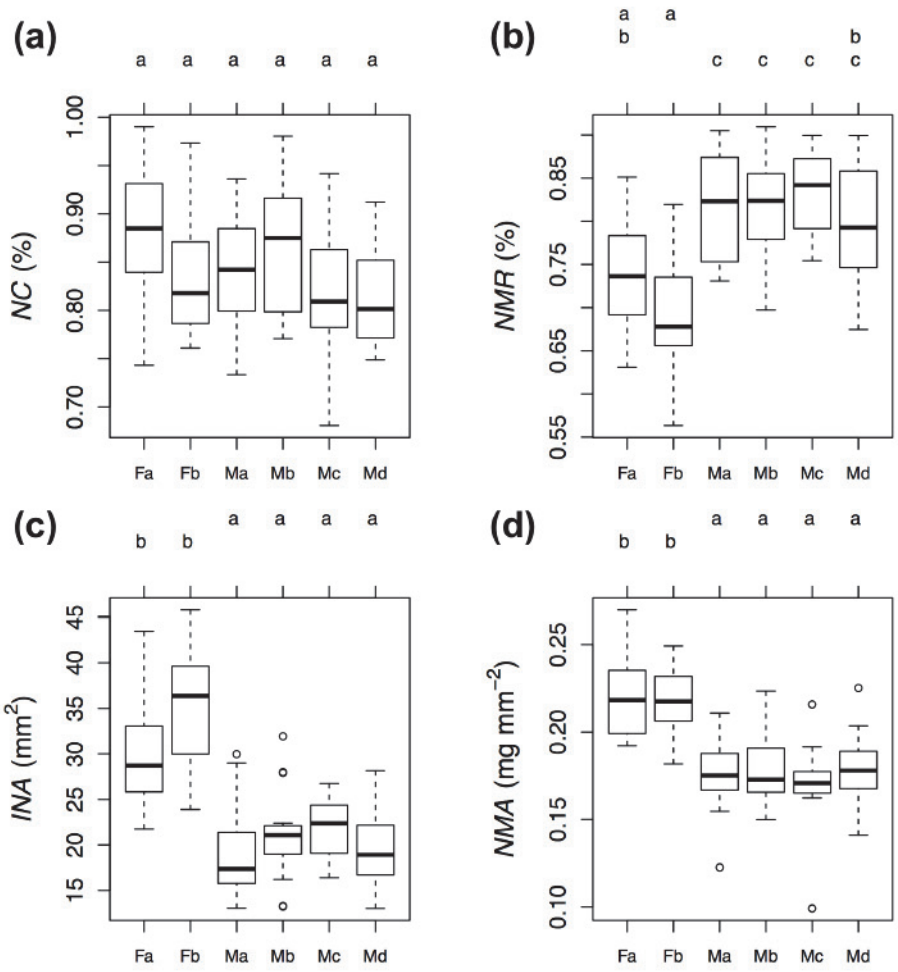

Figure 2.3.3: Comparison of morphological parameters measured from one-year shoot samples. Morphological parameters are: (a) needle cover (NC), (b) needle mass ratio (NMR), (c) individual needle area (INA), and (d) needle mass per unit area (NMA). One-way ANOVA was use to compare the differences in morphological parameters among the study sites. Boxes with different letters above are significantly different according to Tukey's HSD test.

2.4.1 Subalpine moorlands as sinks of genetic diversity for *A. mariesii*

In this study, we did not find evidence for the presence of significant genetic structures of *A. mariesii* populations in the two eco-habitats. This conclusion is mainly drawn from pairwise population comparisons of F_{ST}, and from the Mantel test based on F_{ST} and relatedness. Overall, F_{ST} was low and no isolation by distance pattern (IBD) was detected (Fig. 2.3.1a). Usually, some spatial scale exists, where the chances of mating are limited so that genetic differentiation begins to occur. Depending on the situation of a given species, the spatial scale could be as small as a few hundred meters (e.g. Arnaud et al. 2001; Watts et al. 2004) or as large as thousands of kilometers (e.g. Sharbel et al. 2000). The lack of a clear pattern of IBD suggests that the pattern of genetic variation at this study site is independent from spatial distance. Phenological isolation, driven by habitat heterogeneity in some cases (e.g. Stanton et al. 1997; Yamagishi et al. 2005), could lead to genetically differentiated populations. Nevertheless, in this study, phenological isolation is also unlikely, because the first flowering in the moorland populations was only slightly delayed (4 days), compared to the forest populations, at least in 2013 (Appendix 4). Neutral markers, like the microsatellite markers we used in this study to investigate population genetic structure, are widely used and becoming more popular due to the relatively high polymorphisms and the codominant inheritance (Ellegren 2004). Still, we cannot completely preclude differentiation detected using different measurements of genetic variation, or differentiation detected at other loci besides the six loci we investigated in this study.

The simulations of gene flow suggest that the best explanation for the observed patterns is that the two eco-habitats were connected with one-directional gene flow, from forest populations to moorland populations (Model 3 in Fig. 2.2.2). Because we found that within-habitat relatedness was significantly higher than among-habitat relatedness, suggesting that individuals within the same eco-habitat are more related than are individuals in different eco-habitats, we believe that levels of gene flow are present but the gene flow is somewhat

restricted. Thus, Model 1, that assumes random mating among all populations, may not necessarily be the best model. The gene flow from the forest habitat to the moorland habitat, though asymmetric, can homogenize the genetic structure and be responsible for the lack of detectable genetic differentiation (Harrison 1991; Mayer et al. 2009). Nevertheless, if the connectivity among populations had decreased very recently, we may not be able to precisely predict whether or not genetically differentiated populations are present since our genetic estimates resemble historical, rather than contemporary, gene flow.

While we did not directly measure pollen and seed dispersal, to explain the contemporary gene flow patterns, similar studies have demonstrated that pollen and seed dispersal can be strongly biased because of habitat heterogeneity (Cortés et al. 2014; Nathan and Muller-Landau 2000). Pollen dispersal is believed to be constrained by snowmelt since heterogeneous snowmelt can shape the flowering time differentiation (Hirao and Kudo 2008; Shimono et al. 2009; Wipf et al. 2009). In any case, seed dispersal is not extensively constrained by the snowmelt gradient, since seeds are released when many of the snow patches have melted. In this study, we did not find a significant delay in the flowering time (Appendix 4), though the difference in individual abundance could shape the asymmetric pollen dispersal pattern among forest populations and the patchily distributed moorland populations (Ellstrand and Elam 1993; Kwak et al. 1998). In addition, due to the wind dispersal of seeds, the seed dispersal of *A. mariesii* could be affected by small-scale topographic variations as well (Nathan and Muller-Landau 2000). The impacts of wind and topography on pollen and seed dispersal could shed light on the contemporary gene flow patterns of *A. mariesii*. On the whole, a source-sink system could exist among the *A. mariesii* populations in the two eco-habitats.

Trees such as conifers are considered relatively unlikely to lose genetic diversity in heterogeneous or fragmented habitats, because of their longevity, fecundity, and strong dispersal abilities (Hamrick 2004). Nevertheless, a recent meta-analysis (Vranckx et al. 2012) argued that trees can undergo a loss of genetic diversity equal to that of herbal species under severely fragmented habitat

conditions. When populations get smaller and more fragmented, they are more likely to be subjected to genetic stochasticity, which can lead to a loss of genetic variation through genetic drift (Allendorf et al. 2012; Frankham et al. 2010). In our study of *A. mariesii*, the continuously distributed forest populations were compared to the moorland populations, which were found to be small and patchy. Nevertheless, we did not find evidence for a reduction in genetic diversity of the moorland populations. Instead, allelic richness (A) and number of alleles corrected by rarefaction (A_S), were both found to be significantly higher in the moorland habitat, rather than the forest habitat. The moorland populations may have an increased genetic diversity due to asymmetric gene flow from the surrounding populations, including the forest populations as we detected asymmetric migration from the forest to the moorland populations. The lower individual relatedness in moorland populations, compared to forest populations, further confirmed this conclusion. This pattern of genetic diversity and the direction of gene flow was also observed among microhabitats with different snowmelt timing in another woody species, *Salix herbacea* (Cortés et al. 2014). Although much of the incoming within-habitat pairwise individual relatedness is higher than among-habitat comparisons, we did not find any overall patterns that might suggest population inbreeding (Fig. 2.3.1b-c). Conifers usually express strong inbreeding depression (Koelewijn et al. 1999; Remington and O'Malley 2000), while inbred individuals are usually purged at the seedling stage (Ferriol et al. 2011; Petit and Hampe 2006). Thus, adult trees would not be expected to show significant inbreeding values. Furthermore, conifers generally produce abundant wind-dispersed pollen and are typically outbreeding (Nystedt et al. 2013), so that the high gene flow may rescue moorland populations from isolation and subsequent inbreeding.

The bottleneck results suggest that none of the *A. mariesii* populations have experienced changes in population size over the long- or short-term (Table 2.2.1). Bottlenecks often occur during the founding of new and relatively isolated populations; they have reduced genetic diversity and higher levels of inbreeding (Frankham et al. 2010). Our findings indicating a lack of systematic

bottlenecks adds more evidence to the likelihood of moorlands being sinks of genetic diversity. A similar conclusion regarding moorland-like habitats near mountain ridges acting as a reservoir of genetic diversity was reached by Cortés et al. (2014).

Genetic variation allows populations to tolerate a wide range of environmental extremes and fluctuations (Frankham et al. 2010; Hoffmann and Parsons 1997). Since woody species other than A. mariesii have generally failed to thrive in the moorlands (Appendix 2), even small and discrete *A. mariesii* population patches can be critically important to other species in the ecosystem that may not be able to afford their loss (i.e. foundation species, Ellison et al. 2005). The population patches are likely irreplaceable considering their role in providing necessary habitats for arthropods or microbes (Ellison et al. 2005), and their contribution to the persistence of associated species (Callaway 2009). Individual and genetic supplies from surrounding, well-established populations into moorland populations are probably crucial for maintaining the current moorland ecosystems.

2.4.2 Morphological responses of A. mariesii to habitat heterogeneity

A. mariesii showed distinct differences in tree architecture and varied shoot- and needle-level morphology across the two eco-habitats. Topography and wind direction contribute to heavier snow accumulation in moorlands (approximately 2-3 m on the upper slopes, and ≥ 4-6 m in the moorlands). While snow cover in many cases can protect plants from frost events early in the growing season (Mori and Komiyama 2008; Rixen et al. 2010) and promote germination rates (Shimono and Kudo 2005), heavy snowpack can also cause mechanical damage in crown development; therefore, limiting the vertical growth of *A. mariesii* (Kajimoto et al. 2002; Seki et al. 2005). Meanwhile, late snowmelt and hydraulic stress, caused by strong moisture regimes, may also lead to slow tree growth and limited photosynthesis (Doi et al. 2007). Thus, branch growth and canopy

development of *A. mariesii* in the moorlands would be expected to occur slowly (Mori and Mizumachi 2009; Yamanaka et al. 1973).

No detectable differences in *NC* were observed across all six study sites (Fig. 2.3.3a). *NC* provides a rough estimate that can be used to quantify the ratio of a tree's silhouette to the projected needle area; so that smaller *NC* values represent reduced mutual shading among needles on a shoot cylinder (Stenberg et al. 1995). Few differences in *NC* were expected because all of the sampled shoots were exposed to good light conditions. Indeed, needle arrangement greatly contributes to enhancing the light interception efficiency of the canopy by reducing mutual shading among needles (Sprugel et al. 1996). Most of the dwarfed *A. mariesii* individuals are buried under snow cover throughout the winter; these individuals would be subjected to higher physical pressure, compared to those in the forest habitats. Therefore, investing more resources to the organs that would help them maintain their above-ground architecture, rather than needles, would seem to be a high priority (Grassi and Bagnaresi 2001; Mori and Hasegawa 2007; Mori et al. 2008; Stenberg et al. 1998). The smaller *INA* (Fig. 2.3.3c) and *NMA* (Fig. 2.3.3d) observed in individuals of *A. mariesii* in snowy moorlands reflects this strategy for biomass allocation. *NMR* indicates biomass allocation between photosynthetic and respiratory tissues within a shoot (Mori and Takeda 2004; Mori and Takeda 2005), where a larger *NMR* leads to the formation of more abundant, densely packed needles within a single shoot. Mori and Mizumachi (2009) suggested that the needle packing pattern could prevent excess evaporation from shoots and maintain mild temperatures within each shoot. As a result, most of the moorland individuals showed higher *NMR* (Fig. 2.3.3b) values within one-year shoots, a finding that agrees with our assumption.

Phenotypic plasticity and local adaptation are possible mechanisms for the morphological variation in *A. mariesii*. In this study; however, we cannot draw conclusions about the origins of observed variation in the *A. mariesii* tree morphology, as the distinction between phenotypic plasticity and local adaptation is based primarily on a combination of genetic analyses and

transplantation experiments (DeWitt and Scheiner 2004). Both plasticity and adaptation are outcomes of a plant's interaction with the surrounding environment on which it relies. Regardless of the origin, the ability to adjust tree morphology across different organs is supposed to strengthen the competitiveness in *A. mariesii*, and make a tangible contribution to the maintenance of populations in contrasting habitats.

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Appendix

Appendix 1

Photo S1 Panorama photo showing the two kinds of habitats with heterogeneous snow accumulation. Letter "a" indicates snowless forested upper slope habitats; letter "b" indicates snowy moorland habitats nearby. Forest *A. mariesii* individuals are larger in size, and usually stand above snow cover throughout winter, while moorland *A. mariesii* are mostly dwarfed individuals and mostly buried under snow cover until snowmelt in late spring.

Appendix 2

Table S1 Summary of woody species composition in six study sites

Habitat	Number of sites	Species	Number of Individuals	Total BA (m^2ha^{-1})
Forest	2	*Abies mariesii*	92	28.861
		Sorbus commixta	43	1.007
		Acer sp.	23	0.434
		Betula ermanii	13	5.757
Moorland	4	*Abies mariesii*	185	3.959
		Pinus sp.	14	0.110
		Betula ermanii	6	0.073
		Sorbus commixta	2	0.012

Individuals with DBH size smaller than 5 cm were not included.

Appendix 3 Description of (a) microsatellite marker development process for *A. mariesii*, (b) primer characteristics, and (c) PCR amplification mixtures and thermal profile.

(a) Development of microsatellite markers for *Abies mariesii*

Microsatellite marker was developed using an improved technique for isolating codominant compound microsatellite markers (Kaneko et al. 2008; Lian and Hogetsu 2002; Lian et al. 2006). Total genomic DNA of *A. mariesii* was extracted from leaves using a modified CTAB method (Murray and Thompson 1980). An adaptor-ligated, restricted DNA library of *A. mariesii* was then constructed according to the following procedure: DNA was digested with the blunt-end restriction enzyme, *Afa* I. The restriction fragments were then ligated with a

specific blunt adaptor (consisting of the 48-mer:
5'-GTAATACGACTCACTATAGGGCACGCGTGGTCG
ACGGCCCGGGCTGGT-3' and an 8-mer with the 3'-end capped with an amino residue: 5'-ACCAGCCC-NH_2-3') using the DNA ligation kit (Takara). Fragments were amplified from the *Afa* I DNA library using compound SSR primer $(AC)_6(AG)_7$, $(AG)_6(AC)_7$ or $(AC)_6(TC)_7$ and an adaptor primer (5'-CTATAGGGCACGCGTGGT-3'). The amplified fragments, ranging from 400 to 800 bp, were then separated on a 1.5% LO_3 agarose gel (Takara) and purified using the QIAquick Gel Extraction Kit (Qiagen). The purified DNA fragments were subsequently cloned using the QIAGEN PCR Cloning plus Kit (Qiagen) according to the manufacturer's instructions. In brief, polymerase chain reaction products were ligated into the pDrive vector, and transformed into QIAGEN EZ competent cells. Transformants were identified by blue/white screening on LB agar plates containing ampicillin, X-gal and IPTG. The cloned fragments were amplified using the M13 forward and reverse primers from the plasmid DNA of positive clones. Amplified fragments were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). For each fragment containing $(AC)_6(AG)_5$, $(AG)_6(AC)_5$ or $(AC)_6(TC)_5$ compound SSR sequence at one end, a specific primer was designed from the sequence flanking the compound SSR using Primer3 software (Version 0.4.0, Rozen and Skaletsky 2000).

(b) Characteristics of six microsatellite loci for *A. mariesii*

(c) PCR amplification mixtures and thermal profile used for genotyping *A. mariesii* samples

PCR amplification mixtures were prepared in a final volume of 6.0 µl, which contained 2.13 µl of ultrapure water, 0.06 µl of each 20 µM forward and reverse primer, 3 µl of Multiplex PCR Master Mix (Qiagen) and 0.75 µl of DNA template (37.5-75 ng). The PCR thermal profile involved an initial denaturation step at 95 °C for 15 min followed by 30 cycles at 94 °C for 0.5 min, 57 °C for 1.5 min and 72

Locus	DDBJ accession no.	Size Range (bp)	Repeat motif	Primer sequence (5'–3')	T_a (°C)	H_O	H_E
Am003	AB911247	191-235	$(AC)_6(AG)_{22}$	ACACACACACACAGAGAGAGAG AATGTGGAATGCTTAAAGTGAA	57	0.932	0.865
Am247	AB911250	110	$(AC)_6(TC)_7$	ACACACACACACTCTCTCTCTC TTATTCTGTCTTAAGCATTTAGTTCT	57	0.000	0.000
Am351	AB911248	133-157	$(AG)_6(AC)_{17}$	AGAGAGAGAGAGACACACACAC GCCTATTGGCAAAAGCTTAG	57	0.715	0.693
Am364	AB911249	126-140	$(AG)_6(AC)_9$	AGAGAGAGAGAGACACACACAC GAACCCGAGTAGGGGATG	57	0.291	0.326
Am418	AB911252	215-269	$(AC)_6(AG)_{26}$	ACACACACACACAGAGAGAGAG CAACTGGTTTGTGCTTGATT	57	0.890	0.901
Am548	AB911251	118-132	$(AC)_6(TC)_7$	ACACACACACACTCTCTCTCTC AGCCATTGAAGGATTTACTGA	57	0.464	0.509

T_a, annealing temperature of primer pair; H_O, observed heterozygosity; H_E, expected heterozygosity. H_O and H_E were summarized over all 179 samples for each locus. DDBJ accession numbers are available to the public after manuscript being accepted for publication.

°C for 1.0 min, with a final extension at 60 °C for 30 min.

Appendix 4

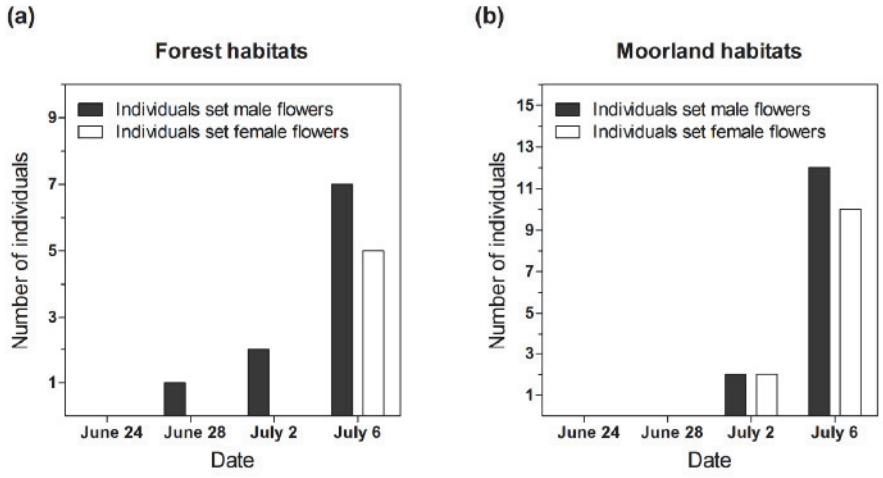

Fig. S1 Observed first flowering in 2013 in (a) forest habitats and (b) moorland habitats. Dark-colored bars show *A. mariesii* individuals found with male flowers within study sites; light-colored bars show *A. mariesii* individuals found with male flowers within study sites. Observations began in mid-June 2013 to ensure that they were ahead of the initial flowering stage of *A. mariesii*. Starting from 24 June 2013, observations were made every four days by direct counting and

recording of the number of individuals within each study site. Observations ended on 6 July 2013, resulting in a total of four counts of flowering individuals within each study site.

3

Causes and consequences of intraspecific functional variation

3.1 Introduction

Species that are locally abundant, whose structural or functional characteristics create habitat for a large number of associated species, and which regulate key ecosystem processes such as energy and nutrient fluxes, are referred as foundation species (Dayton, 1972, Ellison et al., 2005). Experimental and comparative studies have revealed that, changes in foundation species can lead to severe consequences in local biodiversity and ecosystem functions (Peters and Yao, 2012, Block et al., 2012, Martin and Goebel, 2013, St John et al., 2012, Kane et al., 2011, Cáceres et al., 2014). As foundation species in many cases are a single dominant species within an ecosystem (e.g., Block et al., 2012, Cáceres et al.,

2014, Kane et al., 2011), and the environmentally mediated plastic responses of a species can generate varied intraspecific patterns (Sultan, 2004), looking into these intraspecific patterns will have important significance for studies centred on foundation species.

Foundation species may express intraspecific variations in physiological traits (e.g., vegetative height, leaf area and leaf nutrient content) in the face of heterogeneous habitat quality (Albert et al., 2010, Gedan and Bertness, 2010, Cochrane et al., 2014, Grassein et al., 2010, Qian et al., 2014, Kumordzi et al., 2014). To date, considerable effort has been devoted to documenting the causes of variations in physiological traits of plants, leading to the identification of several axes of variation, of which one major axis is related to the nutrient use strategy of a particular species (Chapin, 1980, Diaz et al., 2004, Reich et al., 2003). For example, the efficiency of withdrawing nutrients from plant leaves prior to abscission (i.e. nutrient resorption efficiency), and the ability of a plant to retain plant leaves and foliar nutrient, are found to vary among plant species; this represents a variation in conservative or acquisitive-oriented nutrient use strategies at the species level (Chapin III et al., 2012, Tang et al., 2013, Vergutz et al., 2012, Yuan and Chen, 2009). Variations in nutrient use strategies are also likely to occur within a single species, and could be an important source of variation in physiological traits of a foundation species.

Intraspecific variation in physiological traits, which could be driven by variations in nutrient use strategies of foundation species, may further affect ecological processes in local ecosystems. Nutrient content of senesced plant leaves (i.e. leaf litter), for instance, could be reduced in stressful and less productive sites in line with the conservative nutrient use strategy, compared with those fast-growing individuals in productive sites (e.g., Güsewell, 2005). Hence, production of plant litter that differed in litter nutrient content would be expected within the single species (e.g., Covelo et al., 2008). Consequently, the breakdown rates of plant leaf litter that vary in chemical properties (e.g., litter nutrient and lignin content) are expected to differ as well (Cornwell et al., 2008, Lecerf and Chauvet, 2008, Wang et al., 2014). Although it remains poorly

understood, a growing awareness has been raised related to the importance of the effects of intraspecific variation on ecological processes (Crutsinger et al., 2009, Jackson et al., 2013). The decomposition of leaf litter and the release of litter nutrients back to soil directly affects the level of nutrients available to plants in the soil, leading to further interactions between plants and soil (van der Putten et al., 2013). In addition to litter properties, site factors such as soil properties, as well as the interaction between litter and soil organisms, also play decisive roles in determining litter turnover and nutrient release rates (Austin et al., 2014, Freschet et al., 2012, Wang et al., 2014, Liu et al., 2006), yielding more complex mechanisms of decomposition and nutrient release patterns within local-scale ecosystems. Studying the effects of intraspecific variation on ecological processes will therefore offer indispensable details on how foundation species contribute to the functioning of local ecosystems.

We conducted this study in a local-scale subalpine ecosystem within an area of about 3 km² that includes forest habitats near a wind-blown mountain ridge and habitats in the nearby moorlands (hereafter referred to as the two "eco-habitats") in central Japan. Local topography and wind direction results in heavier snow accumulation in moorlands, compared with the forest habitat. Earlier investigations have suggested that *Abies mariesii*, a coniferous foundation species, showed considerable intraspecific variation in physiological traits across the two eco-habitats (Qian et al., 2014). Here, we set out to find the causes and consequences of the variation in physiological traits of *A. mariesii*. First, we investigated the nutrient use strategies of *A. mariesii* distributed across the two eco-habitats (i.e. the forest habitat and the moorland habitat) by quantifying the nitrogen (N)-resorption efficiency and the mean residence time (*MRT*) of needles as well as foliar N. We focused on N-related traits and strategies in this study because N is an essential element that limits plant growth in many biomes and is an important element in ecosystem nutrient cycling processes (Lambers et al., 2008, Berg and McClaugherty, 2014). Second, we asked whether intraspecific variation in physiological traits can cause impacts to litter decomposition, an important ecosystem process, using an *in situ* litterbag transplant experiment.

3.2 Materials and methods

3.2.1 Site description and field survey

We conducted this study in a subalpine ecosystem that includes areas from the upper slopes near a mountain ridge and in relatively low moorland sites with gentle slopes (36°33′–34′N, 137°32–33′E; elevations ranged from 1850 to 2010 m) in the Tateyama Mountain Range of central Japan. Bedrock of the study areas is mainly volcanic andesite (Honda, 1965). Large *A. mariesii* trees dominate on the snowless and forested upper slope habitat, while short individuals do so in the adjacent moorlands (Qian et al., 2014). We selected two 30 × 30 m sites, one each in the forest and moorland habitats, and divided each site into nine 10 × 10 m subplots to collect samples and conduct a litterbag transplant experiment.

We harvested one-year shoots from ten *A. mariesii* individuals in each site in Aug. 2013 to measure nutrient concentrations of mature needles. As for nutrient concentrations, we mainly focused on the concentrations of carbon (C) and N, and the C:N ratio. Shoot samples we collected were those exposed to full sunlight conditions. We also harvested two primary branches per individual from a total of three mature *A. mariesii* individuals in each site to estimate age-dependent variables (Supplementary Data Fig. S1). We collected senesced needle samples from each 10 m × 10 m subplot to investigate nutrient concentrations of senesced needles and to construct litterbags ("senesced needles" were treated as "freshly fallen litter" in this study).

To explore the patterns of litter decomposition, with consideration of separating the effects of litter quality and habitat-associated factors, we designed and carried out a reciprocal litterbag transplant experiment in this study. We prepared 10 × 10 cm litterbags with 1 × 1 mm polyethylene mesh, each containing a total of 2 g of air-dried litter. Two types of litterbags were prepared in this study, containing litter collected from either the forest (hereafter, F litter) or moorland (hereafter, M litter) habitat. We deployed litterbags in pairs (each pair had one containing F and another containing M litter) to meet with our

reciprocal litterbag transplant experiment design (Supplementary Data Fig. S2). Six pairs of litterbags were set within each 10 m × 10 m subplot in late Oct. 2013 right before it started snowing. Then we retrieved one pair of litterbag a time from each subplot after nine months (immediately after snowmelt) and 12 months, respectively. The litterbags were immediately transported to the laboratory and kept in refrigerators.

We collected fresh soil samples with soil cores in the upper soil layer (about 0-10 cm depth) from each 10 m × 10 m subplot for the analyses of soil properties. We transported soil samples using a cooler bag and kept at 5 °C until the analyses of soil WC, pH, total C, total N and inorganic N (NH_4^+-N and NO_3^--N).

3.2.2 Laboratory measurements

We oven-dried both mature and senesced needle samples at 70 °C for 72 h and measured nutrient concentrations with a CHNOS elemental analyser (Elementar, Hanau, Germany). We measured age-dependent variables based on the primary branch system (Supplementary Data Fig. S1) to estimate MRT (described later). The list of age-dependent variables used in this study can be found in Supplementary Data Appendix S1.

To measure soil WC, we weighed fresh soil WC samples, and oven-dried the samples at 105 °C for 48 h to determine the dry weight. We sieved soil samples with a 2 mm mesh to remove the root and rocks, and extracted each 8 g sieved soil sample with 40 mL deionized water for one hour to determine soil pH. We then extract soil again using 8 g sieved soil sample with a 40 mL of 2M KCL solution for one hour to analyse ammonium N (NH_4^+-N) and nitrate (NO_3^--N). We analysed NH_4^+-N of the extracts using indophenol blue absorptiometry, and NO_3^--N using naphthyl ethylenediamine dihydrochloride spectrophotometry, with an auto-analyser (AACS-4, BL-TEC, Inc., Japan). The remains of the sieved soil samples were dried at 70 °C and were used to measure total C, total N and C:N ratio using the CHNOS elemental analyser.

To stay consistent in our method of weighing litter, we air-dried litter from

retrieved litterbags to constant mass, and then weighed the remaining litter mass for the calculation of decomposition rates. We selected retrieved litterbags (12-month of field exposure) that were deployed in the five subplots in each habitat (i.e. subplot 1, subplot 3, subplot 5, subplot 7 and subplot 9; Supplementary Data Fig. S2), and sub-sampled remaining litter in the litterbags to quantify litter N loss during the decomposition process.

3.2.3 Calculations and statistical analyses

We firstly calculated N-resorption efficiency (r) as $r = (N_m - N_s)/N_m \times 100$, and did not correct it for mass loss (Hayes et al., 2014, Aerts and Chapin III, 2000, Vergutz et al., 2012). In the equation, N_m and N_s are the concentrations of N in mature and senesced needles, respectively. Then, we estimated *MRT*, the duration of time that plant tissues and nutrient are retained in a plant, using a model suggested by Hirose (2012).We estimated MRT as $MRT_i = \frac{1}{f_i(T)} \int_0^T [f_i(t) - g_i(t)]dt$, in which $f_i(t) - g_i(t)$ represents the amount of standing needles or foliar N at time t. The subscript 'i' represents either needle quantities (i.e. mean needle residence time; MRT_{Needle}) or foliar N (i.e. mean foliar N residence time; $MRT_{FoliarN}$). A detailed description for the estimation of *MRT* based on measured age-dependent variables can be found in Supplementary Data Appendix S1. Lastly, we investigated the relationship between MRT_{Needle} and $MRT_{FoliarN}$ using major axis (MA) regression.

We compared the whole-year (12-month period) litter mass and N losses using two-way analysis of variance (ANOVA), and calculated the decomposition rate (k) using a single negative exponential decay model: $x_t/x_0 = e^{-kt}$, where x_t/x_0 is the fraction of mass remaining at time t, with t being the time elapsed in years and k being the annual decay constant (Olson, 1963).

We used t-test to compare the mean differences in the majority of measured and estimated parameters, namely N_m, N_s, MRT and soil variables, at the habitat level. All statistical analyses were performed in R version 3.1.1 (R Core Team, 2014).

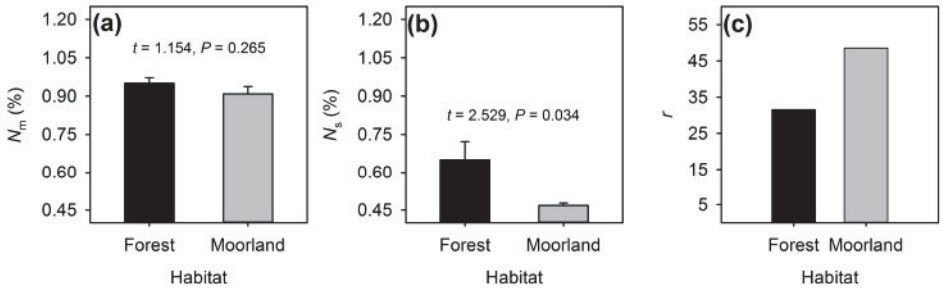

Figure 3.3.1: N concentration of (a) mature *A. mariesii* needles (N_m) and (b) senesced *A. mariesii* needles (N_s). In (c), N-resorption efficiency (r) was calculated according to Aerts and Chapin (2000), and was uncorrected for leaf mass loss. Error bars indicate the standard error of the mean.

3.3 Results

3.3.1 Habitat characteristics and soil properties of the two eco-habitats

Table 3.3.1 summarizes the habitat characteristics and soil properties of the two *A. mariesii*-dominated eco-habitats. Large-sized *A. mariesii* individuals dominated the wind-blown forest habitat; therefore, this habitat had greater amounts of aboveground biomass and litterfall production (the proportion of *A. mariesii* litterfall to the total amount of collected litterfall of non-herbaceous species) than was observed in the moorland habitat. The soil was considerably moist in both habitats; however, the soil WC was even higher in the moorlands. Compared with moorland soil, forest soil was relatively more acidic. The total C and N concentrations in soils were lower in the forest habitat than in the moorlands. However, the soil C:N ratio did not differ between the two habitats. The concentration of NH_4^+-N was significantly higher in moorland soil, but the concentration of NO_3^--N in soil was nearly identical across the two habitats.

Table 3.3.1: Habitat characteristics and soil properties of the two *A. mariesii*-dominated eco-habitats

Habitat	Elevation (m a.s.l.)	Slope (°)	Proportion of *A. mariesii* individuals (%)	*A. mariesii* total basal area ($m^2 ha^{-1}$)	Proportion of *A. mariesii* litterfall (%)	Soil properties						
						WC (%)	pH	Total C (%)	Total N (%)	C : N	NH_4^+-N ($mg kg^{-1}$)	NO_3^--N ($mg kg^{-1}$)
Forest	2007	31	52.0	32.03	60.8	397.89 ± 30.35	4.54 ± 0.06	32.48 ± 3.73	1.65 ± 0.14	19.41 ± 1.39	36.97 ± 7.31	0.60 ± 0.28
Moorland	1920	12	93.6	3.24	22.9	618.70 ± 21.51	4.87 ± 0.08	38.30 ± 1.02	2.10 ± 0.08	18.47 ± 0.97	67.30 ± 10.41	0.57 ± 0.31
t-test												
t	–	–	–	–	–	−5.936	−3.086	−1.507	−2.845	0.555	−2.385	0.076
P-value	–	–	–	–	–	**<0.001**	**0.008**	0.166	**0.014**	0.588	**0.031**	0.941

Proportion of *A. mariesii* individuals and *A. mariesii* total basal area were measured and calculated within each of the 30 m × 30 m core site; individuals with diameter at breast height smaller than 5 cm were not included. Litterfall was quantified at habitat-level using litter traps (Doi unpublished data); the proportion of *A. mariesii* litterfall was defined as the mass of *A. mariesii* litterfall devided by the totall mass of litterfall collected within each habitat. Soil properties were masured within each 10 m × 10 m subplot; values for soil properties are means ± standard errors (n = 9); values in bold are significant at $P < 0.05$

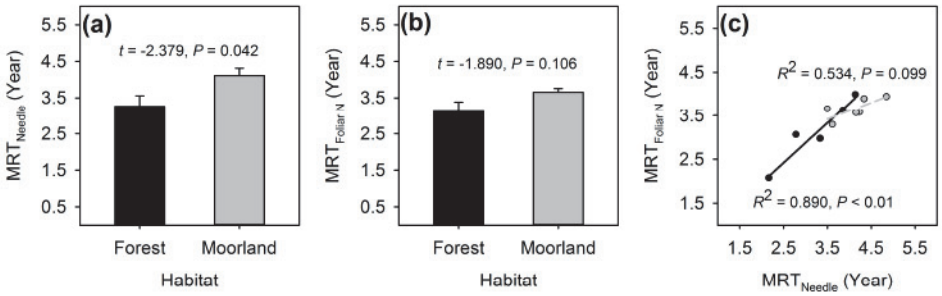

Figure 3.3.2: Estimated (a) mean needle residence time (MRT_{Needle}) and (b) mean foliar N residence time ($MRT_{FoliarN}$). In (c), MA regression was used to check the correlation between MRT_{Needle} and $MRT_{FoliarN}$; black dots indicate primary branches harvested from forest-type *A. mariesii* individuals, and grey dots indicate primary branches harvested from moorland-type *A. mariesii* individuals; error bars indicate the standard error of the mean; solid line suggests significant relationship at $P < 0.05$, and dashed line suggests marginal significant relationship at $P < 0.10$.

3.3.2 Nutrient use strategies of *A. mariesii* in the two eco-habitats

No differences were found in N_m across habitats (Fig. 3.3.1a). However, N_s differed significantly at the habitat-level (Fig. 3.3.1b). We calculated r using mean N_m and N_s that averaged at habitat-level because of the separated sampling procedures of N_m and N_s. The r tended to be higher in the moorland habitat (Fig. 3.3.1c).

The estimated results of MRT showed that, the residence time that *A. mariesii* needles remain attached to the stems was 0.84 years longer in the moorland habitat (Fig. 3.3.2a). Meanwhile, *A. mariesii* foliar N also had a longer residence time (approximately half-year longer) in the moorland habitat, though the difference is only near marginal significant (Fig. 3.3.2b). Finally, the longer needle residence time could expand the residence of foliar N, and the regression slope of MRT_{Needle} and $MRT_{FoliarN}$ estimated for forest individuals was larger than for moorland individuals (Fig. 3.3.2c).

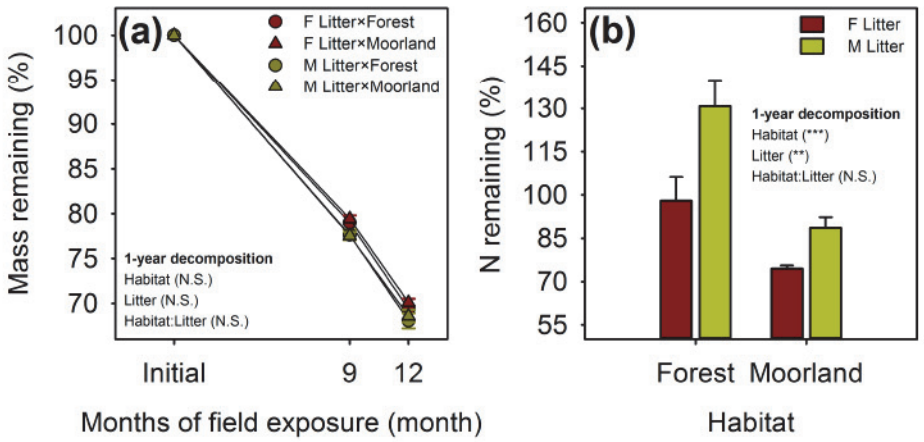

Figure 3.3.3: Percentage of *A. mariesii* (a) litter mass remaining and (b) N remaining. In (a), both data for the 9-month period and 1-year period of field exposure are shown; in (b), only data for the 1-year period of field exposure are shown; error bars indicate the standard error of the mean; significance levels are derived from ANOVA; ***, $P < 0.001$; **, $P < 0.01$; N.S., not significant.

3.3.3 The decomposition of *A. mariesii* litter

M litter decomposed slightly more rapidly than F litter in terms of mass loss during the first nine-month period; however, no significant difference in litter mass loss was observed among both litter and habitat types for the whole-year decomposition period (Fig. 3.3.2a; Table 3.3.2). In contrast, whole-year litter N loss varied significantly between F litter and M litter, and between forest and moorland habitat (Fig. 3.3.3b; Table 3.3.2). For F litter, the loss of litter N occurred more rapidly in the moorlands than in the forest habitat, and within the moorlands, the loss of litter N was more rapid for F than for M litter. In the forest habitat, after one year of field exposure, litter N showed an increase for M litter, compared to its initial litter N content. Calculated k for F litter decomposed in

Table 3.3.2: Levels of significance (P-values) from the two-way ANOVA for comparison of influences of habitat, litter type and their interaction on whole-year litter mass loss and N loss

Source	Mass		N	
	$F_{d.f.}$	P	$F_{d.f.}$	P
Habitat	$0.566_{1,16}$	0.4573	$25.964_{1,8}$	**<0.001**
Litter	$2.903_{1,16}$	0.0981	$13.208_{1,8}$	**0.0022**
Habitat × Litter	$0.018_{1,16}$	0.8943	$2.108_{1,8}$	0.1659

Values in bold are significant at $P < 0.05$

the forest and moorland habitat was 0.35 and 0.34, respectively, while k was 0.37 both habitats for M litter.

3.4 Discussion

3.4.1 Varied nutrient use strategies of *A. mariesii* in contrasting eco-habitats

In this study, both higher concentration of total N and plant-available N (inorganic N) were found in moorland soil than in forest soil. Faster N mineralization rate in the moorlands might be possible factor contributed to the higher N availability. Meanwhile, strong topographic variation is also likely to have caused the accumulated inorganic N in moorland soil due to the water flow path towards the moorlands, as slopes are gentler here than in the forest habitat (Enoki et al., 1996, Swanson et al., 1988, Enoki et al., 1997). Topographic variation resulted in heterogeneous snow accumulation patterns across the local scale (approximately 2–3 m in the forest habitat, and 4–6 m in the moorlands), which further led to contrasting hydraulic conditions across the two eco-habitats. The extremely high water content (soil WC > 600%; Table 3.3.1) in the moorlands could go beyond the field capacity (i.e. gravitational water), making water unavailable for plant uptake (Rodriguez-Iturbe, 2000, Schwinning, 2010,

Figure 3.4.1: Examples of *A. mariesii* crown damages due to heavy snow accumulation in the moorlands

Rose et al., 2003). The N-resorption efficiency of *A. mariesii* increased in the moorlands. Because resorption from senescing leaves enables plants to reduce the losses of nutrients associated with leaf turnover (Aerts and Chapin III, 2000), this could be important for the survival of plants under nutrient-poor conditions (Aerts, 1999, Güsewell, 2005). In the case of *A. mariesii* in this study, variations in N-resorption efficiency have been observed within the species. Although N availability is higher in the moorlands than in the forest habitat, it is possible that plant absorbable nutrients could be limited due to excessive soil water content; a conservative nutrient use strategy (e.g., increased N-resorption efficiency) for *A. mariesii* individuals in the moorlands is therefore reasonable. In addition, habitat heterogeneity (e.g., snow accumulation) may cause variations in the strategies of N resorption. As snow accumulation is much heavier in the moorlands than in the forest habitat, and most of the dwarfed *A. mariesii* are generally buried under the snow throughout the winter, moorland *A. mariesii* individuals are therefore subjected to higher physical pressures and potential damages on tree architectures (Fig. 3.4.1; Mori and Mizumachi, 2009). Allocating more resources

to supportive organs (i.e. non-photosynthetic organs) that would help *A. mariesii* individuals maintain their above-ground architecture would seem to be a high priority in the snowy moorlands (Grassi and Bagnaresi, 2001, Mori et al., 2008, Mori and Hasegawa, 2007, Stenberg et al., 1998). Regardless, in this study estimated *MRT* was found to vary across habitats. Moorland *A. mariesii* individuals retained needles for a longer time than the forest ones, which enables *A. mariesii* to increase the duration of foliar N for possible resorption and re-use in the moorlands (Fig. 3.3.2a-c). Furthermore, longer needle residence time will limit C allocation to foliage relative to organs that support the crown (King, 1997, Mori and Takeda, 2004, Mori and Hasegawa, 2007), echoing the hypothesis that moorland individuals could be more conservative in using and allocating nutrients. It could also be possible that, the habitat-associated drivers did not promote higher resorption efficiency in the moorlands, but rather low N concentration in litter (i.e. resorption proficiency; Killingbeck, 1996) or longer needle residence time (Eckstein et al., 1999, Wright and Westoby, 2003). Reich et al. (2014) has reported the intraspecific variation in the ability of retaining needles across the biogeographical gradients, demonstrating the successful ecological strategies regarding the way evergreen conifers use acquired resources. In addition to the reported intraspecific phenomenon at the large or global scale, our study, which demonstrated the plasticity of nutrient use strategies of *A. mariesii* among contrasting habitats, highlights the significance of this ecological strategy over the local scale. In particular for long-lived species such as subalpine conifers, as the mountainous environments being steep and complex, the variability in the way to use acquired resources could be the "bag of tricks" available for future environmental conditions over the next several hundred years.

3.4.2 Intraspecific nutrient use strategies and litter decomposition process

The varied nutrient use strategies observed here in *A. mariesii* distributed in contrasting eco-habitats led to the production of needle litter that varied in

quality. Litter quality is mostly related to the chemical characteristics of the litter, such as C:N ratio and/or lignin content (Aber et al., 1990, Aerts, 1997); in this study, we emphasized N, as it is important for litter decomposition especially during the early stage of decomposition (Berg, 2000, Berg and McClaugherty, 2014). In contrast to the almost identical pattern of mass loss after a whole year of field exposure, the loss of N during the first-year decomposition showed remarkable differences, between both the two types of litter and the two eco-habitats (Fig. 3.3.3; Table 3.3.2). Within the moorlands, N was released from F litter more rapidly than M litter; this meets our early expectation that nutrient-rich *A. mariesii* litter may contribute to a more rapid release of nutrients through litter decomposition, compared with that of the nutrient-poor *A. mariesii* litter. Soil decomposers access to N sources to meet their needs in the early stage of decomposition; *A. mariesii* litter with higher N content is therefore easier to be accessed by decomposers, thus resulting in a more rapid release of N through litter decomposition (Aerts and Caluwe, 1997, Berg and McClaugherty, 2014). For M litter in the forest habitat, we found an increase in the litter N content after one year of field exposure, probably due to the immobilization by microbes. When nutrient-poor M litter was transplanted into the forest habitat, it may fail to meet the stoichiometric requirements of the local decomposers that may have adapted to the nutrient-rich F litter thus lead to the observed net immobilization (Manzoni et al., 2008, Parton et al., 2007).

Our results demonstrated that, the intraspecific variation in litter nutrient quality, which was driven by differed within-species nutrient use strategies due to contrasting environments, can affect the speed of nutrient release back to the soil environment. When the performance of a plant is greatly influenced by soil environment (i.e. abiotic or biotic soil properties), and the plant in turn also affects and modifies the soil environment, a feedback mechanism may occur (i.e. plant–soil feedbacks; Bever et al., 1997, Ehrenfeld et al., 2005, Kulmatiski et al., 2008). Nutrient conservative *A. mariesii* individuals in the moorlands produce slowly decomposed (in terms of nutrient release speed) litter, while nutrient acquisitive individuals in the forest habitats produce rapidly decomposed litter;

this points to a mechanism that is analogous to the positive plant–soil feedback, which indicates potentially amplified effect of *A. mariesii* on soil and then amplified effect of the soil on *A. mariesii* in both of the two eco-habitats.

Intraspecific plant–soil feedbacks can act as important factors contributing to vegetation dynamics such as species replacement during succession process (e.g., van de Voorde et al., 2011). In the long run, *A. mariesii*, this foundation species could benefit from this likely existed positive plant–soil feedback to maintain itself across contrasting habitats (Ehrenfeld et al., 2005, Wardle and Jonsson, 2013). Furthermore, subordinate species that associated to this foundation species (e.g., Callaway, 2009, Ellison et al., 2005) may also subject to changes due to intensified habitat heterogeneity driven by the plant–soil feedbacks. In this study, habitat-related factors were also important in affecting the decomposition of litter (e.g., transplanted F litter in the moorlands showed accelerated nutrient release, compared with F litter decomposing in the forest habitats; Fig. 3.3.3b). Although further investigations are required to precisely predict the future changes in both *A. mariesii* individuals and in ecosystem properties, this study makes it clear that intraspecific variation of foundation species, mediated by the environment while simultaneously affecting the environment by modifying critical ecological processes, bears great significance and cannot be neglected. Quantitative investigation of the source of intraspecific variation, together with an investigation of its potential impacts on ecological processes, has rarely been done in the past in foundation species-centred studies. Therefore, our study will bring in valuable opportunities to learn the interplay between foundation species and the ecosystem it belongs, leading to a more complete and accurate understanding of the functional role of foundation species.

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Supplementary Data

Supplementary Data Fig. S1

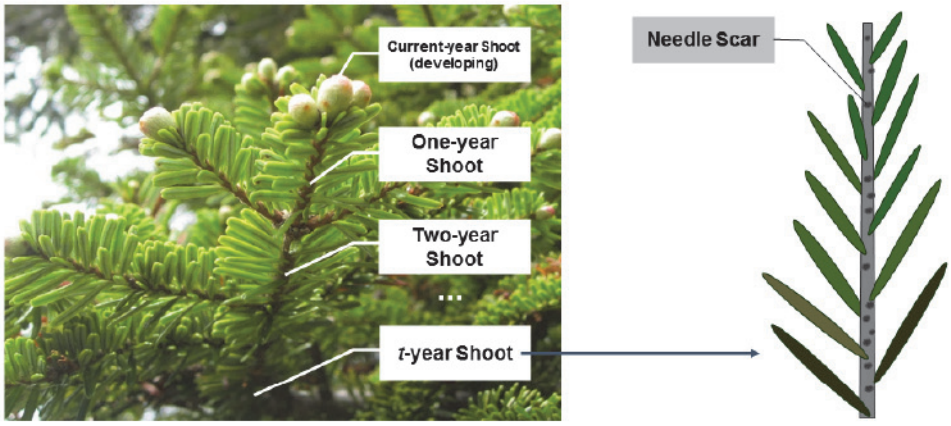

Fig. S1 *A. mariesii* branches and an illustration of one primary shoot separated from the branch

Supplementary Data Fig. S2

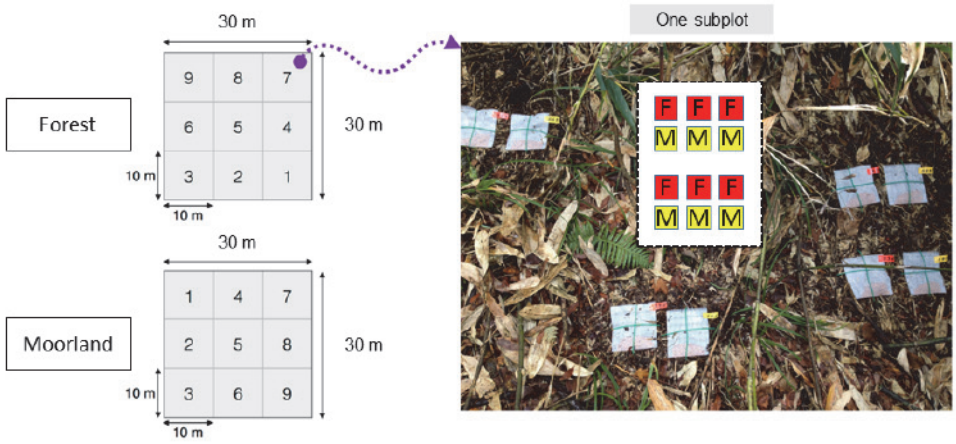

Fig. S2 Reciprocal litterbag transplant experiment design in the two eco-habitats. Letter "F" represents litterbags that contain F litter, and letter "M" represents litterbags that contain M litter.

Appendix S1

Appendix S1 Measurements of age-dependent variables and the estimation of mean residence time (MRT) based on *A. mariesii* primary branch system

We firstly separated primary shoots from each harvested *Abies mariesii* primary branch. We labeled each primary shoot with shoot ages (e.g. current-year, one-year and two-year etc.) for each individual branch (Fig. S1).

We then recorded the standing needle number by year (NN_t), and removed needles to measure standing needle mass (NM_t) and calculated mass-based standing Needle N by year (NC_t) for each primary shoot. The subscript 't' in all parameters means the t-year aged primary shoot. We approximated the total amount of standing needles accumulated during t years as $\sum_{i=0}^{t} NN_i$, and the total amount of standing needle N accumulated during t years as $\sum_{i=0}^{t} NC_i$. We

recorded the total needle number (TNN_t) by counting the number of needle scars for each primary shoot after the removal of needles (Fig. S1). We estimated the total needle mass (TNM_t) for each primary shoot as: $TNM_t = NM_t/NN_t \times TNN_t$, and estimated the mass-based total amount of needle N (TNC_t) using TNM_t. Finally, we approximated the amount of needles produced during t years as sum of TNN_t for all shoots ranged from current-year to t-year aged shoot as $\sum_{i=0}^{t} TNN_i$, and the amount of needle N produced during t years as sum of TNC_t for all shoots ranged from current-year to i-year aged shoot as $\sum_{i=0}^{t} TNC_i$.

We then estimated MRT according to Hirose (2012) as $MRT_i = \frac{1}{f_i(T)} \int_0^T [f_i(t) - g_i(t)]dt$, in which $f_i(t) - g_i(t)$ represents the amount of standing needles or foliar N at time t. The subscript 'i' represents either needle quantities (i.e. mean needle residence time; MRT_{Needle}) or foliar N (i.e. mean foliar N residence time; $MRT_{FoliarN}$). The total amount of needles or foliar N that are produced by the end ($t = T$) is $f_i(T)$ (equal to $\sum_{i=0}^{t} NN_i$ or $\sum_{i=0}^{t} TNC_i$). We assumed a linear increase in $f_i(t)$ and $g_i(t)$ between successive censuses as suggested by Hirose and Oikawa (2012), and calculated the function of $f_i(t) - g_i(t)$ from $\sum_{i=0}^{t} NN_i$ or $\sum_{i=0}^{t} NC_i$. We computed the integrals using a seven-year time period ($T = 7$; $t = 0$ stands for the shoot age of "current-year"), and calculated MRT individually for each of the 12 primary branches.

4

Intraspecific patterns to bridging the gaps between ecological studies: a review

4.1 Introduction

Defined earlier by Krebs (1972), ecology was viewed as the study of the interactions that determine the distribution and abundance of organisms. To date, as far as the subject matter of ecology is concerned, this definition has been expanded to the next levels: the *individual organism*, the *population* and the *community*. When the flow of matter and energy among living and nonliving elements are considered, it brings in a further level of organization: the *ecosystem*, which is composed of the community together with its physical environment (Begon et al. 2006). At each level, specific questions are addressed to describe and explain the observed biological patterns. The ecological systems, in which

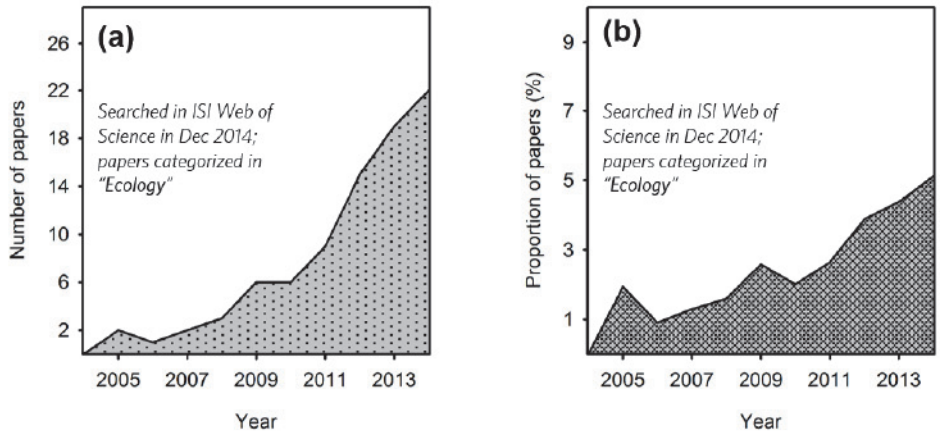

Figure 4.1.1: Relative number (a) and proportion (b) of studies which emphasized the within-species level patterns (i.e. among population and/or among individual patterns) in the studies of functional responses of plant communities to environment.

individual organisms, *populations*, *communities* and the abiotic environment interact with each other, form a hierarchy; this points to the fact that, none component of the highly complex systems exists isolated. However, ecologists often address unique set of questions that specific to one level of organization, disregarding the hierarchical view of the system and the patterns and processes associated with each level. For instance, studies that deal with communities, the unit of which are usually taxonomic species, tend to emphasize community-wide patterns and trends while ignoring variations within each taxonomic identities (i.e. variations among *population* and/or among *individual organisms*; Fig. 4.1.1).

Starting with a pattern at one level, while integrating knowledge of pattern and process at the other level, brings in invaluable opportunities for systematically investigation of the complex ecological systems. In the case of community-centered studies, it is widely accepted that focusing at a single level of organization (i.e. the *community*) would be easier to draw clearer and more ambitious conclusions, rather than considering multiple levels of organization at

a time based on limited ecological observations. Nevertheless, empirical evidence have emerged recently and have pushed the idea that, the effects originated from *individual organism* or *population* -level differences cannot be neglected when learning how every component (i.e. the *individual organism*, the *population* and the *community*) interact with each other in the context of ecosystem (e.g. Crutsinger et al. 2009; Jung et al. 2014; Kumordzi et al. 2014; Lecerf and Chauvet 2008; Souza et al. 2011). A hierarchical view of the system, that is considering multiple levels of organization, is going to become a prerequisite when developing solid hypotheses and methodologies for the rapidly evolving fields in ecology.

I proposed a conceptual framework in this review, and demonstrated how levels of organization in ecology, ranged from *individual organisms* and *populations* to *ecosystems*, can be linked and considered in a more systematic perspective based on this framework.

4.2 Intraspecific patterns to bridging the gaps between ecological studies

The conceptual framework I proposed here emphasized the key role of intraspecific patterns (i.e. individual- and population-level patterns) of plants (Fig. 4.2.1). I prioritized intraspecific patterns because it is a bridge to effectively link the rapidly developing but fragmenting study areas across levels of organization in ecology. I also incorporated the evolutionary context in this framework; an increasing number of studies (e.g. Crawford and Rudgers 2012; Crawford and Whitney 2010; Johnson et al. 2006) has indicated that, links between evolutionary dynamics and community- and ecosystem-level dynamics are likely to be common. Yet, there is little understanding regarding this kind of links and how they are functioned (Pelletier et al. 2009; Whitham et al. 2006). Our conceptual framework highlights some pathways which have not received enough attentions in current ecological studies, or some novel pathways which

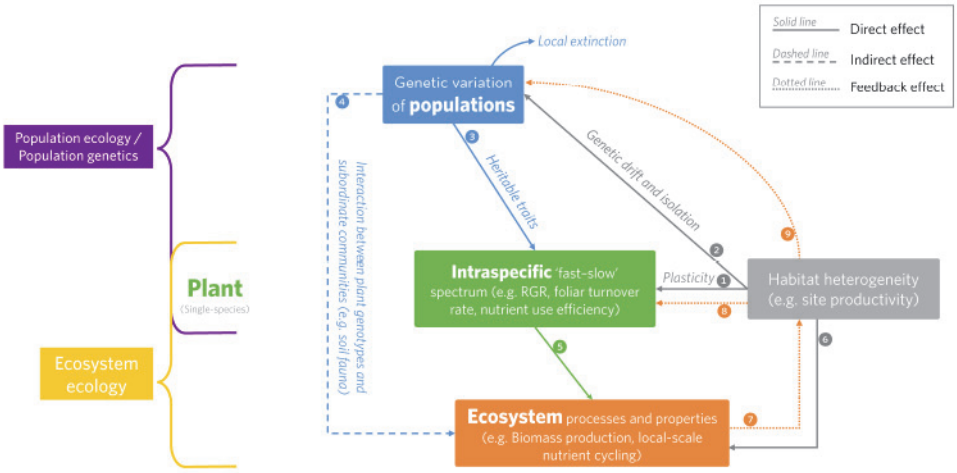

Figure 4.2.1: Schematic diagram showing a systematic and unified framework to link areas of ecology (i.e. population ecology, population genetics and ecosystem ecology) through intraspecific patterns of plants.

may bare great significance in future. Our aims are to call for more attentions on those critical but easy-to-be ignored novel pathways as proposed in this framework.

4.2.1 Novel pathways connected through intraspecific patterns

Generally, ecosystem properties or processes are inferred by ecologists from several aggregated measurements of plant functional characteristics (e.g. Cohen et al. 2014; Garnier et al. 2004; Grigulis et al. 2013; Tardif et al. 2014), by which taxonomic species is usually treated as the minimum unit. However, emerging empirical evidence suggested that, variations within each taxonomic species are also essential to explain the observed overall changes in processes and properties of ecosystems (Path 5 in Fig. 4.2.1), especially at the local spatial scale (e.g. Crutsinger et al. 2009; Jung et al. 2014; Kumordzi et al. 2014; Lecerf and Chauvet 2008; Souza et al. 2011). These changes, mediated by intraspecific functional characteristics (better known as the 'fast-slow' economic spectrum),

can have genetic basis (Path 3 in Fig. 4.2.1), or solely caused by plastic response of plants (Path 1 in Fig. 4.2.1) against abiotic conditions.

In the case of genetic heritable trait of plants, it is a key factor to bridge the interaction of evolutionary dynamics at individual organisms- and populations-level, with their impacts on ecosystems (Path 3→Path 5 in Fig. 4.2.1). Genetically based intraspecific 'fast-slow' economic spectrum in terms of tree growth, wood density and foliar chemical properties has seen reports recently (e.g. Madritch et al. 2006; Sotelo Montes and Weber 2009a; Sotelo Montes and Weber 2009b; Weber and Sotelo Montes 2008; Weber et al. 2011). Despite that most studies that have reported the genetically based intraspecific 'fast-slow' economic spectrum of plants, few of them have examined further regarding its potential impacts on ecosystem processes and properties. Only a few studies, for instance Hines et al. (2014), have emphasized the pathway (i.e. Path 3→Path 5 in Fig. 4.2.1) in my framework. According to Hines et al. (2014), changes in abiotic conditions could greatly alter ecosystem process (e.g. decomposition process) if it causes loss of genetic variation associated with key trait values (e.g. high- or low-nutrient content in plant leaf).

Intraspecific differences in a plant's functional characteristics that resulted from plant heritable traits can repeatedly influence correlated ecosystem process. This could be an output of genotype × environment ($G \times E$) interaction, through which variations in crucial functional traits are expressed (e.g. LeRoy et al. 2007; Madritch et al. 2006; Pregitzer et al. 2013). In the future, genetically-based responses of plants to changing abiotic conditions, not only help to understand the biotic and abiotic interactions at the individual and population-level, it will also add ecosystem-wide understandings to existing knowledge considering how processes are regulated across multiple levels of organization in ecological systems.

4.2.2 The indirect effects that linking together genetic background of plants and ecosystem processes

Plant genetic variation can generate impacts on ecosystem processes and properties through heritable plant traits, it can also affect the ecosystem indirectly, meaning that genes in a focal plant may affect ecosystems by altering the phenotype of interacting (i.e. neighboring) individuals (Path 4 in Fig. 4.2.1; Table 4.2.1). Despite the increased attentions on this pathway, it is still poorly understood. At the individual- and population-level, that is within a taxonomic species, indirect genetic effects (IGEs) occur when the fitness and phenotype of one individual changes due to the genetic identity of interacting individuals (Bailey et al. 2009; Moore et al. 1997; Wolf et al. 1998). While at the community- or ecosystem-level, interspecific indirect genetic effects (IIGEs; Shuster et al. 2006) represent the fundamental unit of the co-evolutionary process and hold essential information for the understanding of interactions across levels of organizations, such as the plant-plant, plant herbivore and plant-soil interactions.

Table 4.2.1: Representative studies of indirect effects of intraspecific plant genetic variation on ecosystem process

Species	Interspecific interaction	Ecosystem	Ecosystem processes	Study
Oenothera biennis	Not tested	Terrestrial	TAPP	Cook-Patton et al. (2010)
Solidago altissima	Herbivore, predator	Terrestrial	ANPP	Crutsinger et al. (2006)
Orchesella cincta	Not tested	Terrestrial	Primary productivity	Ellers et al. (2011)
Populus sp.	Not tested	Terrestrial	Soil nitrogen dynamics	Fischer et al. (2010)
Solidago sp.	Arthropod pollinator	Terrestrial	Biomass production	Genung et al. (2012)
Populus sp.	Fungi	Terrestrial	Decomposition	LeRoy et al. (2007)
Populus tremuloides	Not tested	Terrestrial	Decomposition	Madritch et al. (2006)
Hordeum vulgare	Not tested	Terrestrial	Biomass production	Schöb et al. (2014)
Populus sp.	Not tested	Terrestrial	Soil N mineralization	Schweitzer et al. (2004)
Phragmites australis	Becteria	Terrestrial	Productivity, denitrification	Tomimatsu et al. (2014)

Ecosystem functions (i.e. the biological, geochemical and physical processes and components that take place or occur within an ecosystem) such as aboveground biomass production and decomposition rate of leaf litter, have been frequently used as indices to investigate the consequences of biotic interaction on

ecosystems. The investigations based on these indices are mainly rely on manipulation experiments; whether the existing theories such as complementarity and selection effects derived from manipulation experiments of different taxonomic identities or functional identities are appropriately applicable to the manipulation experiments using different genotypic individuals, is still remain open to be discussed. Further, as evolutionary dynamics provides more information on historical backgrounds and processes, new hypotheses regarding the consequences of biotic interactions across multiple levels of organization are therefore to be expected.

4.2.3 The feedback effects that most often get overlooked

Qian et al. Manuscript (also see Chapter 3) provided some insights into possible intraspecific positive plant-soil feedback for *Abies mariesii* in a local-scale subalpine ecosystem. Due to intraspecific variation in resource usage, needle litter that varied in chemical properties was produced, and further nutrient release during the decomposing process was seen different (possibly Path 1→Path 5 in Fig. 4.2.1). In this case, *A. mariesii* has been recognized as the foundation species; if the intraspecific positive plant-soil feedback is to be expected, increased local-scale heterogeneity will probably occur and changes in subordinate communities that associated to this foundation species are also likely (Callaway 2009; Ellison et al. 2005). This feedback effect (Path 1→Path 5→Path 7→Path 8 in Fig. 4.2.1) has been considered as important factors contributing to vegetation dynamics such as species replacement during succession process, and has been recognized in some other studies (e.g. van de Voorde et al. 2011). However, how will the feedback mechanisms influence the plants, especially under the 'fast-slow' economic spectrum framework, as well as how community-level interactions and ecosystem changes will occur, require more coming researches to uncover.

At the same time, there is little information that current available for the investigation of the feedbacks of IGEs (Path 4→Path 7→Path 9 in Fig. 4.2.1).

Although theoretical discussions have been tentatively made regarding this kind of feedback (Bailey et al. 2014), due to the lack of sufficient empirical data, many of the details are right now missing. Because when IGEs occur along abiotic gradients, both positive and negative feedbacks can evolve, resulting in regions of strong local adaptation and competition. Further, such evolutionary dynamics will impact how individuals interact and evolve across multiple levels of organization. Importantly, it could be essential to infer future extinction risks of individuals and populations considering this feedback effect on the fitness of organisms against changing abiotic conditions.

4.3 Conclusions and implications

Because of anthropogenic landscape modification and habitat fragmentation, genetic connectivity of many plant species have been threatened. Combining the climate change scenario, evolutionary dynamics not only help to understand individual and population survival and extinction, it also generate great values to insights into possible changes in ecosystem properties and processes either through IGEs or through the production of functionally varied plant organs (i.e. heritable plant traits). What's more, plants can show plastic response to changing environmental conditions and climates; this adds to the pathway through which plants can influence the ecosystem.

It is to be noted that, as my framework proposed here emphasized intraspecific pattern, and use it to bridge the gaps between areas in ecological studies, it could be limited to apply to all studies, particularly studies focus on scopes beyond the ecosystem (i.e. biome and global biosphere). Nevertheless, studies that incorporated intraspecific patterns to improve their models and simulations of broad-scale biotic interactions and changes (e.g. Oney et al. 2013; Reich et al. 2014; Swab et al. 2014; Valladares et al. 2014), have begun to emerge. This proved that ecologists should consider more than one single level of organization to learn the biotic and abiotic interactions within the complex ecological systems. To archive this, I expect more examples and proposals to update and improve

current methodological frameworks in future through the integration of hierarchical view of levels of organization.

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5

General discussion

I focused on the patterns of *Abies mariesii*, a foundation coniferous species, in a snowy subalpine ecosystem in our early studies. I found that this conifer showed considerable intraspecific variation, which may contribute to the maintenance of *A. mariesii* populations in heterogeneous environments. Further, our study showed that the observed intraspecific variation was important in determining critical ecological processes, such the nutrient release speed from litter during the decomposition process. In the beginning, I was interested in the within-species patterns of *A. mariesii*. That is, investigations and discussions were mainly carried out at the population level or individual -level. Then, I started with the observed intraspecific patterns, and showed their possible impacts on the ecosystem (Fig. 5.0.1). Indeed, my studies involves the topics in areas both in population ecology and ecosystem ecology, which in general are unfortunately studied

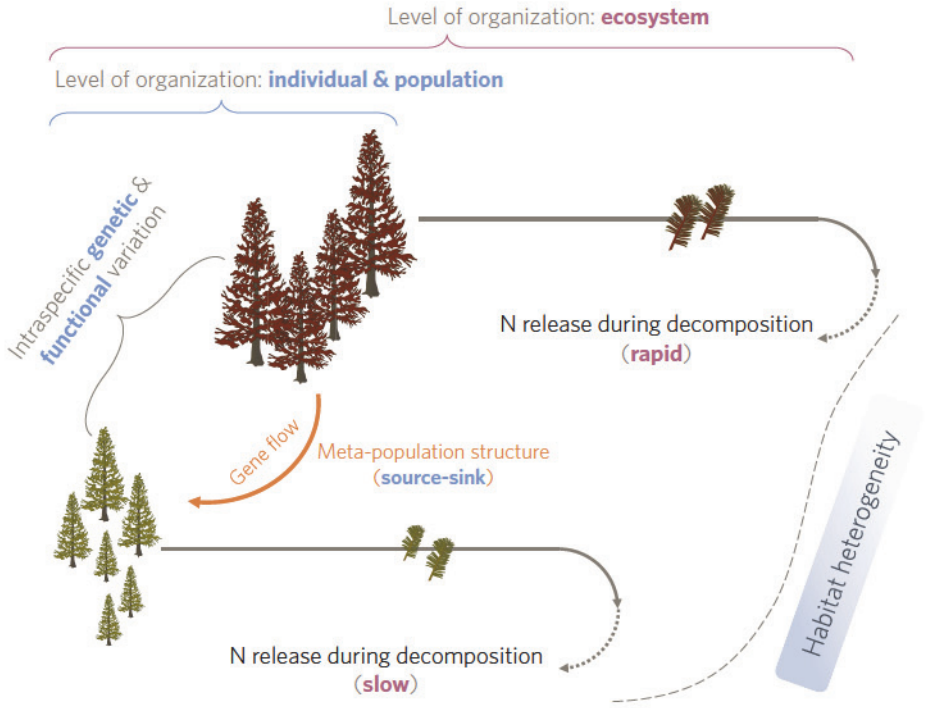

Figure 5.0.1: Investigation of patterns of *A. mariesii* across multiple levels of organization in this dissertation.

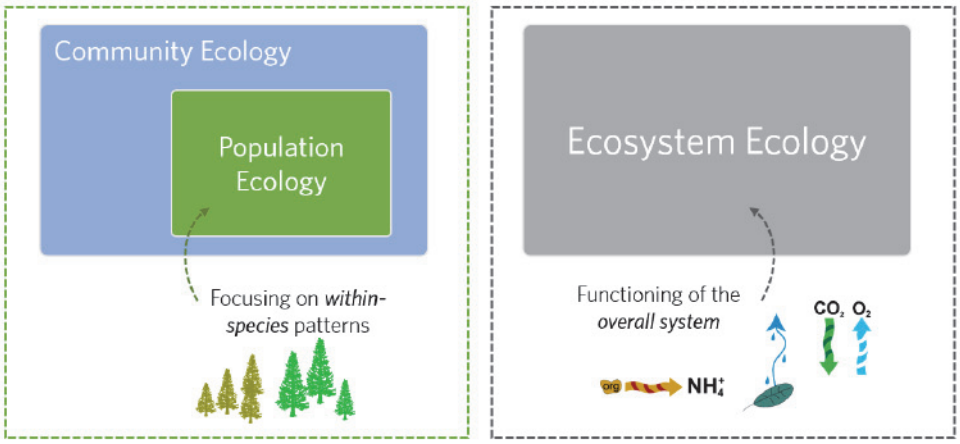

Figure 5.0.2: Population ecology and ecosystem ecology as examples of diverged study areas in ecology.

separately.

As Loreau (2010) wrote in his book and argued that, the conceptual frameworks of the various areas in ecology tend to become increasingly divergent over time. This divergence is nowhere more apparent than between two of the major subdisciplines of ecology, i.e. community ecology and ecosystem ecology (Fig. 5.0.2). Community ecology is to a large extent an outgrowth of population ecology. It is mainly concerned with the dynamics, evolution, diversity, and complexity of the biological components of ecosystems, while ecosystem ecology is mainly concerned with the functioning of the overall system composed of biological organisms and their abiotic environment (Loreau 2010).

Like what I have reviewed and synthesized in Chapter 4, I am looking forward to using intraspecific patterns as the proxy for the study of interaction between plants and the environments. By incorporating intraspecific variation, important details can be added for understanding how plants response to the environment variability, and how plants in turn affect and modify the environments (i.e. the effects of plants on ecosystem functioning). Populations and communities do not exist in isolation; they are parts of ecosystems, and are subjected to

constraints arising from ecosystem functioning. On the other hand, ecosystems do not exist without their biological components; the latter impose their own constraints on ecosystem processes. Investigations such as my studies (see Chapter 2 and Chapter 3) are likely to be able to draw implications based on population-level patterns and ecosystem processes. That is, a link between the population ecology and ecosystem ecology -related topics and be expected. My investigations of population-level patterns and ecosystem processes, are mainly based on natural observational data. Similar studies which also emphasized the link between plant populations and ecosystem functions are also emerging with the help of manipulation experiments (e.g. Crutsinger et al. 2006; Crutsinger et al. 2008; Pregitzer et al. 2013; Schöb et al. 2014). All these efforts have been made to make clear the missing details in current ecological studies, so that hidden mechanisms can be uncovered regarding how biological components of ecosystems interact with each other and change through space and time in a more systematic perspective.

It is not the first time for ecologists other than Loreau (2010) who have called for integrated frameworks and unified approaches in ecological studies. For instance, in the past decades has seen a major resurgence of interest in explicit integration of ecological and evolutionary studies (e.g. Lavergne et al. 2010; Schoener 2011; Vellend and Geber 2005; Wiens and Graham 2005). While some others, such as Meiners et al. (2015) which gave a comprehensive and integrative framework for understanding successional dynamics, are trying to bring in contemporary knowledge to classic ecological theories (i.e. theories regarding the successional dynamics of plant communities). Great progress has been in recent years to understand the underlying mechanisms of biodiversity patterns and the functioning of ecosystems, during which knowledge from a single area of ecology are no longer sufficient to explore the complexity of nature. Therefore, in the future, integrating knowledge from multiple areas of ecology, or further integrating knowledge from fields other than the ecology (i.e. evolutionary biology), would be of great help to accelerate the development of comprehensive ecological frameworks for better understanding of complex mechanisms behind

the observed biodiversity patterns, as well as understanding of plant-mediated effects on critical ecosystem processes that would cause future impacts on ecosystem functioning.

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