Ecological Research Monographs

Gaku Kudo
Editor

Structure and Function of Mountain Ecosystems in Japan

Biodiversity and Vulnerability to Climate Change

Springer

Ecological Research Monographs

Series editor
Yoh Iwasa

More information about this series at http://www.springer.com/series/8852

Gaku Kudo
Editor

Structure and Function of Mountain Ecosystems in Japan

Biodiversity and Vulnerability to Climate Change

Springer

Editor
Gaku Kudo
Faculty of Environmental Earth Science
Hokkaido University
Sapporo, Hokkaido
Japan

ISSN 2191-0707 ISSN 2191-0715 (electronic)
Ecological Research Monographs
ISBN 978-4-431-55952-8 ISBN 978-4-431-55954-2 (eBook)
DOI 10.1007/978-4-431-55954-2

Library of Congress Control Number: 2016930102

Front cover: Alpine meadow and lake system in the Taisetsu Mountains, Hokkaido, Japan (photo by Gaku Kudo, July 2012). *Back cover: Left*: Upper limit of subalpine conifer (*Abies mariesii*) forest at the summit (about 1,400 m a.s.l.) in the Hakkoda Mountains, Aomori, Japan (photo by Tohru Nakashizuka, July 2009). *Center*: Fritillary butterflies (Lepidoptera, Nymphalidae) visiting *Cimicifuga simplex* (Ranunculaceae) flowers in the Utsukushigahara highlands, Nagano, Japan (photo by Takao Itino, September 2009). *Right*: Lake Komaike as a typical mountain lake (1,340 m a.s.l.) in Akita Prefecture, Japan (photo by Manabu Fukui, July 2006).

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Japan KK

Preface

High-mountain ecosystems are an interesting field for a wide area of ecological research, such as population ecology, community ecology, landscape ecology, evolutionary ecology, physiological ecology, biological interactions, and global change biology. Mountain ecosystems in mid-latitudes are characterized by clear seasonality and dynamics of a snow-thawing regime during the summer season. Especially in Japanese mountain systems, in Far East Asia, the mild, wet summer and cold, snowy winter contribute to the formation of a diverse community structure on a regional scale.

An altitudinal transition from subalpine forest to alpine zone reflects a clear gradient of thermal conditions and the length of the growth period for plants and animals. Along the altitudinal ecotone in high mountains, species composition changes quickly, which influences biological interactions within communities. In the subalpine zone, not only forests but also moorland and lake systems are important components of landscape features forming a spatial structure of communities. In the alpine zone above the timberline, various habitats exist as mosaic patterns reflecting topographic undulation that affects snow distribution and soil moisture on a microscale. Snowmelt time is a crucial factor determining the distribution and phenology of alpine plants. Therefore, environmental heterogeneities along the altitudinal and snowmelt gradients are key structures maintaining the biodiversity of high-mountain ecosystems. Furthermore, the existence of environmental gradients and diverse habitats may accelerate the microscale evolution among neighboring populations of the same species. This results in the formation of genetic differentiation and diversity on a local scale.

In general, high-mountain ecosystems are strongly susceptible to climate change because a cool climate and short growth season limit the distribution of organisms at high altitudes. Climate warming may accelerate the migration of plants and animals upward, while earlier snowmelt affects edaphic condition and the growth period which may cause the distribution shift of plants along the snowmelt gradient. Furthermore, the acceleration of snowmelt modifies the growth schedule and phenological events of terrestrial organisms. Changes in the land–water environment also affect the species composition and life cycle of aquatic organisms in lake systems. Therefore, global warming is predicted to disturb the spatiotemporal structures of high-mountain ecosystems, and it should modify the biodiversity and community structure on various scales. To predict the global change impacts on high-mountain ecosystems, understanding of the ecosystem structure, significance of the environmental gradients, and the mechanisms maintaining and creating biodiversity and life history strategies of organisms are crucial.

The aim of this book is to broaden the understanding of high-mountain ecosystems, especially focusing on the structure and function of Japanese mountain systems. So far, there has been no book that synthesizes and introduces the general features of mountain ecosystems in Far East Asia located in the monsoonal region. This book is composed of seven chapters. Chapter 1 analyzes the recent forest dynamics along altitudinal gradients and predicts the distribution of subalpine forests based on the warming scenario. Chapter 2 introduces the eco-physiological approach analyzing the significance of functional diversity of moorland plants for the prediction of vegetation change under climate warming. Chapter 3 synthesizes the dynamics of the phenological structure of alpine plant communities over the years, its ecological meaning, and the vulnerability of plant–pollinator interactions in an alpine environment. Chapter 4 introduces the evidence of genetic differentiation and adaptation of floral traits along altitudinal gradients responding to the differences in pollinator composition. Chapter 5 explains the geographical distribution of coniferous forests along altitudinal gradients that constitute the main vegetation structure in the subalpine zone. Chapter 6 demonstrates the terrestrial–aquatic linkage in terms of carbon supply to the mountain lake ecosystems. Finally, Chapter 7 deals with the composition of bacteria communities in mountain lakes over wide geographical regions to clarify the factors determining the aquatic microbial diversity in mountain ecosystems.

Throughout these diverse topics, we expect that readers can develop a better understanding of the structure and ecological function of Japanese high-mountain ecosystems and their vulnerabilities to climate change. We sincerely hope that this book is a step toward the further promotion of ecological studies of high-mountain ecosystems.

Sapporo, Japan Gaku Kudo

Contents

1 **Influences of Climate Change on the Distribution and Population Dynamics of Subalpine Coniferous Forest in the Hakkoda Mountains, Northern Japan** 1
Tohru Nakashizuka, Masaya Shimazaki, Takehiro Sasaki, Takahisa Tanaka, Hiroko Kurokawa, and Kouki Hikosaka

2 **Trait-Based Approaches for Understanding Species Niche, Coexistence, and Functional Diversity in Subalpine Moorlands** 17
Kouki Hikosaka, Takehiro Sasaki, Chiho Kamiyama, Masatoshi Katabuchi, Shimpei Oikawa, Masaya Shimazaki, Hiroshi Kimura, and Tohru Nakashizuka

3 **Landscape Structure of Flowering Phenology in Alpine Ecosystems: Significance of Plant–Pollinator Interactions and Evolutionary Aspects** 41
Gaku Kudo

4 **Plant Genetic Diversity and Plant–Pollinator Interactions Along Altitudinal Gradients** 63
Takao Itino and Akira S. Hirao

5 **Vegetation Zonation and Conifer Dominance Along Latitudinal and Altitudinal Gradients in Humid Regions of the Western Pacific** 89
Shin-ichiro Aiba

6 **Roles of Terrestrial Carbon Subsidies to Aquatic Community Metabolism in Mountain Lake Ecosystems** 115
Tomoya Iwata, Naoka Mochizuki, Takao Suzuki, Ayato Kohzu, Hisaya Kojima, Manabu Fukui, and Jotaro Urabe

7 **Planktonic Bacterial Communities in Mountain Lake Ecosystems** . . . 145
Masanori Fujii, Toshihide Hirao, Hisaya Kojima, and Manabu Fukui

Index 171

Chapter 1
Influences of Climate Change on the Distribution and Population Dynamics of Subalpine Coniferous Forest in the Hakkoda Mountains, Northern Japan

Tohru Nakashizuka, Masaya Shimazaki, Takehiro Sasaki, Takahisa Tanaka, Hiroko Kurokawa, and Kouki Hikosaka

Abstract Recent research in the Hakkoda Mountains has shown that climate change during recent decades has caused a shift in the distribution of subalpine coniferous forest, which is dominated by *Abies mariesii*. To examine this shift, we analyzed past and present aerial photographs and found population dynamics evidence that suggests a shift of this species' distribution to higher elevations. A fine-scale habitat suitability model for the species elucidated the environmental conditions that determine its present distribution, and this model can be applied to predict the future distribution under the predicted warming scenarios. The model showed that not only temperature but also topography and snow deposition will regulate the future distribution. One important suggestion is that habitats at the periphery of moorlands will act as refugia for *A. mariesii* during the warming process. The fine-scale habitat model may therefore guide the management by providing a measure of how populations will adapt to environmental change.

Keywords *Abies mariesii* • Altitudinal distribution • Aerial photographs • Distribution shift • Population change • Refugia • Snowfall

---

T. Nakashizuka (✉) • M. Shimazaki • T. Tanaka • K. Hikosaka
Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi 980-8578, Japan
e-mail: toron@m.tohoku.ac.jp

T. Sasaki
Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba, Japan

Department of Biology, Faculty of Science, Chiba University, 1-33 Yayoicho, Inage-ku, Chiba 263-8522, Japan

H. Kurokawa
Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi 980-8578, Japan

Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_1

1.1 Introduction

The ongoing climate change caused by global warming is greatly affecting terrestrial ecosystems. Warming has shifted the distributions of many plant and animal species poleward and upward (Parry et al. 2007). An increasing number of studies on the upward shift of the range of plants in alpine ecotones have been conducted in Europe and North America (e.g., Beckage et al. 2008; Lenoir et al. 2008). Climate change is expected to decrease or even eliminate the habitats of some alpine and subalpine plants because these plants are isolated in high-elevation patches near mountain summits (Horikawa et al. 2009), leaving them nowhere to move, and ecosystems at higher latitudes may be similarly vulnerable to climate change.

Many studies have predicted the effects of climate change on the distributions of wild plants and vegetation (Araújo et al. 2005; Huntley et al. 1995; Iverson and Prasad 1998; Thuiller et al. 2005). However, most of these predictions have been based on coarse-grained (e.g., 50-km grid) climate response surfaces or idealized scenarios that assumed uniform warming and which therefore failed to consider spatially heterogeneous warming at local and landscape scales (Ashcroft et al. 2009). Trivedi et al. (2008) also suggested that recent large-scale modeling studies may overestimate the ability of montane plant species to adapt to global warming because the input climate data had coarse resolution and was biased against cold, high-altitude habitats.

Studies at finer resolution in Japan have used a Japanese grid coding system with 1-km^2 cells, referred to as the "Third Mesh," that can be used to predict potential refugia for some dominant species, such as stone pine (Horikawa et al. 2009), beech forests (Matsui et al. 2009), and dwarf bamboo (Tsuyama et al. 2011). Each of the Third Mesh cells measures 3000 latitude by 4500 longitude (191 km^2), and their positions were defined by the Geospatial Information Authority of Japan (Japan Map Center 1998). However, because the variation in elevation within a 1-km cell can be potentially large in Japan's many complex and precipitous mountainous areas, the temperature variation in these cells must be large. The influences of geographical factors, such as topography, are also difficult to be incorporated in climate models (Matsui et al. 2004). Therefore, the 1-km resolution (i.e., using one representative value per 1-km cell) is insufficient for practical conservation management, including measures to encourage adaptation of species to climate change at a regional or local scale. Some population parameters may also be able to be detected in fine-scale analyses.

The Hakkoda Mountains are the peaks of volcanoes at the northern end of Japan's Honshu Island and retain areas of well-preserved natural vegetation that includes ecosystems ranging from cool temperate to alpine zones. They are therefore suitable for investigating the impacts of climate change on ecosystems and specifically on changes in their altitudinal distribution. In this study, we review studies on subalpine forests in these mountains that are dominated by *Abies mariesii* and summarize the present evidence about the impacts of climate change on the forest and its future distribution under predicted climate change scenarios. Our results show the effectiveness of fine-scale analyses using aerial photographs to support planning of adaptation measures in response to climate change.

1.2 Climate and Vegetation in the Hakkoda Mountains

The Hakkoda Mountains are located in Aomori Prefecture, near the northern end of Honshu Island (40°39′N, 140°51′E). The highest point in the mountains is 1585 m a.s.l. Natural forests remain at elevations higher than 400 m a.s.l. in the mountains. Most of the land above 700 m a.s.l. has been designated a part of the Towada-Hachimantai National Park where harvesting and other forms of forest management are prohibited.

One of the original vegetation types, cool-temperate (montane) deciduous forests dominated by beech (*Fagus crenata*), is distributed from lowland areas to an elevation of about 1000 m a.s.l (Fig. 1.1). Few primeval cool deciduous forests remain at elevations below 400 m a.s.l., whereas sites above 700 m a.s.l. form a mosaic pattern created by a mixture between cool deciduous forests, secondary deciduous forests (dominated by *F. crenata*), and coniferous plantations (*Cryptomeria japonica* and *Larix kaempferi*).

Subalpine coniferous forests dominated by *A. mariesii* are distributed above 800 m a.s.l (Fig. 1.1). The forests between 800 and 1000 m a.s.l. form a transitional zone between cool deciduous forests and subalpine coniferous forests. The subalpine coniferous forests in the Hakkoda Mountains are mostly primeval and well preserved, though some at high elevations form a mosaic pattern with the

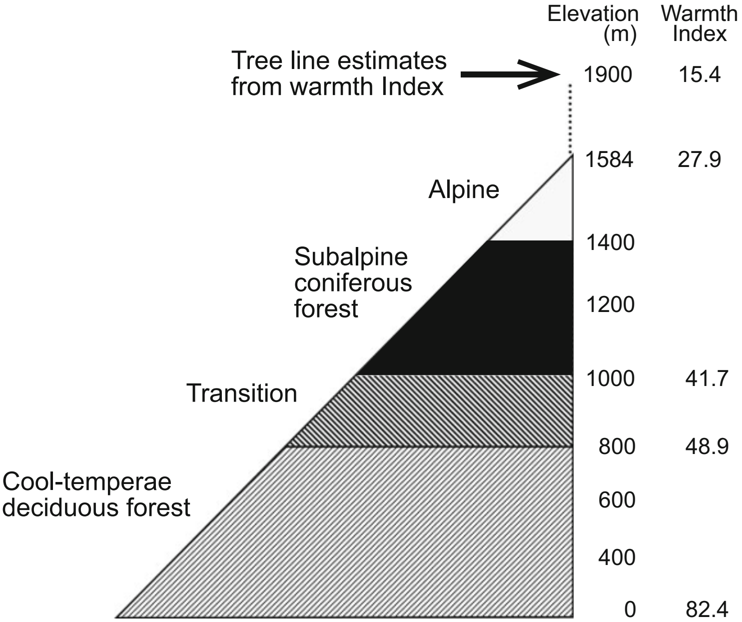


Fig. 1.1 Vertical distribution of vegetation types in the Hakkoda Mountains. The warmth index (see the text for details) is shown for the upper and lower boundaries of the vegetation types

high-altitude vegetation described later in this section. This kind of distribution along a temperature gradient is well explained by the warmth index (WI) that Kira (1977) proposed for the classification of climatic vegetation zones in humid East Asia. WI equals the annual sum of the positive differences between monthly mean temperatures and +5 °C; the index serves as a measure of the effective warmth that sustains plant growth during the growing season.

Several vegetation types other than cool deciduous forests and subalpine coniferous forests are present in the mountains. The types that grow to up to 3-m tall and that are distributed at elevations higher than 1400 m a.s.l. are dwarf forests of Japanese stone pine (*Pinus pumila*), shrubby deciduous vegetation, dwarf bamboo grasslands, and alpine meadows. These form mosaic landscapes with each other and with subalpine coniferous forests and form in response to local environmental conditions such as high elevation, steep topography, and the associated climatic conditions. Alpine meadows grow at the summits in the Hakkoda Mountains. At around 1400 m, *A. mariesii* forms a relatively abrupt tree line (Fig. 1.1).

At elevations of 600–1400 m a.s.l., many patches of moorland are found in depressions filled with volcanic ash created by a massive eruption during the Pleistocene epoch (Muraoka and Takakura 1988; Koike et al. 2005). *Abies mariesii* are distributed at the fringes of the moorland, even when this is lower than the usual lower limit of subalpine coniferous forests (Yamanaka et al. 1988).

The Hakkoda Mountains have among the deepest accumulations of snow in the world, reaching a depth of 3–5 m during the winter. The annual mean temperature and annual precipitation at the Sukayu weather station, in the middle of the mountain range (890 m a.s.l.), are 4–6 °C and 1300–2300 mm (Japan Meteorological Agency 1996).

Abies mariesii is a subalpine conifer that is endemic to Japan and is adapted to habitats with heavy snowfall (Kaji 1982; Sugita 1990). Although *A. mariesii* was not the dominant species during the last glacial period, when snowfall was light, its distribution expanded when snowfall began to increase during the Hypsithermal period (Morita 1985). Since other subalpine coniferous species gradually became locally extinct in many Japanese subalpine forests, particularly those in snowy regions, the snowy subalpine coniferous forests now consist only of *A. mariesii*. However, these forests are sparse or absent in some regions with extreme snowfall (Shidei 1956; Sugita 1992), even when thermal conditions (e.g., WI) are suitable. In the Hakkoda Mountains, *A. mariesii* only grows up to about 1400 m a.s.l (Fig. 1.2), even though its temperature tolerance could allow it to grow at elevations up to about 1900 m (Nogami 1994).

As many reports have suggested, temperatures have been rising in recent decades. At the Aomori weather station (2.8 m a.s.l.), on the northern coast of Honshu, the mean annual temperature has increased by about 0.7 ± 0.5 °C from 1977 to 2003 based on the mean linear trend with 95 % confidence limits (Fig. 1.3a). Annual rainfall at Sukayu (890 m a.s.l.) has increased in recent decades, but at Aomori, there has been no clear trend (Fig. 1.3b). The maximum snow depth showed no clear trend at either station, although the interannual fluctuation at

Fig. 1.2 Forest at the upper limit of *Abies mariesii*. The tree line is fairly sharp at about 1400 m a.s.l.; vegetation communities dominated by shrub species, dwarf bamboo, or alpine plants are distributed above the tree line

Sukayu has tended to increase since 2000 (Fig. 1.3c). However, since the mean temperature has increased, the snowmelt date may have become earlier, and this change would affect tree physiology through its effects on phenological responses.

1.3 Population Dynamics of Subalpine Coniferous Trees in Recent Decades

We investigated differences in the population density and canopy height of *A. mariesii* between 1967 and 2003 by analyzing high-resolution aerial photographs of the Hakkoda Mountains (Shimazaki et al. 2011). We defined 712 plots, each 25 × 25 m, in the photographs and analyzed which environmental variables (elevation, slope aspect, wetness, and distance from the nearest moorland) accounted for the observed changes in the distribution of this species. Since *A. mariesii* is almost the only evergreen tree species in this area, it was easy to identify the species in the photographs.

Our key finding from this analysis is that the population density of *A. mariesii* has decreased significantly in the plots below 1000 m a.s.l. and has increased significantly in the plots above 1300 m a.s.l. since 1967 (Fig. 1.4). The mean elevation, weighted by population density, was 1190 m a.s.l. in 1967 versus 1209 m a.s.l. in 2003; this represents an increase of 0.53 m per year. Of the other

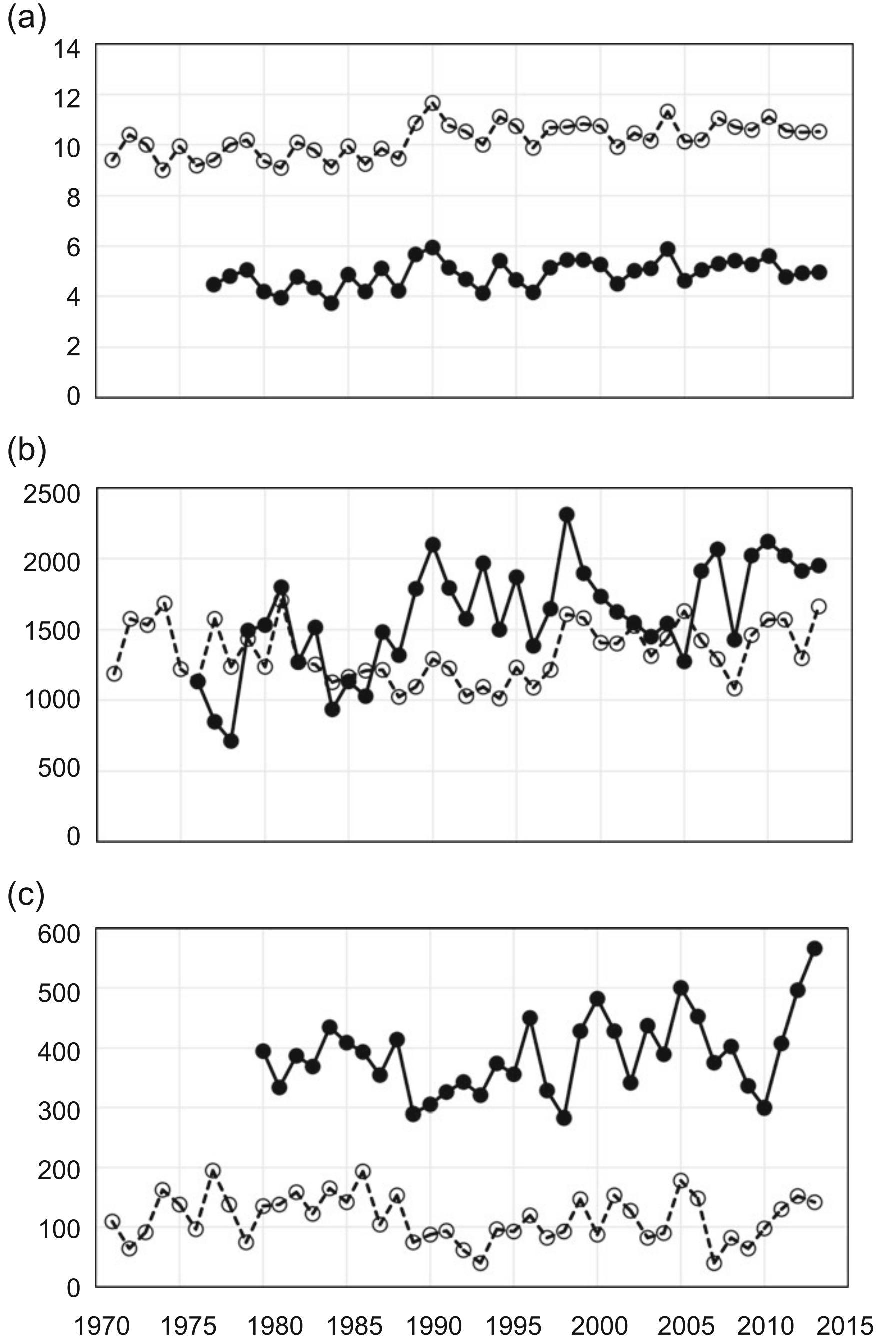


Fig. 1.3 The changes in climatic conditions around the Hakkoda Mountains since 1970. (**a**) Annual mean temperature, (**b**) annual rainfall, and (**c**) maximum snow depth since the 1970s at Aomori (2.8 m a.s.l., *open circles*), a coastal station north of the study area, and at Sukayu (890 m a.s.l., *solid circles*), in the center of the mountainous study area

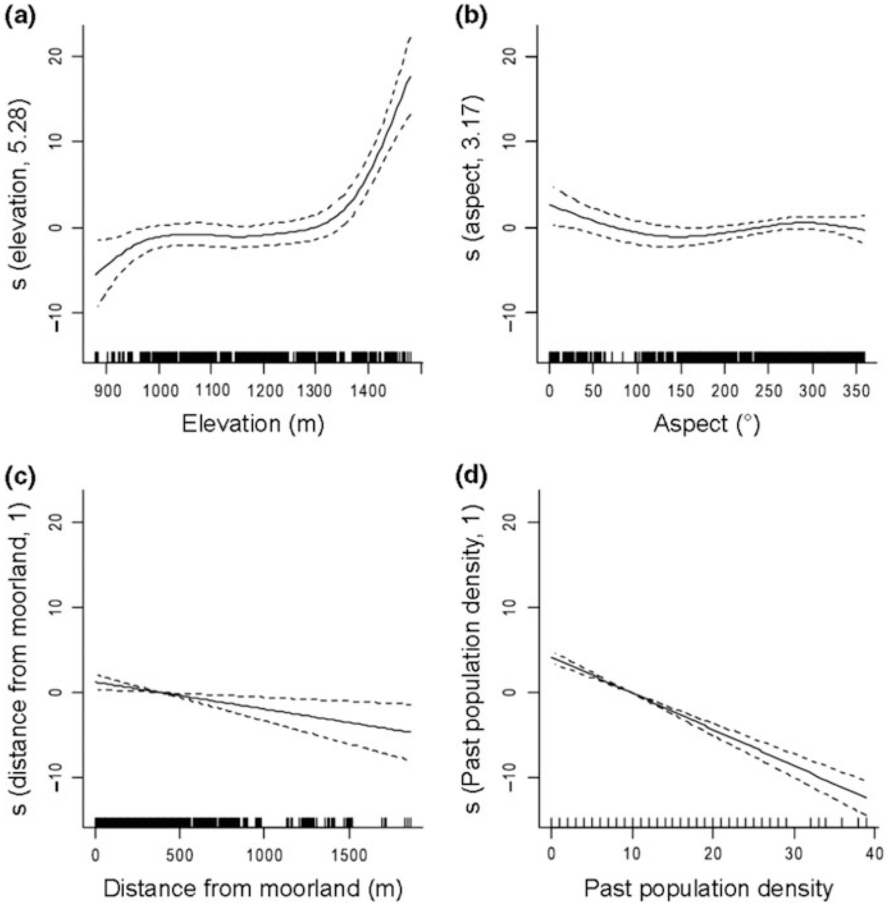


Fig. 1.4 Partial residual plots of the smoothed relationships between changes in the population density of *Abies mariesii* in a generalized additive mixed model as a function of (**a**) elevation, (**b**) slope aspect, (**c**) distance from the nearest moorland, and (**d**) the past population density. *Dashed lines* indicate the 95 % confidence intervals (Modified after Shimazaki et al. 2011)

factors in our analysis, slope aspect did not seem to affect population density, but the population density decreased significantly with increasing distance from moorland and with increasing past population density. Such upward shifts of mountain plants have been commonly reported in Europe and North America (Beckage et al. 2008; Lenoir et al. 2008). Although the cause of the shift in the distribution limits is not clear, Gehrig-Fasel et al. (2007) suggested that a significant increase in forest cover at the upper distribution limit of forests in the Swiss Alps was partly attributable to climate change.

In the Hakkoda Mountains, the decrease in tree density below 1000 m a.s.l. seems to be most strongly associated with climate change, although the factors that contributed to the density increase above 1300 m a.s.l. are debated. Pollen analysis suggests that *A. mariesii* forests in the Hakkoda Mountains became established about 600 years ago (Morita 1985), and the upper limit of the species' distribution predicted based on temperature is expected to be much higher than the current distribution limit (Nogami and Ohba 1991). At high altitudes, an increase in temperature and a decrease in snowfall or in the duration of snow cover have promoted the expansion of *A. mariesii* into areas that were previously unsuitable for the species due to heavy snowfall (Daimaru and Taoda 2004).

The increase in the population density of *A. mariesii* was particularly pronounced at high elevation (Fig. 1.4a) and near moorlands (Fig. 1.4c). The latter finding suggests that the zones around the moorlands represent potential refugia for *A. mariesii* (Fig. 1.5). If *A. mariesii* growing around the moorlands represents remnant populations that have survived since the last glacial period, their population density should not have increased. Since *A. mariesii* has a higher tolerance for the perhumid (and probably more acidic) soil environments of the moorlands than *F. crenata*, which is dominant at warmer, lower elevations (Sugita 1992), *A. mariesii* may thrive better than *F. crenata*, which usually outcompetes *A. mariesii* in low-elevation environments (Yamanaka et al. 1988). Murach and Ulrich (1988) observed a similar relationship between European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*): root growth of *F. sylvatica* was much more sensitive to low pH than that of *P. abies*. These results indicate that acidic mineral soils strongly inhibit the growth of beech trees (Marschner 1991).

Fig. 1.5 An *Abies mariesii* population distributed around a moorland, located at about 800 m a.s.l. The deciduous broadleaf trees (mostly *Fagus crenata*) are distributed farther from the moorland

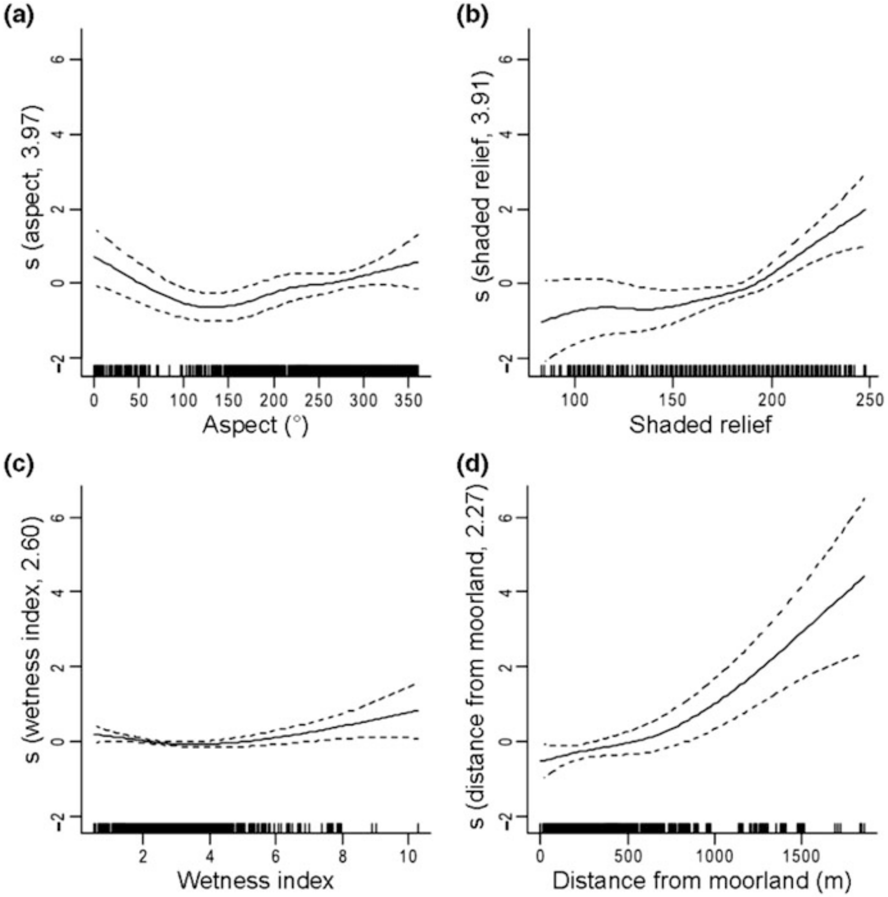


Fig. 1.6 Partial residual plots of the smoothed relationships between changes in the canopy height of *Abies mariesii* in a generalized additive mixed model as a function of (**a**) slope aspect, (**b**) shaded relief, (**c**) the wetness index, and (**d**) distance from the nearest moorland. *Dashed lines* indicate the 95 % confidence intervals (Modified after Shimazaki et al. (2011))

Thus, the growing conditions around moorlands would have prevented the growth of beech trees and provided unoccupied habitats for *A. mariesii*.

We also found that the rate of increase in canopy height was lowest on southeast-facing slopes and close to moorlands (Fig. 1.6a–d). The slow height growth of *A. mariesii* on the southeast-facing slopes (Fig. 1.6a) may be due to the detrimental effect of snowdrifts, which form during winter monsoons that approach from the northwest and that deposit large amounts of snow on the opposite side of the peaks as a result of orographic effects. *Abies mariesii* cannot survive the pressure created by deep snowdrifts as they shift downhill under the influence of gravity because it cannot adopt a creeping (prostrate) growth form (Shidei 1956). In addition, the growing season of *A. mariesii* tends to be shorter in areas with deep snow deposits.

Thus, even if snowfall has decreased since 1967 at a landscape scale, snowdrifts at local scales caused by interactions between the winter monsoon and the mountain topography are likely to limit the height growth of *A. mariesii*.

Interestingly, although the population density of *A. mariesii* increased near moorlands (Fig. 1.4c), the trees grew more slowly there (Fig. 1.6d). Takaoka (1999) investigated the age structure of *A. mariesii* thickets in forest–meadow ecotones in snowy mountains and found that the short canopy height of *A. mariesii* did not suggest an early stage of succession, but instead suggested the effects of adverse environmental conditions, such as heavy snow. As a result, mature *A. mariesii* trees in the forest–meadow ecotones were shorter than those in neighboring forests. We hypothesize that a similar mechanism has probably inhibited the height growth of *A. mariesii* trees near moorlands, where the soil is poorly drained and acidic. These areas do not provide *A. mariesii* with favorable growing conditions. Yet, despite the unfavorable conditions, the much higher sensitivity of *F. crenata* to such conditions probably allowed the moorlands to offer potential refugia for *A. mariesii* populations.

Thus, the populations around the moorlands do not seem to be remnants, but rather increasing populations, which suggest that the areas surrounding the moorlands are potential refugia for *A. mariesii*. However, *A. mariesii* grew more slowly in these areas. On this basis, we suggest that it is not necessary for potential refugia to provide better growing conditions for a species so long as they promote its survival.

1.4 Predicted Distribution Shift of the Subalpine Forest

We constructed a habitat model for *A. mariesii* in the Hakkoda Mountains at a 50-m spatial scale using a decision-tree model with one climate variable (WI), two topographic variables (slope and slope aspect), and two variables (distance and presence/absence) related to moorlands (Shimazaki et al. 2012; Figs. 1.7 and 1.8). In our model (Fig. 1.7), the most important explanatory variable that determined the distribution of *A. mariesii* was WI. The first divergence at WI = 46.5 °C was the dominant factor that defined the boundary between the distributions of *A. mariesii* and *F. crenata*, and this value was in close agreement with the value (WI = 47) estimated by Nogami and Ohba (1991) for this boundary at the scale of Honshu Island.

The second most important explanatory variable was the slope angle. The downhill creep of snow under gravity, which occurs on open slopes steeper than 15° (Leitinger et al. 2008), creates high pressure on tree stems and thus limits the distribution of *A. mariesii*. The third-most-important explanatory variable was the slope aspect. The distribution probability was lower on east-facing slopes, suggesting the detrimental effect of snowdrifts, which form under the influence of winter monsoons that approach from the northwest. *Abies mariesii* cannot survive the high pressures created by snowdrifts (Shidei 1956). In addition, the growing season for *A. mariesii* is shorter in areas with deep snow.

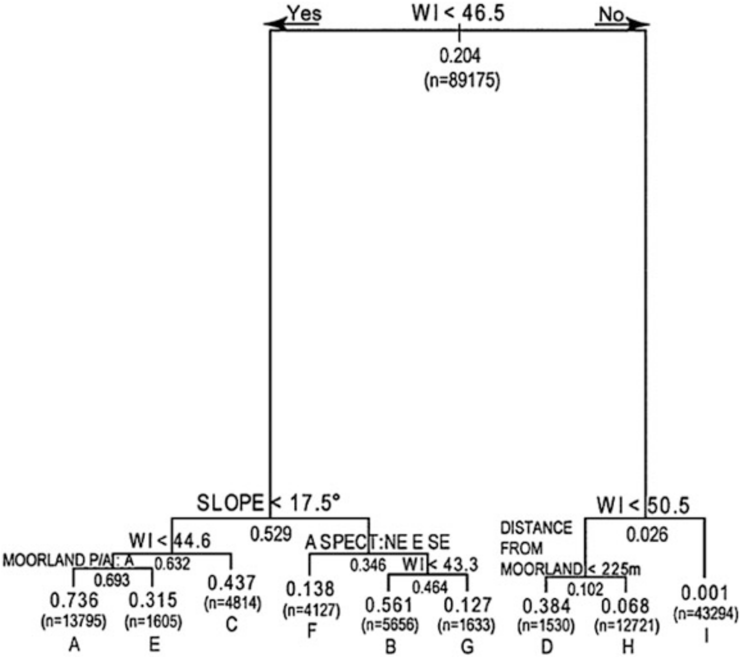


Fig. 1.7 The classification tree model for *Abies mariesii* at a 50-m spatial resolution. The conditions, occurrence probabilities, and number of cells (*n*) are shown at each node. If the condition is met, the left branch is followed; otherwise, the right branch is followed. The length of the *vertical lines* below each true–false split corresponds to the change in the magnitude of the deviance between the parent node (*above*) and the child node (*below*). The warmth index (*WI*), slope, slope aspect, distance from the nearest moorland, and moorland presence/absence (*P*/*A*) data were used as explanatory variables; other environmental variables were not included in this model (Modified after Shimazaki et al. (2012))

Previously, Yamanaka et al. (1988) suggested that *A. mariesii* might have extended its distribution toward lower elevations if there were no competitors. In fact, *A. mariesii* has a higher tolerance for the moist and acidic soil environments around moorlands than beech (Sugita 1992), which would enable it to thrive around moorlands even at WI values higher than 46.5. Shimazaki et al. (2012) applied this classification tree model to several scenarios: warming scenarios (up to +4 °C above present temperatures) and independently for scenarios in which the distribution of moorland decreased or remained stable in response to predicted climate change. The scenario with decreasing moorland was based on a habitat model of moorland as well as on the distribution of *A. mariesii*. Although the suitable areas were notable in the +2 °C scenario, all suitable habitats disappeared in the +4 °C scenario. The distribution of *A. mariesii* will also decrease with a lower temperature increase if the moorland areas simultaneously decrease (Fig. 1.9). Based on the results of this modeling, we conclude that it will be effective to conserve the

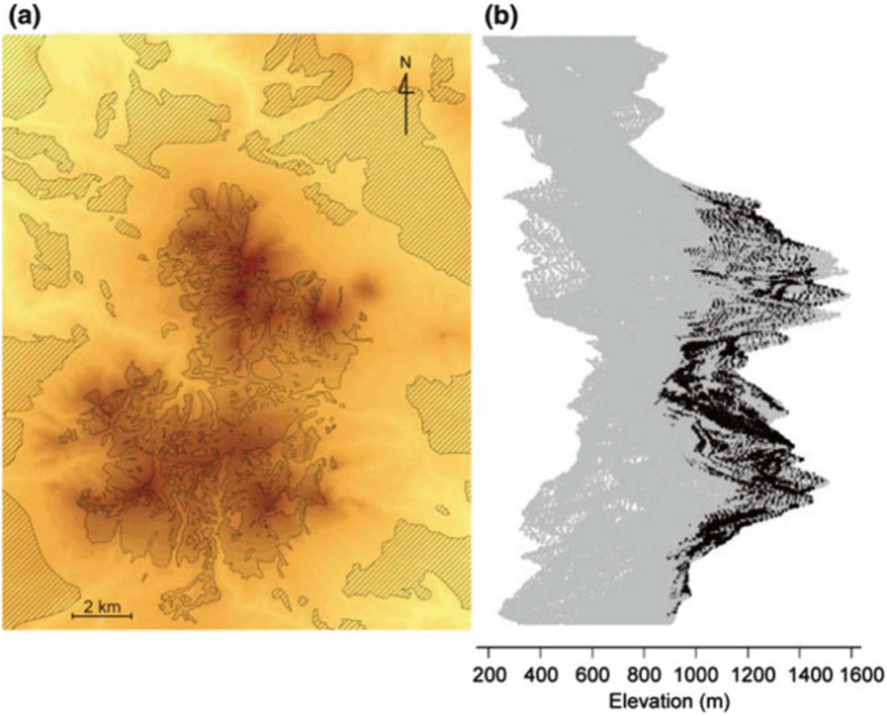


Fig. 1.8 Horizontal and vertical distributions of cells where *Abies mariesii* was present (**a**, *dotted polygons*; **b**, *black dots*) or absent (**a**, outside of the *black dotted polygons*; **b**, *gray dots*) based on aerial photographs. The *background shading* represents the topography of the study area; *darker shades* indicate higher elevations. *Crosshatched areas* depict farms or tree plantations (Modified after Shimazaki et al. (2012))

A. mariesii populations growing around the moorlands that are most likely to persist after global warming, as well as to conserve the moorlands themselves.

1.5 Conclusions

It is clear that the climate change that has occurred since 1967 has already affected the *A. mariesii* populations of subalpine forests in the Hakkoda Mountains. Since 1967, the distribution of *A. mariesii* has shifted about 20 m toward higher elevations. Our high-resolution model revealed the most important factors that are determining the distribution of *A. mariesii* and that can be used to predict its future distribution. Analyses of population and community changes suggested the importance of temperature, mesoscale topography, and fine-scale local environmental gradients associated with snow deposition. Our results also suggest that the

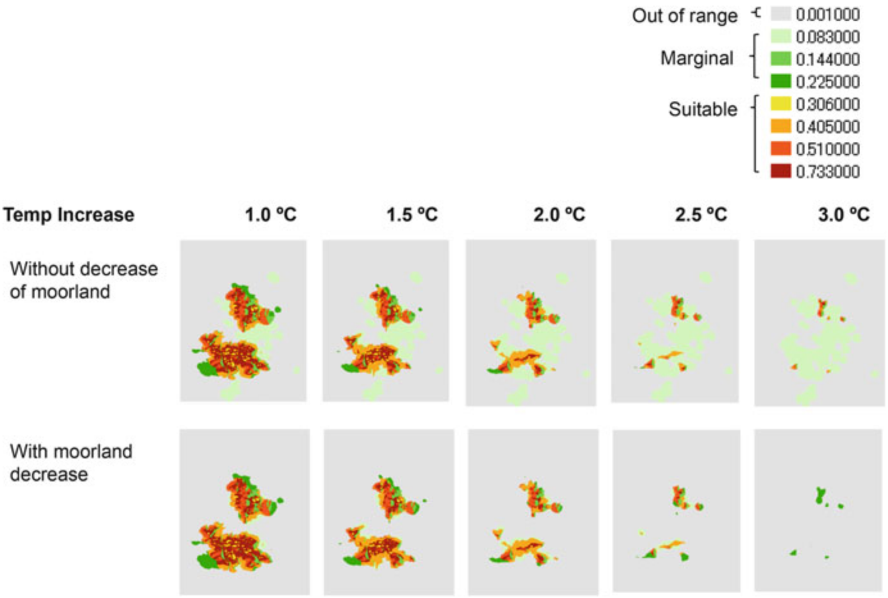


Fig. 1.9 Predicted distributions of potential habitats for *Abies mariesii* under climate change scenarios with temperature increases of 1.0, 1.5, 2.0, 2.5, and 3 °C from present levels and the simultaneous effects of stable (*top*) and decreasing (*bottom*) moorland. The values in the scale represent the probability that a site is suitable for *A. mariesii* (Modified after Shimazaki et al. (2012))

peripheries of moorlands provide effective potential refugia for *A. mariesii* in the Hakkoda Mountains.

Many assessments of the impacts of climate change on vegetation and animals have been made at coarse spatial scales. However, the present results show the importance of accounting for the influence of factors that operate at finer scales and that the assessment and application of adaptation measures will require high-resolution models that incorporate non-climatic variables such as topography and the heterogeneity of landscape units in addition to climatic variables. Although most subalpine areas of Japan have been protected by the government as nature conservation areas, some of these ecosystems will be very sensitive to climate warming (Nogami 1994; Tanaka et al. 2009), and one of the most important adaptation measures for these ecosystems will be the identification of likely population refugia. The present results and other findings from recent studies need to be applied to guide future conservation efforts that account for these and other adaptation measures.

Acknowledgments This study was financially supported by the Ministry of Education, Culture, Sports, Science and Technology (Sousei D), Japan Society for the Promotion of Science (26281049), and The Mitsui & Co., Ltd. Environment Fund (R11-G4-1046).

References

Araújo MB, Thuiller W, Williams PH, Reginster I (2005) Downscaling European species atlas distributions to a finer resolution: implications for conservation planning. Glob Ecol Biogeogr 14:17–30

Ashcroft MB, Chisholm LA, French KO (2009) Climate change at the landscape scale: predicting fine-grained spatial heterogeneity in warming and potential refugia for vegetation. Glob Chang Biol 15:656–667

Beckage B, Osborne B, Gavin DG, Pucko C, Siccama T, Perkins T (2008) A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. Proc Natl Acad Sci U S A 105:4197–4202

Daimaru H, Taoda H (2004) Effect of snow pressure on the distribution of subalpine *Abies mariesii* forests in northern Honshu Island, Japan. J Agric Meteorol 60:253–261

Gehrig-Fasel J, Guisan A, Zimmermann NE (2007) Tree line shifts in the Swiss Alps: climate change or land abandonment? J Veg Sci 18:571–582

Horikawa M, Tsuyama I, Matsui T, Kominami Y, Tanaka N (2009) Assessing the potential impacts of climate change on the alpine habitat suitability of Japanese stone pine (*Pinus pumila*). Landsc Ecol 24:115–128

Huntley B, Berry PM, Cramer W, McDonald AP (1995) Special paper: modelling present and potential future ranges of some European higher plants using climate response surfaces. J Biogeogr 22:967–1001

Iverson LR, Prasad AM (1998) Predicting abundance of 80 tree species following climate change in the eastern United States. Ecol Monogr 68:465–485

Japan Map Center (1998) Numerical map user guide, 2nd version. Japan Map Center, Tokyo

Japan Meteorological Agency (1996) Climate normals for Japan. Japan Meteorological Agency, Tokyo

Kaji M (1982) Studies on the ecological geography of subalpine conifers: distribution pattern of *Abies mariesii* in relation to the effect of climate in the postglacial warm period. Bull Tokyo Univ For 72:31–120 (in Japanese with English abstract)

Kira T (1977) A climatological interpretation of Japanese vegetation zone. In: Miyawaki A, Tuexen R (eds) Vegetation science and environmental protection. Maruzen, Tokyo, pp 21–30

Koike K, Toshikazu T, Chinzei K, Miyagi T (2005) Regional geomorphology of Japanese Islands, geomorphology of Tohoku region, vol 3. University of Tokyo Press, Tokyo

Leitinger G, Ho¨ller P, Tasser E, Walde J, Tappeiner U (2008) Development and validation of a spatial snow-glide model. Ecol Model 211:363–374

Lenoir J, Gegout JC, Marquet PA, de Ruffray P, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. Science 320:1768–1771

Marschner H (1991) Mechanisms of adaptation of plants to acid soils. Plant Soil 134:1–20

Matsui T, Yagihashi T, Nakaya T, Taoda H, Yoshinaga S, Daimaru H, Tanaka N (2004) Probability distributions, vulnerability and sensitivity in *Fagus crenata* forests following predicted climate changes in Japan. J Veg Sci 15:605–614

Matsui T, Takahashi K, Tanaka N, Hijioka Y, Horikawa M, Yagihashi T, Harasawa H (2009) Evaluation of habitat sustainability and vulnerability for beech (*Fagus crenata*) forests under 110 hypothetical climatic change scenarios in Japan. Appl Veg Sci 12:328–339

Morita Y (1985) The vegetational history of the subalpine zone in northeast Japan II. The Hachimantai mountains. Jpn J Ecol 35:411–420 (in Japanese with English abstract)

Murach D, Ulrich B (1988) Destabilization of forest ecosystems by acid deposition. GeoJournal 17:253–259

Muraoka H, Takakura S (1988) Explanatory text of the geological map of the Hakkôda Geothermal area, Geological Survey of Japan, Tsukuba

Nogami M (1994) Thermal condition of the forest vegetation zones and their potential distribution under different climates in Japan. J Geogr 103:886–897 (in Japanese with English abstract)

Nogami M, Ohba H (1991) Japanese vegetation seen from warmth index. Kagaku 61:39–49 (in Japanese)

Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) (2007) IPCC, 2007: Climate Change 2007: Impacts, adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, 976pp

Shidei T (1956) A view on the cause of the lack of coniferous forest zone in subalpine area on some mountains in the Japan sea side. J Jpn For Soc 38:356–358 (in Japanese)

Shimazaki M, Sasaki T, Hikosaka K, Nakashizuka T (2011) Environmental dependence of population dynamics and height growth of a subalpine conifer across its vertical distribution: an approach using high-resolution aerial photographs. Glob Chang Biol 17:3431–3438

Shimazaki M, Tsuyama I, Nakazono E, Nakao K, Konoshima M, Tanaka N, Nakashizuka T (2012) Fine-resolution assessment of potential refugia for a dominant fir species (*Abies mariesii*) of subalpine coniferous forests after climate change. Plant Ecol 213:603–612

Sugita H (1990) Consideration on the history of the development of the *Abies mariesii* forest during postglacial time based on its distributional character. Jpn J Hist Bot 6:31–37 (in Japanese with English abstract)

Sugita H (1992) Ecological geography of the range of the *Abies mariesii* forest in northeast Honshu, Japan, with special reference to the physiographic conditions. Ecol Res 7:119–132

Takaoka S (1999) Stability of subalpine forest-meadow boundary inferred from size and age structure of *Abies mariesii* thickets on a Japanese snowy mountain. J For Res 4:35–40

Tanaka N, Nakazono E, Tsuyama I, Matsui T (2009) Assessing impact of climate warming on potential habitats of ten conifer species in Japan. Glob Environ Res 14:153–164

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. Proc Natl Acad Sci U S A 102:8245–8250

Trivedi MR, Berry PM, Morecroft MD, Dawson TP (2008) Spatial scale affects bioclimate model projections of climate change impacts on mountain plants. Glob Chang Biol 14:1089–1103

Tsuyama I, Nakao K, Matsui T, Higa M, Horikawa M, Kominami Y, Tanaka N (2011) Climatic controls of a keystone understory species, *Sasamorpha borealis*, and an impact assessment of climate change in Japan. Ann For Sci 68:689–699

Yamanaka M, Sugawara K, Ishikawa S (1988) A historical study of the Abies mariesii forest found in the montane zone in the south Hakkoda mountains, northeast Japan. Jpn J Ecol 38:147–157 (in Japanese with English abstract)

Chapter 2
Trait-Based Approaches for Understanding Species Niche, Coexistence, and Functional Diversity in Subalpine Moorlands

Kouki Hikosaka, Takehiro Sasaki, Chiho Kamiyama, Masatoshi Katabuchi, Shimpei Oikawa, Masaya Shimazaki, Hiroshi Kimura, and Tohru Nakashizuka

Abstract Moorland is an interesting vegetation because (1) it has high diversity with various coexisting species of different functional groups; (2) the coexistence of species is conserved for long time without large disturbances; (3) it is isolated from other moorlands by forest areas, where dispersal of species is expected to be influenced by spatial distribution and size of moorlands; and (4) the environmental factors vary among moorlands, which enable us to determine how these environmental factors affect species composition. We analyzed species diversity of plant communities in subalpine moorlands as well as underlying mechanisms using trait-based approaches to reveal that landscape-level species diversity is mainly a function of between-moorland level species diversity. Species composition and

---

K. Hikosaka (✉)
Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan

CREST, Japan Science and Technology Agency, Tokyo, Japan
e-mail: hikosaka@m.tohoku.ac.jp

T. Sasaki
Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba, Japan

Department of Biology, Faculty of Science, Chiba University, 1-33 Yayoicho, Inage-ku, Chiba 263-8522, Japan

C. Kamiyama
Institute for the Advanced Study of Sustainability, United Nations University, Shibuya, Tokyo, Japan

M. Katabuchi
Department of Biology, University of Florida, Gainesville, FL, USA

S. Oikawa
College of Science, Ibaraki University, Mito, Ibaraki, Japan

M. Shimazaki • T. Nakashizuka
Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan

H. Kimura
Tohoku Ryokka Kankyohozen Co., Ltd, Sendai, Japan

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_2

community-level functional traits were influenced by environmental factors such as temperature and soil water characteristics. We analyzed light acquisition efficiency of species belonging to different functional groups and found a seasonal niche separation for light use between functional groups, facilitating species coexistence. Furthermore, we found that species diversity in moorland plant communities conforms to a spatially nested structure. By assuming that the nested rank of species is the potential order of species loss, our simulation analysis demonstrated that vulnerability is considerably different among moorlands, which implies that functional diversity can be a useful measure for the planning of conservation strategies.

Keywords Functional groups • Functional traits • Leaf traits • Moorland • Resource acquisition efficiency • Species coexistence

2.1 Introduction

More than 200,000 vascular plant species coexist on Earth. This fact appears to contradict the principle of competitive exclusion. If all other ecological factors are constant, this principle dictates that two species competing for the same resources cannot coexist (Gause 1934; Hardin 1960), because most of plant species require common resources for growth and reproduction, as has been indicated for phytoplankton (Hutchinson 1961). There is no single ubiquitously dominant species, and the distributions of all species have limits. Furthermore, many plant species often coexist within the same habitat. The question of why a particular plant species can or cannot coexist with other species is one of the most fundamental questions in plant ecology.

An approach based on traits of plant species may be one of the most useful methods to solve this question. Many researchers have recently characterized species by values of functional traits and attempted to understand community assembly. This approach may successfully incorporate ecophysiological knowledge into community ecology. In the present paper, we discuss how the trait-based approach has been developed and then introduce our recent studies on plant communities in subalpine moorlands. We have studied species composition and functional traits in moorlands, which were found to be modified by environmental variables. Analyses on resource acquisition efficiency revealed that functional traits contribute to niche separation within and between plant communities. We also studied functional diversity as a function of species extinction, which may be useful as a tool for conservation.

2.2 Functional Classification and Trait-Based Approach

One of the most accepted explanations for the coexistence of various species is the niche separation. The morphological and physiological traits vary among species considerably. Such functional differentiations result in niche separation among species, leading to a limitation in the distribution of a species. The first functional classification of plant species was conducted by Raunkiær (1904, 1937), who classified plants according to the position of the growth point (i.e., bud) of plant during seasons with adverse conditions. His classification was modified to life form, which is currently one of the most important functional classifications of plants. In general, species are broadly classified into woody, herbaceous, and other plants. Woody species are further categorized into trees and shrubs according to their maximal height. Herbaceous species are divided into forbs and grasses according to the shape of their leaves. The concept of functional type or functional group (hereafter FG) has been recently developed (McIntyre et al. 1999). FG considers not only life form but also other physiological and phenological traits, for example, photosynthetic systems (C_3/C_4/CAM), nitrogen fixation, and leaf habits (evergreen or deciduous).

Although attempts at plant classification have considered FG and other concepts in a qualitative or discrete manner, many researchers have tried to characterize plants in a quantitative manner. In community ecology, organisms have been characterized by intrinsic rates of population growth (r) and carrying capacity (K), assuming that population dynamics follow logistic function (MacArthur and Wilson 1967). This idea may be applicable to successional change in species composition; in other words, early successional species may be associated with a higher r value, whereas late successional species may have a higher K value. However, r and K values have not necessarily been used to characterize plant species.

Grime (1977) proposed that plant strategy is a blend of three strategies labeled as C (competitive), S (stress tolerant), and R (ruderal). Competitors are species that are dominant in a habitat characterized by a low intensity of stress and disturbance. Stress tolerators are species that can survive in habitat characterized by a high intensity of stress and less frequent disturbance. Ruderals are species that rapidly occupy disturbed areas. Grime (1977) innovated a method to classify species in a quantitative manner, where species were plotted in a triangle and the measures for the three strategies were quantitatively expressed on each side of the triangle. Grime et al. (1990) characterized many plant species in the United Kingdom based on this concept. However, his method requires intensive experimental procedures such as a growth experiments and was not easy for application to a number of species.

Advances in the field ecophysiology have enabled us to accumulate data of various traits of plants growing in the field. Reich and his coworkers determined leaf traits for a number of species in various biomes and demonstrated that there are tight correlations among leaf traits across species (Reich et al. 1991, 1992, 1997). Furthermore, these traits were also correlated with functional variables at the whole plant and ecosystem scales, such as plant growth rates and annual net primary production (Reich et al. 1992). Wright et al. (2004) compiled a data set from more than 2000 species (1 % of vascular plant species) and demonstrated that such correlation is conserved at a global scale. Thus, variation in leaf traits can roughly be expressed on an axis (leaf trait economics); on the one end of the axis, there are species with higher photosynthetic capacity per unit leaf mass and higher leaf nitrogen concentration, stomatal conductance and respiration rate, lower leaf mass per area (LMA), and shorter leaf life span (LLS), and the opposite on the other end of the axis. This axis is correlated with FGs; for example, herbaceous species tend to have higher photosynthetic rates than woody species and ruderals tend to have higher photosynthetic rates but shorter LLS than competitors and stress tolerators.

Similar concepts are applicable to other plant tissues (Westoby 1998; Westoby and Wright 2006). For stems, maximum height and stem density may express competitiveness and stress tolerance, respectively. For seeds, seed size may express a trade-off between dispersal ability and establishment (larger seeds may store more resources for survival after germination but are less dispersed because of weight). Since these variables are easily measured, we can obtain values from a number of species. Several data sets have been presented (e.g., Kattage et al. 2011).

Ackerly and Cornwell applied a trait-based approach to community ecology (Ackerly and Cornwell 2007; Cornwell and Ackerly 2009). They characterized species by trait values and expressed plant communities as a collection of species with particular trait values. They plotted species trait values against environmental variables of the community, which successfully and quantitatively express how trait values are related to variations in species niches. This idea enables us to discuss community assembly based on trait values of species.

2.3 Characteristics of Moorland Plant Communities

Moorland is a type of wetland in the broad classification of the Ramsar Convention. Moorland generally occurs in cool climate in which peat is accumulated with low pH (Proctor and Maltby 1998; Tsuyuzaki et al. 2004) because of a low decomposition rate caused by anaerobic and cool temperature conditions (Brady and Weil 1998). Peat accumulation in moorlands contributes sustainably to global carbon sinks (Matthews and Fung 1987; Aselmann and Crutzen 1989), but these sinks are vulnerable to environmental change, especially to global warming (Gorham 1991; Chapin et al. 2000).

Ecosystems associated with moorland are very interesting field for ecological research from several viewpoints. First, moorlands are associated with high species diversity. In general, moorland ecosystems contain unique communities, often with a large number of endemic and threatened species (Wahren et al. 1999; Lienert et al. 2002; Galeuchet et al. 2005; Hajkova et al. 2006). Moorlands generally possess different vegetation types, bog and fen, depending on the hydrology, pH, and trophic state even within one site (Schimper 1903; Wheeler and Proctor 2000). Bog is characterized by oligotrophic conditions and dominance of acidophilic mosses, particularly *Sphagnum* species, while fen is characterized by mesotrophic conditions and dominance of grasses and/or sedges (Mitsch and Gosselink 2007). Species composition also varies between sites (Mochida et al. 1997). Many species belonging to different functional groups coexist, such as evergreen/deciduous, woody/herbaceous, and grass/forb species.

Second, species composition has been remained over a long period without large disturbances. Pollen analyses have suggested that similar species composition has been maintained for a thousand year in some moorlands (Yonebayashi 1996, 2001). This suggests that species interaction may be in a steady state, in contrast to forest communities, where disturbance plays an important role for species composition in the communities. Therefore, the moorland plant community presents a good opportunity to investigate mechanisms of species coexistence.

Third, moorlands are often isolated by forested areas and are therefore comparable to islands isolated by the ocean. Many moorland species cannot inhabit forest, and therefore, dispersion of such species between moorlands is strongly limited. We can expect that spatial distribution and size of moorlands strongly influence species composition in the moorlands, similar to islands (MacArthur and Wilson 1967).

Finally, moorlands in mountainous ranges are located within various elevations, where environmental factors such as nutrient availability and pH vary among moorlands. These variations enable us to study relationships between vegetation and environmental factors. In particular, elevation is one of the most powerful "natural experiments" for testing ecological and evolutionary responses of biota to geophysical influences, such as low temperature (Körner 2007).

2.4 Case Studies of Moorland in the Hakkoda Mountain Range

2.4.1 Study Site

The Hakkoda mountain range (the highest point is 1584 m above sea level at 40°39′N, 140°52′E) locates in Aomori Prefecture, northern Japan (Fig. 2.1). There are many moorlands in this area, most of which were created in depressions in the volcanic ash layer that settled on the ground subsequent to a huge eruption during the Pleistocene and parts of which were created in snowy patches along gentle slopes (Muraoka and Takakura 1988; Koike et al. 2005). Moorlands in this region are located at various elevations ranging from 600 to 1300 m, which provide a variation of air temperature of more than 3 °C. This altitudinal variation corresponds to an increase in global temperature until the end of this century predicted by the Intergovernmental Panel on Climate Change (2013). Thus, we can use this temperature gradient as a natural experiment of plant responses to global warming.

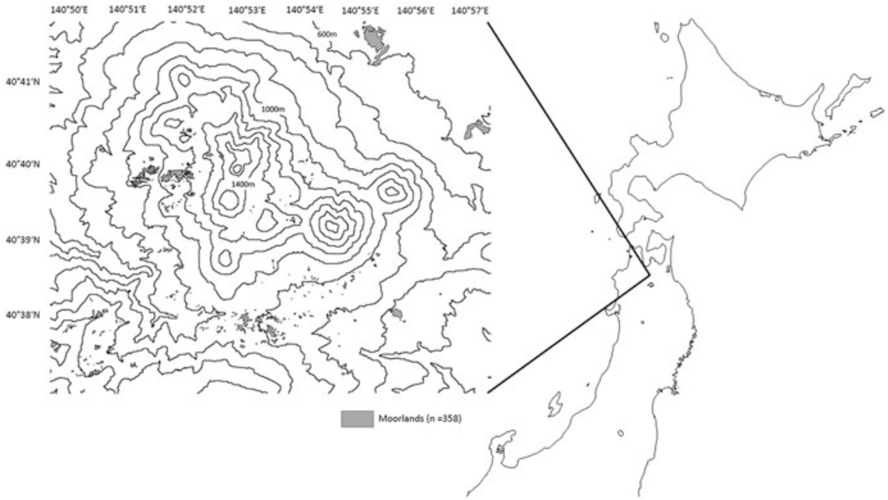


Fig. 2.1 Map showing the distribution of moorlands ($n = 358$) in the Hakkoda mountain range, in Aomori Prefecture, northern Japan. *Contour lines* represent 100-m increments in altitude (Modified from Sasaki et al. (2013))

2.4.2 Plant Species Diversity in Moorlands

In the Hakkoda area, several studies have described species composition of individual moorlands (e.g., Mochida et al. 1997). However, there is no comprehensive study of species diversity at a landscape level. We selected 28 moorlands in the Hakkoda area. Five quadrates (1 × 1 m) in each of six transects per moorland (total 30 quadrats per moorland) were established, and coverage of species was recorded in each quadrat. The studied moorlands differed markedly in size (range 1,000–160,000 m^2) and were isolated from each other to varying extents within an inhospitable matrix (i.e., forests). We used an additive diversity partitioning framework and quantified diversity patterns of moorland plant communities across hierarchical spatial scales, within-sample transects (α diversity) and between-sample transects (β_1 diversity), and between sites (β_2 diversity) and evaluated the contribution of each diversity measure to landscape-level diversity (γ diversity). In total, 98 vascular plant species were identified in the studied moorlands (γ diversity). Figure 2.2 shows the contribution of α, β_1 and β_2 diversity to the γ diversity. Contribution of β_2 diversity was greater than that of α and β_1 diversity, indicating that the landscape-level species diversity in this area was mainly explained by the between-moorland diversity (Sasaki et al. 2012a).

We studied relationships between species composition and environmental factors (Sasaki et al. 2013). From the cluster analysis, we identified six plant community types. Indicator species analysis identified characteristic species for each community type: (1) *Sieversia pentapetala* and *Rhynchospora yasudana*, (2) *Primula nipponica* and *Juncus kamschatcensis*, (3) *Schizocodon soldanelloides* and *Narthecium asiaticum*, (4) *Gaultheria adenothrix* and *Nephrophyllidium crista-*

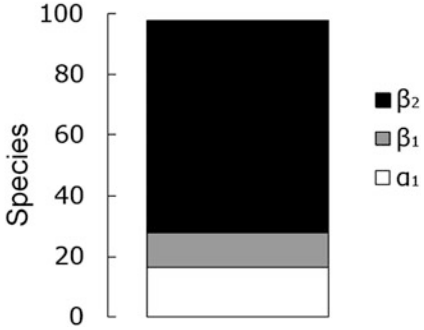


Fig. 2.2 Additive partitioning of diversity (expressed as species richness) across hierarchical spatial scales (Drawn based on data shown in Sasaki et al. (2012a))

galli, (5) *Moliniopsis japonica* and *Coptis trifolia*, and (6) *Vaccinium oxycoccos* and *Eriophorum vaginatum*. The differences in species composition among these community types were mainly related to the variations in soil solution pH and electrical conductivity (EC) and in elevation and temperature (Fig. 2.3), indicating that species composition varies depending on environmental factors.

2.4.3 Community Ecology of Functional Traits: Variations in Functional Traits Within and Among Moorland Plant Communities

Ackerly and Cornwell (2007) studied functional traits of species in plant communities along a precipitation gradient and analyzed relationships between traits and environmental factors. They applied a concept of habitat filtering to their analysis, which assumes that only species that have a trait value within a certain range can survive in the environment and that the successful range differs depending on environmental factors (Cornwell and Ackerly 2009). We applied this concept to our moorland sites (Kamiyama et al. 2014a).

We determined three leaf traits, namely, maximum leaf height, leaf mass per area (LMA), and leaf size, for 72 out of 98 species found in 27 moorlands. Maximum leaf height represents the ability of species to capture light in the community (Westoby 1998). LMA represents an investment of leaf resources in stress tolerance and is strongly correlated with photosynthetic traits such as photosynthetic capacity and leaf nitrogen concentration (Westoby 1998; Hikosaka 2004; Hikosaka and Shigeno 2009). Leaf size as related to boundary layer resistance helps to maintain favorable leaf temperatures and is subject to allometric constraints of plant size, shoot size, leaf morphology, or size of reproductive organs (Milla and Reich 2011). In this study, we also classified species into four FGs according to presence of wintering leaves (evergreen or deciduous) and wintering stems (woody or herbaceous). Values of functional traits may classify species quantitatively, whereas FG classifies species qualitatively or discretely. We characterized the

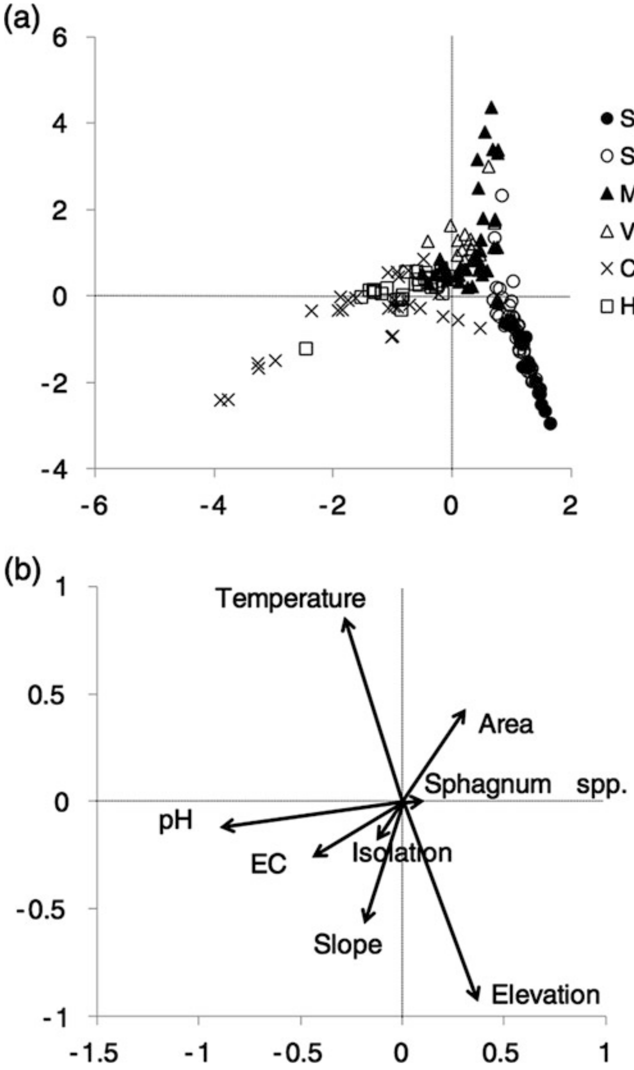


Fig. 2.3 Diagrams of canonical correspondence analysis ordination: (**a**) species composition of each community type and (**b**) environmental vectors (including the total cover of Sphagnum spp.). Eigenvalues for the first and second axis were 0.50 and 0.23, respectively. The abbreviations for community types are *Sipe-Rhya Sieversia pentapetala* and *Rhynchospora yasudana*, *Scso-Naas Schizocodon soldanelloides* and *Narthecium asiaticum*, *Moja-Cotr Moliniopsis japonica* and *Coptis trifolia*, *Vaox-Erva Vaccinium oxycoccos* and *Eriophorum vaginatum*, *Cath-Phau Carex thunbergii* and *Phragmites australis*, and *Hosi-Liho Hosta sieboldii* and *Ligularia hodgsonii* (Redrawn from Sasaki et al. (2013))

environments of moorlands using principle component analysis (PCA) with four environmental variables: elevation, air temperature, soil pH, and EC of soil water. The first axis mainly represented altitude and air temperature, and the second axis represented pH and EC. Here we describe analysis on axis 1.

In Fig. 2.4, the *X* and *Y* axes represent the score of the first axis of PCA (higher values represent lower altitude and higher air temperature) and trait values,

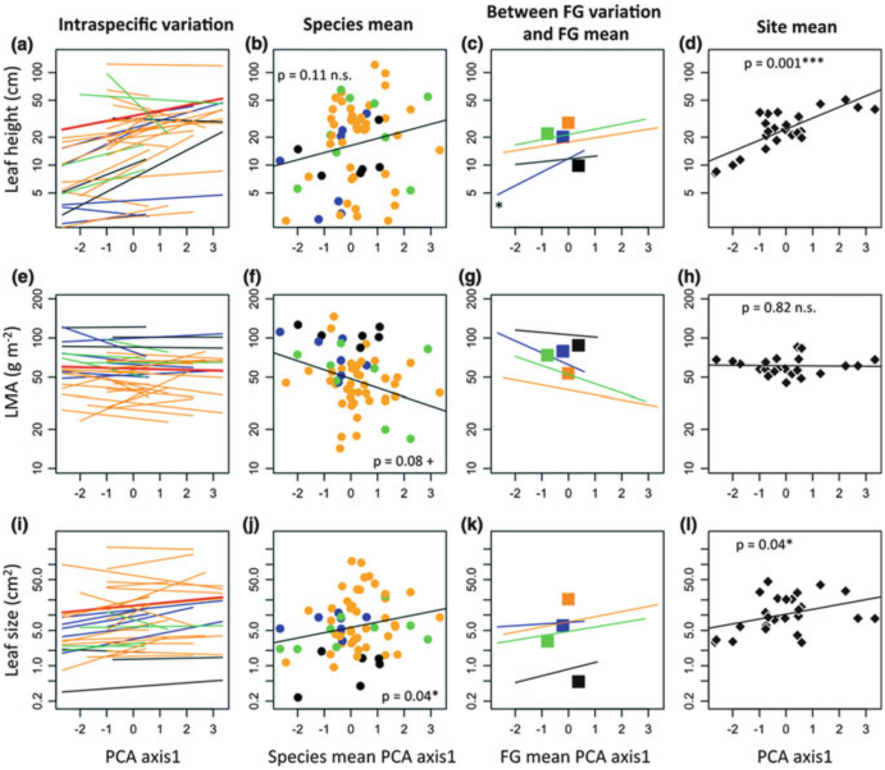


Fig. 2.4 The relationship between PCA 1 score and maximum leaf height (**a**–**d**), LMA (**e**–**h**) and leaf size (**i**–**l**). Panels (**a**), (**e**), and (**i**) show intraspecific variation in leaf traits in each species, where each *line* represents the least-squares fit across the PCA 1 score. Panels (**b**), (**f**), and (**j**) show interspecific variation in species-mean leaf traits with species-mean PCA 1, and each *line* shows the least-squares regression for the species-mean trait. Panels (**c**), (**g**), and (**k**) show within-functional group (*FG*) and among-FG variation in leaf traits, where each *line* represents the least-squares fit across the mean PCA 1 score of the species belonging to a certain FG and symbols represent FG-mean PCA 1 and FG-mean traits, respectively. Panels (**d**), (**h**), and (**l**) show site-mean trait value across PCA 1, and each *line* shows the least-squares regression. The significance of regression lines was tested with a *t*-test (+$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Colors of lines and symbols denote FGs: *orange*, deciduous herbs; *green*, deciduous shrubs; *blue*, evergreen herbs; *black*, evergreen shrubs. *Red lines* in panels (**a**), (**e**), and (**i**) denote the dominant species with the widest range of PCA 1, *Moliniopsis japonica* (deciduous graminoid) (Redrawn from Kamiyama et al. (2014a))

respectively. In Fig. 2.4a, e, and i, each curvilinear line denotes a linear regression for a single species that appeared in more than four moorlands. The slope of the curve signifies intraspecific variation along the environmental gradient. Many species exhibited significant environmental dependence in leaf trait values. In Fig. 2.4b, f, and j, each data point denotes a single species, where the mean value of the leaf traits of the species is plotted against the mean value of the PCA score that the species achieved. Species inhabiting higher altitudes tended to have higher LMA and smaller leaf size. In Fig. 2.4d, h, and l, each data point denotes single moorland and demonstrates the relationship between the mean trait value of a species in the community and the PCA score. The mean leaf height and leaf size decreased with increasing elevation. In Fig. 2.4c, g, and k, each data point and the curvilinear line denote mean values and regression line of each FG. The mean value of the PCA score did not vary among FGs, whereas there was environmental dependence in leaf traits within FGs.

Changes in trait values along an environmental gradient may involve intraspecific variation (phenotypic plasticity and/or between-population variations) and interspecific variation (replacement by species that have different trait values), both of which result in changes in the community traits related to ecosystem functions along an environmental gradient (Ackerly 2003; Ackerly and Cornwell 2007; Cornwell and Ackerly 2009). We were also interested in how much FG-level replacement and interspecific variation within FGs (within-FG variation) contribute to the change in community-level traits along environmental gradients. To quantify the relative contribution of intraspecific variation, species replacement, within-FG variation, and FG replacement to the community variation, we calculated hypothetical scaling slopes of the community variation, where no variation in trait values within species or within FGs was assumed. Contribution of within-species or within-FG variations was calculated as the difference between the actual and the hypothetical slopes. Figure 2.5 shows the relative contributions of intraspecific variation, species replacement, and FG replacement to the community-level slope of the relationship between leaf trait values and the PCA scores. Their contributions varied depending on the case. For maximum leaf height versus PCA 1, species replacement within FGs explained 65.3 % of the variation in the community-level slope and intraspecific variation explained most of the remainder; FG replacement contributed little to the community-level slope. Species replacement within FGs was in addition most responsible for leaf size versus PCA 1, whereas intraspecific variation accounted for most of LMA versus PCA 1. This trend was similar to the trait gradient along PCA 2, which mainly reflected from soil pH and EC. The contribution of FG replacement was greater along PCA 2 than PCA 1. In particular, for leaf size versus PCA 2, FG replacement made a greater contribution than that of intraspecific variation. These results suggest that all of the within-species variation, within-FG variation, and FG replacement contribute to the environmental dependence of trait values at the community level. However, their relative contribution varies depending on traits and environmental factors.

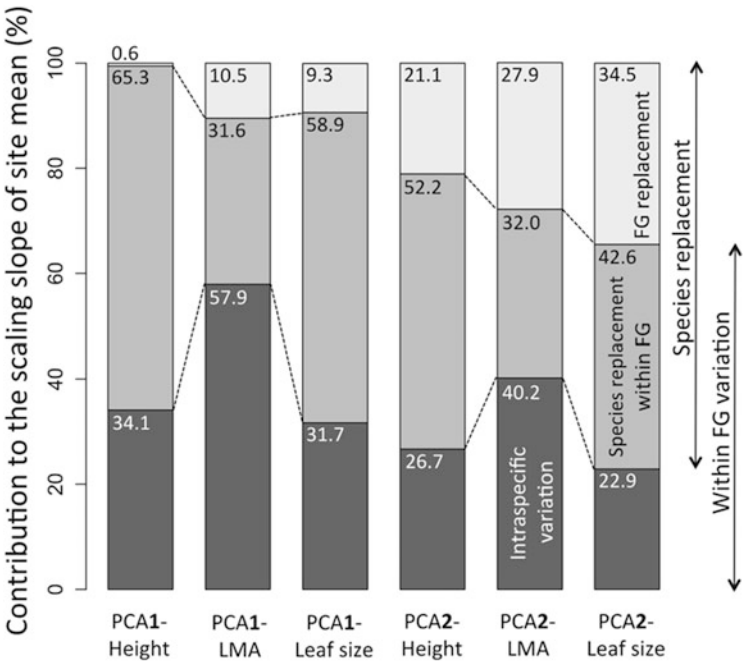


Fig. 2.5 The scaling slopes of each site-mean trait versus environment relationship attributed to intraspecific variation, species replacement, and functional group (*FG*) replacement, which are expressed as percentages of the increment from slopes with no species and FG variation considered (Redrawn from Kamiyama et al. (2014a))

2.4.4 The Ecophysiological Approach: How Do Functional Traits Contribute to the Coexistence and Replacement of Species?

Approaches from community ecology are able to reveal the empirical relationships between species distribution, trait values, and environmental factors, but are unable to clarify the underlying mechanisms. To determine how functional traits contribute to the coexistence in a plant community and to the niche separation, ecophysiological approaches are necessary.

In the field, species interaction is an important factor for survivorship and growth of individual species. For example, we can grow alpine plants at low elevation if the plants are separately grown in pots. However, if we transplant alpine plants to a plant community at low elevation, they would likely be eliminated from the community probably due to competitive exclusion. Because resources needed for plant growth and reproduction are limited in many ecosystems, plants that cannot acquire sufficient resources may be eliminated from the community. However, it should be noted that the absolute amount of acquired resources does not necessarily determine the existence of species within a community. For example, light acquired by understory shrubs is generally much less than that by tall trees, but they can survive and reproduce in the understory because they require much lower light intensity for growth than canopy trees. Plants can survive in a specific environment if the benefit of acquired resource is greater than the cost invested to acquire the resource. Resource acquisition efficiency, the ratio of acquired resource to the invested cost, is a useful measure to evaluate the cost–benefit balance.

Light acquisition efficiency (Φ_{mass}), which is the absorbed photon flux per unit aboveground mass, may be a good indicator of the species strategy for light acquisition, because it represents the ratio of benefit (light) to cost if the aboveground part of the plant is regarded as a light-harvesting structure (Hirose and Werger 1995). In monospecific stands, subordinate individuals have been shown to have a lower Φ_{mass} than dominant individuals (Anten and Hirose 1998; Hikosaka et al. 1999, 2003; Matsumoto et al. 2008), while Hirose and Werger (1995) reported that there was no significant difference in Φ_{mass} between dominant and subordinate species in a tall grassland where the species had coexisted for a long time. Werger et al. (2002) studied stands of a successional series after exclosure in heavily grazed grassland and found that the Φ_{mass} of early successional species was relatively lower than that of late successional species in the late successional stand, probably leading to species replacement. It is suggested that a similar Φ_{mass} may contribute to the coexistence of species in the community (Hirose and Werger 1995; Werger et al. 2002).

Light acquisition efficiency is affected by morphological traits of plants; therefore, Φ_{mass} can be decomposed as follows:

$$\begin{array}{ccccc} \Phi_{\text{mass}} & = & \Phi_{\text{area}} & \times & \text{LAR} \\ \dfrac{\text{Acquired light}}{\text{Aboveground mass}} & & \dfrac{\text{Acquired light}}{\text{Leaf area}} & & \dfrac{\text{Leaf area}}{\text{Aboveground mass}} \end{array}$$

$$\begin{array}{cccccc} = & \Phi_{\text{area}} & \times & \text{SLA} & \times & \text{LMR} \\ & \dfrac{\text{Acquired light}}{\text{Leaf area}} & & \dfrac{\text{Leaf area}}{\text{Leaf mass}} & & \dfrac{\text{Leaf mass}}{\text{Aboveground mass}} \end{array}$$

Φ_{area} (acquired light per leaf area) is the light intensity that the species absorbs, which is generally greater in taller plants in dense stands (Hirose and Werger 1995). SLA (specific leaf area) is the inverse of LMA. LMR (leaf mass ratio) quantifies the biomass allocation to leaves. In a heath stand, taller dominant plants had higher Φ_{area}, whereas shorter, subordinate plants had higher SLA and LMR, leading to a comparable Φ_{mass} values among the coexisting species (Hirose and Werger 1995). LMA and LMR vary among species, especially among FGs (Hirose and Werger 1995; Poorter et al. 2000; Valladares et al. 2002; Pearcy et al. 2005).

In spite of many studies analyzing Φ_{mass}, no study has determined Φ_{mass} in plant communities consisting of coexisting species with different FGs coexisted. As mentioned above, because multiple plant species of varying FGs coexist in the moorland communities, the moorland ecosystem is an ideal case study for investigating Φ_{mass}. We studied Φ_{mass} in moorlands at different elevations (Kamiyama et al. 2010) in which four or five quadrats were established in each of moorlands at 600, 1000, and 1300 m, and the stratified clipping method was applied during August when the stand biomass was at a maximum. The spatial distribution of leaf area and biomass were determined, and intercepted light was estimated. We found that evergreen species had lower Φ_{mass} due to higher LMA and lower leaf height (Fig. 2.6). Woody species had comparable Φ_{mass} to herbaceous species; they had higher Φ_{area} and leaf height, which offset the lower biomass allocation to leaves. These results revealed that Φ_{mass} was significantly different among FGs.

Although evergreen plants have relatively low Φ_{mass}, their longer leaf life span may be advantageous if we consider light acquisition efficiency at a leaf lifetime level. Furthermore, evergreen leaves may use light in a season when there are no deciduous leaves (Karlsson 1985). We studied temporal changes in spatial distribution of leaf area and light acquisition for species coexisting in the moorland communities (Kamiyama et al. 2014b). The results demonstrated that evergreen species received strong light during spring when leaves of deciduous plants were not developed. In addition, there was a trend that shorter species develop their leaves at earlier season in deciduous species. Thus, there was a seasonal partitioning of light between deciduous and evergreen species and between taller and shorter species. Figure 2.7 shows variables related to lifetime light acquisition plotted against maximum leaf height. SLA was greater in deciduous than in evergreen species and taller than in shorter species. Instantaneous light acquisition efficiency ($\Phi_{Dleafmass}$; daily light acquisition per unit leaf mass) during August was greater in deciduous than in evergreen species, whereas leaf life span was longer in evergreen than in deciduous species. Consequently, light acquisition efficiency at a leaf lifetime scale ($\Phi_{Lleafmass}$; lifetime light acquisition per unit leaf mass) was comparable between deciduous and evergreen species and between taller and shorter species. Our results clearly demonstrated that there is a phenological trade-off in light absorption between shorter and taller species and between evergreen and deciduous species. The former mainly utilize light during early spring, whereas the latter utilize light mainly during the summer.

At high elevation (1300 m), the leaf area development of dominant deciduous species was small, probably due to suppression by cool temperature or shorter growing season, and understory plants receive relatively strong light during August. This led to higher $\Phi_{Lleafmass}$ in evergreen than in deciduous species at high elevation (Fig. 2.8). This result suggests that shorter growing season may be advantageous for evergreen species that have photosynthesizing leaves throughout the season and may explain the reason why the number and the biomass of evergreen species were greater at higher elevation. Evergreen leaf habit is thus relatively advantageous in terms of lifetime light acquisition efficiency at higher elevations. Phenological light partitioning between evergreen and deciduous plants partly explains the reason not only for coexistence between species with different functional traits but also for species replacement across environmental gradients.

If the changes in community structure along decreasing elevation can be assumed to be comparable to changes in community structure toward future global

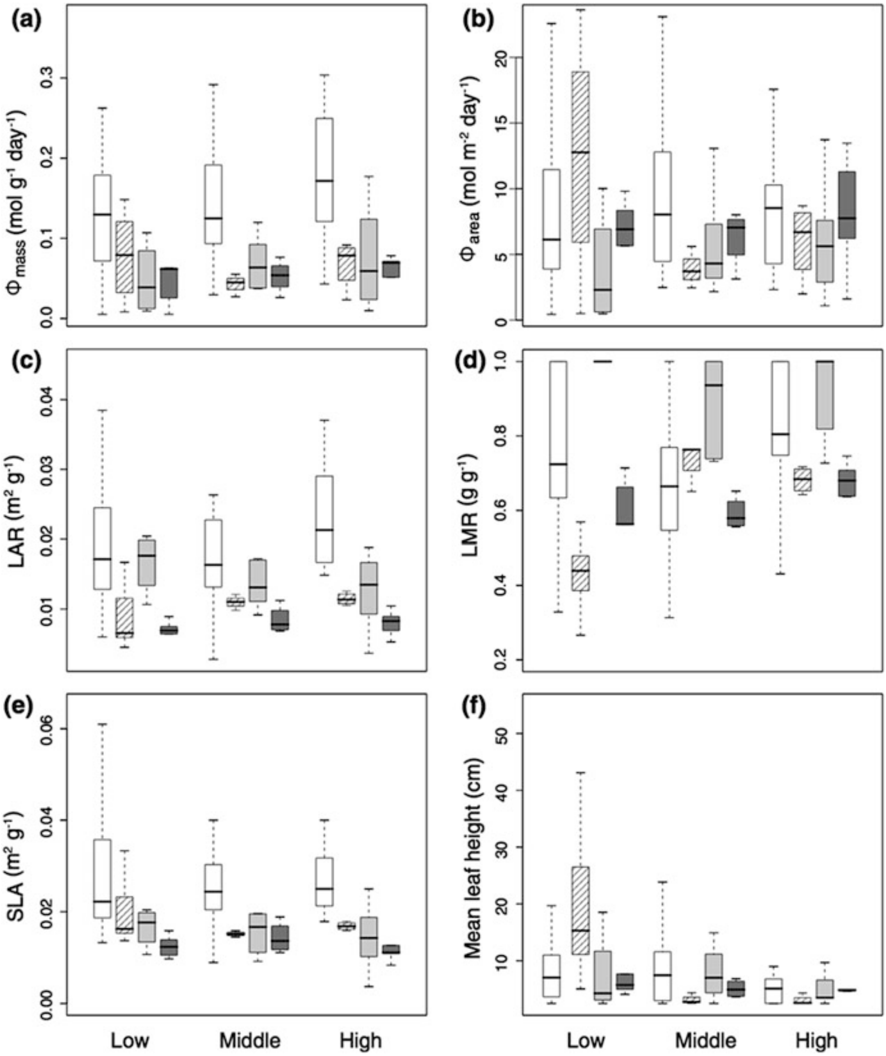


Fig. 2.6 Comparison of Φ_{mass} (photon flux density absorbed per unit aboveground mass of vegetative part, **a**), Φ_{area} (photon flux absorbed per unit leaf area, **b**), *LAR* (leaf area ratio, **c**), *LMR* (ratio of leaf biomass to the aboveground biomass, **d**), *SLA* (leaf area per unit leaf biomass, **e**), and mean leaf height (**f**) of each functional group at different elevations. *Open columns* represent deciduous herbs, *diagonally lined columns* represent deciduous shrubs, *gray columns* represent evergreen herbs, *solid black columns* represent evergreen shrubs at different elevations. The four leftmost columns denote low elevation, the four rightmost columns denote high elevation, and the middle four columns denote middle elevation. The *horizontal lines* of the column denote the 25th, 50th, and 75th percentile values, respectively, while the whiskers represent the range of values (Modified from Kamiyama et al. (2010))

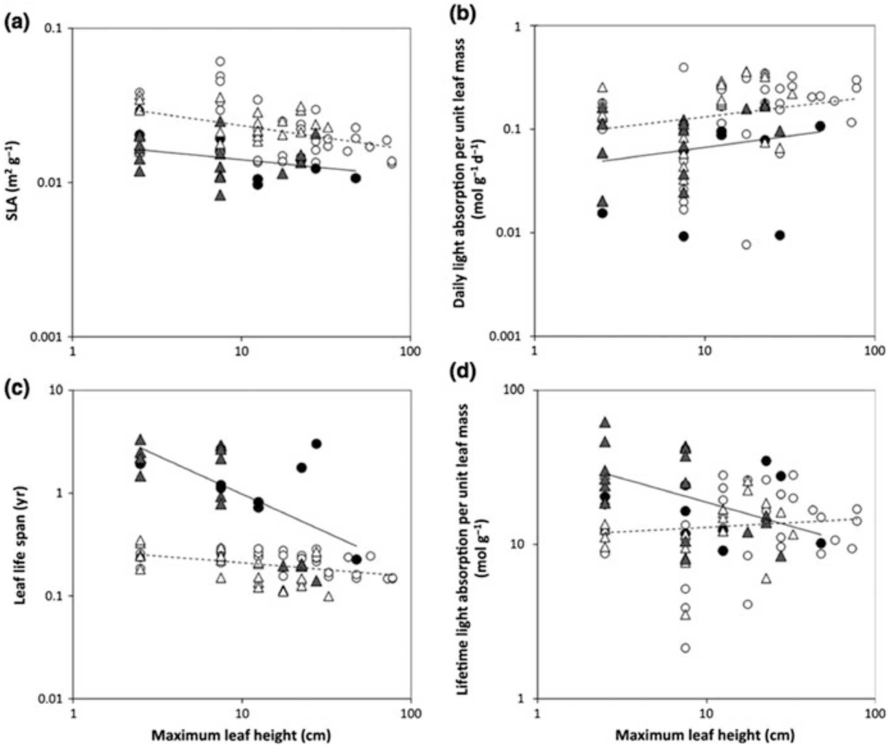
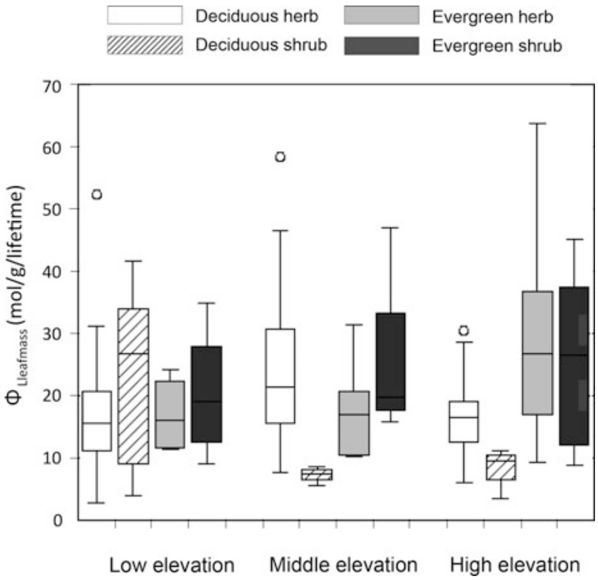


Fig. 2.7 Specific leaf area (*SLA*; **a**), daily light absorption per leaf mass ($\Phi_{Dleafmass}$; **b**), leaf life span (*LLS*; **c**), and lifetime light absorption per leaf mass ($\Phi_{Lleafmass}$; **d**), plotted against leaf maximum height. *Triangles* and *circles* denote species at high elevation and low elevation, respectively. *Solid* and *open symbols* denote evergreen and deciduous species, respectively. The *dashed line* is the regression of deciduous species at all three elevations (**a**, $y = 0.03x^{-0.14}$; **b**, $y = 0.08x^{0.25}$; **c**, $y = 0.29x^{-0.15}$; **d**, $y = 11.28x^{0.06}$), and the *solid line* denotes the regression of evergreen species at all three elevations (**a**, $y = 0.02x^{-0.10}$; **b**, $y = 0.08x^{0.25}$; **c**, $y = 5.76x^{-0.78}$; **d**, $y = 38.34x^{-0.31}$) (Modified from Kamiyama et al. (2014b))

warming, we will present some predictions about the future moorland ecosystem. Under global warming, moorland vegetation will be taller and denser, and leaf size will become larger, resulting in higher LAI. Such changes in community traits will be mainly accompanied by intraspecific variation of dominant species, which are often graminoids with deciduous leaves. Therefore, a few dominant species will continue to be abundant because of their wide distribution range along environmental gradients in moorlands. On the other hand, presence of other species within the plant community will be influenced. Species turnover across moorlands is certainly high, but such turnover will occur mainly in rare subordinate species (Sasaki et al. 2012a). The change in community structure will decrease light availability to a greater degree at the bottom layer of the community. Evergreen

Fig. 2.8 Leaf lifetime light acquisition efficiency of each functional group at different elevations (Modified from Kamiyama et al. (2014b))



species will be disadvantaged due to light competition because of the extremely shaded light condition and may finally be excluded from the community.

2.4.5 Vulnerability Assessment of Moorland Plant Communities

In the face of biodiversity loss under global change, conservation biologists have shifted their focus from single species to entire communities within a landscape, and the maintenance of functional aspects of communities, which is mostly driven by common species, has been considered to be important for the integrity and sustainability of communities (Olson et al. 2002; Grime 1998; Hillebrand et al. 2008). These perspectives necessitate the knowledge of scale-dependent phenomena, particularly spatial patterns of species diversity, because the distributions of organisms are the result of processes operating at both small and large spatial scales (Crawley and Harral 2001; Collins et al. 2002; Freckleton 2004). Hence, understanding of spatial structure of diversity within ecosystems is important for the consideration of conservation and management strategies of individual ecosystems under changing climate. As mentioned above, species diversity in the moorlands of the Hakkoda area is composed of the floristic differences among local moorlands (Fig. 2.2). This suggests that the protection of as many moorlands as possible is the most effective approach for the conservation of total diversity of this area. However, when the protection of all moorlands is not a realistic strategy for conservation, we need another measure that is useful for guiding decisions of conservation strategies.

Communities in isolated habitats are generally surrounded by inhospitable matrices, such as island communities surrounded by ocean, and often form a nested subset pattern. In a landscape with a nested pattern, species richness varies among communities. Species-rich communities include most of the species in the landscape, and species-poor communities consist of nested subsets of more species-rich communities (Atmar and Patterson 1993; Wright et al. 1998; Ulrich et al. 2009). Perfect nestedness occurs when all species in less species-rich sites are to be found in all of the more species-rich sites. Although perfect nestedness is rarely observed in nature, the degree of nestedness can be quantified and statistically evaluated through simulations based on null models (Jonsson 2001; Ulrich and Gotelli 2007; Ulrich et al. 2009). Nestedness analysis contributes to the understanding of mechanisms determining community development. It also provides information on whether a particular species in isolated and fragmented systems are moving toward extinction or colonization of new habitats (Wright and Reeves 1992; Azeria and Kolasa 2008; Ulrich et al. 2009).

In the analysis of nestedness of species diversity (Sasaki et al. 2012b), we detected significant nested structure of moorland plant communities (Fig. 2.9). The pH and moorland kernel density (proxy for spatial clustering of moorlands around the focal site) were the most important predictors of moorland site nested rank in a nestedness matrix (Fig. 2.10). The niche breadths of species (measured as variation in leaf mass area and height) predicted the nested ranks of species. Selective environmental tolerances imposed by environmental harshness and selective extinction caused by declines in site-carrying capacities probably account for the nested subset pattern observed in moorland plant communities. The nested rank of species in the nestedness matrix can therefore be translated into the potential order of species loss explainable by species niche breadths (based on variation in functional traits).

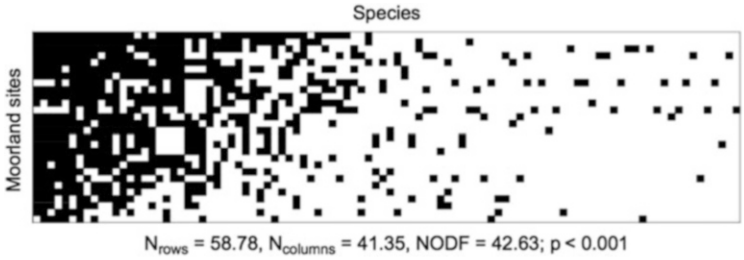


Fig. 2.9 Maximally packed species presence–absence matrix (where species and sites are ordered according to incidence and species richness) for samples from moorland plant communities arrayed across the Hakkoda mountain range, Aomori Prefecture, northern Japan. Filled cells indicate presence; white cells indicate absence. The measure of nestedness NODF varies from 0 (no nestedness) to 100 (perfect nestedness). N_{rows} and N_{columns} are a sum of the nestedness introduced by rows (sites) and by columns (species), representing the independent contributions of sites and species to NODF (total nestedness). The matrix is significantly nested under the constrained null model ($P < 0.001$) (Redrawn from Sasaki et al. (2012b))

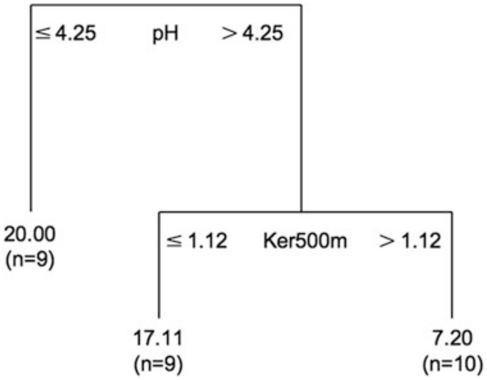


Fig. 2.10 The regression tree explaining nested ranking of moorland sites (the site ordered in the first row in the maximally ordered species presence–absence matrix has the highest rank, and the site ordered in the last row has the lowest rank; 1 = highest rank, 28 = lowest rank) by environmental variables in moorland sites (pH, EC, elevation, temperature, area, kernel densities of moorlands with 500, 1000 and 2000 m radii, isolation index, and coefficients of variation for pH and EC). Splitter variables (and their splitting values for $\log_{10}$-transformed quantitative variables) are given at each node. Values at the terminal ends of branches are mean nested ranks of moorland sites sharing the set of environmental conditions defined by the upper nodes: pH, pH of soil solution; Ker500 m, kernel densities of moorlands with a 500-m radius (Redrawn from Sasaki et al. (2012b))

Assuming the nested rank of species as the potential order of species loss, we assessed how species loss affects functional diversity in each moorland (Sasaki et al. 2014). Functional diversity (FD) is defined as the extent of trait differences among species in a given community (Petchey and Gaston 2002b, 2006) and generally assumed to be one of the best predictors of ecosystem functioning and persistence (Petchey and Gaston 2006; Cadotte et al. 2011). FD can be an especially strong tool for conservation when target ecosystem functions cannot be specifically defined and when comprehensive ecosystem functions and different aspects of biological diversity that cannot necessarily be quantified through direct measurements of functions (e.g., the timing of flowering) are of concern (Cadotte et al. 2011). Quantifying changes in FD according to the loss of species from natural systems can thus help to predict the consequences of species loss on the functioning and persistence of communities and ecosystems (Petchey and Gaston 2002a; Matsuzaki et al. 2013).

FD in each moorland was estimated based on trait data obtained from the above studies and other literatures. We assumed that the rate of decrease in FD caused by species loss in a given community represents its vulnerability to species loss in response to environmental changes, because a decrease in FD suggests a decline in the overall functioning and persistence of communities. We estimated FD in each moorland and simulated the vulnerability based on two patterns of species loss: (1) an ecologically plausible order of species loss derived from the nested subset pattern in the communities and (2) a randomized species loss order. The form of response of FD to species loss differed among moorlands (Fig. 2.11). In site A,

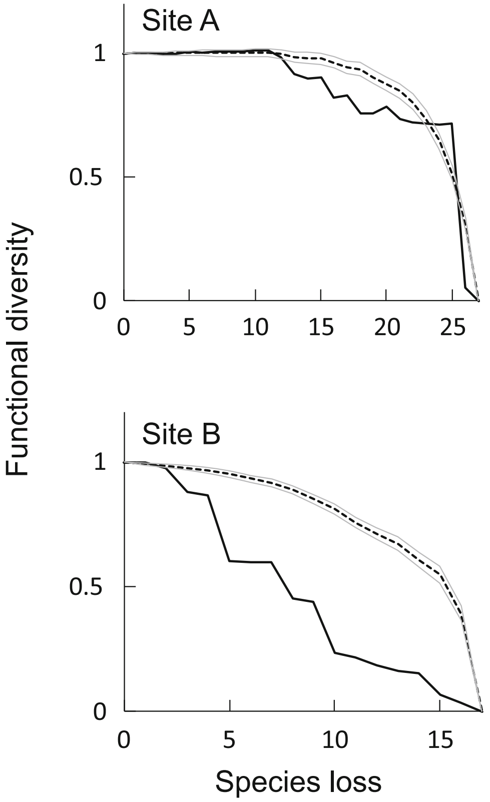


Fig. 2.11 Two examples of changes in functional diversity caused by simulated species loss at each moorland site based on two species loss orders: (1) an ecologically plausible order derived from the nested subset pattern in the moorland communities (*solid lines*) and (2) a randomized order (mean trajectories; *broken lines*). *Grey solid lines* indicate the 95 % CIs for the FD trajectories by random species loss. FD is expressed relative to its initial value, which was set to 1. See text for detail

ordered species loss caused a relatively small decrease in FD until a certain number of species were lost, and subsequently, FD decreased rapidly. The form of the responses of FD to random species loss was generally similar to that of ordered species loss at these sites. In contrast, at site B, ordered species loss caused an approximately proportional decrease in FD, whereas random species loss caused a relatively small decline in FD until a certain number of species were lost and an accelerating decline occurred thereafter. The vulnerability of plant communities is considered to be higher in site B than in site A.

The rate of decrease in FD by simulated species loss by the ecologically plausible order can be considered to represent the vulnerability of the community in each site to future environmental changes. We defined FD half-life as an index of vulnerability; the index is calculated as the number of species that must be lost to halve the original FD value (S′) divided by the original number of species at each site (S). An FD half-life close to 0 means a shorter half-life (Fig. 2.11, site B; in the case of a perfectly linear response), whereas that close to 1 means a longer half-life. We further analyzed the relationship between FD half-life and environmental variables. The best-fit model included elevation, kernel density of moorlands within a

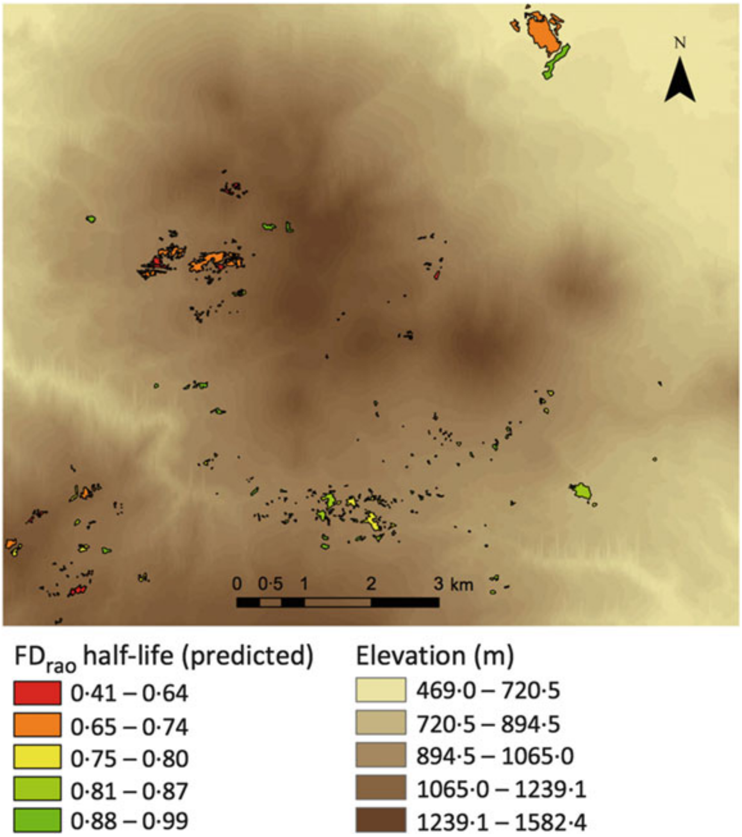


Fig. 2.12 Map showing the predicted value of FD half-life for each moorland within the Hakkoda mountain range (Redrawn from Sasaki et al. (2014))

500-m search radius, and isolation index as explanatory variables. There were significantly negative relationships between the FD half-life and elevation and kernel density and a positive relationship between the FD half-life and the isolation index. By applying the model to environmental data for individual moorlands, we mapped the predicted value of FD half-life across the entire landscape (Fig. 2.12).

In this study, we conducted fine-scale assessments of the vulnerability of moorland plant communities to species loss that is likely to occur under future environmental changes. Our key finding on the contrasting consequences of realistic species loss on FD among the sites agreed with previous studies, which suggested that the impact of species loss on ecosystem functioning depends on the interspecific differences in functional traits and in abundance within a given community (Petchey and Gaston 2002a, 2006). The vulnerability assessments of communities determined by combining FD and species loss simulations in an applied context, as presented here for moorland communities, can provide urgently needed information guiding how to act to conserve ecosystems in the face of global loss of biodiversity under environmental change.

2.5 Conclusions

Trait information enabled us to connect community ecology with ecophysiological knowledge. We studied species diversity in moorland communities using plant functional traits. Species composition was different between moorlands along environmental gradient (Sasaki et al. 2012a, 2013). We successfully described species replacement within and between functional groups by values of leaf traits (Sasaki et al. 2012b; Kamiyama et al. 2014a). Variation in leaf traits was related to the strategy of light acquisition (Kamiyama et al. 2010, 2014b). Evergreen and short-stature species tended to utilize light mainly during spring, whereas deciduous and tall-stature species utilized light predominately during summer, which enabled coexistence of species with different functional traits. However, the degrees of advantages of traits were different depending on the environment, which partly explain species replacement along the environmental gradient. We found that species diversity in moorland communities was significantly nested (Sasaki et al. 2012b). Assuming the nested rank of species as the potential order of species loss, we assessed vulnerability of moorland plant communities using a simulation of functional diversity loss (Sasaki et al. 2014). We found that vulnerability is considerably different among moorlands, which implies that functional diversity can be a useful measure for conservation strategy.

How functional traits are related to ecosystem functions is yet to be studied. In particular, the moorland ecosystem is an important sink of carbon. Since decomposition processes are also related to functional traits, functional variation in species diversity may affect the carbon cycle in moorland ecosystems. Furthermore, since peat accumulation is related to cool temperatures, responses to global warming are also an important issue in moorland studies. A combination of studies using altitudinal gradient and warming experiments may provide effective implications for predicting future changes in biodiversity and ecosystem functions of subalpine moorlands.

Acknowledgments We thank Koji Yonekura for advice on species identification, our laboratory colleagues for helping with field work, and land owner (Tashiro Farm) and government for permission of the study. This work was funded by the Global Environmental Research Fund of Japan's Ministry of the Environment (F-052, F-092 and D-0904), the Mitsui & Co., Ltd. Environment Fund, Tohoku University's Global COE program "Ecosystem Adaptability Science for the Future" (J03), JSPS research fellowship, and KAKENHI.

References

Ackerly DD (2003) Community assembly, niche conservatism, and adaptive evolution in changing environments. Int J Plant Sci 164:S165–S184

Ackerly DD, Cornwell WK (2007) A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. Ecol Lett 10:135–145

Anten NPR, Hirose T (1998) Biomass allocation and light partitioning among dominant and subordinate individuals in *Xanthium canadense* stands. Ann Bot 82:665–673

Aselmann I, Crutzen PJ (1989) Global distribution of natural freshwater wetlands and rice paddies, their net primary productivity, seasonality and possible methane emissions. J Atmos Chem 8:307–358

Atmar W, Patterson BD (1993) The measure of order and disorder in the distribution of species in fragmented habitat. Oecologia 96:373–382

Azeria ET, Kolasa J (2008) Nestedness, niche metrics and temporal dynamics of a metacommunity in a dynamic natural model system. Oikos 117:1006–1019

Brady N, Weil R (1998) The nature and properties of soils, 12th edn. Macmillan, New York

Cadotte MW, Carscadden K, Mirotchnick N (2011) Beyond species: functional diversity and the maintenance of ecological processes and services. J Appl Ecol 48:1079–1087

Chapin F, Zavaleta ES, Eviner V et al (2000) Consequences of changing biodiversity. Nature 405:234–242

Collins SL, Glenn SM, Briggs JM (2002) Effects of local and regional processes on plant species richness in tallgrass prairie. Oikos 99:571–579

Cornwell WK, Ackerly DD (2009) Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecol Monogr 79:109–126

Crawley MJ, Harral JE (2001) Scale dependence in plant biodiversity. Science 291:864–868

Freckleton RP (2004) The problems of prediction and scale in applied ecology: the example of fire as a management tool. J Appl Ecol 41:599–603

Galeuchet DJ, Perret C, Fischer M (2005) Performance of *Lychnis flos-cuculi* from fragmented populations under experimental biotic interactions. Ecology 86:1002–1011

Gause GF (1934) The struggle for existence. Williams & Wilkins, Baltimore

Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to climate warming. Ecol Appl 1:182–195

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169–1194

Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J Ecol 86:902–910

Grime JP, Hodgson JG, Hunt R (1990) Comparative plant ecology. Chapman & Hall, London

Hajkova P, Hajek M, Apostolova I (2006) Diversity of wetland vegetation in the Bulgarian high mountains, main gradients and context-dependence of the pH role. Plant Ecol 184:111–130

Hardin G (1960) The competitive exclusion principle. Science 131:1292–1297

Hikosaka K (2004) Interspecific difference in the photosynthesis-nitrogen relationship: patterns, physiological causes, and ecological importance. J Plant Res 117:481–494

Hikosaka K, Shigeno A (2009) The role of Rubisco and cell walls for the interspecific variation in photosynthetic capacity. Oecologia 160:443–451

Hikosaka K, Sudoh S, Hirose T (1999) Light acquisition and use by individuals competing in a dense stand of an annual herb, *Xanthium canadense*. Oecologia 118:388–396

Hikosaka K, Yamano T, Nagashima H, Hirose T (2003) Light- acquisition and use of individuals as influenced by elevated CO_2 in even-aged monospecific stands of *Chenopodium album*. Funct Ecol 17:786–795

Hillebrand H, Bennett DM, Cadotte MW (2008) Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. Ecology 89:1510–1520

Hirose T, Werger MJA (1995) Canopy structure and photon flux partitioning among species in a herbaceous plant community. Ecology 76:466–474

Hutchinson GE (1961) The paradox of the plankton. Am Nat 95:137–145

Intergovernmental Panel on Climate Change (IPCC) (2013) In: Stocker TF et al (eds) Climate change 2013 the physical science basis. Cambridge University Press, Cambridge

Jonsson BG (2001) A null model for randomization tests of nestedness in species assemblages. Oecologia 127:309–313

Kamiyama C, Oikawa S, Kubo T, Hikosaka K (2010) Light interception in species with different functional types coexisting in moorland plant communities. Oecologia 164:591–599

Kamiyama C, Katabuchi M, Sasaki T, Shimazaki M, Nakashizuka T, Hikosaka K (2014a) Leaf-trait responses to environmental gradients in moorland communities: plasticity, species replacement and functional group replacement. Ecol Res 29:607–617

Kamiyama C, Oikawa S, Hikosaka K (2014b) Seasonal change in light partitioning among coexisting species of different functional groups along elevation gradient in subalpine moorlands. New Phytol 204:913–923

Karlsson PS (1985) Photosynthetic characteristics and leaf carbon economy of a deciduous and an evergreen dwarf shrub: *Vaccinium uliginosum* L. and *V. vitis-idaea* L. Holarct Ecol 8:9–17

Kattage J, Díaz S, Lavorel S et al (2011) TRY—a global database of plant traits. Glob Chang Biol 17:2905–2935

Koike K, Toshikazu T, Chinzei K, Miyagi T (2005) Regional geomorphology of the Japanese Islands, vol 3, Geomorphology of Tohoku Region. University of Tokyo Press, Tokyo (in Japanese)

Körner C (2007) The use of 'altitude' in ecological research. Trends Ecol Evol 22:569–574

Lienert J, Fischer M, Diemer M (2002) Local extinctions of the wetland specialist *Swertia perennis* L. (Gentianaceae) in Switzerland: a revisitation study based on herbarium records. Biol Conserv 103:65–76

MacArthur R, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton

Matsumoto Y, Oikawa S, Yasumura Y, Hirose T, Hikosaka K (2008) Reproductive yield of individuals competing for light in a dense stand of an annual, *Xanthium canadense*. Oecologia 157:185–195

Matsuzaki SS, Sasaki T, Akasaka M (2013) Consequences of the introduction of exotic and translocated species and future extirpations on the functional diversity of freshwater fish assemblages. Glob Ecol Biogeogr 22:1071–1082

Matthews E, Fung I (1987) Methane emissions from natural wetlands: global distribution, area, and environmental characteristics of sources. Global Biogeochem Cycles 1:61–86

McIntyre S, Díaz S, Lavorel S, Cramer W (1999) Plant functional types and disturbance dynamics. Introduction. J Veg Sci 10:604–608

Milla R, Reich PB (2011) Multi-trait interactions, not phylogeny, fine-tune leaf size reduction with increasing altitude. Ann Bot 107:455–465

Mitsch WJ, Gosselink JG (2007) Wetlands, 4th edn. Wiley, London

Mochida Y, Sato M, Kikuchi T (1997) The vegetation of the south Hakkoda mountains, northern Honshu, Japan. Ecol Rev 23:295–300

Muraoka H, Takakura S (1988) Explanatory text of the geological map of the Hakkoda geothermal area. Geological Survey of Japan, Tsukuba (in Japanese)

Olson DM, Dinerstein E, Powell GVN, Wikramanayake ED (2002) Conservation biology for biodiversity crisis. Conserv Biol 16:1–3

Pearcy RW, Muraoka H, Valladares F (2005) Crown architecture in sun and shade environments: assessing function and trade-offs with a three-dimensional simulation model. New Phytol 166:791–800

Petchey OL, Gaston KJ (2002a) Extinction and the loss of functional diversity. Proc R Soc Lond Ser B Biol Sci 269:1721–1727

Petchey OL, Gaston KJ (2002b) Functional diversity (FD), species richness and community composition. Ecol Lett 5:402–411

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9:741–758

Poorter L, Kwant R, Hernandez R, Medina E, Werger MJA (2000) Leaf optimal properties in Venezuelan cold forest trees. Tree Physiol 20:519–526

Proctor M, Maltby E (1998) Relations between acid atmospheric deposition and the surface pH of some ombrotrophic bogs in Britain. J Ecol 86:329–340

Raunkiær C (1904) Om biologiske Typer, med Hensyn til Planternes Tilpasninger til at overleve ugunstige Aarstider. Bot Tidskr 34:1–13

Raunkiær C (1937) Plant life forms. Clarendon Press, Oxford

Reich PB, Walters MB, Ellsworth DS (1991) Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. Oecologia 86:16–24

Reich PB, Walters MB, Ellsworth DS (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecol Monogr 62:365–392

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. Proc Natl Acad Sci U S A 94:13730–13734

Sasaki T, Katabuchi M, Kamiyama C, Shimazaki M, Nakashizuka T, Hikosaka K (2012a) Diversity partitioning of moorland plant communities across hierarchical spatial scales. Biodivers Conserv 21:1577–1588

Sasaki T, Katabuchi M, Kamiyama C, Shimazaki M, Nakashizuka T, Hikosaka K (2012b) Nestedness and niche-based species loss in moorland plant communities. Oikos 121:1783–1790

Sasaki T, Katabuchi M, Kamiyama C, Shimazaki M, Nakashizuka T, Hikosaka K (2013) Variations in species composition of moorland plant communities along environmental gradients within a subalpine zone in northern Japan. Wetlands 33:269–277

Sasaki T, Katabuchi M, Kamiyama C, Shimazaki M, Nakashizuka T, Hikosaka K (2014) Vulnerability of moorland plant communities to environmental change: consequences of realistic species loss on functional diversity. J Appl Ecol 51:299–308

Schimper AFW (1903) Plant-geography upon a physiological basis. Clarendon Press, Oxford

Tsuyuzaki S, Haraguchi A, Kanda F (2004) Effects of scale-dependent factors on herbaceous vegetation patterns in a wetland, northern Japan. Ecol Res 19:349–355

Ulrich W, Gotelli NJ (2007) Null model analysis of species nestedness patterns. Ecology 88:1824–1831

Ulrich W, Almeida-Neto M, Gotelli NJ (2009) A consumer's guide to nestedness analysis. Oikos 118:3–17

Valladares F, Skillman JB, Pearcy RW (2002) Convergence in light capture efficiencies among tropical forest understory plants with contrasting crown architectures: a case of morphological compensation. Am J Bot 89:1275–1284

Wahren CH, Williams RJ, Papst WA (1999) Alpine and subalpine wetland vegetation on the Bogong High Plains, southeastern Australia. Aust J Bot 47:165–188

Werger MJA, Hirose T, During HJ et al (2002) Light partitioning among species and species replacement in early successional grasslands. J Veg Sci 13:615–626

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199:213–227

Westoby M, Wright IJ (2006) Land-plant ecology on the basis of functional traits. Trends Ecol Evol 21:261–268

Wheeler BD, Proctor MCF (2000) Ecological gradients, subdivisions and terminology of north-west European mires. J Ecol 88:187–203

Wright DH, Reeves JH (1992) On the meaning and measurement of nestedness of species assemblages. Oecologia 92:416–428

Wright DH, Patterson BD, Mikkelson GM, Cutler A, Atmar W (1998) A comparative analysis of nested subset patterns of species composition. Oecologia 113:1–20

Wright IJ, Reich PB, Westoby M et al (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Yonebayashi C (1996) Reconstruction of the vegetation at AD 915 at Ohse-yachi Mire, northern Japan, from pollen, present-day vegetation and tephra data. Plant Ecol 125:111–122

Yonebayashi C (2001) Reconstruction of local vegetation in AD 915 at an oligotrophic mire in northern Japan from pollen, present-day vegetation and tephra data. Ecol Res 16:275–288

Chapter 3
Landscape Structure of Flowering Phenology in Alpine Ecosystems: Significance of Plant–Pollinator Interactions and Evolutionary Aspects

Gaku Kudo

Abstract Spatiotemporal variation in flowering phenology at community scale is an important structure in alpine ecosystems. The composition of flowering species at a regional scale changes drastically on a weekly scale during the short summer. In early snowmelt year, flowering more often overlapped among species, and the whole flowering period was shorter than in usual year. The major pollinators in Japanese alpine ecosystems are flies and bees. Approximately 50 % and 30 % of insect-pollinated plant species were predominantly visited by flies and bees, respectively. Bee-pollinated flowers showed a dispersed flowering pattern in which anthesis of early bloomers corresponded with the active period of queen bumblebees, while that of late bloomers with the active period of worker bumblebees. Fly-pollinated flowers showed a peak in their flowering in the middle of the season when the ambient temperature was high. In an extremely warm summer, the flowering season finished earlier, and the synchrony of flowers and pollinators was disrupted because the life cycle of bees did not keep pace with the rapid progression of the flowering season. This phenological mismatch suggested a fragile relationship between plants and bees. Phenological isolation of pollen-mediated gene flow between local plant populations caused a spatial genetic structure within a local area. The restriction of gene flow could accelerate local adaptation. Because pollinator activity often increased as the season progressed, both fruit-set success and outcrossing rate increased in populations in late-snowmelt habitats. Such variations in reproductive success may cause life history variation at the local scale.

Keywords Alpine ecosystem • Flowering pattern • Landscape phenology • Local adaptation • Pollination • Snowmelt time

---

G. Kudo (✉)
Faculty of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan
e-mail: gaku@ees.hokudai.ac.jp

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_3

3.1 Introduction

Alpine ecosystems are characterized by cool temperature, strong winds, high radiation, and short growth period that restrict plant growth and reproduction (Billings and Bliss 1959; Körner 2003). Usually, phenological progress of alpine plants is largely determined by species-specific thermal requirement (Kudo and Suzuki 1999; Huelber et al. 2006; Wipf 2010; Kudo and Yokosuka 2012). In snowy mountain regions, however, snowmelt regime directly determines the time of growth initiation and growth period of alpine plants when snow remains even after the ambient temperature has increased above the lower limit for plant growth. Therefore, leafing and flowering of alpine plants progress sequentially along the local snowmelt gradient (Holway and Ward 1965; Kudo 1991). Furthermore, the species composition of plant communities varies highly in response to local snow conditions. Wind-exposed *fellfield* habitat located on ridges and summits with little snow and *snowbed* habitat with lingering snow are two basic components of alpine ecosystems, in which different types of vegetation are present (Miller 1982; Kudo and Hirao 2006). The species composition of alpine vegetation changes clearly along the snowmelt gradient (Kudo and Ito 1992; Heegaard 2002; Schöb et al. 2009), and this vegetation pattern creates complex and dynamic phenological structures at the landscape level (abiotic process in Fig. 3.1). However, the ecological significance of community-scale phenology structure has scarcely been examined in alpine ecosystems.

Time of flowering strongly influences the seed-set success of alpine plants. Because pollinator activity and availability usually increase as the season progresses, early flowering often results in pollen limitation for seed production due to low pollination service (e.g., Kudo 1993; Kudo and Suzuki 2001; Kudo and Hirao 2006; Thomson 2010). Furthermore, early flowering may increase the risk of frost damage early in the season (Inouye 2008; Wheeler et al. 2014). On the other hand, flowering too late may restrict the period for fruit development after flowering, resulting in the failure of seed production (Galen and Stanton 1991; Kudo 1991; Molau 1993). Therefore, both abiotic and biotic factors affect the seed-set success of alpine plants in association with variation in flowering phenology. There have been many studies on the variation in seed-set success of alpine plants with reference to flowering phenology, including both inter- and intraspecific comparisons. However, there have been few studies on pollination ecology in terms of the landscape structure of flowering phenology. Here, the term “landscape phenology” is used to indicate temporal variations in flowering time both among local populations of a single species and among plant communities within an area in which pollinators can move spatially. Because pollinators can move spatially over multiple plant communities within a region, plant species may compete for pollinator acquisition not only within a community but also between different communities if their flowering period overlaps with each other. Therefore, consideration of the landscape phenological structure is necessary to understand plant–pollinator interactions at the community scale (biotic process in Fig. 3.1).

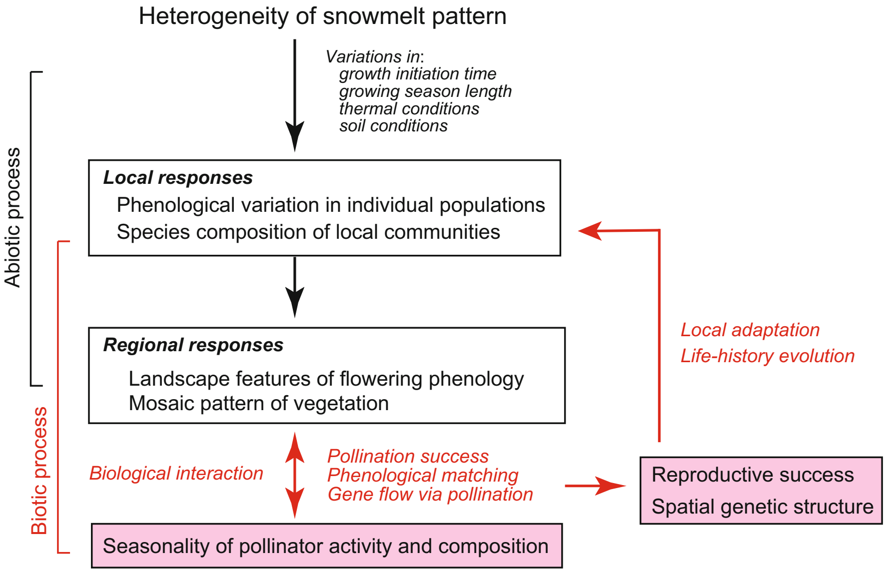


Fig. 3.1 Formation of landscape phenology and its importance for plant–pollinator interactions in alpine ecosystems. Heterogeneity of snowmelt patterns reflecting topographic changes causes vegetational complexity and spatiotemporal variation in the phenology of plants in both local and regional communities (abiotic process). These landscape features of plant communities, in addition to the seasonality of pollinator availability and activity, create diverse interactions between alpine plants and pollinators, resulting in variations in reproductive success and acceleration of local adaptation (biotic process)

The major pollinator insects in alpine ecosystems are flies and bees (especially bumblebees), and their life cycles are different. The availability and activity of bumblebees changes greatly as the season progresses, reflecting colony development because of their eusocial life cycle (Heinrich 1975). In contrast, flies commonly have short life cycles, and their activity may be more opportunistic in comparison with social bees (Kevan and Baker 1983; Kearns and Inouye 1994; Larson et al. 2001; Bischofe et al. 2013). Therefore, plants dependent on fly pollination may have a different flowering pattern from plants dependent on bee pollination, i.e., pollination syndrome on phenological traits. This possibility was suggested in a previous study conducted at an alpine snow meadow in central Japan (Yumoto 1986), but quantitative analyses of flowering structure over communities have not yet been performed.

Alpine ecosystems are the most sensitive to climate change, and there have been many studies on the responses of plant growth, species composition, and phenology based on long-term monitoring and experiments with environmental factors (e.g., Pauli et al. 2007; Oberbauer et al. 2013). Interactions between alpine plants and pollinators may also be influenced by climate change if the phenologies of plants and pollinators respond differently to climate variations, i.e., phenological mismatch (Memmott et al. 2007; Hegland et al. 2009; Forrest 2015). The effects of climate change on plant–pollinator interactions in alpine ecosystems, however, should be evaluated with consideration of the landscape features of flowering structures along the snowmelt gradient.

In this chapter, I provide an overview of the structure and function of flowering phenology at the landscape level, the sensitivity of plant–pollinator interactions to climate variations, and the evolutionary significance of landscape phenology for plant adaptations in alpine ecosystems. Most studies in this chapter were conducted in the Taisetsu Mountains in Hokkaido (43° 13–45′ N, 142° 32′–143° 19′ E), which is the largest alpine ecosystem in Japan.

3.2 Phenological Responses of Alpine Plants to the Abiotic Environment

3.2.1 Temperature Dependence and Snowmelt Regime

Ambient temperature is the most common determinant factor for the growth initiation and period for most alpine plants. Variation in flowering onset time reflects species-specific thermal requirements that are commonly expressed as the cumulative temperature above a threshold (usually 5 °C), i.e., growing degree days (GDD; Kudo and Suzuki 1999; Wipf 2010; Kudo and Yokosuka 2012). In fellfield habitats with little snow cover, spring air temperature largely determines flowering onset of early bloomers, whereas in snowbed habitats where snow remains until summer, the time of snowmelt determines the time of flowering onset because the soil surface is constantly kept at 0 °C under snow cover and exposed to warm temperatures soon after snowmelt. Therefore, regional phenological dynamics in alpine ecosystems are governed by the combination of temperature and snowmelt regime (Holway and Ward 1965; Kudo 1991; Huelber et al. 2006; Kudo and Hirao 2006).

Thermal requirements for flowering onset (GDD during pre-flowering period) vary widely among species (Kudo and Suzuki 1999; Kudo and Yokosuka 2012) in both fellfield and snowbed species (Fig. 3.2), but few snowbed species have a very large thermal requirement (>400° days). This may reflect the short growth season in snowbed habitats, in which flowering too late could increase the risk of leaving insufficient time for fruit development. Such a diverse pattern of thermal requirements may contribute to reduce the flowering overlap among species within communities.

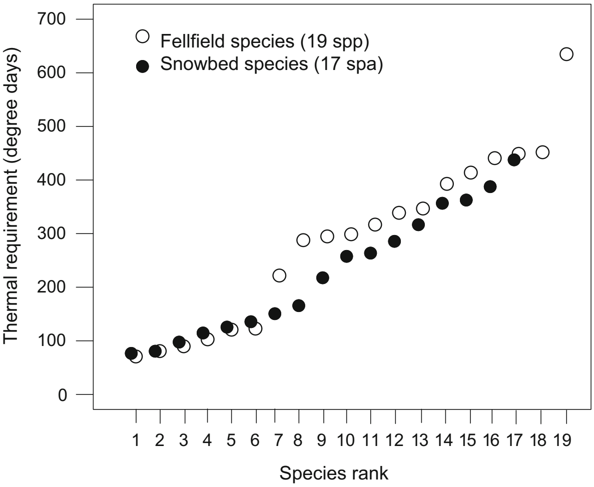
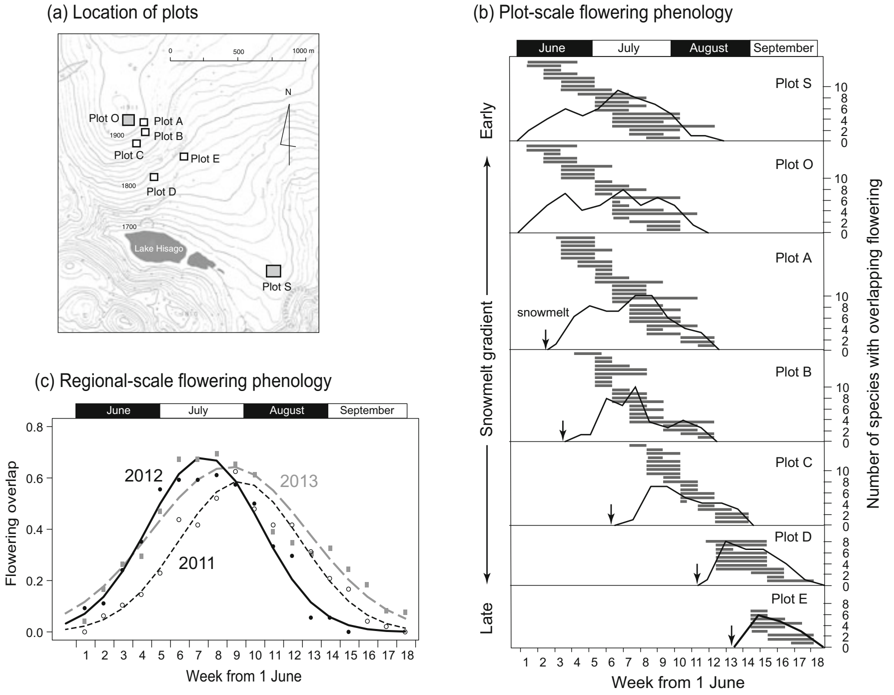


Fig. 3.2 Distributions of thermal requirement for flowering onset (growing degree days) of fellfield species (19 spp.) and snowbed species (17 spp.) observed in the Taisetsu Mountains, Japan (Data from Kudo and Yokosuka 2012)

3.2.2 Landscape Structure of Flowering Phenology

The combination of flowering patterns in fellfield and snowbed communities represents the flowering structure at the landscape level (Kudo and Hirao 2006). The flowering phenology of fellfield communities is relatively stable from year to year, in which flowering onset starts around the beginning of June and the flowering season lasts until mid-August. In contrast, the flowering phenology of snowbed communities fluctuates substantially from year to year owing to large yearly variation in snowmelt time. Thus, the total flowering period at the regional scale strongly depends on the flowering progress of snowbed communities.

The qualitative structure of flowering phenology in each community can be expressed as a seasonal transition of overlapping flowering among species (Fig. 3.3b), whereas the phenological structure at the regional scale can be expressed as a combination of the community phenology along the snowmelt gradient (Fig. 3.3c). This landscape feature of flowering structure is useful to quantify the phenological responses to climate variations of alpine ecosystems. In a usual summer (2011), flowering progressed gradually from fellfield to snowbed communities along the snowmelt gradient, and the total flowering period was approximately 3 months. In 2012, which was an unusually warm summer with early snowmelt, however, the flowering period largely overlapped among the communities, and the total flowering period was 1 month shorter than in a usual summer. In 2013, when the early season was warm but snowmelt progressed slowly, the total flowering period was similar to 2011, but flowering overlap was greater throughout the season. These yearly variations in landscape phenology express the fluctuation in floral resources and influences on the competitive situation for pollinator acquisition among plant species.


(a) Location of plots

(b) Plot-scale flowering phenology

(c) Regional-scale flowering phenology

Fig. 3.3 (**a**) Location of plots within the research area in the Taisetsu Mountains, Japan. Plots S and O were located in a fellfield habitat and plots A–E were arranged along a snowmelt gradient in a snowbed habitat. (**b**) Flowering periods of major plant species (individual *horizontal bars*) and the number of species with overlapping flowering periods (*lines*) in each plot (example of measurements from 2011). (**c**) Yearly variation in the seasonal trends in flowering overlaps between species across seven plots during 2011–2013. Week 1 corresponds to 1 June

3.3 Pollination Syndrome of Flowering Phenology

3.3.1 Pollinator Types for Alpine Plants

In alpine ecosystems, flies (Diptera) and bees (Hymenoptera) are the major flower visitors (Arroyo et al. 1982; Kevan and Baker 1983; Yumoto 1986; Totland 1993; Bergman et al. 1996). In particular, bumblebees (*Bombus* spp.) are the most effective pollinators for many alpine plants (Bingham and Orthner 1998). In addition, in Japanese alpine regions, the most common flower visitors are flies (60 %) and bees (35 %), and the proportions of visits by butterflies and moths (Lepidoptera) and other insects are small ($\leq$5 %). These compositions are similar between alpine ecosystems in northern and central Japan (Table 3.1). Reflecting the composition of flower visitors, insect-pollinated alpine plant species can be largely classified into three groups: bee-pollinated flowers (B type), fly-pollinated flowers (F type), and bee- and fly-pollinated flowers (BF type). Observations of flower

Table 3.1 Compositions of flower visitors to alpine plants in the Taisetsu Mountains (northern Japan, N43.5°, 1700–1900 m) and Mt. Tateyama (central Japan, N36.6°, 2400–2800 m)

Order	Diptera (%)	Hymenoptera (%)	Lepidoptera (%)	Others (%)	No. of obs.
Taisetsu Mts.	61.4	35.8	2.0	0.8	$N = 4275$
Mt. Tateyama[a]	64.4	31.1	1.6	2.9	$N = 4955$

[a] Unpublished data from H.S. Ishii, Toyama University

Table 3.2 Proportion of plant species predominantly visited by bees, flies, and both bees and flies in the alpine plant communities from two Japanese mountain regions

	Bee flowers (%)	Fly flowers (%)	Bee and fly flowers (%)	No. of species
Taisetsu Mts.	27.8	46.3	25.9	54 spp.
Mt. Tateyama[a]	27.1	51.4	21.4	70 spp.

[a] Unpublished data from H.S. Ishii, Toyama University

visitors in alpine regions located in northern and central Japan revealed that 46–51 % of plant species were predominantly visited by flies (Anthomyiidae, Syrphidae, Empididae, and others), 27–28 % were predominantly visited by bees (mostly bumblebees), and the remaining 21–26 % were visited by both (Table 3.2). These similarities in flower visitor compositions and flower types between geographically isolated mountain regions imply a possibility that the flowering patterns of alpine plant communities may be formed under the selection of seasonality and/or foraging patterns of bees and flies.

Pollination syndrome of floral traits, such as morphology, color, odor, and reward, has been well studied (e.g., Fenster et al. 2004; Ollerton et al. 2009). However, the flowering patterns within communities have scarcely been studied in terms of pollination syndrome. In this section, I compare the flowering patterns of bee- vs. fly-pollinated plants and their consistency between years based on the seasonal activity of flower visitors.

3.3.2 *Seasonal Activity of Bees and Flies*

Seasonal patterns of foraging activity of bumblebees reflect the life cycle of colony development (Kudo et al. 2011). In the early season, only overwintered queen bees are available at a low frequency. The timing of queen emergence in spring is determined by thermal conditions of the overwintering habitat, i.e., soil temperature (Alford 1969). In alpine regions of northern Japan, queen bees usually appear around the beginning of June, whereas worker bees usually emerge in mid-July. The active period of worker bees lasts until late August, after which new queens and male bees also emerge; bee activity then decreases and is finished by mid-September (Kudo 2014). This clear seasonality in the life cycle of bees creates a seasonal trend in pollination efficiency for bee-pollinated plants, in which

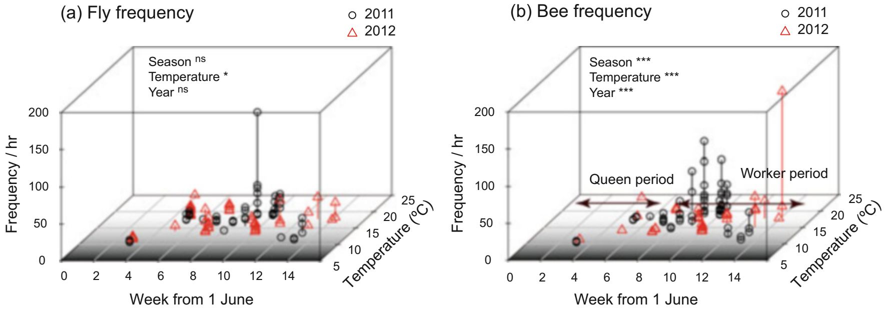


Fig. 3.4 Seasonal patterns in visitation frequency of flies (**a**) and bees (**b**) to patches of flowering species in the Taisetsu Mountains, Japan. Measurements were conducted on calm days in 2 × 2 m flowering patches that were arbitrarily selected each time. Most bee visitors were bumblebees, and their overwintered queen active period and worker active period are indicated. *Circles*: 2011, *triangles*: 2012. Results from a generalized linear model (GLM) postulating a negative binomial error distribution are indicated, in which the frequency of insects was an objective variable and observation week, ambient temperature, and year were explanatory variables (ns >0.01, * <0.005, *** <0.0001)

pollination success often increases with a delay in flowering time in both inter- and intraspecific populations (Kudo 1993; Kudo and Suzuki 2001).

There have only been a few studies on the seasonal activity of flies in alpine ecosystems despite their importance. In alpine Norway, where flies are the predominant flower visitors, the abundance of flower visitors tended to decrease gradually from mid-July to September, but this was more related to temperature and floral resources (Totland 1994). In the Rocky Mountains in North America, the abundance of syrphid flies responded to the yearly variation in floral resources (Iler et al. 2013). These reports suggest that flies may exhibit flexible activity responding to weather conditions and floral resources more than bumblebees.

In the Taisetsu Mountains, the abundance of anthophilous flies was positively related to ambient temperature but independent of season and year (Fig. 3.4a). This indicated that the availability of fly pollinators is potentially high throughout the summer. In contrast, the abundance of bumblebees strongly depended on both ambient temperature and season, with a significant difference between years (Fig. 3.4b), indicating a clear seasonality of pollinator availability for bee-pollinated plants. Therefore, the selective forces acting on flowering time of fly-pollinated species may be more moderate than that of bee-pollinated species.

3.3.3 Flowering Pattern of Bee-Pollinated and Fly-Pollinated Plants

In addition to the seasonal availability of pollinators, the foraging behavior and pollination efficiency of flower visitors may be an important selective force affecting the flowering pattern of plants depending on different pollinators. There have been many studies on the floral constancy of bumblebees during foraging flights irrespective of their pollinator habit as a generalist (Heinrich 1976; Waser 1986; Wilson and Stine 1996; Ishii 2005). The floral choice of bumblebees is generally determined by the abundance of floral reward and its distribution in plant communities. This means that bee-pollinated flowers with overlapping flowering time may compete with each other for pollinator acquisition; thus, sequential flowering among species within communities may be beneficial to reduce competition (Pleasants 1980; Rathcke and Lacey 1985; Rathcke 1993). In fact, sequential flowering among bee-pollinated species was detected in some plant communities (Heinrich 1975; Pleasants 1980; Yumoto 1986), indicating a possibility of "*exploitative competition*" among bee-pollinated species.

Foraging behavior of fly pollinators has rarely been quantified in alpine ecosystems (see Kearns 1992; Kearns and Inouye 1994). Generally, flies have been recognized as faithless pollinators in comparison with bees (Bischofe et al. 2013), although some studies reported a significant flower color preference by syrphid flies (Goulson and Wright 1998; Campbell et al. 2010). If flies exhibit less floral constancy and frequently induce pollen transfer among flowers of different species, "*interference competition*" may occur between co-flowering species, resulting in potential selection for segregated flowering among species. On the other hand, if interspecific flower movements of flies are not common and/or if pollen deposition during a single flower visit by a fly is small, interference competition between co-flowering plants may be limited (Kearns 1992). Furthermore, if higher flowering overlap among species contributes to attract and/or maintain pollinators in a community, simultaneous flowering may even be beneficial (Rathcke 1993), especially under the conditions of pollinator limitation (Moeller 2004). Because seed production of alpine plants is commonly restricted by pollen limitation, high overlap of fly-pollinated flowers is expected.

The flowering patterns of alpine plant communities in the Taisetsu Mountains are shown in Fig. 3.5. Flowering overlaps among species are expressed as the proportion of species flowering at same time, and this varied significantly between flower types and between years (statistics not shown). B-type flowers tended to have a dispersed flowering pattern within a growth period, whereas F-type flowers showed a concentrated pattern in the middle of the season when the ambient temperature was high. The flowering pattern of BF-type flowers was similar to F-type flowers in the early period, but higher flowering overlap was retained from the middle to late period. In 2012, which was a warm year with early snowmelt, the flowering periods were shortened, especially in B-type flowers. Snowmelt progressed slowly in 2013, and the flowering periods of B- and BF-type flowers were extended in the later season. In comparison with these flowers, the flowering period and overlap of F-type flowers were relatively stable between years.

The flowering periods of individual populations were 17.8 ± 1.9 days (mean ± SE) in B-type species ($n = 17$), 20.7 ± 2.6 days in BF type, and 25.9 ± 2.1 days in F type (unpublished data), and there was a significant difference between B-type and

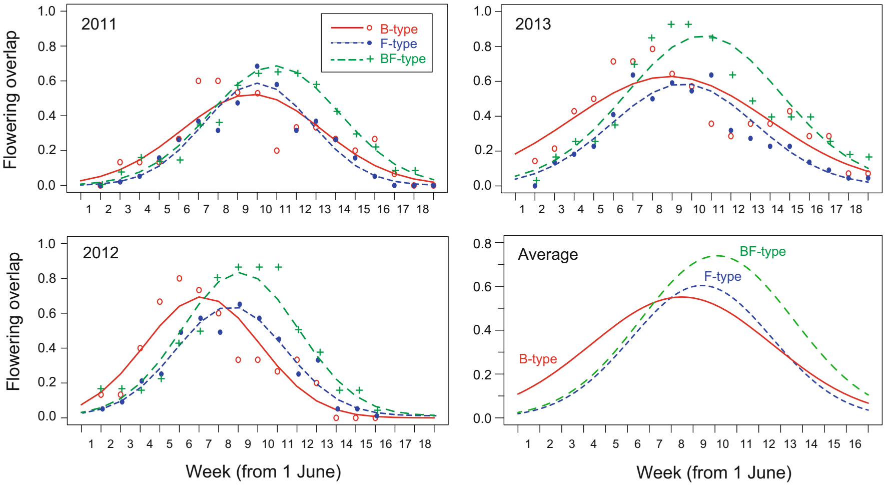


Fig. 3.5 Seasonal patterns in flowering overlap among species of bee-pollinated (B-type; *solid line*), fly-pollinated (F-type; *short broken line*), and bee- and fly-pollinated (BF-type; *long broken line*) plants over 3 years (2011–2013) in the Taisetsu Mountains, Japan (Data from seven plots arranged along the snowmelt gradient are pooled (see Fig. 3.3))

F-type species ($t_{44} = 2.59$, $P = 0.013$). Shorter flowering period with low flowering overlap in B-type species may reflect the selective force to reduce the competition for pollinators among bee-pollinated species.

For the precise assessment of floral resources, not only the qualitative description of phenological structure but also the quantification of flower production is crucial. Thus, flower densities were measured across seven plots from a fellfield habitat to the bottom of a snowbed habitat in early (2012) and late snowmelt years (2013, Fig. 3.6). The seasonal trend of B-type flowers varied highly between years. Floral densities were low in 2012, in which a unimodal flowering pattern was detected. In contrast, a clear bimodal pattern was detected in 2013. This was because mass flowering of fellfield shrubs (*Arctous alpinus* and *Arcterica nana*) occurred in early June and mass flowering of snowbed shrubs (*Phyllodoce aleutica* and *P. caerulea* f. *yesoensis*) occurred in late August. In 2012, an early flowering peak was not detected because of very low flower production in the fellfield habitat, and flowering of snowbed plants occurred very early owing to early snowmelt. As a result, the flowering pattern of bee-pollinated plants showed a unimodal shape with a peak in late July.

Flower production of BF-type plants was similar between years, and a unimodal flowering pattern was detected with a peak in late July to early August in both years. The pattern of F-type flowers was also similar between years, but the seasonal fluctuation was much smaller in comparison with B-type and BF-type flowers. Therefore, yearly variations in flower production and flowering pattern in this area were mainly formed by the flowering performance of bee-pollinated plants. This trend may reflect the differences in growth form between B-type and F-type species.

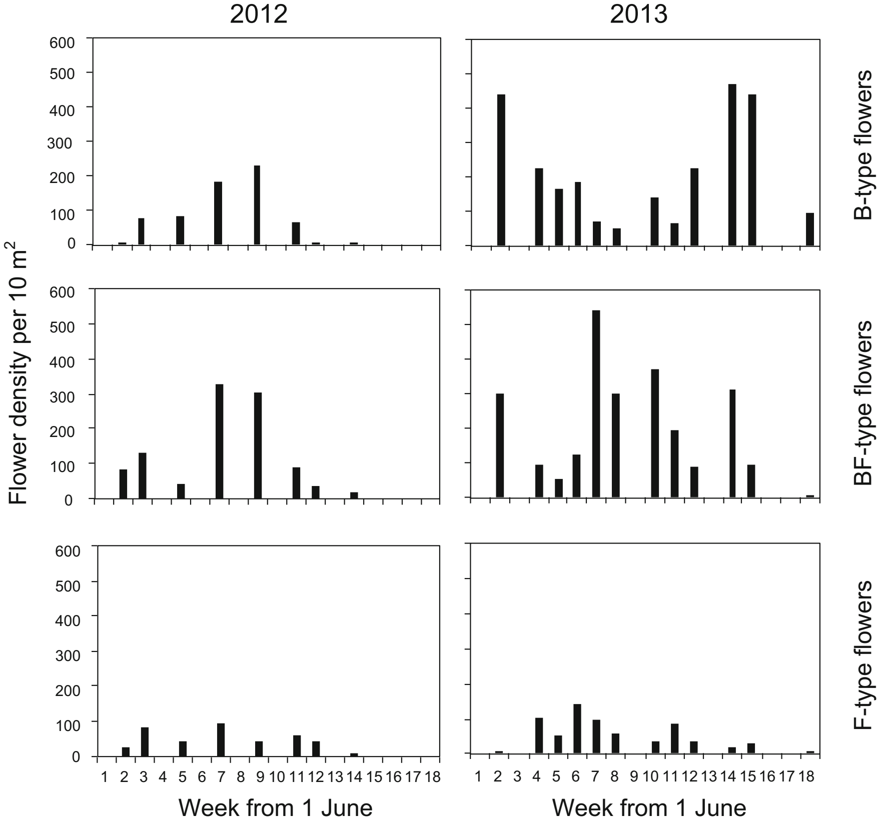


Fig. 3.6 Seasonal patterns in floral resources of the bee-pollinated (B-type), fly-pollinated (F-type), and bee- and fly-pollinated (BF-type) plants in years of early (2012) and late snowmelt (2013) in the Taisetsu Mountains, Japan. For a quantification of floral resources, 10 quadrats (2 × 2 m) were arbitrarily set in each of seven plots (see Fig. 3.3), and the number of flowers of every insect-pollinated species was counted (70 quadrats in total) throughout the flowering season, mostly at weekly intervals. Then floral resources at the regional scale are expressed as flower density per unit area (10 m^2)

Bees commonly prefer flowers of ericaceous shrubs, and flower production of these shrubs tends to show large yearly variation (Kudo 1991). In contrast, most fly-pollinated plants are herbs, and they commonly show stable flower production over years. Large fluctuations in floral resources may be related to the yearly variation in bee frequency (Fig. 3.4). The relationship between floral resources and population dynamics of pollinators is an important issue for future studies in alpine ecosystems.

3.4 Phenological Matching of Flowers and Pollinators

3.4.1 Phenological Shift in Response to Climate Change

There is a large body of evidence of phenological shifts in various organisms responding to recent climate change (Root et al. 2003; Parmesan 2007). Phenological shifts are most apparent in arctic and alpine ecosystems where cold winter climates restrict the growth of plants and insects. In snowy alpine ecosystems, snow conditions are an important driving force of flowering phenology of alpine plants (Kudo and Hirao 2006; Wipf 2010). Recently, snowmelt time has been advancing in many alpine regions (Mote et al. 2005; Kudo and Hirao 2006; Rixen et al. 2012). In a warm climate, alpine plant communities tend to shorten the whole flowering period due to earlier completion of the flowering season. It is an important question whether the phenologies of plants and pollinators respond similarly to climate change (Forrest 2015). Phenological mismatch between flowering time and pollinator activity may disrupt plant–pollinator interactions within a pollination network of an individual ecosystem. Memmott et al. (2007) predicted that phenological shifts caused by global warming would reduce the floral resources available to 17–50 % of pollinator species owing to reduced overlap between flowers and pollinators.

To evaluate the ecological impacts of climate change on the interaction between plants and pollinators, the following three approaches are important: (1) detection of phenological shifts in flowering time and pollinator activities responding to climate variation, (2) quantification of the effects of phenological mismatch on fitness and population dynamics of plants and pollinators, and (3) prediction of the dynamics of community structure and species diversity caused by the disruption of plant–pollinator interactions. There have been several studies on the first approach based on observations and experimental manipulation of phenology (Forrest and Thomson 2011; Iler et al. 2013). For the second approach, there have only been a few studies on the evaluation of plant fitness, but there have been no studies of plant population dynamics, and studies on pollinator fitness and population dynamics are completely lacking. For the third approach, there are only a few theoretical predictions based on historical records of the phenological shifts of many plant and insect phenologies (Memmott et al. 2007; Hegland et al. 2009).

Predictions in previous studies on phenological shifts have not been consistent. There have been several studies that suggested similar phenological shifts in plants and pollinating insects (Bartomeus et al. 2011; Ovaskainen et al. 2013; Rafferty and Ives 2011; Burkle et al. 2013). In contrast, some studies reported significant phenological mismatch between plants and insects (Gordo and Sanz 2006; Kudo and Ida 2013), and these mismatches affected plant fitness in some cases (Thomson 2010; Kudo and Ida 2013). These inconsistent conclusions may partly reflect differences in climatic conditions of the research sites and types of pollinators in the various studies. Generally, phenological mismatch was more common in the early season or under cool climatic conditions (Miller-Rushing et al. 2010;

McKinney et al. 2012). Previous studies conducted in the Arctic and alpine environments reported good synchrony between fly abundance and community-wide flowering even in unusually warm summers (Høye et al. 2013; Iler et al. 2013). In contrast, a significant mismatch between bee abundance and flowering time was detected in an extremely warm summer for a Japanese alpine site (Kudo 2014).

3.4.2 Phenological Mismatch Between Alpine Plants and Bumblebees

During 3 years of observation (2011–2013) of flowering pattern and bumblebee abundance at Mt. Akadake (northern part of the Taisetsu Mountains), the synchrony of flowering phenology and bumblebee activity was apparently disrupted in the unusually warm summer of 2012 (Fig. 3.7; Kudo 2014 for details). In a normal year, 2011, flowering of a fellfield community started in early June and that of a snowbed community started around mid-July depending on snowmelt conditions, and the flowering season lasted until early September. Overwintered queen bees emerged in early June, and the active period of workers was from late July to mid-August, and then worker activity decreased toward early September. The number of flowering species was retained at a high level during the active period of workers. Owing to the warm and early snowmelt conditions in 2012, flowering started in late May and early July in fellfield and snowbed communities, respectively, and the flowering period had almost finished by late August. In contrast to the early shift in flowering period, the appearance of worker bees was late and their activity was highest in late August after the main flowering season. Thus, a clear phenological mismatch was apparent. In the third year, 2013, temperature conditions were rather normal but snowmelt progressed slowly. Therefore, the flowering period of snowbed community was extended toward late September. Responding to the extended flowering period, bee activity was also maintained until mid-September.

These yearly variations in flowering patterns across plant communities and bee activity suggest a fragile relationship between plants and bees in alpine ecosystems under conditions of climate change. A delay in worker bee emergence was an unexpected response to the warm weather conditions. In 2012, soil thawing in fellfield habitats occurred 3 weeks earlier than in a usual year. Because overwintering queen bees emerge when soil temperature reaches around 5–9 °C (Alford 1969), queens seemed to emerge before the flowering of fellfield community species in this year. Food limitation conditions soon after emergence might cause physical damage to queens, which might influence subsequent colony establishment. Experimental studies revealed that diapause duration and thermal conditions affect the preoviposition period and subsequent colony development of bumblebees (Beekman et al. 1998; Amin et al. 2007). In ground-nesting bees, soil temperature in overwintering and nesting habitats may be a crucial factor determining the life cycle.

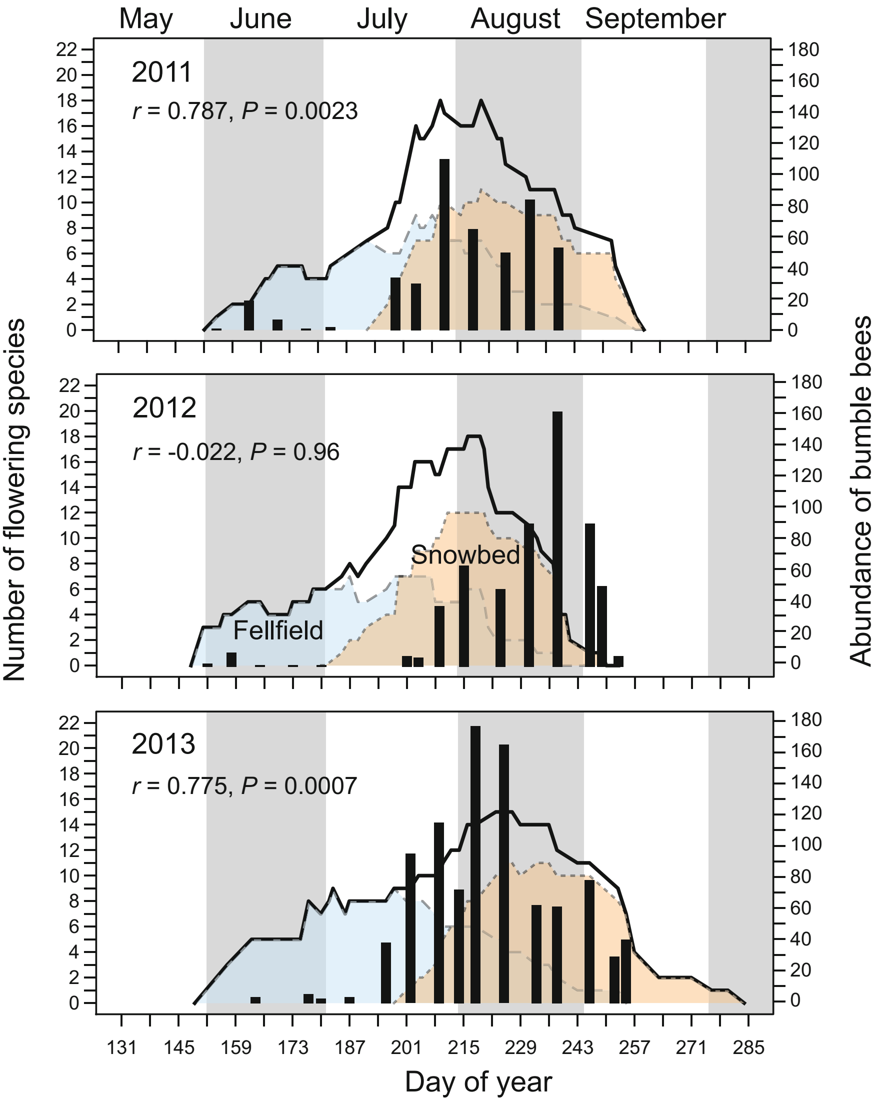


Fig. 3.7 Relationship between the number of flowering species and the abundance of bumblebees over 3 years at site on Mt. Akadake in the northern part of the Taisetsu Mountains, Japan. The total number of flowering species (*solid line*) was composed of fellfield plants (area surrounded by a *broken line*) and snowbed plants (area surrounded by a *dotted line*). Bee frequency (histogram) is expressed as the number of observed bees along a fixed route between fellfield and snowbed plots (see Kudo 2014 for details). Correlation coefficient (r) between flowering species and bee frequency is indicated with a significant value (P) for each year. Only in an unusually warm summer, 2012, was there no positive correlation between them

Responses of bumblebees to yearly variation in flowering phenology were highly species specific (Kudo 2014). Among four *Bombus* species observed at this site, only *B. hypocrita sapporoensis* remained in the alpine environment for its whole life cycle from overwintering to breeding, i.e., it was a resident pollinator. *Bombus beaticola moshkarareppus* was commonly observed in alpine regions from late June to early September, but queen bees were rare in the early season. This species can also breed in alpine habitats, but its major overwintering site may be in subalpine habitats. The other two species, *B. hypnorum koropokkrus* and *B. yezoensis*, showed large fluctuations in abundance between years. It seems that they visit alpine regions only for foraging, i.e., migrant pollinators, because their active periods in alpine sites were short. Interestingly, the active period of *B. hypocrita sapporoensis*, a resident pollinator, was synchronized with the major flowering period every year, i.e., short active period in 2012 and long active period in 2013. Therefore, the life cycle of this species can be regulated flexibly depending on the availability of floral resources. In contrast, the abundance of *B. beaticola moshkarareppus* and *B. yezoensis* increased continuously toward late August in 2012. In early September, when the flowering of most species had finished, these bees (workers, males and queens) were commonly visiting the sporadic remaining flowers. Further study is necessary to clarify the late-season mismatch effects on subsequent bee population dynamics.

3.5 Evolutionary Aspects on Flowering Phenology in Alpine Ecosystems

3.5.1 Significance of Phenological Isolation

Previous studies on the evolutionary implications of mountain ecosystems often focused on the significance of altitudinal gradient with reference to the variation in pollinator guilds (see Chap. 4 and references therein). Under different pollination situations, selective forces may act differently on floral characteristics to improve the pollination efficiency in each population, which may accelerate local adaptations and ecotype differentiation along the altitudinal ecotone. Similarly, phenological differences along the snowmelt gradient in addition to the clear seasonality of alpine ecosystems may act as selective forces for the creation of gene flow, reproductive success, and local adaptation between neighboring populations of alpine plants at a smaller scale rather than the altitude gradient. First, phenological isolation of pollen-mediated gene flow between neighboring populations may cause a spatial genetic structure within a restricted area of not only spatially isolated populations but also within a continuous patch when flowering time varied due to heterogenetic snowmelt pattern (Hirao and Kudo 2004, 2008). Such a microscale genetic structure may reflect the short pollen dispersal distance in insect-pollinated alpine plants (Hirao et al. 2006; Buehler et al. 2012). Because spatial snowmelt pattern is stable reflecting topographic features, flowering sequence along the snowmelt gradient often causes a directional and restricted gene flow. This sorting function of flowering structure may contribute to form a meta-population structure within a local area even when natural selection does not exist.

Second, when selective forces acting on morphological, physiological, and ecological characteristics exist at a local scale, restriction of gene flow due to phenological isolation may accelerate the local adaptation even when flowering time is not directly related to the selective force. For example, flowering, germination, and growth form traits of *Potentilla matsumurae* were significantly different between fellfield and snowbed populations within a local area (Shimono and Kudo 2003; Shimono et al. 2009). Plants in the fellfield populations showed sequential flowering within each plant, sporadic germination, and prostrate leaf morphology in comparison with the snowbed populations. These traits specific to the fellfield populations may reflect windy, dry, and exposed situations in the fellfield habitat. Restriction of gene flow between the habitats owing to phenological isolation may accelerate and/or maintain habitat-specific ecotypes within local areas.

Third, in addition to the phenological isolation between local populations, habitat-specific selective forces may directly act on phenological traits (Elzinga et al. 2007). In late snowmelt habitats, for example, early flowering soon after snowmelt is beneficial to complete the life cycle within the short growth season, especially for late bloomers (Kudo 1991; Kudo and Suzuki 1999). The thermal requirement of the late-blooming snowbed herb *Gentiana nipponica* was smaller in late snowmelt populations, and this trait was retained even after transplantation to an early snowmelt habitat (Kawai and Kudo 2011). Because this species is a monocarpic herb, directional selection toward earlier flowering in late snowmelt habitats might act strongly on phenological traits. Intraspecific variation in the thermal requirement for reproductive phenology has been detected among populations with different snowmelt regimes in several species (Kudo and Suzuki 1999). Ecological and evolutionary significance of phenological variation is expected in these species.

3.5.2 Significance of Pollinator Seasonality

When pollinator activity and availability vary seasonally, the time of flowering directly affects the pollination success of individual populations. As mentioned previously, the activity of bumblebees has a strong seasonality, in which opportunistic visits by queen bees are common in the early season, whereas frequent visits by worker bees are common in the latter half of the season. Reflecting this clear seasonal trend in pollinator availability, not only fruit-set success but also outcrossing rate varies depending on the flowering time (Kameyama and Kudo 2009). For instance, outcrossing rates of the snowbed dwarf shrub *Phyllodoce aleutica* varied from 10 % in an early snowmelt habitat to 88 % in a late snowmelt habitat among neighboring populations (Fig. 3.8). Because of the high inbreeding

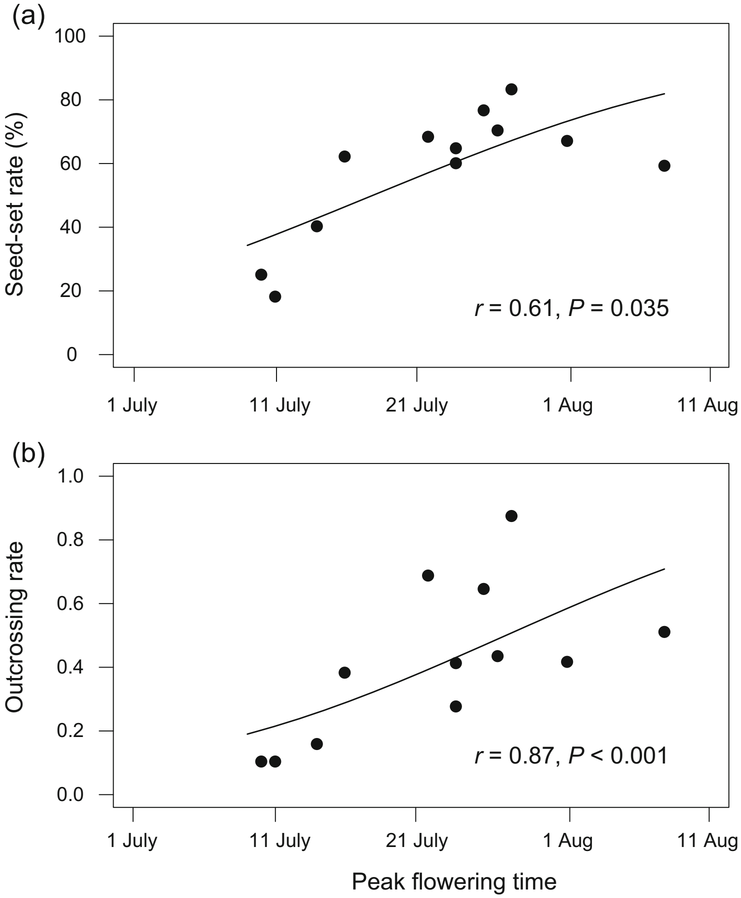


Fig. 3.8 Relationships between flowering time and seed-set success (**a**) and between flowering time and outcrossing rate (**b**) of *Phyllodoce aleutica* in the Taisetsu Mountains (Data from six populations over 2 years are pooled. See Kameyama and Kudo (2009) for details)

depression, however, selfed offspring in this species might not contribute to the maintenance of populations. Therefore, early snowmelt populations suffering from low pollinator service may be maintained by vegetative growth and occasional establishment of outcrossed progenies. In our previous study (Kameyama et al. 2008), the clonal patch size of *P. aleutica* was much larger (>10 m in diameter) in an early snowmelt plot, and it was estimated to be a few thousand years old. In comparison, in late snowmelt plots, the clonal patch size was usually smaller (2–3 m in diameter). It seemed that populations at the early snowmelt habitat were mainly maintained by vegetative growth. Therefore, habitat-specific pollinator situations may influence the population structure of clonal plants.

Low seed-set success of early bloomers is a common trend for bee-pollinated species inhabiting fellfield sites (Kudo and Suzuki 2001). This is because flowering times of fellfield communities are rather stable between years in comparison with snowbed communities (Kudo and Hirao 2006). Therefore, some fellfield species may have evolved life history traits that tolerate low seed production, such as long life span and/or vegetative propagation. On the other hand, the flowering time of snowbed plants varies highly depending on the fluctuation in snowmelt time between years, in which seed-set success also varied depending on pollinator availability (Galen and Stanton 1991; Kudo and Hirao 2006). Especially for short-lived herbaceous species, continuous mismatch between flowering time and pollinator availability may cause a serious effect on population dynamics (Miller-Rushing et al. 2010). Thus, phenological variations among local populations may cause a large variation in life history traits (Kawai and Kudo 2011).

Seed-set success of plants should depend on the mating system of each species and the type of pollinators. When autogamous selfing is common, seed production may be independent of pollinator availability. Even in self-compatible species, however, pollinator availability may affect the seed production if pollen deposition on stigmas increases owing to pollinator visits. Furthermore, fly pollinators tend to have less clear seasonality in comparison with social bees. Therefore, fly-pollinated plants may have stable pollination service irrespective of flowering time in alpine ecosystems. For clarification of the dynamics of plant–pollinator interactions in alpine ecosystems, information on mating system, life history traits, pollinator type, and seed-set pattern for major component species are still required.

3.6 Conclusion

The structure of landscape phenology shows substantial fluctuation between seasons and years in alpine ecosystems. These dynamics reflect the combination of phenological responses between fellfield communities (governed by temperature regime) and snowbed communities (governed by snowmelt regime). Although the activity and availability of pollinators vary temporally, there are clear differences in seasonal trends between flies and bees. Detected phenological patterns specific to fly-pollinated and bee-pollinated plants may be caused by plant–pollinator interactions in alpine ecosystems. Global warming could cause a serious impact on the phenological structure of alpine ecosystems. The risk of phenological mismatch is suggested between plants and bees, but these may include species-specific responses. For more precise prediction of global change impacts, understanding of the key factors that determine the phenology of individual species is necessary, in addition to the prediction of regional climate conditions, especially temperature and snowmelt pattern.

Phenological structure at a regional scale may act as a major driving force to create and maintain the biodiversity of alpine ecosystems. However, the evolutionary contribution of landscape phenology has rarely been studied. Because both flowering structure and pollinator activity vary strongly with seasonal progress, the features of interactions between co-flowering species (competition, facilitation, or neutrality) may vary spatiotemporally. For example, the relationships of pollination success between neighboring species vary from competitive to facilitative depending on the abundance of pollinators (Ye et al. 2014). For clarification of the interaction web in alpine ecosystems, better understanding of ecological connections between keystone species is crucial. Thus, studies on the ecosystem function of landscape phenology will be an important focus for both global change biology and evolutionary ecology.

Acknowledgements I am grateful to Yuka Kawai, Yukihiro Amagai, and Yuki Mizunaga for their support in field surveys, Hiroshi Ishii for offering data of pollinator assemblages on Mt. Tateyama, and Takao Itino for comments on an early version of the manuscript. This study was funded by the Global Environmental Research Fund of the Ministry of the Environment, Japan (F-092 and D-0904) and by JSPS KAKENHI (23405006, 24570015).

References

Alford DV (1969) A study of the hibernation of bumblebees (Hymenoptera: Bombidae) in southern England. J Anim Ecol 38:149–170

Amin MR, Suh SJ, Kwon YJ (2007) Impact of artificial photoperiodism on the colony development of the bumblebee *Bombus terrestris*. Ecol Sci 10:315–321

Arroyo MTK, Primack R, Armesto J (1982) Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. Am J Bot 69:82–97

Bartomeus I, Ascher JS, Wagner D, Danforth BN, Colla S, Kornbluth S, Winfree R (2011) Climate-associated phenological advances in bee pollinators and bee-pollinated plants. Proc Natl Acad Sci U S A 108:20645–20649

Beekman M, van Stratum P, Lingeman R (1998) Diapause survival and post-diapause performance in bumblebee queens (*Bombus terrestris*). Entomol Exp Appl 89:207–214

Bergman P, Molau U, Holmgren B (1996) Micrometeorological impacts on insect activity and plant reproductive success in an alpine environment, Swedish Lapland. Arct Alp Res 28: 196–202

Billings WD, Bliss LC (1959) An alpine snowbank environment and its effects on vegetation, plant development, and productivity. Ecology 40:388–397

Bingham RA, Orthner AR (1998) Efficient pollination of alpine plants. Nature 391:238–239

Bischofe M, Campbell DR, Lord JM, Robertson AW (2013) The relative importance of solitary bees and syrphid flies as pollinators of two outcrossing plant species in the New Zealand alpine. Austral Ecol 38:169–176

Buehler D, Graf R, Holderegger R, Gugerli (2012) Contemporary gene flow and mating system of *Arabis alpina* in a central European alpine landscape. Ann Bot 109:1359–1367

Burkle LA, Marlin JC, Knight TM (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence and function. Science 339:1611–1615

Campbell DR, Bischoff M, Lord JM, Robertson AW (2010) Flower color influences insect visitation in alpine New Zealand. Ecology 91:2638–2649

Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G (2007) Time after time: flowering phenology and biotic interactions. Trends Ecol Evol 22:432–439

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. Ann Rev Ecol Evol Syst 35:375–403

Forrest JRK (2015) Plant-pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? Oikos 124:4–13

Forrest JRK, Thomson JD (2011) An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecol Monogr 81:469–491

Galen C, Stanton ML (1991) Consequences of emergence phenology for reproductive success in *Ranunculus adoneus* (Ranunculaceae). Am J Bot 78:978–988

Gordo O, Sanz JJ (2006) Temporal trends in phenology of the honey bee *Apis mellifera* (L.) and the small white *Pieris rapae* (L.) in the Iberian Peninsula (1952–2004). Ecol Entomol 31:261–268

Goulson D, Wright NP (1998) Flower constancy in the hoverflies *Episyrphus balteatus* (Degeer) and *Syrphus ribesii* (L.) (Syrphidae). Behav Ecol 9:213–219

Heegaard E (2002) A model of alpine species distribution in relation to snowmelt time and altitude. J Veg Sci 13:493–504

Hegland SJ, Nielsen A, Lázaro A, Bjerknes A-L, Totland Ø (2009) How does climate warming affect plant-pollinator interactions? Ecol Lett 12:184–195

Heinrich B (1975) Bee flowers: a hypothesis on flower variety and blooming times. Evolution 29:325–334

Heinrich B (1976) The foraging specializations of individual bumblebees. Ecol Monogr 46: 105–128

Hirao AS, Kudo G (2004) Landscape genetics of alpine-snowbed plants: comparisons along geographic and snowmelt gradients. Heredity 93:290–298

Hirao AS, Kudo G (2008) The effect of segregation of flowering time on fine-scale spatial genetic structure in an alpine-snowbed herb *Primula cuneifolia*. Heredity 100:424–430

Hirao AS, Kameyama Y, Ohara M, Isagi Y, Kudo G (2006) Seasonal changes in pollinator activity influence the pollen dispersal and seed production of the alpine shrub *Rhododendron aureum* (Ericaceae). Mol Ecol 15:1165–1173

Holway JG, Ward RT (1965) Phenology of alpine plants in northern Colorado. Ecology 46:73–83

Høye TT, Post E, Schmidt NM, Trøjelsgaard K, Forchhammer MC (2013) Shorter flowering seasons and declining abundance of flower visitors in a warmer Arctic. Nat Clim Chang 3: 759–763

Huelber K, Gottfried M, Pauli H, Reiter K, Winkler M, Grabherr G (2006) Phenological responses of snowbed species to snow removal dates in the central Alps: implications for climate warming. Arct Antarct Alp Res 38:99–103

Iler AM, Inouye DW, Høye TT, Miller-Rushing AJ, Burkle LA, Johnston EB (2013) Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. Glob Chang Biol 19:2348–2359

Inouye DW (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89:353–362

Ishii HS (2005) Analysis of bumblebee visitation sequences within single bouts: implication of the overstrike effect on short-term memory. Behav Ecol Sociobiol 57:599–610

Kameyama Y, Kudo G (2009) Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, *Phyllodoce aleutica*: effects of pollinators and self-incompatibility. Ann Bot 103:1385–1394

Kameyama Y, Kasagi T, Kudo G (2008) A hybrid zone dominated by fertile F_1s of two alpine shrub species, *Phyllodoce caerulea* and *Phyllodoce aleutica*, along a snowmelt gradient. J Evol Biol 21:588–597

Kawai Y, Kudo G (2011) Local differentiation of flowering phenology in an alpine-snowbed herb *Gentiana nipponica*. Botany 89:361–367

Kearns CA (1992) Anthophilous fly distribution across an elevation gradient. Am Midl Nat 127: 172–182

Kearns CA, Inouye DW (1994) Fly pollination of *Linum lewisii* (Linaceae). Am J Bot 81: 1091–1095

Kevan PG, Baker HG (1983) Insects as flower visitors and pollinators. Ann Rev Entomol 28: 407–453

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, Berlin

Kudo G (1991) Effects of snow-free period on the phenology of alpine plants inhabiting snow patches. Arct Alp Res 23:436–443

Kudo G (1993) Relationship between flowering time and fruit set of the entomophilous alpine shrub, *Rhododendron aureum* (Ericaceae), inhabiting snow patches. Am J Bot 80:1300–1304

Kudo G (2014) Vulnerability of phenological synchrony between plants and pollinators in an alpine ecosystem. Ecol Res 29:571–581

Kudo G, Hirao AS (2006) Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: implications for global-change impacts. Popul Ecol 48: 49–58

Kudo G, Ida TY (2013) Early onset of spring increases the phenological mismatch between plants and pollinators. Ecology 94:2311–2320

Kudo G, Ito K (1992) Plant distribution in relation to the length of the growing season in a snow-bed in the Taisetsu Mountains, northern Japan. Vegetatio 98:165–174

Kudo G, Suzuki S (1999) Flowering phenology of alpine plant communities along a gradient of snowmelt timing. Polar Bios 12:100–113

Kudo G, Suzuki S (2001) Relationship between flowering phenology and fruit-set of dwarf shrubs in alpine fellfields in northern Japan: a comparison with a subarctic heathland in northern Sweden. Arct Antarct Alp Res 34:185–190

Kudo G, Yokosuka K (2012) Spatiotemporal variations in flowering phenologies of alpine plant communities: long-term volunteer monitoring in an alpine ecosystem. Jpn J Constr Ecol 17: 49–62 (in Japanese with English summary)

Kudo G, Kawai Y, Hirao AS (2011) Pollination efficiency of bumblebee queens and workers in the alpine shrub *Rhododendron aureum*. Int J Plant Sci 172:70–77

Larson BMH, Kevan PG, Inouye DW (2001) Flies and flowers: taxonomic diversity of anthophiles and pollinators. Can Entomol 133:439–465

McKinney AM, Caradonna PJ, Inouye DW, Barr B, Bertelsen CD, Waser NM (2012) Asynchronous changes in phenology of migrating broad-tailed hummingbirds and their early-season nectar resources. Ecology 93:1987–1993

Memmott J, Craze PG, Waser NM, Price MV (2007) Global warming and the disruption of plant-pollinator interactions. Ecol Lett 10:710–717

Miller PC (1982) Environmental and vegetational variation across a snow accumulation area in montane tundra in central Alaska. Holarct Ecol 5:85–98

Miller-Rushing AJ, Høye TT, Inouye DW, Post E (2010) The effects of phenological mismatches on demography. Phil Trans R Soc B 365:3177–3186

Moeller DA (2004) Facilitative interactions among plants via shared pollinators. Ecology 85: 3289–3301

Molau U (1993) Relationships between flowering phenology and life history strategies in tundra plants. Arct Alp Res 25:391–402

Mote PW, Hamlet AF, Clark MP, Lettenmaier DP (2005) Declining mountain snowpack in western north America. Bull Am Meteorol Soc 86:39–49

Oberbauer SF, Elmendorf SC, Troxler TG et al (2013) Phenological response of tundra plants to background climate variation tested using the International Tundra Experiment. Phil Trans R Soc B 368:20120481

Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter CI, Rotenberry J (2009) A global test of the pollination syndrome hypothesis. Ann Bot 103: 1471–1480

Ovaskainen O, Skorokhodova S, Yakovleva M et al (2013) Community-level phenological response to climate change. Proc Natl Acad Sci U S A 110:13434–13439

Parmesan C (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Glob Chang Biol 13:1860–1872

Pauli H, Gottfried M, Reiter K, Klettner C, Grabherr G (2007) Signals of range expansion and contributions of vascular plants in the high Alps: observations (1994–2004) at the GLORIA master site Schrankogel, Tyrol, Austria. Glob Chang Biol 13:147–156

Pleasants JM (1980) Competition for bumblebee pollinators in rocky mountain plant communities. Ecology 61:1446–1459

Rafferty NE, Ives AR (2011) Effects of experimental shifts in flowering phenology on plant–pollinator interactions. Ecol Lett 14:69–74

Rathcke B (1993) Competition and facilitation among plants for pollination. In: Real L (ed) Pollination biology. Academic, Orlando, pp 305–325

Rathcke B, Lacey EP (1985) Phenological patterns of terrestrial plants. Ann Rev Ecol Syst 16: 179–214

Rixen C, Dawes MA, Wipf S, Hagedorn F (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO_2 enrichment and soil warming. Oikos 121:1532–1543

Root TL, Price JF, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming in wild animals and plants. Nature 421:57–60

Schöb C, Kammer PM, Choler P, Veit H (2009) Small-scale plant species distribution in snowbeds and its sensitivity to climate change. Plant Ecol 200:53–66

Shimono Y, Kudo G (2003) Intraspecific variations in seedling emergence and survival of *Potentilla matsumurae* (Rosaceae) between alpine fellfield and snowbed habitats. Ann Bot 91:21–29

Shimono Y, Watanabe M, Hirao AS, Wada N, Kudo G (2009) Morphological and genetic variations of *Potentilla matsumurae* (Rosaceae) between fellfield and snowbed populations. Am J Bot 96:728–737

Thomson JD (2010) Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. Phil Trans R Soc B 365:3187–3199

Totland Ø (1993) Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. Can J Bot 71:1072–1079

Totland Ø (1994) Influence of climate, time of day and season, and flower density on insect flower visitation in alpine Norway. Arct Alp Res 26:66–71

Waser NM (1986) Flower constancy: definition, cause and measurement. Am Nat 127:593–603

Wheeler JA, Hoch G, Cortés AJ, Sedlacek J, Wipf S, Rixen C (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. Oecologia 175:219–229

Wilson P, Stine M (1996) Floral constancy in bumble bees: handling efficiency or perceptual conditioning? Oecologia 106:493–499

Wipf S (2010) Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. Plant Ecol 207:53–66

Ye Z-M, Dai W-K, Jin X-F, Gituru RW, Wang Q-F, Yang C-F (2014) Competition and facilitation among plants for pollination: can pollinator abundance shift the plant-plant interactions? Plant Ecol 215:3–13

Yumoto T (1986) The ecological pollination syndromes of insect-pollinated plants in an alpine meadow. Ecol Res 1:83–95

Chapter 4
Plant Genetic Diversity and Plant–Pollinator Interactions Along Altitudinal Gradients

Takao Itino and Akira S. Hirao

Abstract Alpine plants are thought to be particularly vulnerable to extinction as a result of global warming because their habitat ranges are expected to shift upward until, eventually, no higher habitats remain into which they can escape. Moreover, even mountain plants distributing across wide altitudinal ranges are likely to experience range shifts, raising the possibility of local extinctions, especially of genetically and ecologically differentiated types with narrow distribution ranges. In this chapter, we examine to what extent plants genetically and ecologically differentiate along altitude and how altitudinal changes in pollinator assemblages affect floral evolution. First, by literature survey, we demonstrate that although altitudinal patterns of species and genetic diversity have been extensively investigated, few clear-cut examples of altitudinal genetic differentiation of neutral markers have been identified. On the other hand, many studies have shown that adaptive traits of plants differentiate along altitude, although their adaptive genetic background is yet to be uncovered. We then briefly introduce three case studies of mountain herbaceous plant species displaying cryptic neutral genetic or adaptive trait differentiation. The first two case studies show that the floral size of both *Campanula punctata* and *Prunella vulgaris* differs among populations along altitude and is adapted to altitudinally variable pollinator size. The third case study demonstrates that *Cimicifuga simplex* is composed of three genetically differentiated ecotypes that are distributed parapatrically or allopatrically along an altitudinal gradient. To meet the challenges posed by future climate warming, it is essential to clarify the genetic and ecological differentiation of mountain plants along altitude.

Keywords Alpine plants • Bumblebees • Climate warming • Conservation unit • Floral size • Species interaction

---

T. Itino (✉)
Department of Biology, Faculty of Science, Shinshu University, Nagano, Japan

Institute of Mountain Science, Shinshu University, Nagano, Japan
e-mail: itinot@shinshu-u.ac.jp

A.S. Hirao
Sugadaira Montane Research Center, University of Tsukuba, Tsukuba, Japan

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_4

4.1 Introduction

High mountain plants are expected to react sensitively to climate warming. In particular, plants living near the top of elevation gradients are expected to become extinct as plant species shift upward under a warming climate (Theurillat and Guisan 2001; Colwell et al. 2008). Consequently, a reduction of biodiversity in alpine habitats is predicted (Chen et al. 2011). Results from a standardized monitoring network of 60 sites on European mountain summits support these inferences: Pauli et al. (2012) reported that between 2001 and 2008, species moved upslope on average, and Gottfried et al. (2012) presented evidence for a decline in cold-adapted species and an increase in warm-adapted species at mountain summits.

Most studies, however, have focused on species- and community-level effects of climate warming, and few studies have examined intraspecific diversity (Pauls et al. 2013). Because taxonomically recognized morphospecies are often not evolutionarily or ecologically relevant units (Bálint et al. 2011), morphospecies-based approaches may seriously underestimate the effects of climate warming on biodiversity. Therefore, accurate assessment of such effects requires identification of cryptic intraspecific differentiation (Bálint et al. 2011; Pauls et al. 2013).

In mountain ranges, steep environmental clines along altitude can strongly influence adaptive traits and, potentially, lead to adaptive diversification within a narrow geographic range (Byars et al. 2007; Milá et al. 2009). This adaptive diversification may in turn lead to genetic differentiation, especially when adaptation of the reproductive organs is involved. Thus, mountain ecosystems are ideal for exploring adaptive and genetic differentiation over short distances. For a full understanding of mountain biodiversity and how to conserve it in the face of climate change, elucidation of altitudinal differentiation between populations living at higher and lower elevations is essential.

In this chapter, we examine to what extent plants genetically and ecologically differentiate along altitude and how altitudinal changes in pollinator assemblages affect adaptive differentiation of the floral traits of plants. We first present a literature overview, and then we examine the details in some case studies. We then propose a framework for the conservation of mountain plants. Our literature survey, presented in Sect. 4.2, shows that, despite numerous investigations of altitudinal patterns of species and genetic diversity, few clear-cut examples of altitudinal genetic differentiation of neutral markers in plants have been identified. However, numerous studies have reported adaptive phenotypic divergence along altitude, and recently, some pioneering eco-genomic studies have examined the genetic background of such altitudinal adaptive diversification. In Sect, 4.3, we introduce three case studies of cryptic genetic and ecological differentiation of mountain herbaceous plant species that inhabit a wide altitudinal range in the mountains of central Japan. The cases of *Campanula punctata* var. *hondoensis* (case 1) and *Prunella vulgaris* (case 2) show that the floral size of these species differs among populations along altitude, and they are locally adapted to altitudinally variable pollinators of different size. The third case study demonstrates that in central Japan, *Cimicifuga simplex* comprises at least three genetically differentiated ecotypes that are distributed parapatrically or allopatrically along altitudinal gradients.

4.2 Global Pattern of Biodiversity Along Altitudinal Gradients

4.2.1 Altitudinal Gradients in Ecological Research

Altitudinal gradients in biodiversity are nearly as ubiquitous as latitudinal gradients, and they have some characteristics that make them, perhaps, more suitable for uncovering the causes underlying the ecological and evolutionary responses of biota to such gradients (Körner 2007). First, changes in ecological phenomena occur over a shorter distance along an altitudinal gradient than along a latitudinal gradient, because temperature often decreases rapidly, by as much as 5.5–6.5 °C per 1000 m, with increasing altitude. In contrast, a comparable temperature decline occurs over ~1000 km of latitude (Jump et al. 2009). Thus, field data can be collected more readily along altitudinal gradients than along latitudinal gradients simply because the spatial extent of an altitudinal gradient is small compared to that of a latitudinal gradient. Second, it is possible to study many replicates of altitudinal diversity gradients, because each mountain or mountain range is a potential replicate, and to thereby assess the generality of the causes underlying the responses of biota. Third, manipulative experiments such as reciprocal transplants along altitudinal gradients can test theories of evolutionary adaptation over short distances.

Environmental factors are either physically linked to altitude, such as land area and climatic variables such as temperature, atmospheric pressure, and UV radiation, or they are correlated with altitude, such as vegetation zones and land use (Körner 2003, 2007). Thus, a suite of environmental gradients accompanies changes in altitude. By exploring an organism’s response to this suite of co-varying factors, ecological researchers have investigated the underlying causes of biodiversity. In this section, we present a literature review of patterns of plant biodiversity along altitude at the species and gene level.

4.2.2 Species Diversity Along Altitudinal Gradients

Altitudinal trends in species biodiversity are generally thought to mirror latitudinal trends, with biodiversity decreasing both at higher altitudes and higher latitudes. Consistent with this assumption, some studies have found a decreasing trend in plant species richness with altitude and have attributed this plant diversity trend to corresponding gradients in climate and primary productivity (Kitayama 1992;

Odland and Birks 1999; Grytnes and Vetaas 2002; Grytnes 2003). However, species biodiversity does not always show a decreasing trend with altitude. Rahbek (1995) reviewed biodiversity patterns in relation to altitude and showed that the prevailing pattern is for species richness to peak at an intermediate altitude. The occurrence of peak diversity at intermediate altitudes has since been reported across a wide range of taxa (Rowe 2009; Wang et al. 2009; reviewed in Sanders and Rahbek 2012). Notably, in alpine regions, either diversity decreases along altitudinal gradients or the diversity gradient has a humped shape, with peak diversity at intermediate altitude (Rahbek 1995; Grytnes 2003; Nogues-Bravo et al. 2008). In addition, anthropogenic factors such as grazing can disturb natural altitudinal diversity patterns (Speed et al. 2013).

The occurrence of a humped relationship between species richness and altitude is presumably attributable to complex factors such as climate and productivity, geographic constraints on the ranges of species, the disturbance history of the landscape, and the evolutionary history of the species (Grytnes and Vetaas 2002). In addition, altitudinal gradients in the phylogenetic structure of communities can reflect the interplay of biotic and abiotic constraints on diversity (Machac et al. 2011). For example, Shrestha et al. (2014) reported significant phylogenetic clustering of flowering plant communities along an altitudinal gradient with phylogenetically related species showing more variety in flower color than predicted by the null evolution model. Evolutionary history may also influence the mix of species that contributes to the diversity and structure of biotic communities (Webb et al. 2002).

4.2.3 Altitudinal Patterns of Genetic Diversity

The causes underlying intraspecific genetic diversity can also be explored along altitudinal gradients. A steep, continuous, altitudinal gradient can maximize both environmental differences and the potential for gene flow, thus providing opportunities for studying the relative effects of gene flow and natural selection on spatial patterns of genetic variation in populations. Altitudinal gradients have already been used by evolutionary biologists to obtain evidence for population differentiation in response to environmental change (Clausen et al. 1940; Meinzer et al. 1985; Gurevitch 1988; Galen et al. 1991). The application of molecular markers to the measurement of genetic variation has revealed extremely complex distributional patterns of genetic variation within and among populations along altitudinal gradients that are highly variable among species (Herrera and Bazaga 2008; Byars et al. 2009; Hahn et al. 2012).

After reviewing published studies of 48 plant species, Ohsawa and Ide (2008) reported that genetic variation along altitudinal gradients on mountains is generally categorized and explained as follows. First, intermediate-altitude populations sometimes have higher levels of genetic variation than either low- or high-altitude populations because, in accordance with the central–marginal hypothesis (Eckert et al. 2008), habitats at intermediate altitude are optimal. Low- and high-altitude populations tend to occur near the outer boundaries of a species' range, where limitations on gene flow, population size, and founder effects all promote genetic drift, thereby reducing genetic variation and increasing differentiation among populations. Second, low-altitude populations may have the highest level of genetic diversity, which decreases with altitude probably because optimal low-altitude habitats and founder effect through upward expansion occur. Third, the highest genetic diversity may occur at high altitudes because of decreased human disturbance, a historical downward range shift due to climate change, or adaptation. Lastly, genetic variation is sometimes largely constant along a given altitudinal gradient because of extensive gene flow between populations.

Overall, variations in the genetic drift–gene flow balance along altitudinal gradients are expected to result in altitudinal variations in the genetic structures and diversity of populations. However, other factors potentially influence the genetic diversity of populations on mountains. First, biogeographic history affects the genetic diversity of populations. At a large spatial scale in Europe, the genetic structure of populations of mountainous species mainly reflects historical factors such as the locations of glacial refugia and subsequent recolonization during postglacial expansion (Hewitt 2000; Petit et al. 2003). For example, genetic diversity of high-mountain plants in the Alps and the Carpathians does not reflect altitude, and it differs between these two mountain systems because of their divergent phylogeographic history (Thiel-Egenter et al. 2009). In contrast to Europe, where continental glaciation occurred during the Quaternary, far eastern Asia, including the Japanese archipelago, was not covered by a continuous ice sheet during the Pleistocene (Frenzel 1968; Svendsen et al. 2004), and several studies have documented the resulting distinctive biogeographic history (Fujii and Senni 2006; Ohsawa and Ide 2011; Hirao et al. 2015). Life history traits have also long been hypothesized to influence genetic diversity (Hamrick and Godt 1989; Nybom and Bartish 2000), and significant changes in life history traits such as the breeding system, the pollen and seed dispersal mode, and successional status along altitude have the potential to influence the genetic diversity of populations. For example, it has been suggested that the presence of fewer and less active pollinators at high compared with low altitude (Arroyo et al. 1985; Bingham and Orthner 1998; Malo and Baonza 2002) leads to clonality and selfing in plants at high altitude (Jacquemyn et al. 2005 but see also Wirth et al. 2010). Because inbreeding is well known to reduce genetic diversity, these life history trait changes might cause plant species to experience a decrease in genetic diversity along altitudinal gradients. The processes underlying these patterns can be either neutral, such as genetic drift and bottleneck effects due to demographic history, or selective, if they are caused by environmental clines related to altitude changes.

4.2.4 Altitudinal Genetic Differentiation of Neutral Markers

Numerous studies of mountain plants have found significant genetic differentiation and structuring among plant populations along altitudinal gradients (Ohsawa and Ide 2008; Byars et al. 2009; Shi et al. 2011). However, studies attempting to quantify the degree of genetic differentiation along altitude are relatively scarce. To consider differential patterns of population genetic structures along altitudinal gradients, we reviewed published studies that investigated altitudinal differentiation of neutral molecular markers. In all, we found 25 papers that examined a total of 26 species (Table 4.1), but few of these studies demonstrated clear-cut altitudinal genetic differentiation in plant populations. In most cases, the genetic variance component explained by altitudinal differences was subtle or weak, ranging from none to 10 % with a mean of 3.1 %. This result is somewhat counterintuitive, because one would expect many mountainous species to exhibit phenological differences along altitudinal gradients (Dittmar and Elling 2006; Ziello et al. 2009). For example, altitudinal differences in the timing of flowering should promote reproductive isolation and, thus, genetic differentiation along altitude. Gene flow across altitudinal gradients hindering complete reproductive isolation among populations inhabiting different altitudes might, however, explain the reported subtle or weak altitudinal differentiation (e.g., Hahn et al. 2012). In fact, Matter et al. (2013) demonstrated that contemporary pollen-mediated gene flow between populations occurs in two herb species across an altitudinal gradient. Similarly, effective gene flow across an altitudinal gradient has been shown to explain the high levels of genetic diversity found in the alpine species *Campanula rotundifolia* (Bingham and Ranker 2000) and *Arabis serrata* (Okuyama et al. 2012). In mountain regions, levels of gene flow between populations appear to be highly variable. In some species, restricted gene flow ($N_e m < 1$, where N_e is population size and m is the fraction of migrants) has been detected between altitudinal populations (Barbara et al. 2007), whereas in other species a moderate level of gene flow ($N_e m \geq {\sim}1$) has been found (e.g., Byars et al. 2009). A paternity analysis of *Arabis alpina* in an alpine landscape revealed that several long-distance (≥1 km) pollen flow events had occurred (Buehler et al. 2012). Garcia-Fernandez et al. (2012) also reported a substantial level of gene flow within altitudinal gradients and suggested that seed and, especially, pollen dispersal is more likely to occur between populations along an altitudinal gradient on the same mountain than between populations located at the same altitude on different mountains, although occasional horizontal movement cannot be ruled out. Overall, these results indicate that several populations distributed across altitudinal gradients on a mountain or in a mountain range can form a metapopulation with a single genetic structure.

Sexton et al. (2014) have proposed that gene flow follows one of three patterns with respect to spatial and environmental gradients. Gene flow may be higher between geographically close populations, between populations in similar environments, or between populations in dissimilar environments (counter-gradient flow). If the gene flow rate is higher among populations at similar altitudes rather than

Table 4.1 Published studies of altitudinal genetic differences in plant populations

Species	Life form	Altitudinal range (m)		Area (km^2)	No. populations	Overall F_{ST} or F_{ST} analogue	Variance component of genetic variation explained by altitudinal differences	Marker type	Reference
Athyrium filix-femina	Fern	450	1800	8000	20	0.040	0.001	RAPD	Schneller and Liebst (2007)
Briza media	Forb	1200	1800	22,000	20	0.099	0.059	AFLP	Hahn et al. (2012)
Geum reptans	Forb	2070	3080	12,500	20	0.150	0.024	RAPD	Pluess and Stöcklin (2004)
Isodon umbrosus	Forb	320	1730	62,500	15	0.360	–	Isozymes	Dohzono and Suzuki (2010)
Lilium longiflorum	Forb	720	2820	9100	7	0.066	0.051	RAPD	Wen and Hsiao (2001)
Polemonium viscosum	Forb	3500	4000	–	2	0.015–0.069	0.015–0.069	Isozymes	Galen et al. (1991)
Primula farinosa	Forb	811	1940	32	10	0.170	0.085	RAPD	Reisch et al. (2005)
Ranunculus bulbosus	Forb	1200	1800	14,000	18	0.071	0.043	AFLP	Hahn et al. (2012)
Saxifraga oppositifolia	Forb	2480	3020	2500	10	0.04–0.05	0.000	RAPD	Gugerli et al. (1999)
Trifolium montanum	Forb	1200	1800	22,000	20	0.118	0.054	AFLP	Hahn et al. (2012)
Trifolium montanum	Forb	682	2066	600	61	0.044	–	AFLP	Hahn et al. (2013)
Festuca eskia	Grass	1582	2604	20,000	9	0.380	0.040	Microsatellites	Gonzalo-Turpin and Hazard (2009)

(continued)

Table 4.1 (continued)

Species	Life form	Altitudinal range (m)		Area (km^2)	No. populations	Overall F_{ST} or F_{ST} analogue	Variance component of genetic variation explained by altitudinal differences	Marker type	Reference
Hordeum vulgare	Grass	1798	3324	720,000	106	0.105	0.100	Microsatellites	Hadado et al. (2010)
Poa hiemata	Grass	1700	1880	180	11	0.096	0.045	Microsatellites	Byars et al. (2009)
Sorghum bicolor	Grass	530	2890	1,248,000	48	0.410	0.030	Isozymes	Ayana et al. (2001)
Hippophae rhamnoides	Shrub	1800	3400	80,000	5	0.097	–	Microsatellites	Chen et al. (2008)
Lavandula latifolia	Shrub	990	1540	22,500	21	0.298	0.000	AFLP	Herrera and Bazaga (2008)
Castanopsis eyrei	Tree	251	920	81	24	0.032	–	Microsatellites	Shi et al. (2011)
Fagus orientalis	Tree	600	1900	100	6	0.015–0.046	–	Isozymes and microsatellites	Shanjani et al. (2011)
Fagus sylvatica	Tree	992	1640	12	3	0.0135–0.0156	0.015	AFLP	Jump et al. (2006)
Larix kaempferi	Tree	1300	2700	108	6	0.009	0.0023	Microsatellites	Nishimura and Setoguchi (2011)
Pinus brutia	Tree	275	1050	20,000	9	0.053	–	Isozymes	Kara et al. (1997)
Pinus canariensis	Tree	1035	2135	375	6	0.013–0.019	0.001–0.006	Microsatellites	Navascués et al. (2008)
Pinus pallasiana	Tree	150	900	–	4	0.017	–	Isozymes	Korshikov and Mudrik (2006)

Podocarpus parlatorei	Tree	1040	3100	90,000	18	0.104	–	Isozymes	Quiroga and Premoli (2007)
Quercus crispula	Tree	800	1800	2800	19	0.013	0.000	Microsatellites	Ohsawa et al. (2007)
Quercus serrata	Tree	140	1200	2800	15	0.013	0.000	Microsatellites	Ohsawa et al. (2008)

F_{ST} a measure of population differentiation due to genetic structure, *RAPD* random amplified polymorphic DNA, *AFLP* amplified fragment length polymorphism among those at dissimilar altitudes, altitudinal adaptation might be promoted by the spread of favorable alleles (Byars et al. 2009). In contrast, a prevailing counter-gradient gene flow across altitude might limit adaptation because gene swapping would prevent the maintenance of locally adapted individuals (Matter et al. 2013). Despite the large amount of literature on spatial genetic variation in mountainous species, we know little about actual patterns of gene flow with respect to altitudinal gradients. Therefore, the question remains: Which is more common – isolation by elevation or counter-gradient gene flow across altitude?

4.2.5 Differentiations in Adaptive Traits and Non-neutral Genes Along Altitudinal Gradients

Natural selective pressures along an altitudinal gradient can also affect genetic variation in mountain plants. Since the classic studies of Clausen and his colleagues (Clausen et al. 1940; Clausen 1951), who established field experimental approaches to detect genetic differentiation in phenotypic traits and used common garden plants and reciprocal transplantation experiments to detect local adaptation, substantial evidence for adaptive genetic variation in plants along altitudinal gradients has accumulated. Intraspecific adaptation across altitudinal gradients has been reported in various plants, from woody conifers (Conkle 1973; Kitzmiller 2005; Ishizuka and Goto 2012) to herbs and grasses (Galen et al. 1991; Byars et al. 2007; Gonzalo-Turpin and Hazard 2009; Kenta et al. 2011). In addition, Gonzalo-Turpin and Hazard (2009) combined a field experimental study with a molecular approach to show that local adaptation can occur along altitude even in the presence of gene flow as estimated by neutral markers. In general, behavioral trends of neutral and adaptive genes differ both within and among populations because natural selection can contribute in various ways to heterogeneous genomic divergence. Genome scans have shown that usually only a small proportion of scored loci, generally 5–10 %, consists of outliers that might be linked to genes implicated in the adaptive divergence (reviewed by Nosil et al. 2009), and some empirical studies have reported that only a few loci show non-neutral behavior associated with altitude that might drive natural selection (Jump et al. 2006; Shi et al. 2011; Manel et al. 2012). These studies observed very strong allele frequency differences along altitudinal gradients. However, very little is known about the locations and functions of specific genes involved in altitudinal adaptation (a notable exception is the albumin gene in deer mice; Stortz and Nachman 2003). The outlier genes mentioned above are appropriate targets for future investigations of linked functional genes, which can now be carried out by using high-throughput sequencing technology.

4.3 Case Studies: Altitudinal Trait Differentiation in Three Plant Species

4.3.1 Altitudinal Floral Adaptation to Local Pollinator Assemblages

Ever since the seminal work of Clausen et al. (1940) in the Rocky Mountains, plants have been known to be physiologically and ecologically differentiated along altitudinal gradients (Byars et al. 2007; Angert 2009; Montesinos-Navarro et al. 2011). Typically, altitudinal changes in abiotic environments (e.g., meteorological changes) often cause the decrease in overall plant size with increasing elevation (Körner 2003; Hautier et al. 2009), but altitudinal changes in biotic interactions can also influence plant traits (Dohzono and Suzuki 2010). Studies exploring plant trait variations caused by such biotic interactions are scarce, however, though there are some exceptions (Galen 1985, 1989; Malo and Baonza 2002; Dohzono and Suzuki 2010; Sun et al. 2014). Because the modification of floral traits can easily lead to reproductive isolation and genetic differentiation in plants, studies of floral trait variations along altitude are particularly important for gaining understanding of altitudinal plant differentiation.

In angiosperms, selective pressure imposed by pollinators has played an important role in floral evolution (Stebbins 1970; Harder and Johnson 2009). Therefore, if pollinator assemblages differ among populations, local adaptations of floral traits may also be observed. In fact, floral traits have been shown to change geographically in relation to differences in pollinator assemblages (Galen 1996; Gómez et al. 2009; Nattero et al. 2010; Hattori et al. 2015; see Herrera et al. 2006 for a review), and this ecological differentiation of floral traits can potentially lead to genetic divergence among populations that become isolated as a result of specialization to specific pollinators (Nosil 2012).

Bumblebees are the dominant pollinators in the sub-alpine zone of Japanese mountains, and their species composition is known to change along altitude (Tomono and Sota 1997). Because a small bumblebee, *Bombus beaticola*, is strongly dominant above 1700 m a.s.l. in central Japan, bumblebee-pollinated plants at high altitude should have relatively smaller flowers than those growing at lower elevations, which are pollinated by larger bumblebee species. In the following sections, we describe two bumblebee-pollinated herbaceous species that show changes in floral size along an altitudinal gradient: *Campanula punctata* var. *hondoensis* and *Prunella vulgaris*.

4.3.1.1 *Campanula punctata*

Campanula punctata var. *hondoensis* (Campanulaceae) grows across a wide altitudinal range (0–2300 m a.s.l.) in central Japan. Nagano et al. (2014) studied altitudinal changes in floral size and pollinator assemblages of 12 populations

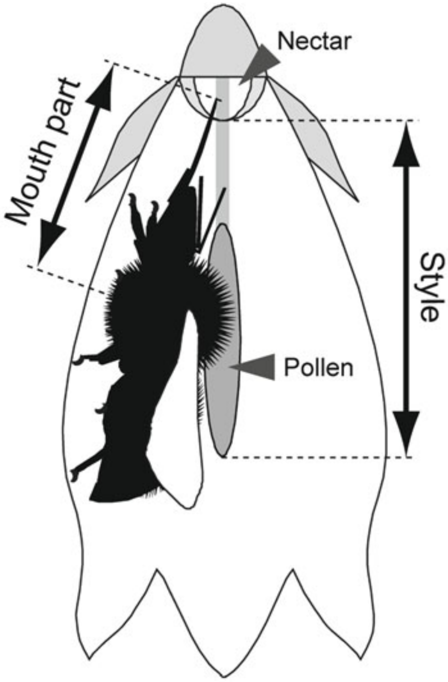


Fig. 4.1 Male-phase *Campanula punctata* flower and a visiting bumblebee. In this phase, the pollen grains adhere to the lateral surface of the style. Several days later, in the female phase, the tip of the style unfolds and the stigmatic lobes appear. The match between floral style length and pollinator mouthpart length is important for plant male fitness (i.e., removal of pollen during a bee's visit). See text for details (After Nagano et al. 2014)

situated at elevations from 744 to 2269 m a.s.l. in three mountain ranges and examined the affect of the flower–pollinator size match on plant fitness.

In *Campanula* flowers (Fig. 4.1), pollen grains are not transferred directly from the anthers to the pollinator; rather, pollen grains are shed onto the style while it is still tightly surrounded by the anthers in the young bud. By the time the flower opens, the stamens have shriveled and the pollen adhering to the style is ready for transfer to visiting pollinators as they crawl into the corolla (male phase; Fig. 4.1). The stigma does not become receptive (female phase) until about 2 days after the flower opens.

The size match between flowers and bumblebees affects the male fitness of the plants but not their female fitness. Among different size-match indices investigated, only the ratio of the pollinator's mouthpart length (PL) to the floral style length (SL) (PL:SL; see Fig. 4.1) affected plant male fitness (defined as pollen removal from the flower style onto bees). While the bee is collecting nectar, pollen grains stick to the dorsal surface of its thorax. Thus, the PL:SL ratio determines the position of the bee's thorax on the style while the bee is collecting nectar. Because most grains adhere to the middle part of the style (about one-third of the distance from the tip), the optimal PL:SL should be around 2:3. Nagano and his colleagues in fact found that when the PL:SL ratio is larger or smaller than this value, pollen removal by the bumblebees is decreased. These results suggest that a large floral size should be selected for in locations (at altitudes) where larger bumblebee species are dominant.

Nagano et al. (2014) also investigated the visitation frequency of bumblebee species to *C. punctata* flowers in the peak flowering season at sites at different elevations (Fig. 4.2, top) and calculated the average pollinator mouthpart length at each site from the relative abundance and average mouthpart length of each bumblebee species (Fig. 4.2, middle). They then examined whether bee size corresponded to floral size at different geographic locations (altitudes).

At high-altitude sites, where the small bumble bee *B. beaticola* was dominant (Fig. 4.2, top), the average pollinator mouthpart length was relatively short (Fig. 4.2, middle) and the style length of *C. punctata* also tended to be short (Fig. 4.2, bottom). However, bee and floral size did not simply decrease with elevation; rather, they changed in parallel although there is an exceptional site (circled by a dotted line in Fig. 4.2). Thus, floral size was large at a middle elevation site where the largest bumblebee species, *B. consobrinus*, was dominant (circled by a solid line in Fig. 4.2). By using generalized linear mixed models, Nagano et al. (2014) were able to show that the floral style length significantly correlated only with pollinator mouthpart length ($P < 0.05$); it did not correlate with altitude ($P = 0.40$) or with the number of flowers per plant (an index of plant size, $P = 0.38$).

4.3.1.2 *Prunella vulgaris*

Prunella vulgaris (Labiatae) is a perennial plant that grows in sunny mountain meadows of the temperate zone throughout the Northern Hemisphere. In central Japan, it blooms from June to August and is pollinated by bumblebees (Fig. 4.3). Kuriya et al. (2015) investigated geographical variations in the floral size of *P. vulgaris* and variations in the pollinator assemblage among seven *P. vulgaris* populations in the northern Japanese Alps, central Japan (1150–2050 m a.s.l.).

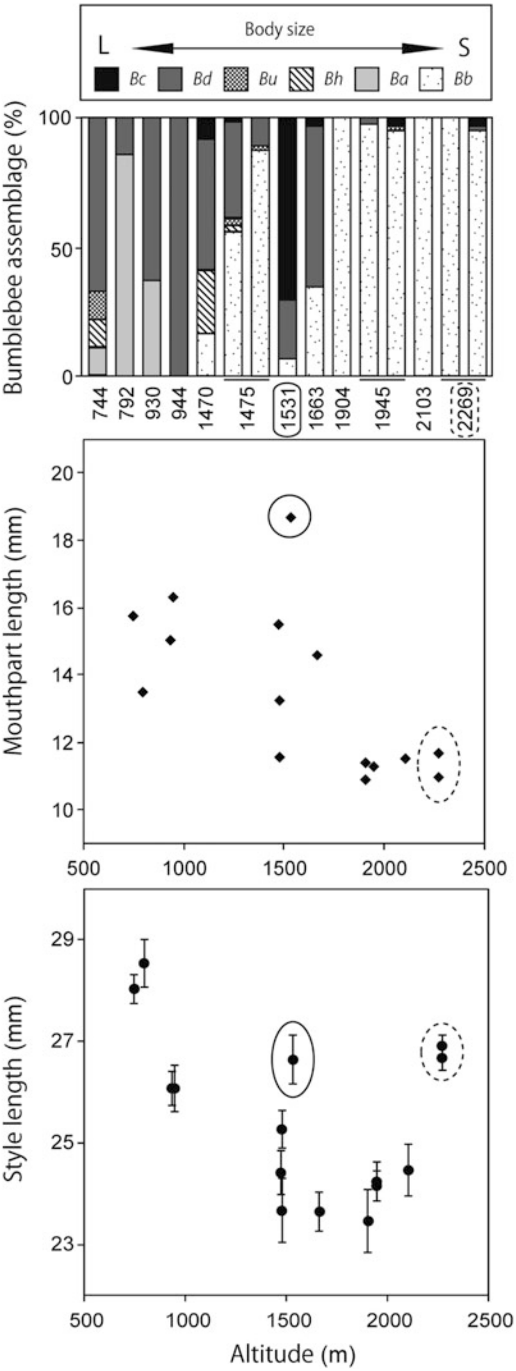


Fig. 4.2 *Top*: Altitudinal change in assemblages of *C. punctata* pollinators (bumblebee). *Bb B. beaticola* workers and males, *Ba B. ardens* males, *Bh B. honshuensis* workers, *Bu B. ussuriensis* workers, *Bd B. diversus* workers, *Bc B. consobrinus* workers. In the figure key, mouthpart length is used as the index of bumble bee body size. The altitude of each population (m a.s.l.) is also indicated. Three populations were surveyed in 2 years. The largest bumblebee, *B. consobrinus* (worker), was the main visitor to the population encircled by the solid line. In the populations encircled by the dashed line, there was a mismatch between floral size and bumblebee size. *Middle*: Altitudinal variation of the average mouthpart length (see Fig. 4.1) of the pollinators of the 12 populations. Each diamond represents a population year. *Bottom*: Altitudinal variation (mean ± SE) of floral style length (see Fig. 4.1) (After Nagano et al. 2014)

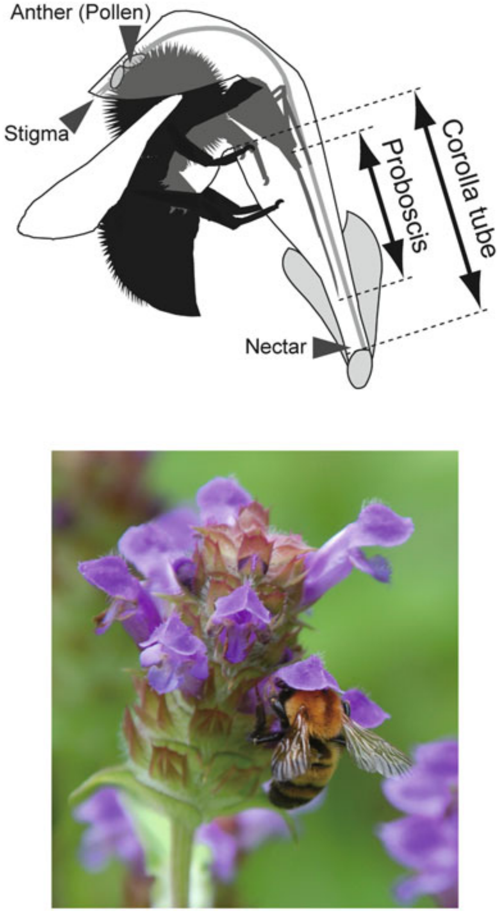


Fig. 4.3 A *Prunella vulgaris* flower and a visiting bumblebee. The anthers and stigma of *P. vulgaris* are at the top of the corolla tube (After Kuriya et al. 2015)

The flowers of *P. vulgaris* are tubular with an upper hooded lip and a lower fringed lip, and the anthers and stigma lie along the top lip (Fig. 4.3). When a bumblebee visits a flower, the top of its head will touch the anthers and stigma if its proboscis is similar in length to the tubular corolla (Fig. 4.3, bottom). If a smaller bumblebee visits the same flower, however, it must crawl into the corolla to reach the nectar, and the dorsal side of its thorax will touch the anthers and stigma (Fig. 4.3, top). In either case, the pollen seems to be successfully transferred. Therefore, the bee–flower size match would not appear to be as important for plant fitness as it is in the case of *C. punctata*. Nevertheless, Kuriya et al. (2015) found that pollen removal by bees (an estimate of male fitness) and pollen deposition from a bee onto the stigma (female fitness) were both strongly affected by the bee–flower size match ($P < 0.001$, generalized linear model). Plant fitness was maximized when the length of the corolla tube was a little bit shorter than the length of the bee's proboscis, that is, when the bee's thorax could just touch the anthers and stigma.

Kuriya and her colleagues also found that the primary visitor to high-altitude *P. vulgaris* populations was the small bumblebee *B. beaticola*, whereas the dominant pollinators at low-altitude sites were the medium-to-large bumblebees *B. diversus* and *B. ussuriensis* (Fig. 4.4, top). Overall, the proboscis length of the bees decreased along the altitudinal gradient (Fig. 4.4, middle), as was also the case with *C. punctata* pollinators (Fig. 4.2, middle).

Just as in *C. punctata* the style length tended to be shorter at higher elevations (Fig. 4.2, bottom), in *P. vulgaris* the corolla tube tended to be shorter at higher elevations (Fig. 4.4, bottom). One flower population at high elevation, however, had long corolla tubes (Fig. 4.4, bottom), and the large bumblebee *B. consobrinus* was the dominant visitor to this *P. vulgaris* population (Fig. 4.4, top). Kuriya and her colleagues showed by a generalized linear model that the corolla tube length of each population was significantly correlated only with bee proboscis length ($P < 0.001$) and not with inflorescence length ($P = 0.74$) or altitude ($P = 0.44$).

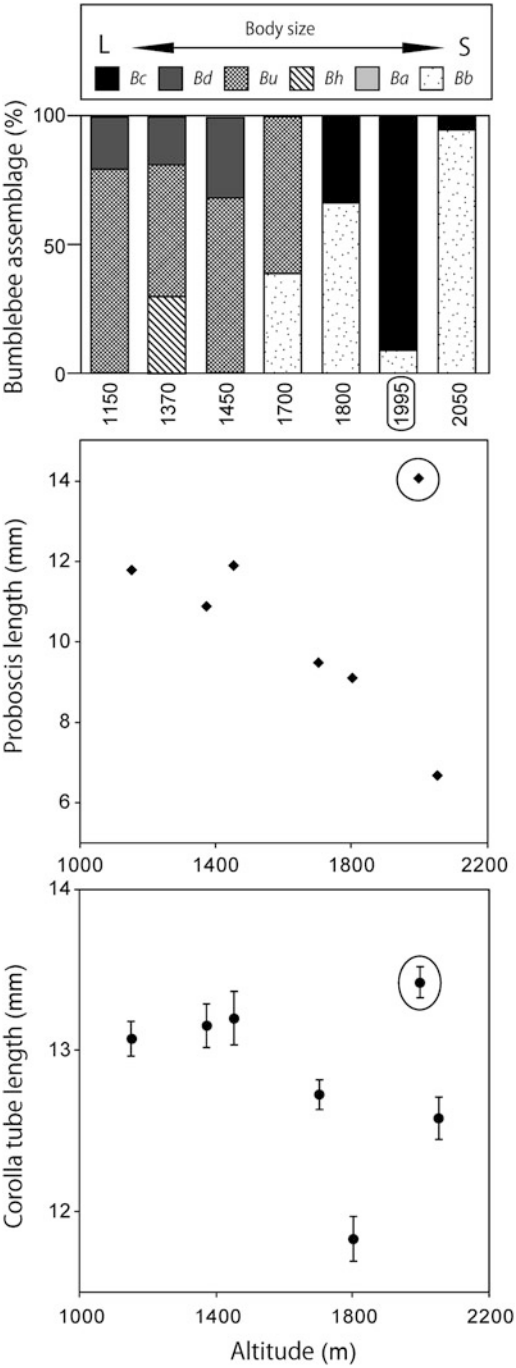
4.3.1.3 Synthesis

The local floral size of both *C. punctata* and *P. vulgaris* correlated only with the local pollinator size. At least two possible mechanisms might account for this correlation: (1) an adaptive floral size may be selected for by the local bee size (bees-came-first hypothesis), or (2) local floral size may vary because of unknown (climatic or edaphic) factors and each bumblebee species preferentially visits the right-sized flowers (flowers-came-first hypothesis).

If the flowers-came-first hypothesis is correct, then the larger bees such as *B. diversus* should prefer larger *C. punctata* (*P. vulgaris*) flowers and smaller bees such as *B. beaticola* should prefer smaller flowers in the same *C. punctata* (*P. vulgaris*) population. However, Nagano et al. (2014) detected no floral size preference of *B. diversus* or *B. beaticola* in populations of *C. punctata* visited by the both bumblebee species. This result suggests that the floral size of *C. punctata* does not influence the flower visitor assemblage of *C. punctata*. In addition, as described in Sects 4.3.1.1 and 4.3.1.2, in each population of *C. punctata* and *P. vulgaris*, floral size correlated only with the pollinator mouthpart length and not with altitude, number of flowers per plant, or inflorescence length. This finding suggests that floral size is not influenced by climatic or edaphic factors but by bee size. Further, plant fitness was maximized when floral size was matched to bee size, which suggests adaptive local floral evolution. These results strongly suggest that variation in local bee assemblages along altitude is exerting selective pressure on the floral size of *C. punctata* and *P. vulgaris*.

The altitudinal variation of bumblebee assemblages (Figs. 4.2 and 4.4, top) found in these two case studies generally reflects their altitudinal distribution in central Japan (Tomono and Sota 1997), whereas variation detected within the same

Fig. 4.4 *Top*: Altitudinal change in assemblages of *P. vulgaris* pollinators. The abbreviations of the bumblebee species are the same as in Fig. 4.2. The largest bumblebee, *B. consobrinus* (worker), was the main visitor to the population encircled by a solid line. *Middle*: Altitudinal variations of the average proboscis length (see Fig. 4.3) of the pollinators of the seven populations. Each diamond represents a population year. *Bottom*: Altitudinal variation (mean ± SE) of floral corolla length (see Fig. 4.3) (After Kuriya et al. 2015)



altitudinal range (Figs. 4.2 and 4.4, top) may reflect the influence of bumblebee nest location, which depends on the distribution of floral resources during the nest-initiating period of bumblebee queens (Suzuki et al. 2007, 2009). The correlation of pollinator (bumblebee) assemblages and floral traits of these two plant species along altitude suggests that the floral traits of other bumblebee-pollinated plants might also be influenced by local bumblebee assemblages along altitude. Future studies, therefore, should focus not on a single plant species but on multiple sympatric species to elucidate how local pollinator assemblages influence the formation of concerted floral trait evolution along altitude (Hattori et al. 2014, 2015).

4.3.2 **Cimicifuga simplex**: *Ecological and Genetic Differentiation Along Altitude*

Cimicifuga simplex (Ranunculaceae) is a perennial herb widely distributed in East and Northeast Asia. In Japan, it grows from sea level to over 3000 m (Pellmyr 1986). Pellmyr (1986) grouped Japanese *C. simplex* into three pollination morphs based on their pollinator guilds and other traits. Around Nikko in central Honshu, morph I occurs at altitudes over 1300 m and typically reaches 1.5–2.5 m in height (Pellmyr 1986). Morph II occurs at or below 950 m and is as tall as morph I. Morph III, which grows in very dark, moist habitats within and below the altitudinal range of morph II, is less than 1.0 m tall. Morphs I and III are pollinated by bumblebees (Hymenoptera, Apidae), whereas morph II is frequently visited by fritillary butterflies (Fig. 4.5; Lepidoptera, Nymphalidae). Morph II is distinguishable from morphs I and III by its floral fragrance (Pellmyr 1986; Groth et al. 1987), whereas morph III can be distinguished from the other two morphs by its lower plant height and later flowering season.

Working independently of Pellmyr (1986), Yamaji et al. (2005) divided *C. simplex* into seven genotypes based on polymorphism of ITS sequences of nuclear ribosomal DNA. Whether any of these genotypes correspond to the pollination morphs of Pellmyr (1986) has not yet been investigated. However, because the three pollination morphs of Pellmyr (1986) are distributed parapatrically or allopatrically along altitude and have clearly different morphological and

Table 4.2 Floral characters of the three pollination morphs of *Cimicifuga simplex* (after Kuzume and Itino 2013)

Pollination morph	Floral fragrance	Flowering period	Major flower visitors	Altitudinal distribution (m, a.s.l)
I	Not sweet	August–September	Bumblebees	1500–2400
II	Sweet	September	Butterflies	1100–1500
III	Not sweet	September–October	Flies and syrphids	800–1000

Fig. 4.5 Fritillary butterflies (Lepidoptera, Nymphalidae) visiting a *Cimicifuga simplex* plant (pollination morph II)

ecological traits, it is reasonable to hypothesize that they are genetically differentiated as well.

Accordingly, Kuzume and Itino (2013) surveyed the altitudinal distribution of the three pollination morphs of *C. simplex* in 16 populations along an altitudinal gradient (800–2400 m) in the northern Japanese Alps and in the Utsukushigahara highlands of central Japan, determined the genotypes and compared the morphological traits of the three morphs. In these two regions, which are about 50 km apart, the altitudinal distributions of the pollination morphs were basically the same, and they also were in accord with Pellmyr's (1986) description (Table 4.2). Morphs I, II, and III were found at high, medium, and low elevations, respectively, although the different morphs sometimes coexisted in the same population in the Utsukushigahara highlands (Fig. 4.6). In these mixed populations, individuals with intermediate morphological characteristics suggesting hybridization were not observed, indicating that the morphs are probably reproductively isolated.

Kuzume and Itino (2013) determined the ITS sequences of the three pollination morphs and found that, without exception, morphs I, II, and III corresponded to three of the seven ITS genotypes described by Yamaji et al. (2005); they did not detect the other four genotypes. In accordance with the genetic differentiation of the pollination morphs, they also found that the degree of pubescence of the leaf margin was a diagnostic external character that could be used to discriminate pollination morph II from morphs I and III.

Thus, in central Japan, the *C. simplex* populations are composed of at least three genetically differentiated ecotypes, which are distributed parapatrically or allopatrically along an altitudinal gradient.

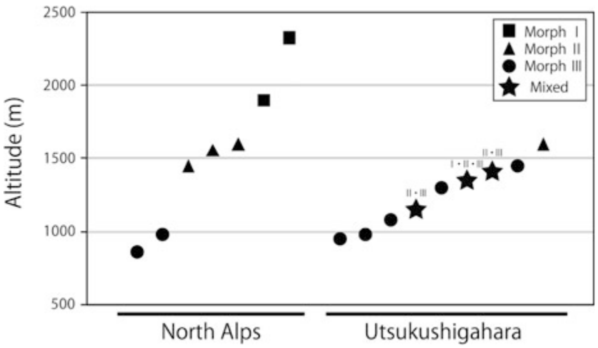


Fig. 4.6 Altitudinal distribution of the three pollination morphs of *Cimicifuga simplex* in the northern Japanese Alps and the Utsukushigahara highlands of central Japan

4.4 Concluding Remarks: Conserving Intraspecific Variation Along Altitudes

Steep altitudinal environmental changes cause remarkable clinal changes in species composition and species diversity. Although possible effects of climate warming on diversity at the species or community level along an altitudinal (or latitudinal) gradient have attracted considerable attention recently (Sanders and Rahbek 2012), those on intraspecific diversity have attracted less attention (Pauls et al. 2013). Studies of intraspecific diversity have mainly investigated altitudinal patterns of genetic diversity, and few examples of neutral genetic differentiation along altitude have been identified (Table 4.1). Rather, the prevailing counter-gradient gene flow appears to hinder altitudinal differentiation of neutral genes.

In contrast, a substantial number of studies have shown that, regardless of the frequency of gene flow, altitudinal differences in selective pressure cause adaptive differentiation of physiological, morphological, or life history traits along altitude (Kenta et al. 2011; Ishizuka and Goto 2012 and references therein). Our survey of the literature suggests that intraspecific differentiation of neutral genes is relatively uncommon whereas that of adaptive traits is more common. In either case, for effective conservation management of mountain plants, it is important to elucidate the degree and intensity of intraspecific differentiation along altitude.

The three case studies on genetic (*C. simplex*) and phenotypic (*C. punctata* and *P. vulgaris*) differentiations along altitudes suggest that recognized taxonomic species are often composed of several different evolutionary or ecological units, thus providing new insight into our understanding of biodiversity in mountain ecosystems. From the viewpoint of conservation, the intraspecific diversification documented here broadens the scope of our understanding of the threat posed by climate warming to mountain biodiversity. Previous investigations focused on the potential loss of species- and community-level diversity (Gottfried et al. 2012; Pauli et al. 2012), whereas here we have shown that climate warming may lead to a loss of intraspecific diversity. Because the distribution range of intraspecifically differentiated types (e.g., the high-altitude morph of *C. simplex*) along altitude is typically narrower than that of recognized taxonomic species, populations are more likely to be extirpated, or, in the worst case, the type may become extinct in the face of climate warming and the resultant range shift. In addition, intraspecific types are more likely to lose genetic variation because they are relatively less abundant than the species as a whole; thus, their ability to adapt is limited.

The altitudinal phenotypic variations in *C. punctata* and *P. vulgaris* suggest that inter-altitudinal gene flow occurs and sharply defined ecotypes are thus absent. However, the observed ecological variation along altitude in these species still has important implications for their conservation. For example, if the small bumblebee, *B. beaticola*, were to become extinct because of climate warming, the adaptive floral variation (small-sized flower) of the high-altitude plant variants that are specially adapted to this bee species would be lost, and the type would become extirpated.

To ensure the conservation of mountain plants in the face of future climate warming, it is first necessary to clarify to what extent such plants are genetically and ecologically differentiated along altitude. Then, further studies should identify intraspecific types or variants and their predicted range shift (Chen et al. 2011), adaptive potential (Till-Bottraud and Gaudeul 2002; Byars et al. 2007; Hoffmann and Sgrò 2011), and plastic phenotypic changes in response to climate change (Canale and Henry 2010). The results of these studies could then be used to develop a framework for the conservation of intraspecifically and altitudinally variable mountain plant species.

Acknowledgments We thank Yusuke Nagano, Satoko Kuriya, and Haruka Kuzume for their preparation of the figures. This study was supported by the Global Environmental Research Fund (D-0904) of the Ministry of the Environment, Japan, and the Japanese Alps Inter-University Cooperative Project, and by the Research and Education Funding for Japanese Alps Inter-Universities Cooperative Project, Ministry of Education, Culture, Sports, Science and Technology, Japan. We thank the Chubu Regional Office for Nature Conservation and the Nagano Prefectural government for their permission to work in those areas.

References

Angert AL (2009) The niche, limits to species' distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. PNAS 106(Suppl 2):19693–19698

Arroyo MTK, Armesto JJ, Primack RB (1985) Community studies in pollination ecology in the high temperate Andes of central Chile II. Effect of temperature on visitation rates and pollination possibilities. Plant Syst Evol 149:187–203

Ayana A, Bryngelsson T, Bekele E (2001) Geographic and altitudinal allozyme variation in sorghum (*Sorghum bicolor* (L.) Moench) landraces from Ethiopia and Eritrea. Hereditas 135:1–12

Bálint M, Domisch S, Engelhardt CHM, Haase P, Lehrian S, Sauer J, Theissinger K, Pauls SU, Nowak C (2011) Cryptic biodiversity loss linked to global climate change. Nat Clim Chang 1:313–318

Barbara T, Martinelli G, Fay MF, Mayo SJ, Lexer C (2007) Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude 'inselbergs', *Alcantarea imperialis* and *Alcantarea geniculata* (Bromeliaceae). Mol Ecol 16:1981–1992

Bingham RA, Orthner AR (1998) Efficient pollination of alpine plants. Nature 391:238–239

Bingham RA, Ranker TA (2000) Genetic diversity in alpine and foothill populations of *Campanula rotundifolia* (Campanulaceae). Int J Plant Sci 161:403–411

Buehler D, Graf R, Holderegger R, Gugerli F (2012) Contemporary gene flow and mating system of *Arabis alpina* in a Central European alpine landscape. Ann Bot 109:1359–1367

Byars SG, Papst W, Hoffmann AA (2007) Local adaptation and cogradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradients. Evolution 61:2925–2941

Byars SG, Parsons Y, Hoffmann AA (2009) Effect of altitude on the genetic structure of an Alpine grass, *Poa hiemata*. Ann Bot 103:885–899

Canale CI, Henry P-Y (2010) Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. Clim Res 43:135–147

Chen G, Wang Y, Zhao C, Korpelainen H, Li C (2008) Genetic diversity of *Hippophae rhamnoides* populations at varying altitudes in the Wolong Natural Reserve of China as revealed by ISSR markers. Silvae Genet 57:29–36

Chen I-C, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. Science 333:1024–1026

Clausen J (1951) Stages in the evolution of plant species. Cornell University Press, New York

Clausen J, Keck DC, Hiesey WM (1940) Experimental studies on the nature of species I. Effect of varied environments on western north American plants. Carnegie Institution of Washington Publication, Washington, DC

Colwell RK, Brehm G, Cardelús CL, Gilman AC, Longino JT (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322:258–261

Conkle MT (1973) Growth data for 29 years from the California elevational transect study of ponderosa pine. For Sci 19:31–39

Dittmar C, Elling W (2006) Phenological phases of common beech (*Fagus sylvatica* L.) and their dependence on region and altitude in Southern Germany. Eur J For Res 125:181–188

Dohzono I, Suzuki K (2010) Morphological and genetic differentiation in *Isodon umbrosus* by altitudinal variation in bumblebee pollinator assemblages. Plant Species Biol 25:20–29

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. Mol Ecol 17:1170–1188

Frenzel B (1968) The Pleistocene vegetation of Northern Eurasia. Science 161:637

Fujii N, Senni K (2006) Phylogeography of Japanese alpine plants: biogeographic importance of alpine region of Central Honshu in Japan. Taxon 55:43–52

Galen C (1985) Regulation of seed-set in *Polemonium viscosum*: floral scents, pollination, and resources. Ecology 66:792–797

Galen C (1989) Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. Evolution 43:882–890

Galen C (1996) Rates of floral evolution: adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. Evolution 50:120–125

Galen C, Shore JS, Deyoe H (1991) Ecotypic divergence in alpine *Polemonium viscosum*: genetic-structure, quantitative variation, and local adaptation. Evolution 45:1218–1228

Garcia-Fernandez A, Segarra-Moragues JG, Widmer A, Escudero A, Iriondo JM (2012) Unravelling genetics at the top: mountain islands or isolated belts? Ann Bot 110:1221–1232

Gómez J, Perfectti F, Bosch J, Camacho J (2009) A geographic selection mosaic in a generalized plant-pollinator-herbivore system. Ecol Monogr 79:245–263

Gonzalo-Turpin H, Hazard L (2009) Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. J Ecol 97:742–751

Gottfried M, Pauli H, Futschik A et al (2012) Continent-wide response of mountain vegetation to climate change. Nat Clim Chang 2:111–115

Groth I, Bergstrom G, Pellmyrt O (1987) Floral fragrances in *Cimicifuga*: chemical polymorphism and incipient speciation in *Cimicifuga simplex*. Biochem Syst Ecol 15:441–444

Grytnes JA (2003) Species-richness patterns of vascular plants along seven altitudinal transects in Norway. Ecography 26:291–300

Grytnes JA, Vetaas OR (2002) Species richness and altitude: a comparison between null models and interpolated plant species richness along the Himalayan altitudinal gradient, Nepal. Am Nat 159:294–304

Gugerli F, Eichenberger K, Schneller J (1999) Promiscuity in populations of the cushion plant *Saxifraga oppositifolia* in the Swiss Alps as inferred from random amplified polymorphic DNA (RAPD). Mol Ecol 8:453–461

Gurevitch J (1988) Variation in leaf dissection and leaf energy budgets among populations of *Achillea* from an altitudinal gradient. Am J Bot 75:1298–1306

Hadado TT, Rau D, Bitocchi E, Papa R (2010) Adaptation and diversity along an altitudinal gradient in Ethiopian barley (*Hordeum vulgare* L.) landraces revealed by molecular analysis. BMC Plant Biol 10:121

Hahn T, Kettle CJ, Ghazoul J, Frei ER, Matter P, Pluess AR (2012) Patterns of genetic variation across altitude in three plant species of semi-dry grasslands. PLoS ONE 7, e41608

Hahn T, Kettle CJ, Ghazoul J, Hennig EI, Pluess AR (2013) Landscape composition has limited impact on local genetic structure in mountain clover, *Trifolium montanum* L. J Hered 104:842–852

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics breeding and genetic resources. Sinauer, Sunderland, pp 43–63

Harder LD, Johnson SD (2009) Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. New Phytol 183:530–545

Hattori M, Nagano Y, Itino T (2014) Geographic flower trait variation of *Aquilegia buergeriana* Sieb. et Zucc. var *buergeriana* on Mt. Norikura and the Utsukushigahara Highland, Japan. Ame J Plant Sci 5:2686–2692. doi:10.4236/ajps.2014.518283

Hattori M, Nagano Y, Itino T (2015) Geographic variation in flower size and flower-visitor composition of two bumblebee-pollinated, spring-flowering herbs, *Lamium album* L. var. *barbatum* (Lamiaceae) and *Meehania urticifolia* (Lamiaceae). Am J Plant Sci 6:737–745. doi:10.4236/ajps.2015.65079

Hautier Y, Randin CF, Stöcklin J, Guisan A (2009) Changes in reproductive investment with altitude in an alpine plant. J Plant Ecol 2:125–134

Herrera CM, Bazaga P (2008) Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes. Heredity 100:275–285

Herrera CM, Castellanos MC, Medrano M (2006) Geographical context of floral evolution: towards an improved research programme in floral diversification. In: Harder LD, Barrett SCH (eds) Ecology and evolution of flowers. Oxford University Press, New York, pp 278–294

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913

Hirao AS, Watanabe M, Liu Q-J, Li X, Masuzawa T, Ohara M, Wada N (2015) Low genetic diversity and high genetic divergence in southern rear-edge populations of Dryas octopetala in mountain sky islands of the Far East. Acta Phytotax Geobot 66:11–22

Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. Nature 470:479–485

Ishizuka W, Goto S (2012) Modeling intraspecific adaptation of *Abies sachalinensis* to local altitude and responses to global warming, based on a 36-year reciprocal transplant experiment. Evol Appl 5:229–244

Jacquemyn H, Micheneau C, Roberts DL, Pailler T (2005) Elevational gradients of species diversity, breeding system and floral traits of orchid species on Reunion Island. J Biogeogr 32:1751–1761

Jump AS, Hunt JM, Martinez-Izquierdo JA, Penuelas J (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Mol Ecol 15:3469–3480

Jump AS, Matyas C, Penuelas J (2009) The altitude-for-latitude disparity in the range retractions of woody species. Trends Ecol Evol 24:694–701

Kara N, Korol L, Isik K, Schiller G (1997) Genetic diversity in *Pinus brutia* Ten.: altitudinal variation. Silvae Genet 46:155–161

Kenta T, Yamada A, Onda Y (2011) Clinal variation in flowering time and vernalisation requirement across a 3000-m altitudinal range in perennial *Arabidopsis kamchatica* ssp. *kamchatica* and annual lowland subspecies *kawasakiana*. J Ecosyst Ecogr S6:001. doi:10.4172/2157-7625.S6-001

Kitayama K (1992) An altitudinal transect study of the vegetation on Mount Kinabalu, Borneo. Vegetatio 102:149–171

Kitzmiller JH (2005) Provenance trials of ponderosa pine in northern California. For Sci 51:595–607

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, Berlin

Körner C (2007) The use of ‘altitude’ in ecological research. Trends Ecol Evol 22:569–574

Korshikov I, Mudrik E (2006) Elevation-dependent genetic variation of plants and seed embryos in the Crimea Mountain population of *Pinus pallasiana* D. Don. Russ J Ecol 37:79–83

Kuriya S, Hattori M, Nagano Y, Itino T (2015) Altitudinal flower size variation correlates with local pollinator size in a bumblebee-pollinated herb, *Prunella vulgaris* L. (Lamiaceae). J Evol Biol 28:1761–1769. doi:10.1111/jeb.12693

Kuzume H, Itino T (2013) Congruence between pollination morphs and genotypes based on internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA in *Cimicifuga simplex* (Ranunculaceae). J Jpn Bot 88:176–181

Machac A, Janda M, Dunn RR, Sanders NJ (2011) Elevational gradients in phylogenetic structure of ant communities reveal the interplay of biotic and abiotic constraints on diversity. Ecography 34:364–371

Malo JE, Baonza J (2002) Are there predictable clines in plant–pollinator interactions along altitudinal gradients? The example of *Cytisus scoparius* (L.) Link in the Sierra de Guadarrama (Central Spain). Divers Distrib 8:365–371

Manel S, Gugerli F, Thuiller W, Alvarez N, Legendre P, Holderegger R, Gielly L, Taberlet P (2012) Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation. Mol Ecol 21:3729–3738

Matter P, Kettle CJ, Ghazoul J, Pluess AR (2013) Extensive contemporary pollen-mediated gene flow in two herb species, *Ranunculus bulbosus* and *Trifolium montanum*, along an altitudinal gradient in a meadow landscape. Ann Bot 111:611–621

Meinzer F, Goldstein G, Rundel P (1985) Morphological changes along an altitude gradient and their consequences for an Andean giant rosette plant. Oecologia 65:278–283

Milá B, Wayne RK, Fitze P, Smith TB (2009) Divergence with gene flow and fine-scale phylogeographical structure in the wedge-billed woodcreeper, *Glyphorynchus spirurus*, a Neotropical rainforest bird. Mol Ecol 18:2979–2995

Montesinos-Navarro A, Wig J, Pico FX, Tonsor SJ (2011) *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. New Phytol 189:282–294

Nagano Y, Abe K, Kitazawa T, Hattori M, Hirao AS, Itino T (2014) Changes in pollinator fauna affect altitudinal variation of floral size in a bumblebee-pollinated herb. Ecol Evol 4:3395–3407

Nattero J, Cocucci AA, Medel R (2010) Pollinator-mediated selection in a specialized pollination system: matches and mismatches across populations. J Evol Biol 23:1957–1968

Navascués M, Vendramin GG, Emerson BC (2008) The effect of altitude on patterns of gene flow in the endemic Canary Island pine, *Pinus canariensis*. Silvae Genet 57:357–363

Nishimura M, Setoguchi H (2011) Homogeneous genetic structure and variation in tree architecture of *Larix kaempferi* along altitudinal gradients on Mt. Fuji. J Plant Res 124:253–263

Nogues-Bravo D, Araujo MB, Romdal T, Rahbek C (2008) Scale effects and human impact on the elevational species richness gradients. Nature 453:216–219

Nosil P (2012) Ecological speciation. Oxford University Press, New York

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. Mol Ecol 18:375–402

Nybom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol Evol Syst 3:93–114

Odland A, Birks H (1999) The altitudinal gradient of vascular plant richness in Aurland, western Norway. Ecography 22:548–566

Ohsawa T, Ide Y (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. Glob Ecol Biogeogr 17:152–163

Ohsawa T, Ide Y (2011) Phylogeographic patterns of highland and lowland plant species in Japan. Alp Bot 121:49–61

Ohsawa T, Tsuda Y, Saito Y, Sawada H, Ide Y (2007) Altitudinal genetic diversity and differentiation of *Quercus crispula* in the Chichibu Mountains, central Japan. Int J Plant Sci 168:333–340

Ohsawa T, Saito Y, Sawada H, Ide Y (2008) Impact of altitude and topography on the genetic diversity of *Quercus serrata* populations in the Chichibu Mountains, central Japan. Flora – Morphol Distrib Funct Ecol Plants 203:187–196

Okuyama Y, Tanabe AS, Kato M (2012) Entangling ancient allotetraploidization in Asian Mitella: an integrated approach for multilocus combinations. Mol Biol Evol 29:429–439

Pauli H, Gottfried M, Dullinger S et al (2012) Recent plant diversity changes on Europe's mountain summits. Science 336:353–355

Pauls SU, Nowak C, Bálint M, Pfenninger M (2013) The impact of global climate change on genetic diversity within populations and species. Mol Ecol 22:925–946

Pellmyr O (1986) Three pollination morphs in *Cimicifuga simplex*; incipient speciation due to inferiority in competition. Oecologia 68:304–307

Petit RJ, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M, Mohanty A, Müller-Starck G, Demesure-Musch B, Palmé A, Martín JP, Rendell S, Vendramin GG et al (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300:1563–1565

Pluess AR, Stöcklin J (2004) Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. Am J Bot 91:2013–2021

Quiroga MP, Premoli AC (2007) Genetic patterns in *Podocarpus parlatorei* reveal the long-term persistence of cold-tolerant elements in the southern Yungas. J Biogeogr 34:447–455

Rahbek C (1995) The elevational gradient of species richness: a uniform pattern? Ecography 18:200–205

Reisch C, Ankea A, Markus R (2005) Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. Basic Appl Ecol 6:35–45

Rowe RJ (2009) Environmental and geometric drivers of small mammal diversity along elevational gradients in Utah. Ecography 32:411–422

Sanders NJ, Rahbek C (2012) The patterns and causes of elevational diversity gradients. Ecography 35:1–3

Schneller J, Liebst B (2007) Patterns of variation of a common fern (*Athyrium filix-femina*; Woodsiaceae): population structure along and between altitudinal gradients. Am J Bot 94:965–971

Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? Evolution 68:1–15

Shanjani PS, Vendramin GG, Calagari M (2011) Altitudinal genetic variations among the *Fagus orientalis* Lipsky populations in Iran. Iran J Biotechnol 9:11–20

Shi M-M, Michalski SG, Chen X-Y, Durka W (2011) Isolation by elevation: genetic structure at neutral and putatively non-neutral loci in a dominant tree of subtropical forests, *Castanopsis eyrei*. PLoS ONE 6, e21302

Shrestha M, Dyer AG, Bhattarai P, Burd M, Shefferson R (2014) Flower colour and phylogeny along an altitudinal gradient in the Himalayas of Nepal. J Ecol 102:126–135

Speed JDM, Austrheim G, Mysterud A (2013) The response of plant diversity to grazing varies along an elevational gradient. J Ecol 101:1225–1236

Stebbins GL (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. Annu Rev Ecol Syst 1:307–326

Stortz JF, Nachman MW (2003) Natural selection drives altitudinal divergence at the albumin locus in deer mouse, *Peromyscus maniculatus*. Evolution 58:1342–1352

Sun M, Gross K, Schiestl FP (2014) Floral adaptation to local pollinator guilds in a terrestrial orchid. Ann Bot 113:289–300

Suzuki Y, Kawaguchi LG, Toquenaga Y (2007) Estimating nest locations of bumblebee *Bombus ardens* from flower quality and distribution. Ecol Res 22:220–227

Suzuki Y, Kawaguchi LG, Munidasa DT, Toquenaga Y (2009) Do bumble bee queens choose nest sites to maximize foraging rate? Testing models of nest site selection. Behav Ecol Sociobiol 63:1353–1362

Svendsen JI, Alexanderson H, Astakhov VI et al (2004) Late Quaternary ice sheet history of northern Eurasia. Quat Sci Rev 23:1229–1271

Theurillat JP, Guisan A (2001) Potential impact of climate change on vegetation in the European Alps: a review. Clim Chang 50:77–109

Thiel-Egenter C, Gugerli F, Alvarez N et al (2009) Effects of species traits on the genetic diversity of high-mountain plants: a multi-species study across the Alps and the Carpathians. Glob Ecol Biogeogr 18:78

Till-Bottraud I, Gaudeul M (2002) Intraspecific genetic diversity in alpine plants. In: Körner C, Spehn EM (eds) Mountain biodiversity: a global assessment. The Parthenon Publishing Group, New York, pp 23–34

Tomono T, Sota T (1997) The life history and pollination ecology of bumblebees in the alpine zone of central Japan. Jpn J Entomol 65:237–255

Wang X, Fang J, Sanders NJ, White PS, Tang Z (2009) Relative importance of climate vs local factors in shaping the regional patterns of forest plant richness across northeast China. Ecography 32:133–142

Webb CO, Ackerly DD, Mcpeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annu Rev Ecol Syst 33:475–505

Wen C, Hsiao J (2001) Altitudinal genetic differentiation and diversity of Taiwan lily (*Lilium longiflorum* var. *formosanum*; Liliaceae) using RAPD markers and morphological characters. Int J Plant Sci 162:287–295

Wirth LR, Graf R, Gugerli F, Landergott U, Holderegger R (2010) Lower selfing rate at higher altitudes in the alpine plant *Eritrichium nanum* (Boraginaceae). Am J Bot 97:899–901

Yamaji H, Sakakibara I, Kondo K, Shiba M, Miki E, Inagaki N, Terabayashi S, Takeda S, Aburada M et al (2005) Phytogeographic analyses of variation in *Cimicifuga simplex* (Ranunculaceae) based on internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA. J Jpn Bot 80:109–120

Ziello C, Estrella N, Kostova M, Koch E, Menzel A (2009) Influence of altitude on phenology of selected plant species in the Alpine region (1971–2000). Clim Res 39:227–234

Chapter 5
Vegetation Zonation and Conifer Dominance Along Latitudinal and Altitudinal Gradients in Humid Regions of the Western Pacific

Shin-ichiro Aiba

Abstract In humid regions of the western Pacific, conifers and deciduous broadleaf trees dominate in colder climate while evergreen broadleaf trees dominate in warmer climate. There are two geographically discontinuous forest zones of conifer-dominated forests, i.e., boreal zone and temperate-to-tropical zone. Between them, there is a deciduous broadleaf forest zone with warm summer and cold winter (Kira's warmth index, WI from 45 to 85 °C, and coldness index, CI <-15 °C). Boreal conifer forest is characterized by continental climate with short summer and cold winter (WI <45 °C and CI <-15 °C), whereas temperate-to-tropical conifer-dominated forests in both hemispheres are characterized by oceanic, moderate climate lacking severe winter (WI <144 °C and CI >-15 °C). Boreal conifer forests are distributed at high latitudes or altitudes in Japan ("subarctic or subalpine forest"). Temperate conifer forests occur in a narrow belt on the Pacific Ocean side in Japan and are more extensive and distributed to warmer climate at high altitudes in Taiwan and in temperate zone of New Zealand, where they are called "conifer-broadleaf" forests. These temperate conifer (or mixed) forests grade into tropical conifer forests at high altitudes in Southeast Asia and New Guinea. Thus, these forests may be collectively called "temperate-to-tropical conifer-mixed forests" in contrast to boreal conifer forest. It appears that relatively cool summer (or year-round low temperature on tropical mountains) limits the growth of evergreen broadleaf trees, while mild winter (or the absence of winter on tropical mountains) gives the competitive advantage to conifers over deciduous broadleaf trees, leading to conifer dominance in the temperate-to-tropical conifer-mixed forests.

Keywords Biogeography • Boreal forest • Broadleaf forest • Coldness index • Conifer forest • Forest zone • Mixed forest • Warmth index

---

S.-i. Aiba (✉)
Graduate School of Science and Engineering, Kagoshima University, Kagoshima 890-0065, Japan
e-mail: aiba@sci.kagoshima-u.ac.jp

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_5

5.1 Introduction

The east and southeast coastal regions of the Asian continent are a unique place on earth in that humid climate continues from equator to high latitudes without being interrupted by dry climate (Kira 1991; Ohsawa 1993, 1995). Similarly, humid regions with annual rainfall >1000 mm are more or less continuously distributed from New Guinea through the east coast of Australia to Tasmania and New Zealand (Wardle 1991; Adam 1992). Moreover, all these regions except northeast Australia have high mountains >2000 m elevation, culminating in the glacier-shouldered summits (up to 4884 m on Puncak Jaya) on New Guinea. Thus, in the western Pacific regions, one can observe how humid vegetation changes across latitudes in both hemispheres and also across altitudes. However, no study has yet been done to summarize the vegetation zonation along latitudinal and altitudinal gradients in the western Pacific including both hemispheres.

Troll (1948) published a diagram depicting vegetation zonation at global scale along both latitudinal and altitudinal gradients to demonstrate "asymmetric vegetation structure" between Northern and Southern Hemispheres. His diagram amalgamates the data from various regions (e.g., tropical zonation primarily from the Andes and northern temperate zonation from Europe) and may not be necessarily applicable to the western Pacific. Ohsawa (1990, 1993, 1995) developed a vegetation template for southern and eastern Asia incorporating both latitudinal and altitudinal gradients, yet he has not extended it to the Southern Hemisphere temperate regions. His template also suffers from combining the data of the Himalayas and coastal Asia at the middle latitudes of 20–30°N, obscuring the pattern in the western Pacific. Dodson et al. (2004) and Hope et al. (2004) presented vegetation maps for the western Pacific including both hemispheres, while they lack the consideration of altitudinal gradient, and the latter was one reconstructed for the last glacial maximum. Here, a new classification system of vegetation zonation along both latitudinal and altitudinal gradients is proposed for the western Pacific regions including both hemispheres.

There are three approaches for the classification of vegetation: ecological, physiognomic, and floristic (Collinson 1988). Ecological and physiognomic approaches are interrelated because vegetation physiognomy reflects underlying ecological factors, and the combination of these approaches is usually employed to define vegetation zones at the global scale (e.g., Walter 1985; Archibold 1995). Yet these two approaches are not entirely compatible. For example, in Archibold's system, physiognomically defined conifer forest biome is distributed in the wide range of climate from boreal to temperate. By contrast, in Walter's system, "zonobiome" of the warm-temperate humid climate (Zonobiome V) includes a range of forest types with different physiognomy (e.g., conifer forest in western North America, mixed conifer-broadleaf forest in Chile and New Zealand, and evergreen broadleaf forest in eastern Asia and southeastern Australia; also see Grubb et al. 2013).

In line with Walter's system, this study primarily adopts an ecological approach, aiming at vegetation classification based on climate. Physiognomic and floristic aspects are also considered, with particular reference to conifer dominance. Conifers are gymnosperms, evolutionarily more ancient than broadleaf trees (angiosperms). With the rise of angiosperms since the Cretaceous, conifers have been losing their dominance in the vegetation on the earth, probably due to the competition with angiosperms. At present, globally, conifers are largely restricted to unproductive habitats at high latitudes and altitudes and on nutrient-poor or dry soils, where growth of angiosperms is reduced. Bond (1989) hypothesized that this reflects less efficient transport system (stereotyped leaves with non-reticulate venation and stems with tracheids) in gymnosperms than in angiosperms. There is a global trend in the humid vegetation that evergreen broadleaf trees dominate in warmer climate, conifers dominate in colder climate, and deciduous broadleaf trees dominate in between. There are many exceptions in individual areas to this global pattern as suggested by Walter's Zonobiome V, and the critical climatic factors for the transition from broadleaf dominance to conifer dominance can be different from place to place. Fortunately, however, climate fairly well explains the pattern of conifer dominance in the western Pacific as will be demonstrated in this study. In particular, this study shows that there are two distinctive zones of conifer-dominated forest under drastically different climates, i.e., boreal versus temperate-to-tropical zones.

5.2 Study Areas and Climate Data

The regions considered in this study are Japan, Taiwan, the Philippines, Borneo, Sumatra, New Guinea, eastern Australia including Tasmania, and New Zealand (Fig. 5.1). All except northeast Australia and Tasmania have high mountains >2000 m elevation, facilitating the simultaneous interpretation of latitudinal and altitudinal vegetation zonation. The monthly mean temperature data (calculated as mean of monthly mean maximum and minimum temperatures if not available) of the highest-elevation climatic stations were collected for the following mountains or islands from the websites of the meteorological agencies of respective countries or regions (Fig. 5.2; Japan Meteorological Agency, Taiwanese Central Weather Bureau, and Australian Bureau of Meteorology) except otherwise noted: Taisetsu Mountains (latitude: 44°N; the elevation of the summit followed by the highest climate station: 2290 m, 1700 m; G. Kudo, unpublished data), Mount Hayachine (40°N; 1914 m, 760 m), Mount Fuji (35°N; 3776 m, 3775 m), Aso Mountains (33°N; 1592 m, 1142 m), Kirishima Mountains (32°N; 1700 m, 1325 m; Kagoshima Meteorological Station 1958), Yakushima Island (30°N; 1936 m, 1800 m; Eguchi 2009), Mount Yushan (23°N; 3997 m, 3845 m), Mount Kinabalu (6°N; 4095 m, 3940 m; Kudo and Suzuki 2004), Mount Wilhelm (6°S; 4508 m, 3480 m; McAlpine et al. 1983), Mount Bellenden Ker (17°S; 1582 m, 909 m; the data of the highest climate station from Herberton), Mount Kosciusko (34°S; 2230 m, 1518 m),

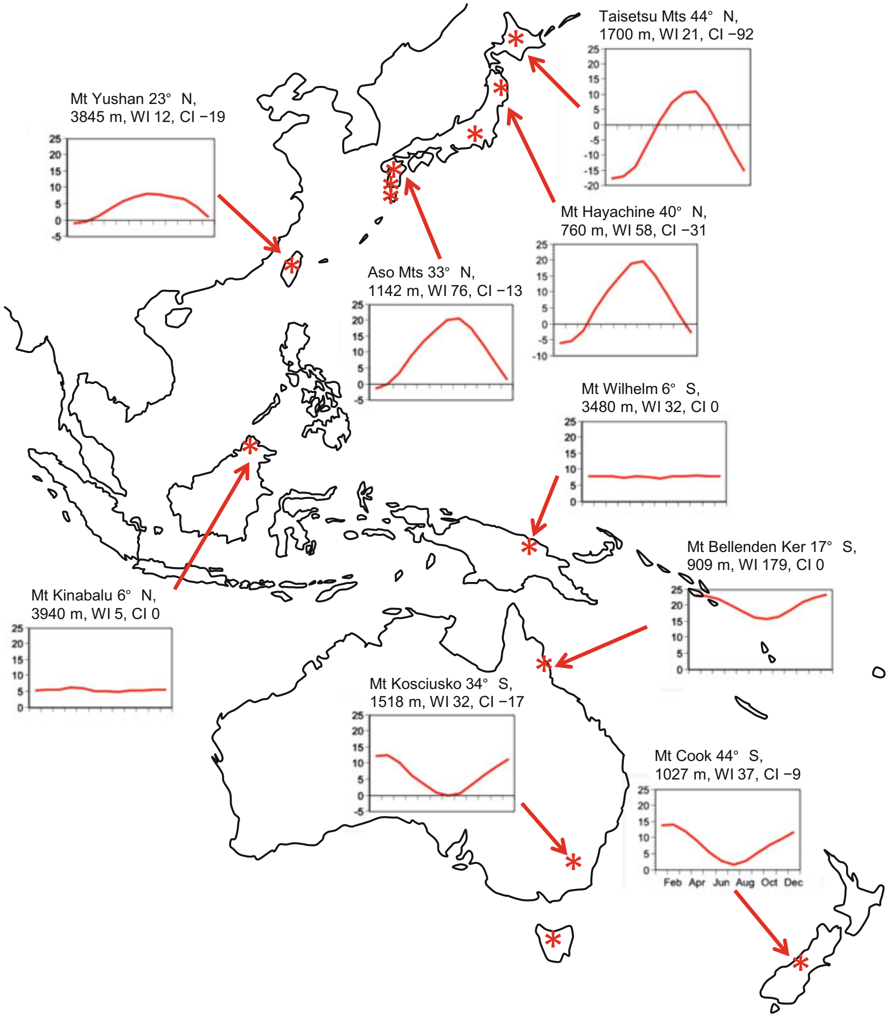


Fig. 5.1 A map of the western Pacific regions showing the locations (*) of mountains where thermal indices were calculated at given altitudes. Seasonal changes in monthly mean temperature are shown for selected climatic stations; the latitude and altitude of the station, the values of warmth index (*WI*), and coldness index (*CI*) are indicated

Tasmania (42°S; 1617 m, 1057 m), and Mount Cook (44°S; 3764 m, 1027 m; Climate-Charts.com 2007). The monthly mean temperature at given elevation was estimated for each mountain or island by assuming a linear lapse rate by using the data of the nearby climate stations at lower elevations. For Japan, only mountains and islands located in the Pacific Ocean side were selected in order to recognize the so-called *Abies firma-Tsuga sieboldii* forest (Yamanaka 1961; Yoshioka 1973; “hemi temperate subzone” of temperate zone, Hämet-Ahti et al. 1974; “temperate

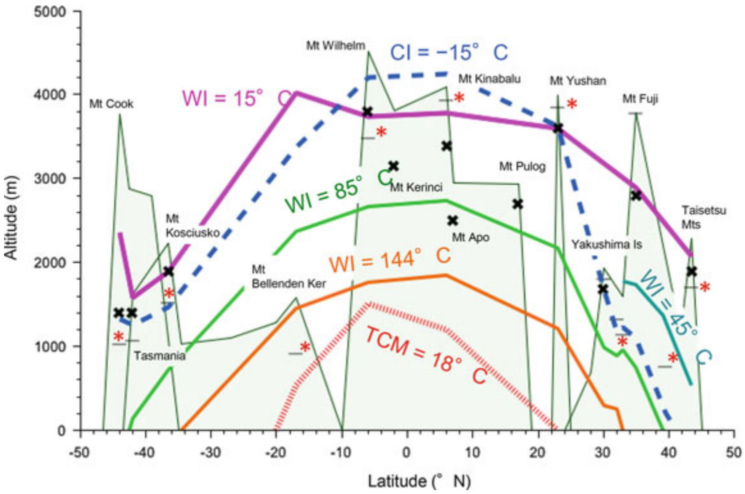


Fig. 5.2 A diagram showing the general topography (*light-colored area with thin outlines*) and the critical values of thermal indices (*thick lines*) along latitudinal and altitudinal gradients in the western Pacific. Thermal indices: *WI* warmth index (*solid lines*), *CI* coldness index (*broken line*), *TCM* monthly mean temperature of the coldest month (*dotted line*). Note that topographic outlines of Japan and Taiwan and those of Australia (including Tasmania) and New Zealand are shown separately. The altitudes of the climatic stations at the highest altitude (−) and treeline (×) on each mountain are also shown. *Asterisks* (*) indicate the climatic station, for which the seasonal changes in monthly mean temperature are shown in Fig. 5.1. The altitude of treeline from Taisetsu Mts, Okitsu (1999); Mt Fuji, Ohsawa (1990); Yakushima Is, Ohsawa (1993); Mt. Yushan, Su (1984); Mt. Pulog (17°N) and Mt. Apo (6°N), Buot and Okitsu (1998); Mt. Kinabalu, Kitayama (1992); Mt. Kerinci, Ohsawa et al. (1985); Mt. Wilhelm, Wade and McVean (1969); Mt. Kosciusko, Williams and Costin (1994); Tasmania, Kirkpatrick (1982); Mt. Cook (northern South Island), Wardle (1991)

conifer forest” *sensu stricto* in this study), which is lacking in the Sea of Japan side, as a distinct forest zone.

5.3 Thermal Indices and Critical Values for the Demarcation of Vegetation Zones

The following thermal indices were calculated from monthly mean temperature t (°C) (Fig. 5.2): warmth index (WI), which is defined as $\Sigma(t–5)$ where the summation is made for months with $t > 5$; coldness index (CI), which is defined as $-\Sigma(5–t)$ where the summation is made for months with $t < 5$ (Kira 1949, 1991); and the monthly mean temperature of the coldest month (TCM). In this study, the following six critical index values were employed, which had been suggested to demarcate vegetation zones in East and Southeast Asia.

1. WI = 15 °C corresponds to forest limit (Kira 1949, 1991; Ohsawa 1990), which is more commonly called “treeline” in recent literature (e.g., Körner 2012).
2. WI = 45 °C corresponds to the lower and southern limit of boreal conifer forest (Kira 1949, 1991).
3. WI = 85 °C corresponds to the lower and southern limit of cool-temperate deciduous broadleaf forest, in particular *Fagus crenata* forest in Japan (Kira 1949, 1991). This value is also effective as a critical value for the transition from tropical montane forest at lower elevations (dominated by evergreen broadleaf trees) to tropical subalpine forests at higher elevations (dominated by conifers) on Mount Kinabalu, Borneo (Kitayama 1992).
4. WI = 144 °C corresponds to the boundary between warm-temperate and subtropical forests. This is taken from the critical value for the transition from *Castanopsis*-*Machilus* forest at lower altitudes to *Quercus* forest at higher altitudes in Taiwan (Su 1984). Kira (1949, 1991) considered WI = 180 °C for the boundary between warm-temperate and subtropical forests; however, this isotherm corresponds to Watase’s line (Hämet-Ahti et al. 1974) where biogeography (historical disjunction between Yakushima Island and Ryukyu Islands) caused drastic species turnover and therefore cannot be considered to be universally significant (though WI = 180 °C seems effective for mainland China as well, Kira 1949, 1991). Notably, WI = 144 °C is close to WI = 140 °C, which corresponds to the boundary of the northern and southern subzones of warm-temperate evergreen broadleaf forest in Japan and to the northern limit of evergreen broadleaf forest in China (Kira 1949, 1991).
5. CI = −15 °C corresponds to the upper limit of canopy species of evergreen broadleaf trees (e.g., *Quercus* spp.) in the absence of heavy snowfall, i.e., in the Pacific Ocean side of Japan (Hattori and Nakanishi 1985). This value is adopted instead of CI = −10 °C (Kira 1949, 1991) representing the northern limit of evergreen broadleaf canopy trees along the coastal areas of Japan because the northern limit may be affected by sea spray (Hattori and Nakanishi 1985).
6. TCM = 18 °C corresponds to the boundary between tropical and extratropical climates (Köppen 1923). This value is also effective in distinguishing tropical lowland forest (dominated by dipterocarps) and tropical montane forest (dominated by non-dipterocarp evergreen broadleaf trees) on Mount Kinabalu (Kitayama 1992).

5.4 Vegetation Zonation in the Western Pacific

According to the above critical values of thermal indices, the following vegetation zones are distinguished as summarized in Table 5.1 and Fig. 5.3. WI = 45 °C is considered only for high-latitude region in the Northern Hemisphere in Fig. 5.3 because this isotherm does not appear to be related to vegetation differentiation in other regions. Each vegetation zone is explained in the following sections. Some of the vegetation zones span from tropical to temperate regions in both hemispheres,

Table 5.1 Vegetation zones in northern temperate (including boreal), tropical, and southern temperate regions, as well as their collective names proposed in this study, of the western Pacific in relation to critical values of thermal index

Thermal index (°C)			Northern temperate/ boreal	Tropical	Southern temperate	Collective name
WI	CI	TCM				
<15	–	–	Alpine zone	Alpine zone	Alpine zone	Alpine zone
15–45	< −15	–	Boreal conifer forest	–	Subalpine forest/ alpine zone[a]	–
45–85	< −15	–	Cool-temperate deciduous broadleaf forest	–	–	–
15–85	> −15	–	Temperate conifer forest	Tropical subalpine forest	Cool-temperate rainforest	Temperate-to-tropical conifer-mixed forests
85–144	–	–	Warm-temperate evergreen broadleaf forest	Tropical upper-montane forest	Warm-temperate rainforest	Temperate-to-tropical conifer-mixed forests
>144	–	<18	Subtropical rainforest	Tropical lower-montane forest	Subtropical rainforest	Tropical-to-subtropical evergreen broadleaf forests
–	–	>18	–	Tropical lowland forest	–	Tropical-to-subtropical evergreen broadleaf forests

WI warmth index, *CI* coldness index, *TCM* monthly mean temperature of the coldest month

[a] Subalpine forest in mainland Australia and alpine zone in Tasmania and New Zealand

but they lack the name inclusive of the whole geographic range because such continuity has rarely been recognized by the previous studies. Therefore, names for each region are listed together in the section headings in the order of northern temperate, southern temperate, to tropical regions (e.g., Sect. 5.4.4). If temperate regions of both hemispheres have the common name, the name for temperate regions is followed by the name for the tropics (e.g., Sect. 5.4.6).

5.4.1 Alpine Zone

"Alpine zone" occurs above treelines in both hemispheres where the climate is too cold for the formation and/or maintenance of forest stands (WI <15 °C). The critical value (WI = 15 °C) for treeline was originally derived from the vegetation pattern in Japan and was usually interpreted as indicating that summer is too short to support tree growth. Interestingly, however, the same critical value appears to

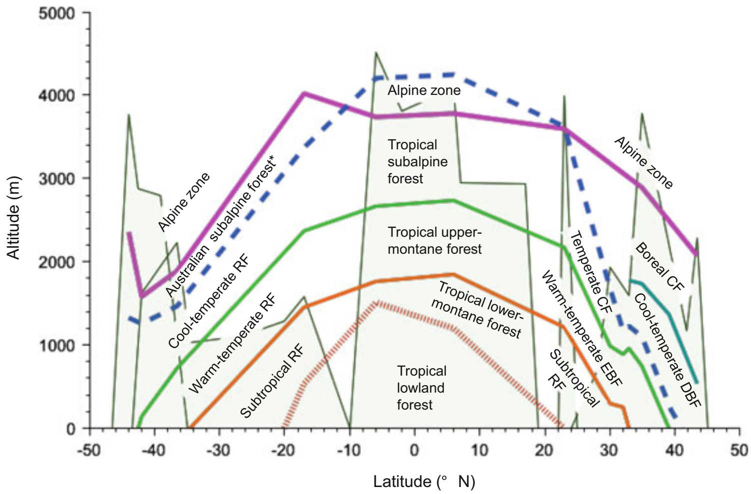


Fig. 5.3 A diagram showing the seven vegetation zones along latitudinal and altitudinal gradients in the western Pacific. Three vegetation zones spanning from tropical montane regions to subtropical and temperate regions in both hemispheres are called by different names in each of these regions. *CF* conifer forest, *DBF* deciduous broadleaf forest, *EBF* evergreen broadleaf forest, *RF* rainforest, *, alpine zone in comparative climate in Tasmania and New Zealand (Fig. 5.2)

explain the treeline on tropical mountains (Kira 1949; Ohsawa 1990) as demonstrated by the data of New Guinea (Fig. 5.2). The lowered treelines on Mount Kerinci and Mount Kinabalu are due to the effects of volcanism and glaciation, respectively (Ohsawa et al. 1985; Kitayama 1992). In the southern temperate regions, the treeline in mainland Australia also corresponds to WI = 15 °C, but those of New Zealand and Tasmania appear to be lower (see Sect. 5.4.2).

The major tree species at treeline are *Abies mariesii*, *A. veitchii,* and *Betula ermanii* in mainland Japan (Kira 1949; Ohsawa 1990; Okitsu 1999), *A. kawakamii* and *Juniperus squamata* in Taiwan (Su 1984), *Dimorphanthera microphylla* and *Rapanea vaccinioides* in New Guinea (Wade and McVean 1969), *Eucalyptus* spp. in Australia and Tasmania (Williams and Costin 1994), and *Nothofagus* spp. in New Zealand (Wardle 1991; Leathwick 2001). The lower treelines in New Zealand and Tasmania may be due to the absence of hardy conifers and deciduous broadleaf trees of the northern origins. In fact, the invasion of introduced *Pinus* spp. has been reported for the alpine zone in New Zealand (Wardle 1985; Froude 2011).

5.4.2 Boreal Conifer Forest and Australian Subalpine Forest

This vegetation zone occurs where summer is short (from 15 to 45 °C in WI) and winter is cold (CI < −15 °C). Globally "boreal conifer forest" is widespread under the continental climate with long, cold winter in the Northern Hemisphere (zonobiome of the cold-temperate boreal climate, Walter 1985). In the western Pacific regions considered in this study, it is restricted at high latitudes and altitudes of Japan, where it is called "subalpine forest" or "subarctic forest" (Kira 1949, 1991; Kira et al. 1976). Although some authors think that there is no or very limited occurrence of true boreal forest in Japan and prefer the name "sub-boreal forest" (Kubota and Hara 1995) or "boreonemoral zone" (*sensu* Walter 1985; Uemura 1994) due to the co-occurrence of cool-temperate deciduous broadleaf trees, climatic conditions corresponding to treeline (WI = 15 °C) do occur in Japan (Fig. 5.2), which may justify the nomenclature adopted here (e.g., Hämet-Ahti et al. 1974; Kojima 1987; Grishin 1995; Okitsu 2000). CI is always <-35 °C in this zone of Japan. Dominant species are *Abies sachalinensis* and *Picea jezoensis* in Hokkaido and *A. mariesii*, *A. veitchii*, *P. jezoensis* (var. *hondoensis*), and *Tsuga diversifolia* in Honshu and Shikoku. This forest zone is absent in Kyushu southward. As will be mentioned below (Sect. 5.4.4), "subalpine forest" dominated by *A. kawakamii* in Taiwan (Su 1984) is not the southern extension of this zone, albeit superficially similar, but should be regarded as "temperate conifer forest" analogous to *A. firma-T. sieboldii* forest in the Pacific Ocean side of Japan. This is counter to the traditional viewpoints regarding Taiwanese subalpine forest as boreal conifer forest (e.g., Tsukada 1966; Su 1984; Ohsawa 1990).

The climatically equivalent areas also occur on high mountains in southern temperate areas in Australia, Tasmania, and New Zealand, but the occurrence of forest is restricted to Snowy Mountains, southeast Australia including Mount Kosciusko (Australian "subalpine forest," Williams and Costin 1994). In Snowy Mountains, the dominant tree is evergreen *Eucalyptus pauciflora*, which may be explained by biogeography (winter hardiness evolved in *Eucalyptus*) and/or climate (winter is less furious). The frost resistance of *E. pauciflora* (−20 °C in bud) is among the highest of trees in Australia, Tasmania, and New Zealand, which is similar to that of the hardiest evergreen broadleaf species in Japan (e.g., −18 °C and −22 °C in bud of *Camellia japonica* and *Pieris japonica*, respectively), but is not as high as deciduous broadleaf trees in Japan (e.g., −30 °C and −50 °C in bud of *Fagus crenata* and *Quercus crispula*, respectively; Sakai et al. 1981; Sakai and Larcher 1987). These suggest that milder winter allows the dominance of evergreen broadleaf trees in subalpine forest of Australia. On Mount Kosciusko 15 °C and 45 °C in WI correspond to −31 °C and −9 °C in CI, respectively, whereas on Taisetsu Mountains, these WI values correspond to −107 °C and −50 °C in CI, respectively. Tree-less alpine vegetation occurs in this zone in Tasmania and New Zealand (Sect. 5.4.1), where treelines appear to coincide with CI = −15 °C (Fig. 5.2), indicating that winter coldness limits the tree distribution in these regions. Wardle (1991) suggested that nocturnal minimum temperature determines treeline in New Zealand, which needs to be interpreted in relation to the absence of hardy trees of the northern origins (Sect. 5.4.1).

5.4.3 Cool-Temperate Deciduous Broadleaf Forest

In the western Pacific, “cool-temperate deciduous broadleaf forest” occurs only in the Northern Hemisphere where summer is warm (45–85 °C in WI) and winter is cold (CI <-15 °C). This vegetation zone is widespread in eastern Asia, Europe, and eastern North America (zonobiome of the temperate-nemoral climate, Walter 1985). It appears that the combination of warm summer and cold winter gives the competitive advantage to deciduous broadleaf trees over both conifers and evergreen broadleaf trees. We need to note that this is the case only in productive habitats on fertile soils. In less productive habitats such as on steep slopes and on immature volcanic soils, conifers may attain high dominance (upper part of “temperate conifer forest” *sensu lato*). This zone does not occur in Taiwan where the climate is oceanic with less thermal seasonality.

The climatically equivalent area to this northern cool-temperate deciduous broadleaf forest does not exist in the southern temperate regions of the western Pacific with oceanic climate similar to Taiwan (compare Mt. Yushan with Mt. Bellenden Ker southward in Fig. 5.1). As was mentioned above (Sect. 5.4.2), WI $=45$ °C corresponds to CI $=-9$ °C on Mount Kosciusko that has the least oceanic climate among humid areas in Australia, Tasmania, and New Zealand.

Deciduous broadleaf forest is virtually absent in Taiwan and in Southern Hemisphere temperate regions with two exceptions: *Fagus hayatae* forest in northern Taiwan (Hukusima et al. 2005) and *Nothofagus gunnii* forest in Tasmania (Veblen et al. 1996), both occurring in warmer climate than this zone. Similar outliers of deciduous forest dominated by *Nothofagus* spp. occur in Chile, South America (Veblen et al. 1996).

5.4.4 Temperate Conifer Forest, Cool-Temperate Rainforest, and Tropical Subalpine Forest

This vegetation zone occurs under oceanic, moderate climate without severe winter (15–85 °C in WI and CI >-15 °C). The forest in this zone is called “temperate conifer forest” (lower part of “temperate conifer forest” *sensu lato*) in northern temperate regions and “cool-temperate rainforest” in the southern temperate regions. Although WI at the threshold CI $=-15$ °C is much higher (i.e., summer is warmer) in Japan (WI $=59$ °C on Yakushima) than in Taiwan (WI $=14$ °C) and southern temperate regions (WI $=35$ °C on Mount Kosciusko), the lack of severe winter characterizes this zone. The climatic equivalence between the temperate forests on both hemispheres can be confirmed by frost resistance of the dominant conifers (Table 5.2).

In Japan, this zone roughly corresponds to *Abies firma-Tsuga sieboldii* forest (Yamanaka 1963; Kira et al. 1976). The lack of severe winter (CI >-15 °C) allows the existence of canopy trees of a few evergreen broadleaf species (e.g., *Quercus* spp.) admixed with conifers. Other dominant conifers include *Cryptomeria*

Table 5.2 Freezing resistance (°C, maximum of leaf or bud) of cut shoots (determined by Sakai and his coworkers) and horticultural hardiness rating (1, hardiest; 11, least hardy; Bannister and Neuner 2001) of conifer species by forest zones

Forest zone and species	Freezing resistance	Hardiness rating	Distribution	Reference
Boreal conifer forest				
Abies mariesii	−70	6	Japan	Sakai and Malla (1981)
A. sachalinensis	−70	5	Japan	Sakai (1983)
A. veitchii	−70	3	Japan	Sakai and Malla (1981)
Picea jezoensis	−70	2	Japan	Sakai (1983)
Tsuga diversifolia	−70	5	Japan	Sakai and Malla (1981)
Temperate conifer forest				
Abies firma	−30	6	Japan	Sakai (1983)
A. kawakamii	−30	6	Taiwan	Sakai (1983)
A. spectabilis	−25	7	Himalaya	Sakai and Malla (1981)
Chamaecyparis obtusa[a]	−20	6	Japan and Taiwan	Sakai and Okada (1971)
Cryptomeria japonica[a]	−25[c]	6	Japan	Sakai and Kurahashi (1975)
Juniperus squamata	−25	5	Taiwan and Himalaya	Sakai (1983)
Picea morrisonicola	−20[c]	8	Taiwan	Sakai (1979)
P. torano[a]	−30	6	Japan	Sakai and Kurahashi (1975)
Tsuga dumosa	−20[c]	8	Himalaya	Sakai and Malla (1981)
T. sieboldii	−25	6	Japan	Sakai and Malla (1981)
Cool-temperate rainforest				
Athrotaxis cupressoides	−20[c]	8	Tasmania	Sakai et al. (1981)
Halocarpus bidwillii	−23	7	New Zealand	Sakai et al. (1981)
Phyllocladus alpinus	−22	8	New Zealand	Sakai et al. (1981)
Podocarpus lawrencei	−22	7	Australia	Sakai et al. (1981)
P. nivalis	−22	7	New Zealand	Sakai et al. (1981)
Warm-temperate rainforest and warm-temperate evergreen broadleaf forest				
Agathis australis	−7[c]	9	New Zealand	Sakai et al. (1981)
Dacrycarpus dacrydioides	−7	9	New Zealand	Sakai et al. (1981)

(continued)

Table 5.2 (continued)

Forest zone and species	Freezing resistance	Hardiness rating	Distribution	Reference
Dacrydium cupressinum	−8	9	New Zealand	Sakai et al. (1981)
Podocarpus hallii	−13	8	New Zealand	Sakai and Wardle (1978)
P. macrophyllus	−13	7	Japan	Sakai and Kurahashi (1975)
P. totara	−7[c]	9	New Zealand	Sakai et al. (1981)
Prumnopitys ferruginea	−10	9	New Zealand	Sakai et al. (1981)
Taiwania cryptomerioides	−15[c]	9	Taiwan	Sakai and Kurahashi (1975)
Subtropical rainforest and tropical lower-montane forest				
Araucaria cunninghamii[b]	−5[c]	10	New Guinea and Australia	Sakai et al. (1981)
Tropical upper-montane forest and tropical subalpine forest				
Dacrycarpus compactus	−5+[c]	NA	New Guinea	Sakai et al. (1981)
Papuacedrus papuana	−5	10	New Guinea	Sakai and Larcher (1987)
Phyllocladus hypophyllus	−3	10	New Guinea	Sakai and Larcher (1987)
Podocarpus brassii	−6	NA	New Guinea	Sakai and Larcher (1987)

NA no data available

[a] Also in cool-temperate deciduous broadleaf forest

[b] Also in tropical upper-montane and tropical lowland forests

[c] Planted trees

japonica, *Chamaecyparis obtusa,* and *Picea torano*, which also occur in unproductive habitats in cool-temperate deciduous broadleaf forest (upper part of "temperate conifer forest" *sensu lato*, Sect. 5.4.3). As mentioned above (Sect. 5.4.2), "subalpine forest" (3100–3600 m) dominated by *A. kawakamii*, as well as "upper-montane forest" (2500–3100 m) dominated by *T. chinensis* and *P. morrisonicola* in Taiwan (Su 1984), also fall in this category. This climatic characterization of species is supported by their frost resistance (Table 5.2).

In cool-temperate rainforest of the Southern Hemisphere, the dominance of conifer may not be necessarily high, e.g., at the treeline in New Zealand showing the mono-dominance of *Nothofagus* spp. (Wardle 1991; Leathwick 2001). However, where *Nothofagus* is absent, mixed conifer-broadleaf forests occur in this zone (see Sect. 5.5). Conifers (e.g., genera *Athrotaxis*, *Lagarostrobos,* and *Phyllocladus*) are also dominant in cool-temperate rainforests in Tasmania (Jarman et al. 1987). Conifers are virtually absent in comparable vegetation in mainland Australia, with only one species (*Podocarpus lawrencei*; Gibson et al. 1995), which may be explained by the past climate (Sect. 5.4.5), although *P. lawrencei* can become dominant in subalpine thickets (Adam 1992).

This zone in tropical forest corresponds to "tropical subalpine forest." Tropical mountains lack winter even at high elevations, so that CI is >0 °C up to treeline (where WI = 15 °C). On non-volcanic high mountains, which show the full development of vegetation, conifers (e.g., genera *Dacrycarpus*, *Phyllocladus,* and *Podocarpus* on Mount Kinabalu and New Guinea and *Papuacedrus* on New Guinea) become dominant occupying the forest upper story (Aiba et al. 2013) or sometime becoming emergents (Grubb and Stevens 1985). Thus, one may call this zone on tropical mountains as "tropical conifer forest" (Tagawa 1995). Ohsawa (1991, 1995) and Buot and Okitsu (1998) suggested that high-altitude forests of Asian tropical mountains showed structure and composition equivalent to the understory of the lower-altitude forests. Their suggestions are based on observations of mountains where conifers are rare at high elevations probably due to the effects of volcanism and/or fires and cannot be accepted as a general rule for the tropical mountains in the western Pacific (Aiba 2011).

5.4.5 Warm-Temperate Evergreen Broadleaf Forest, Warm-Temperate Rainforest, and Tropical Upper-Montane Forest

The climate of this vegetation zone is similar to that of the abovementioned zone (Sect. 5.4.4), but is warmer overall (85–144 °C in WI). The forest in this zone is usually called "warm-temperate evergreen broadleaf forest" in the northern temperate regions and "warm-temperate rainforest" in the southern temperate regions. CI is >-15 °C in Japan and almost always >0 °C in Taiwan and southern temperate regions, leading to the dominance of evergreen broadleaf trees. These thermal conditions largely encompass three regions (Japan, New Zealand, and Australia) of "warm-temperate rain forest" in the western Pacific recognized by Grubb et al. (2013). In limited inland areas of Japan with relatively continental climate, however, winter becomes too cold (CI <-15 °C) to allow the growth of canopy trees of evergreen broadleaf species, where an exceptional "warm-temperate deciduous broadleaf forest" is found (Kira 1949, 1991; Kira et al. 1976).

In the actual vegetation in Japan, warm-temperate evergreen broadleaf forest literally contains few conifers. However, this may reflect historical logging of conifers, especially *Cryptomeria japonica* and *Chamaecyparis obtusa*, dating back to around 600 AC (Totman 1989). There are some areas still showing high abundance of temperate conifers such as *Abies firma* and *Tsuga sieboldii* (e.g., Miyajima Island, Kuroda et al. 2003; Boso Peninsula, Ozaki and Ohsawa 1995), and there used to be more such areas in the past before the large-scale logging started (e.g., the lowland of Yakushima Island, Kira and Yoshino 1967). By contrast, the warm-temperate forest (c. 1500–2500 m) in Taiwan generally shows the greater dominance of conifers such as *Chamaecyparis formosensis*, *C. obtusa* (var. *formosana*), *Pseudotsuga wilsoniana*, *Taiwania cryptomerioides,* and *Cunninghamia konishii*, where historical logging had been less intense (Su 1984; Liao et al. 2014). Kira (1991) proposed the term "warm-temperate lucidophyll forest" for this forest zone in Japan based on leaf properties of the dominant evergreen broadleaf trees with shiny, middle-sized leaves, which are distinct from smaller sclerophyllic leaves and larger tropical leaves.

Like Taiwan, warm-temperate rainforest in New Zealand is also essentially mixed conifer-broadleaf forest including giant conifers such as *Agathis australis*, *Dacrycarpus dacrydioides*, *Dacrydium cupressinum*, *Podocarpus totara,* and *Prumnopitys ferruginea* (Wardle 1991; Leathwick 2001; Lusk 2002). This forest type is characterized by varying degrees of conifer dominance and variously called such as "conifer-broadleaf" (Wardle 1983), "conifer/broad-leaved" (Wardle 1991), or "conifer-broadleaved" forest (Leathwick 2001). In warm-temperate rainforest of eastern Australia, there are two conifer species with highly restricted distributions (the Greater Blue Mountains, 34°S). *Wollemia nobilis* occurs as emergents in the groves in steep canyons (Benson and Allen 2007), while *Microstrobos fitzgeraldii* occurs as a shrub on cliffs generally within the spray zone of waterfalls (Gibson et al. 1995). The paucity of the conifer flora and the lack of conifer dominance in the cool- and warm-temperate rainforests of southeast mainland Australia may be explained by the past climate (Sect. 5.4.4), which will be discussed in Sect. 5.7.2.

Tropical forest in this zone corresponds to "tropical upper-montane forest." This is the lower extension of tropical subalpine forest explained above (Sect. 5.4.4), with reduced dominance of conifers. In the influential book of Whitmore (1984), there is an often-cited diagram of vegetation zones in Malay Peninsula showing that Ericaceae and Myrtaceae, as well as conifers, characterize the upper-montane zone. The review of the literature indicated that conifers and Myrtaceae are by far the dominants and quantitative vegetation data supporting the dominance of Ericaceae (though species richness may be high) is lacking (Aiba 2011).

5.4.6 Subtropical Rainforest and Tropical Lower-Montane Forest

This vegetation zone occurs where climate is generally hot (WI $>$144 °C) but winter (or relatively cooler season in the tropics) can be still recognized (TCM $<$18 °C). Forest in this zone is called "subtropical rainforest" at the middle latitudes of both hemispheres and "tropical lower-montane forest" on the tropical mountains. In this zone, conifer dominance is greatly reduced: only scattered individuals of *Calocedrus* (Taiwan, Liao et al. 2014), *Podocarpus,* and *Nageia* (Japan and Taiwan, Kohyama and Aiba 1997) are found in northern temperate regions, but conifer dominance can become higher in drier areas in southern temperate regions (*Araucaria* forest in Australia, Winter et al 1987; Adam 1992) and on poor soils on tropical mountains (e.g., on ultramafic rocks, Kitayama et al. 2011). Notably, *Araucaria cunninghamii* is distributed in both New Guinea and eastern Australia, demonstrating the climatic equivalence between these two regions. Though this species has a wide distribution range, the centers of the distribution are in lower-montane forest in New Guinea and in subtropical forest in Australia (de Laubenfels 1988; Enright and Hill 1995).

The abovementioned diagram of tropical vegetation zones in Malay Peninsula (Sect. 5.4.5; Whitmore 1984) indicates that Fagaceae and Lauraceae dominate in tropical lower-montane forest. However, the review of the literature revealed the following (Aiba 2011). In the western Malesia (west of Wallace's line, Whitmore 1984), the dominance of Fagaceae is restricted to mountains with nutrient-rich soils affected by volcanism (e.g., the Philippines, Sumatra, and Java), and Myrtaceae and conifers are more dominant on non-volcanic mountains with nutrient-poor soils (e.g., Borneo and Malay Peninsula). In the eastern Malesia (New Guinea, Sulawesi, and Seram), Fagaceae dominates in the lower part of the lower-montane forest, but Myrtaceae and conifers become dominant in the upper part. There is no quantitative vegetation study showing the dominance of Lauraceae (though species richness of Lauraceae may be high as Ericaceae in tropical upper-montane forest, Sect. 5.4.5). Ashton (2003) also pointed out that oak-laurel lower-montane forest is very restricted in Borneo where soils are generally infertile.

5.4.7 Tropical Lowland Forest

"Tropical lowland forest" occurs where climate is hot throughout the year (TCM >18 °C). The northern limit is in the southern lowland of Taiwan (23°N), the southern limit is at around 20°S in eastern Australia, and the upper limit is at around 1500 m in New Guinea. Kira (1991) considered WI = 240 °C as a thermal limit for tropical zone, but TCM = 18 °C roughly corresponds to WI = 210 °C in Taiwan (Lanyu Island), 220 °C in eastern Australia, and 160 °C on New Guinean mountains (note that TCM = 18 °C coincides with WI = 156 °C in perfectly aseasonal climate). The dominant tree family is Dipterocarpaceae in the Philippines and Sundaland (including Borneo and Sumatra), while Dipterocarpaceae is a minor element in New Guinea located in the east of Wallace's line and is absent in Taiwan and Australia. Conifers are essentially absent. The exceptions are *Agathis* and *Araucaria* in New Guinea, *Agathis* in Australia, and *Agathis* and *Dacrydium* on swampy or sandy ("kerangas") soils in Southeast Asia. *Agathis robusta* occurs in lowlands of both New Guinea and Australia, similar to *Araucaria cunninghamii* that tends to occur in cooler climate (Sect. 5.4.6; de Laubenfels 1988). Though not included in the present study, New Caledonia (22°S) is the most notable exception in this zone in having rich conifer flora (Enright and Hill 1995).

5.5 Vegetation Zones in the Tropics and in the Southern Hemisphere

The critical values of thermal indices adopted in this study had been shown effective in explaining the vegetation zones in East and Southeast Asia, especially in the temperate to subtropical regions of the Northern Hemisphere (Japan and Taiwan). Did these fit to the vegetation zones in the tropics and in the Southern Hemisphere?

For the tropical forests below the treelines, the present study recognized four vegetation zones (lowland, lower montane, upper montane, and subalpine) on high mountains (Fig. 5.3). This qualitatively fits the zonation pattern shown by earlier studies conducted for higher mountains (>3000 m) such as New Guinea and Mount Kinabalu (Grubb and Stevens 1985; Kitayama 1992; Ashton 2003), although the subalpine forest is generally lacking on lower mountains <3000 m (Whitmore 1984).

For temperate forests in New Zealand, the present study recognized two vegetation zones, warm-temperate and cool-temperate zones (Fig. 5.3). These zones qualitatively correspond to warm-temperate and cool-temperate belts, the latter including subalpine belt, defined by Wardle (1991). Wardle's subalpine belt is a narrow belt of almost pure stand of *Nothofagu*s spp. marking the treeline. The same *Nothofagus* species are shared by the cool-temperate belt *sensu stricto* (excluding subalpine belt); hence, subalpine belt may be considered to be an upper subzone of the cool-temperate belt *sensu lato*. Leathwick (2001) predicted the potential forest pattern in New Zealand based on extensive datasets and classified forests into five groups: (1) conifer-broadleaf forests in warm, wet climate; (2) mixed forests in cool, wet climate; (3) *Nothofagus*-dominant forests in cool, wet climate, including those at treeline; (4) conifer-dominant forests in warm, dry climate; and (5) *Nothofagu*s-dominant forests in cool, dry climate. The climates for groups (2) and (3) are similar, suggesting that *Nothofagus* dominance is related to biogeographic factors such as the range contraction of *Nothofagus* in the last glaciation (Sect. 5.7.2). This classification, though incorporating the wet-dry axis, also supports the warm versus cool dichotomy of New Zealand forest zones recognized in the present study.

For eastern Australia to Tasmania spanning from tropical to temperate regions, the present study recognized four forest zones excluding the small areas of subalpine zone restricted to high elevations in southeast mainland: tropical lowland, subtropical, warm-temperate, and cool-temperate zones (Fig. 5.3). There has been no consensus as to the classification of Australian rainforest, but the physiognomic system proposed by Webb (1978) has been most widely used (Adam 1992; Bowman 2000). Webb's system may be translated to the classification based on climate, in which four climatic zones are recognized (tropical, subtropical, warm temperate, and cool temperate; Webb 1968; Winter et al 1987; Adam 1992). Thus, the classification proposed in the present study qualitatively matches the zones shown by Australian rainforests as well.

5.6 Tropical-Temperate Dichotomy or Continuity Across Latitudes?

In the present study, boreal conifer forest (Sect. 5.4.2) and cool-temperate deciduous broadleaf forest (Sect. 5.4.3) occur only in the Northern Hemisphere, as was depicted by the Troll's diagram of the "asymmetric vegetation structure" of the earth (Troll 1948). On the other hand, the vegetation zones at the lower latitudes and lower altitudes span from tropical mountains to temperate regions in both hemispheres (Sects. 5.4.4, 5.4.5, and 5.4.6). This is again in line with the general idea of Troll (1948), but is in contrast to Ohsawa (1990, 1993, 1995) who distinguished the altitudinal zonations on tropical versus temperate mountains with transitional zonation at 20–30°N in eastern and southern Asia. Although Ohsawa (1990, 1993, 1995) admitted that tropical lower-montane forest belongs to the same zone with subtropical forest in mid-latitude lowland (corresponding to Sect. 5.4.6 in the present study), he did not take into account the relationship between tropical upper-montane forest (including tropical subalpine forest) and temperate forests. The basis of vegetation zone classifications by Troll and Ohsawa is mostly climate. Based on floristics and physiognomy along with climate, Shimizu (2014) pointed out that tropical upper- and lower-montane forests correspond to warm-temperate and subtropical forests, respectively, which is qualitatively consistent with the present study, but he did not give the detailed explanation about the similarity between tropical and temperate forests.

Should we distinguish tropical versus temperate zones, or should we consider the continuous change in forest vegetation from tropical to temperate regions? The answer will depend on the objectives of the vegetation classification. If we aim to demonstrate the effects of the climate seasonality on vegetation, then tropical and temperate zones should be distinguished. If we aim to clarify the controlling factors for the resemblance between tropical and temperate vegetation, then the continuity between tropical and temperate zones should be explored. As will be discussed below, the continuous system adopted here is effective in understanding the geographic pattern of conifer dominance. Tropical climate, though lacking seasonal change, exhibits diurnal fluctuations in temperature, and the nighttime temperature can become low just like winter in temperate regions (e.g., Kudo and Suzuki 2004). Southern Hemisphere temperate and tropical high-altitude environments are similar in that freeze-thaw event can occur at any time of the year (Feild et al. 2002). Therefore, there seems to be no absolute reason to assume that tropical and temperate zones are fundamentally different.

5.7 Biogeography and Ecology of Conifer Dominance

5.7.1 Geographic Pattern of Conifer Dominance

In the western Pacific regions, there are two forest zones showing conifer dominance (conifer forests or mixed conifer-broadleaf forests), i.e., boreal forest (Sect. 5.4.2) versus temperate-to-tropical forests (Sects. 5.4.4 and 5.4.5), separated by cool-temperate deciduous broadleaf forest zone (Sect. 5.4.3) with warm summer and cold winter (45–85 °C in WI and CI < -15 °C; an example of climate shown for Mount Hayachine in Fig. 5.1). Boreal conifer forest is characterized by continental climate with short summer and long, cold winter (15–45 °C in WI and CI < -15 °C), occurring only at high latitudes and high altitudes (above the cool-temperate deciduous broadleaf forest) in Japan ("subalpine or subarctic forests"). By contrast, temperate-to-tropical forests showing conifer dominance in both hemispheres are characterized by oceanic, moderate climate without severe winter (15–144 °C in WI and CI > -15 °C). These climatic differences between boreal versus temperate-to-tropical forests can be seen if one compare climate of Taisetsu Mountains with those of Aso Mountains southward in Fig. 5.1.

In Japan, at the lower altitudes or latitudes than the cool-temperate deciduous broadleaf forests, the temperate conifer forest (Sect. 5.4.4) is distributed in a narrow geographic belt on the Pacific Ocean side with climatic condition from 45 to 85 °C in WI (so-called *Abies firma-Tsuga sieboldii* forest). However, the restriction of conifer dominance to this narrow belt is probably due to the historical logging (Sect. 5.4.5). If we look at vegetations in Taiwan and New Zealand, the coexistence of conifer and broadleaf trees is the rule in warm-temperate forests in warmer climate (85–144 °C in WI). Thus, temperate forests showing high conifer dominance (conifer forests or mixed conifer-broadleaf forests) are more extensive in temperate regions outside Japan. These temperate conifer or mixed forests grade into tropical conifer forests (tropical subalpine and upper-montane forests) at high elevations in Southeast Asia and New Guinea. Therefore, we may collectively call these forests (Sects. 5.4.4 plus Sect. 5.4.5) as "temperate-to-tropical conifer-mixed forests" in contrast to boreal conifer forest confined to high latitudes and altitudes in the Northern Hemisphere (Fig. 5.4 and Table 5.1). "Conifer-mixed forest" (Devito et al. 1999) rather than "conifer forest" is appropriate for the temperate-to-tropical zone since even so-called temperate conifer forests and tropical conifer forests always accompany broadleaf trees with varying degrees of mixture, especially in the understory.

Similarly, tropical and subtropical forests dominated by evergreen broadleaf trees (Sects. 5.4.6 plus Sect. 5.4.7) can be collectively called "tropical-to-subtropical evergreen broadleaf forests." Subtropical and tropical lower-montane forests (Sect. 5.4.6) can be regarded as impoverished variations of rich, gigantic tropical lowland forest (Sect. 5.4.7) under the suboptimal thermal conditions. On the other hand, Tagawa (1995) merged warm-temperate, subtropical, and tropical montane forests (excluding those dominated by conifers) in Asia and New Guinea

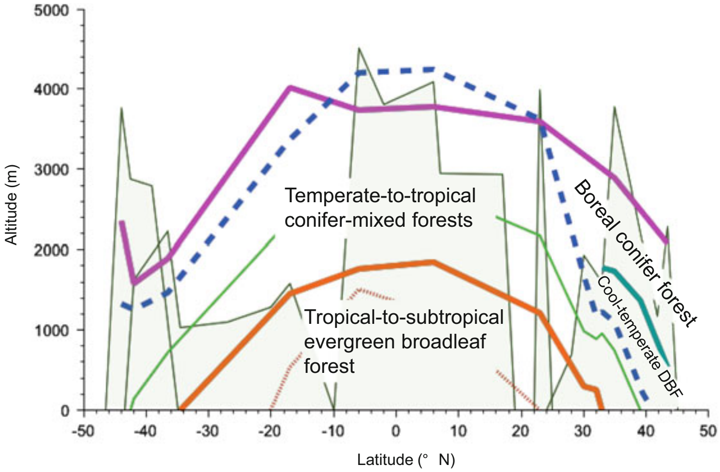


Fig. 5.4 Four forest zones broadly defined by conifer dominance and climate. See Fig. 5.3 for the abbreviation

(Sects. 5.4.5 plus Sect. 5.4.6) into "lucidophyll oak-laurel forest" formation from the physiognomic and floristic viewpoints; however, as was explained earlier (Sect. 5.4.6), the term "oak-laurel forest" is inappropriate as the general name even for tropical lower-montane forest.

What climatic factors make temperate-to-tropical conifer-mixed forests a distinctive vegetation zone from tropical-to-subtropical evergreen broadleaf forests and from cool-temperate deciduous broadleaf forest? It appears that relatively cool summer (or year-round low temperature on tropical mountains) limits the growth of evergreen broadleaf trees while mild winter (or the absence of winter on tropical mountains) gives the competitive advantage to conifers over deciduous broadleaf trees, leading to conifer dominance in the temperate-to-tropical conifer-mixed forests. The latter situation should make photosynthesis possible in winter (or all year round in tropical mountains), as is the case for conifer forests in the western North America (Waring and Franklin 1979; Han and Mukai 1999; Weng et al. 2005). The unproductive habitats on poor soils also help conifers outcompete broadleaf (both evergreen and deciduous) trees and locally modify the vegetation zonation. Aiba et al. (2013) suggested that lower growth of broadleaf trees in cold and/or nutrient-poor environments results in sparser forest canopy, allowing shade-intolerant conifers to dominate the forests.

The boreal conifer forest in Japan is dominated by *Abies mariesii*, *A. sachalinensis*, *A. veitchii*, *Picea jezoensis,* and *Tsuga diversifolia*. Temperate conifer forest is dominated by *A. firma*, *T. sieboldii*, *Cryptomeria japonica,* and *P. torano* in Japan and by *A. kawakamii*, *T. chinensis, and P. morrisonicola* in Taiwan. Therefore, boreal and temperate conifer forests share congeneric species. Assigning these genera to particular climate is therefore not persuasive. *Picea* and *Tsuga* were often considered as purely boreal and cool-temperate elements, respectively, while *Abies* as representing both boreal and cool-temperate zones in the pollen analysis (e g. Takahara et al. 2000; Gotanda et al. 2002). On the other hand, Sakai (1983) demonstrated that these genera showed similar ranges of frost resistance, all with wide interspecific variations (Table 5.2). Therefore, a precaution is needed in interpreting the vegetation reconstructions based on pollen analysis. In particular, it is stressed here that the dominance of *Abies* and *Picea* is not restricted to boreal forest. In this study it was shown that "subalpine forest" dominated by *A. kawakamii* as well as "upper-montane forest" dominated by *T. chinensis* and *P. morrisonicola* in Taiwan are considered as temperate conifer forest analogous to *A. firma*-*T. sieboldii* forest in Japan, not as boreal conifer forest. Though not included in the present study, subalpine forests in the Himalayas dominated by *Abies spectabilis* (and other high-elevation forests including *Abies*, *Picea,* and *Tsuga*) should also be considered as temperate conifer forest, judging from their similar frost resistance to *A. firma*, *A. kawakamii*, *P. morrisonicola,* and *T. sieboldii* (Table 5.2; Sakai and Malla 1981).

5.7.2 Biogeography and Ecology

There is a marked floristic discontinuity in the southern tip of Taiwan (Takhtajan 1986) or between Taiwan and the Philippines (Hsieh 2003; Raes and van Welzen 2009). There, holarctic and paleotropical elements predominate northward and southward, respectively. Dominant conifers in temperate zones of Japan and Taiwan (Pinaceae and some Cupressaceae; hereafter "northern conifers") are probably of northern or Laurasian origins, while those in temperate-to-tropical zones of Southeast Asia, New Guinea, Australia, and New Zealand (Araucariaceae, Podocarpaceae, and other Cupressaceae; hereafter "southern conifers") had been the significant components in southern or Gondowana flora (Hill and Brodribb 1999; LaPage 2003; Mao et al. 2012). Temperate northern conifers such as *Abies firma* and *A. kawakamii* lack extreme cold hardiness, unlike boreal northern conifers (Table 5.2). Contrarily, some southern conifers (e.g., *Halocarpus bidwillii*, *Phyllocladus alpinus*, *Podocarpus lawrencei,* and *P. nivalis*) have evolved to acquire moderate hardiness comparable to temperate northern conifers, although southern conifers are generally less frost resistant than northern conifers (Sakai and Larcher 1987; Bannister and Neuner 2001). Thus, temperate-to-tropical conifers in both hemispheres include exceptions to the global pattern that northern conifers are associated with continental climate in which winter is furious while southern conifers are with maritime climate in which winter is mild or absent (Brodribb and Hill 2004; Bannister 2007).

How almost non-overlapping distributions between northern and southern conifers in the temperate-to-tropical conifer-mixed forest zone arouse and are maintained is an interesting question. Among southern conifers, the genus *Dacrydium* was present in Japan in the past (c. 15 million years ago, Morley 2000), so that its current absence in northern temperate regions (Taiwan and

Japan) at first look seems to be explained by the lack of cold tolerance. However, *D. cupressinum* is distributed to as far south as 47°S in New Zealand, and its leaves can tolerate −8 °C freezing, which is comparable to some evergreen broadleaf trees (e.g., *Elaeocarpus sylvestris* and *Litsea japonica*) in the southwestern part of mainland Japan (Sakai and Larcher 1987; Wardle 1991). This suggests that biogeography (such that *Dacrydium* has not crossed the geographic barriers under the current climate) likely explain the present geographic distribution of *Dacrydium*. Among northern conifers, *Pinus* and *Taxus* have crossed the sea (though probably they moved mostly on land via exposed Sundaland) from Asian continent to insular Southeast Asia (Sumatra and the Philippines for *Pinus* and *Taxus* and Sulawesi for *Taxus*, de Laubenfels 1988), again suggesting that biogeography (geographic barrier) rather than ecology (climatic niche) explains their present ranges of distribution that are biased toward the Northern Hemisphere. However, it is possible that some taxa adapted to seasonal climate cannot sustain their populations in tropical climate (such that flower bud formation requires winter chill).

The possible role of the past climate in determining the current vegetation pattern was suggested for *Nothofagus* dominance in cool-temperate zone in New Zealand (Sect. 5.5). The similar situation may apply to the poor diversity and the lack of dominance of conifers in the cool- and warm-temperate rainforests of southeast mainland Australia (Sects. 5.4.4 and 5.4.5), which had only three species of conifers (*Podocarpus lawrencei*, *Microstrobos fitzgeraldii,* and *Wollemia nobilis*; the latter two species have extremely localized distributions; Gibson et al. 1995; Benson and Allen 2007). The fossil records indicated that the cooler and drier climate in the past have caused the extinction of many rainforest conifer taxa in southeastern Australia (Hill and Brodribb 1999; Kershaw and Wagstaff 2001). This contrasts with relatively rich conifer flora of dry forests and open woodlands in Australia (*Callistris* and *Actinostrobos*; Bowman and Harris 1995). In sum, distributions of tropical-to-temperate conifers should have been constrained by both historical and ecological factors.

The majority of temperate-to-tropical conifers in both hemispheres are shade-intolerant species, which persist in forest overstory due to long lifespan, and they depend on occasional natural disturbances for regeneration (Enright and Hill 1995; Aiba et al. 2013). Some temperate-to-tropical mixed forests with either northern or southern conifers in the western Pacific (Japan, Taiwan, New Guinea, and New Zealand) show “additive basal area” phenomenon, which indicates vertical niche partitioning between shorter, shade-tolerant broadleaf trees and emergent, shade-intolerant conifers (Enright 1982; Ogden 1985; Enright and Hill 1995; Lusk 2002; Aiba et al. 2007; Liao et al. 2014). Thus, temperate-to-tropical conifers of both hemispheres are ecologically similar in many ways irrespective of taxonomy, meaning that these conifers can be considered as a functionally coherent group. These considerations justify the climatically defined vegetation zones proposed here, some of which span from tropical to temperate regions in both hemispheres.

Acknowledgments I thank Gaku Kudo and Takashi Kohyama for giving me the opportunity to write this chapter and for commenting on the manuscript. I-Fang Sun, Yu-Yun Chen, and Yoshiko Iida arranged my visit to Taiwan's magnificent forests.

References

Adam P (1992) Australian rainforests. Oxford University Press, Oxford

Aiba S (2011) Tree composition of tropical mountain forest on Mount Kinabalu, Borneo: comparison with other mountains in Malesian floristic region. Eco-Habitat 18:157–168 (in Japanese)

Aiba S, Hanya G, Tsujino R, Takyu M, Seino T, Kimura K, Kitayama K (2007) Comparative study of additive basal area of conifers in forest ecosystems along elevational gradients. Ecol Res 22: 439–450

Aiba S, Akutsu K, Onoda Y (2013) Canopy structure of tropical and sub-tropical rain forests in relation to conifer dominance analysed with a portable LIDAR system. Ann Bot 112: 1899–1909

Archibold OW (1995) Ecology of world vegetation. Chapman and Hall, London

Ashton PS (2003) Floristic zonation of tree communities on wet tropical mountains revisited. Perspect Plant Ecol Evol Syst 6:87–104

Bannister P (2007) A touch of frost? Cold hardiness of plants in the Southern Hemisphere. N Z J Bot 45:1–33

Bannister P, Neuner G (2001) Frost resistance and the distribution of conifers. In: Bigras FJ, Colombo SJ (eds) Conifer cold hardiness. Kluwer Academic Publishers, Dordrecht, pp 1–21

Benson J, Allen C (2007) Vegetation associated with *Wollemia nobilis* (Araucariaceae). Cunninghamia 10:255–261

Bond WJ (1989) The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence. Bot J Linn Soc 36:227–249

Bowman DMJS (2000) Australian rainforests. Cambridge University Press, Cambridge

Bowman DMJS, Harris S (1995) Conifers of Australia's dry forests and open woodlands. In: Enright NJ, Hill RS (eds) Ecology of the southern conifers. Melbourne University Press, Melbourne, pp 252–270

Brodribb T, Hill RS (2004) The rise and fall of the Podocarpaceae in Australia–a physiological explanation. In: Hemsley AR, Poole I (eds) The evolution of plant physiology. Elsevier, Amsterdam, pp 381–399

Buot IE, Okitsu S (1998) Vertical distribution and structure of the tree vegetation in the montane forest of Mt. Pulog, Cordillera mountain range, the highest mountain in Luzon Is., Philippines. Veg Sci 15:19–32

Climate-Charts.com (2007) World climate. http://www.climate-charts.com/index.html. Accessed 2 Dec 2014

Collinson AS (1988) Introduction to world vegetation, 2nd edn. Unwin Hyman, London

de Laubenfels DJ (1988) Coniferales. Flora Malesiana Ser I 10:337–453

Devito KJ, Westbrook CJ, Schiff SL (1999) Nitrogen mineralization and nitrification in upland and peatland forest soils in two Canadian Shield catchments. Can J For Res 29:1793–1804

Dodson JR, Taylor D, Ono Y, Wang P (2004) Climate, human, and natural systems of the PEP II transect. Quat Int 118–119:3–12

Eguchi T (2009) Regional characteristics and interannual change in the climate of Yakushima. In: Nature Conservation Society of Japan (ed) Report on the evaluation of dynamics and the methods of conservation and management of natural environment in Yakushima World Heritage area. Kyushu Branch, Ministry of Environment, Kumamoto, pp 6–18, in Japanese

Enright NJ (1982) Does *Araucaria hunsteinii* compete with its neighbours? Aust J Ecol 7:97–99

Enright NJ, Hill RS (eds) (1995) Ecology of the southern conifers. Melbourne University Press, Melbourne

Feild TS, Brodribb T, Holbrook NM (2002) Hardly a relict: freezing and the evolution of vesselless wood in Winteraceae. Evolution 56:464–478

Froude VA (2011) Wilding conifers in New Zealand: beyond the status report. Pacific Eco-Logic, Bay of Islands

Gibson H, Barker PCJ, Cullen PJ, Shapcott A (1995) Conifers of southern Australia. In: Enright NJ, Hill RS (eds) Ecology of the southern conifers. Melbourne University Press, Melbourne, pp 223–251

Gotanda K, Nakagawa T, Tarasov P, Kitagawa J, Inoue Y, Yasuda Y (2002) Biome classification from Japanese pollen data: application to modern-day and Late Quaternary samples. Quat Sci Rev 21:647–657

Grishin SY (1995) The boreal forests of north-eastern Eurasia. Vegetatio 121:11–21

Grubb PJ, Stevens PF (1985) The forests of the Fatima Basin and Mt Kerigomna, Papua New Guinea. Australian National University, Canberra

Grubb PJ, Bellingham PJ, Kohyama TS, Piper FI, Valido A (2013) Disturbance regimes, gap-demanding trees and seed mass related to tree height in warm temperate rain forests worldwide. Biol Rev 88:701–744

Hämet-Ahti L, Ahti T, Koponen T (1974) A scheme of vegetation zones for Japan and adjacent regions. Ann Bot Fenn 11:59–88

Han Q, Mukai Y (1999) Cold acclimation and photoinhibition of photosynthesis accompanied by needle color changes in *Cryptomeria japonica* during the winter. J For Res 4:229–234

Hattori T, Nakanishi S (1985) On the distribution limits of the lucidophyllus forest in the Japanese Archipelago. Bot Mag Tokyo 98:317–333

Hill RS, Brodribb TJ (1999) Southern conifers in time and space. Aust J Bot 47:639–696

Hope G, Kershaw AP, van der Kaars S, Sun X, Liew P-M, Heusser LE, Takahara H, McGlone M, Miyoshi N, Moss PT (2004) History of vegetation and habitat change in the Austral-Asian region. Quatern Int 118–119:103–126

Hsieh CF (2003) Composition, endemism and phytogeographical affinities of the Taiwan flora. In: Editorial Committee of the Flora of Taiwan (ed) Flora of Taiwan, vol 6, 2nd edn. National Taiwan University, Taipei, pp 1–14

Hukusima T, Lu SY, Matsui T, Nishio T, Liu CL, Pignatti S (2005) Phytosociology of *Fagus hayatae* forests in Taiwan. Rend Fis Acc Lincei 16:171–189

Jarman SJ, Brown MJ, Kantvillas G (1987) The classification and conservation status of Tasmanian rainforests. In: Werren G, Kershaw AP (eds) The rainforest legacy, vol 1. Australian Government Publishing Service, Canberra, pp 9–22

Kagoshima Meteorological Station (1958) Kagoshima Prefecture meteorological records over 75 years. Kagoshima Meteorological Station, Kagoshima (in Japanese)

Kershaw P, Wagstaff B (2001) The southern conifer family Araucariaceae: history, status, and value for paleoenvironmental reconstruction. Annu Rev Ecol Syst 32:397–414

Kira T (1949) Forest zones of Japan. Nihon Ringyo Gijyutsu Kyokai, Tokyo (in Japanese)

Kira T (1991) Forest ecosystems of east and southeast Asia in a global perspective. Ecol Res 6:185–200

Kira T, Yoshino M (1967) Distribution of Japanese conifers in relation to temperature. In: Morisita M, Kira T (eds) Natural history ecological study. Chuokoron-sha, Tokyo, pp 133–161 (in Japanese)

Kira T, Shidei T, Numata M, Yoda K (1976) Vegetation of Japan. Kagaku 46:235–247 (in Japanese)

Kirkpatrick JB (1982) Phytogeographical analysis of Tasmanian alpine flora. J Biogeogr 9: 255–271

Kitayama K (1992) An altitudinal transect study of the vegetation on Mount Kinabalu, Borneo. Vegetatio 102:149–171

Kitayama K, Aiba S, Ushio M, Seino T, Fujiki Y (2011) The ecology of podocarps in tropical montane forests of Borneo: distribution, population dynamics, and soil nutrient acquisition. In: Turner BL, Cernusak LA (eds) Ecology of the Podocarpaceae in tropical forests. Smithsonian Institution Scholarly Press, Washington, DC, pp 101–117

Kohyama T, Aiba S (1997) Dynamics of primary and secondary warm-temperate rain forests in Yakushima Island. Tropics 6:383–392

Kojima S (1987) Conifer forest zone. In: Ito K (ed) Vegetation of Hokkaido. Hokkaido University Press, Sapporo, pp 200–233 (in Japanese)

Köppen W (1923) Die Klimate der Erde. Walter de Gruyter & Co, Berlin

Körner C (2012) Alpine treelines. Springer, Basel

Kubota Y, Hara T (1995) Tree competition and species coexistence in a sub-boreal forest, northern Japan. Ann Bot 76:503–512

Kudo G, Suzuki S (2004) Flowering phenology of tropical-alpine dwarf trees on Mount Kinabalu, Borneo. J Trop Ecol 20:563–571

Kuroda A, Kai T, Mukai S, Toyohara G (2003) Floristic composition and vertical distribution of *Abies firma-Tsuga sieboldii* forests of the Mt. Misen Primeval Forest on Miyajima Island, SW Japan. Hikobia 14:15–24

LaPage BA (2003) The evolution, biogeography and palaeoecology of the Pinaceae based on fossil and extant representatives. Acta Horticult 615:29–52

Leathwick JR (2001) New Zealand's potential forest pattern as predicted from current species-environment relationships. N Z J Bot 39:447–464

Liao CC, Liu M, Su MH, Wang JC (2014) Compression and overlap of unique vegetation system of subtropical mountain resembling tropical and temperate forests along elevation. J For Res 19:215–225

Lusk CH (2002) Basal area in a New Zealand podocarp-broadleaved forest: are coniferous and angiosperm components independent? N Z J Bot 40:143–147

Mao K, Milne RI, Zhang L, Peng Y, Liu J, Thomas P, Mill RR, Renner SS (2012) Distribution of living Cupressaceae reflects the breakup of Pangea. Proc Natl Acad Sci U S A 109:7793–7798

McAlpine JR, Keig G, Falls R (1983) Climate of Papua New Guinea. CSIRO, Canberra

Morley RJ (2000) Origin and evolution of tropical rain forests. Wiley, Chichester

Ogden J (1985) An introduction to plant demography with special reference to New Zealand trees. N Z J Bot 23:751–772

Ohsawa M (1990) An interpretation of latitudinal patterns of forest limits in South and East Asian mountains. J Ecol 78:326–339

Ohsawa M (1991) Structural comparison of tropical montane rain forests along latitudinal and altitudinal gradients in south and east Asia. Vegetatio 97:1–10

Ohsawa M (1993) Latitudinal pattern of mountain vegetation zonation in southern and eastern Asia. J Veg Sci 4:13–18

Ohsawa M (1995) Latitudinal comparison of altitudinal changes in forest structure, leaf-type, and species richness in humid monsoon Asia. Vegetatio 121:3–10

Ohsawa M, Nainggolan PHJ, Tanaka N, Anwar C (1985) Altitudinal zonation of forest vegetation on Mount Kerinci, Sumatra: with comparison to zonation in the temperate region of east Asia. J Trop Ecol 1:193–216

Okitsu S (1999) Phytogeography of the vegetation of Mt. Taisetsu, central Hokkaido, northern Japan. Actinia 12:103–112 (in Japanese with English summary)

Okitsu S (2000) Phytogeography of the major forests of northern Japan in northeastern Asia. Geogr Rep Kokushikan Univ 9:1–11 (in Japanese with English summary)

Ozaki K, Ohsawa M (1995) Successional change of forest pattern along topographical gradients in warm-temperate mixed forests in Mt Kiyosumi, central Japan. Ecol Res 10:223–234

Raes N, van Welzen PC (2009) The demarcation and internal division of Flora Malesiana: 1857–present. Blumea 54:6–8

Sakai A (1979) Freezing avoidance mechanism of primordial shoots of conifer buds. Plant Cell Physiol 20:1381–1390

Sakai A (1983) Comparative study of freezing resistance of conifers with special reference to cold adaptation and its evolutionary aspects. Can J Bot 63:156–160

Sakai A, Kurahashi A (1975) Freezing resistance of conifers in Japan with special reference to their distributions. Japan J Ecol 25:192–200 (in Japanese with English summary)

Sakai A, Larcher W (1987) Frost survival of plants. Springer, Berlin

Sakai A, Malla SB (1981) Winter hardiness of tree species at high altitudes in the East Himalaya, Nepal. Ecology 62:1288–1298

Sakai A, Okada S (1971) Freezing resistance of conifers. Silvae Genet 20:91–97

Sakai A, Wardle P (1978) Freezing resistance of New Zealand trees and shrubs. NZ J Ecol 1:51–65

Sakai A, Paton DM, Wardle P (1981) Freezing resistance of trees of the south temperate zone, especially subalpine species of Australasia. Ecology 62:563–570

Shimizu Y (2014) Process of the formation of Japanese forest and typification of vegetation zone– from an East Asian viewpoint. Reg Views 27:19–75 (in Japanese with English summary)

Su HJ (1984) Studies on the climate and vegetation types of the natural forests in Taiwan (II) altitudinal vegetation zones in relation to temperature gradient. Q J Chin For 17:57–73

Tagawa H (1995) Distribution of lucidophyll oak-laurel forest formation in Asia and other areas. Tropics 5:1–40

Takahara H, Sugita S, Harrison SP, Miyoshi N, Morita Y, Uchiyama T (2000) Pollen-based reconstructions of Japanese biomes at 0, 6000 and 18,000 ^{14}C yr BP. J Biogeogr 27:665–683

Takhtajan A (1986) Floristic regions of the world. University of California Press, Berkeley

Totman C (1989) The green archipelago. University of California Press, Berkeley

Troll C (1948) Der asymmetrische Vegetations- und Landschaftsbau der Nord- und Südhalbkugel. Göttinger Geogr Abh 1:11–27

Tsukada M (1966) Late Pleistocene vegetation and climate in Taiwan. Proc Natl Acad Sci U S A 55:543–548

Uemura S (1994) Climatic preference and frequent co-occurrence of boreal and temperate plants in Hokkaido Island, northern Japan. Vegetatio 112:113–126

Veblen TT, Hill RS, Read J (eds) (1996) The ecology and biogeography of *Nothofagus* forests. Yale University Press, New Haven

Wade LK, McVean DN (1969) Mt Wilhelm studies 1. The alpine and sub-alpine vegetation. Australian National University, Canberra

Walter H (1985) Vegetation of the earth, 3rd edn. Springer, Berlin

Wardle P (1983) Temperate broad-leaved evergreen forests of New Zealand. In: Ovington JD (ed) Ecosystems of the world 10. Temperate broad-leaved evergreen forests. Elsevier, Amsterdam, pp 33–71

Wardle P (1985) New Zealand timberlines. 3. A synthesis. N Z J Bot 23:263–271

Wardle P (1991) Vegetation of New Zealand. Blackburn Press, Caldwell

Waring RH, Franklin JF (1979) Evergreen coniferous forests of the Pacific Northwest. Science 204:1380–1386

Webb LJ (1968) Environmental relationships of the structural types of Australian rainforest vegetation. Ecology 49:296–311

Webb LJ (1978) A general classification of Australian rainforests. Australian Plants 9:349–363

Weng JH, Liao TS, Sun KH, Chung JC, Lin CP, Chu CH (2005) Seasonal variations in photosynthesis of *Picea morrisonicola* growing in the subalpine region of subtropical Taiwan. Tree Physiol 25:973–979

Whitmore TC (1984) Tropical rain forests of the far East, 2nd edn. Clarendon, Oxford

Williams RJ, Costin AB (1994) Alpine and subalpine vegetation. In: Groves RH (ed) - Australian vegetation, 2nd edn. Cambridge University Press, Cambridge, pp 467–500

Winter JW, Atherton RG, Bell FC, Pahl LI (1987) An introduction to Australian rainforests. In: Werren G, Kershaw AP (eds) The rainforest legacy, vol 1. Australian Government Publishing Service, Canberra, pp 1–7

Yamanaka T (1961) *Abies firma* and *Tsuga sieboldii* forests in Shikoku. Res Rep Kochi Univ (Nat Sci I) 10:19–32

Yamanaka T (1963) The forest vegetation in the temperate ecotone of Shikoku, Japan. Res Rep Kochi Univ (Nat Sci I) 12:17–25 (in Japanese with English summary)

Yoshioka K (1973) Phytogeography. Kyoritsu Shuppan, Tokyo (in Japanese)

Chapter 6
Roles of Terrestrial Carbon Subsidies to Aquatic Community Metabolism in Mountain Lake Ecosystems

Tomoya Iwata, Naoka Mochizuki, Takao Suzuki, Ayato Kohzu, Hisaya Kojima, Manabu Fukui, and Jotaro Urabe

Abstract Terrestrial–aquatic linkages of Japanese mountain lake ecosystems were examined to identify terrestrial carbon input into mountain lakes and the importance of the allochthonous carbon for the ecosystem metabolism of aquatic food webs therein. Stable carbon isotope analyses revealed that the flux of terrestrial dissolved organic matter from the surrounding catchments was relatively larger in smaller lakes with higher drainage ratios and tended to predominate carbon cycling in the epilimnetic water of such lakes. However, the metabolic balance of epilimnetic lake waters was net autotrophic or approximately in balance between gross primary production (GPP) and community respiration (CR) rates, suggesting that allochthonous organic matter generally subsidizes benthic and hypolimnetic communities. Empirical models established based on these observed GPP and CR values predict that climate change may increase carbon flows from both autochthonous and allochthonous pathways in epilimnetic communities of unproductive mountain lakes, directly through the functional dependence of community metabolism on the projected temperature rise and indirectly through the increased supply of terrestrial organic matter.

Keywords Allochthonous organic matter • Carbon isotope ratio • CO_2 supersaturation • Ecosystem metabolism • Heterotrophy • P/R ratio • Terrestrial subsidies

---

T. Iwata (✉)
Faculty of Life and Environmental Sciences, University of Yamanashi, Yamanashi, Japan
e-mail: tiwata@yamanashi.ac.jp

N. Mochizuki
Faculty of Engineering, University of Yamanashi, Kofu, Yamanashi, Japan

T. Suzuki • J. Urabe
Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan

A. Kohzu
National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan

H. Kojima • M. Fukui
The Institute of Low Temperature Science, Hokkaido University, Sapporo, Hokkaido, Japan

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_6

6.1 Introduction

Lake ecosystems are responsive to anthropogenic disturbances occurring at multiple spatial scales such as global climate change, regional atmospheric pollutant deposition, and local human activities of their embedded catchments (Schindler 1997, 2009). Because physical, chemical, and biological characteristics of lake ecosystems respond rapidly to climate-related variables, lakes may serve as a sentinel of climate change and its associated impacts on terrestrial catchments (Schindler 1997, 2009; Adrian et al. 2009). Moreover, recent studies have described that lakes play a major role in global carbon cycling through ecosystem metabolism, CO_2 and CH_4 efflux, carbon burial and sedimentation, and the transformation of organic matter (Cole et al. 2007; Tranvik et al. 2009; Gudasz et al. 2010; Bastviken et al. 2011). Lakes are therefore not merely recipients of materials coming from upstream catchments but they amplify, dampen, or cancel out the catchment inputs, which can influence downstream ecosystems and/or the atmosphere.

A global estimate of lake abundance and size showed that small lakes and ponds (<1 km^2) dominate the numerical and areal cover of the lake surface. In fact, they constitute >99.9 % of the total number of lakes and >50 % of the lake surface area worldwide (Downing et al. 2006). Therefore, small lakes are expected to play a pivotal role in global carbon cycling and in biodiversity maintenance. However, small water bodies are susceptible to climate changes due to their shallow depth and low water volumes (Smol et al. 2005; Smol and Douglas 2007; Thompson et al. 2009). Because small mountain lakes are often located in harsh environments such as high-elevation ecosystems, they may be ultrasensitive to changes in climate-related variables. Several studies predict that global warming may increase lake water temperature, decrease the ice-cover period, and lengthen the growing season, which may consequently affect the biodiversity and ecosystem functioning of alpine and subalpine lake ecosystems (Algesten et al. 2003; Quinlan et al. 2005; Smol and Douglas 2007; Parker et al. 2008; Rosén et al. 2009). Moreover, many such projected changes influence small mountain lakes indirectly through the changes in allochthonous supply of terrestrial organic matter into lakes (Schindler 1997). Therefore, elucidating the linkage between lake communities and the surrounding terrestrial environments through the cross-system transport of terrestrial organic matter is prerequisite for the accurate prediction of climatic change effects on mountain lakes.

Allochthonous inputs of dissolved and particulate organic matter (DOM and POM) from terrestrial surroundings often provide significant subsidies to unproductive lake food webs (del Giorgio et al. 1997; Duarte and Agustí 1998). Many studies have shown that particulate and dissolved forms of terrestrially derived organic matter fuel the secondary production and respiration of heterotrophic organisms (planktonic bacteria, zooplankton, benthic invertebrates, fish, etc.) in unproductive lakes (Pace et al. 2004; Carpenter et al. 2005; Berggren et al. 2010; Cole et al. 2011; Solomon et al. 2011; Karlsson et al. 2012; Scharnweber et al. 2014). As a result of the decomposition of such added allochthonous organic matter, community respiration (CR) usually exceeds the gross primary production (GPP), yielding negative net ecosystem production. Therefore, net heterotrophy has been used as the clue to substantial contributions of allochthonous organic matter to carbon flow in lake food webs (Duarte and Agustí 1998; Cole et al. 2000; Hanson et al. 2003). Moreover, strong dependence of lake communities on terrestrial resources is regarded as the major cause of the prevalence of CO_2 supersaturation and the large amount of CO_2 outgassing in lake ecosystems (Cole and Caraco 2001; Urabe et al. 2011). However, the extent to which unproductive mountain lake ecosystems connect to their surrounding catchments through the flux of allochthonous organic matter remains unclear. It is also not clear how ongoing global warming can affect the degree of allochthony: the dependence of mountain lake communities on terrestrial organic matter.

Tree lines are predicted to advance upslope as temperature rises and precipitation increases if tree line advancement is limited primarily by low-growing season temperature (Macias-Fauria and Johnson 2013). Lake water chemistry will be affected by such expansion of forested vegetation into alpine areas (Kissman et al. 2013). A striking projected change with climate change and resultant tree advancement is the increase of dissolved organic matter concentration in lakes (Kissman et al. 2013). Increased export of dissolved organic matter from terrestrial catchments is known to influence lake acidity, light climate, microbial community structure, primary productivity, heterotrophic respiration, and $p\mathrm{CO}_2$ supersaturation (Sobek et al. 2007; Jansson et al. 2008; Karlsson et al. 2009; Urabe et al. 2011; Fujii et al. 2012). Therefore, the degree of allochthony of mountain lake food webs may also change along the environmental gradients caused by the establishment of forested vegetation across the alpine-to-subalpine-to-montane areas, as well as catchment characteristics and lake morphometry, any of which might affect the supply of terrestrial organic matter (Rasmussen et al. 1989; Hanson et al. 2003; Sobek et al. 2003; Kankaala et al. 2013). However, no such pattern was elucidated until recently, especially in the mountain lakes of mid-latitude areas where alpine ecosystems are likely to be vulnerable to climate change effects.

To evaluate the roles of terrestrial carbon subsidies in mountain lake ecosystems, we investigated the ecosystem metabolism of planktonic communities along the environmental gradients of Japanese alpine, subalpine, and montane lakes with various sizes and catchment characteristics. We specifically aim (i) to show the source of dissolved inorganic and organic carbon in mountain lakes using stable carbon isotope analyses, (ii) to identify the pattern of the size-fractionated and community-based planktonic metabolism of lake ecosystems, and (iii) to analyze environmental factors controlling the community metabolism from which future changes in carbon cycling of mountain lake ecosystems can be predicted.

6.2 Study Area

We performed sampling campaigns in 49 alpine, subalpine, and montane lakes in Japan during 2005 and 2007 (Table 6.1, Fig. 6.1). These lakes lie in temperate or boreal climate regions, located at latitudes from 35°27′N to 43°41′N with elevation of 420–2700 m (Fig. 6.1). The lakes have various morphology in terms of depth (range 0.35–122 m) and area (0.014–1283 ha). Most of the lakes are located within national parks or quasi-national parks. The watershed areas of montane lakes are covered mainly by deciduous forests, whereas those of subalpine lakes are covered by coniferous forests, grasslands, or subalpine wetland vegetation. Alpine lakes are surrounded mainly by alpine dwarf pines (*Pinus pumila*), shrubs, and/or grasslands (Table 6.1). Total phosphorus (TP) concentrations are generally low ranging 0.05–2.11 μM (median = 0.25 μM, Table 6.1). Most of the lakes are classified as oligotrophic (TP <10 mg/m^3, 32 lakes) or mesotrophic (TP $= 10$–35 mg/m^3, 16 lakes) based on the OECD criteria.

6.3 Methods

6.3.1 Field Survey in 2005 and 2006

In each study lake, limnological surveys were made once during the summer (mid-July to August) of 2005 and 2006. At the deepest point of each lake, we collected surface water samples by gently sinking 30-mL glass vials to measure the concentration and stable carbon isotope ratio ($\delta^{13}C$) of dissolved inorganic carbon (DIC). These water samples were sealed with a butyl rubber stopper and an aluminum crimp seal, and 0.2 mL of saturated $HgCl_2$ was added as a preservative. We also measured vertical profiles of the water temperature, conductivity, and pH using a multiparameter sonde (600XLM, YSI). In this study, we defined epilimnion as the warmer surface mixed layer where the vertical temperature gradient does not exceed 1.2 °C m^{-1} (Urabe et al. 2011). For analyzing water chemistry, lake water was also collected at three depths from the epilimnion in stratified lakes or at one to three depths from the entire water column in unstratified lakes. The lake water collected from different depths was mixed evenly to create a single integrated sample and stored for analyses.

6.3.2 Water Sample Analyses

For all lakes examined, partial pressure of CO_2 (pCO_2, μatm) in surface water samples was estimated from the molar concentration of dissolved CO_2 ($[CO_2]_{aq}$, μmol/L) and Henry's law constant (μmol L^{-1} atm^{-1}) proposed by Weiss (1974).

Table 6.1 Characteristics of study lakes surveyed during 2005–2007

No.	Region	Lake	Type	Year	Day of year	Elev. (m)	A_L (ha)	A_C (ha)	DIC (mg/L)	δ^{13}C- DIC (‰)	TP (μM)	DOC (mg/L)	Chl.*a* (μg/L)
1	Aka	Panketo	S	2005	236	461	310	4445	6.5	−2.8	0.10	1.5	0.3
2	Aka	Akan	S	2005	236	420	1283	14,436	9.0	0.9	0.73	2.0	11.6
3	Aka	Jiro	S	2005	237	420	1.3	108	13.5	−2.7	2.11	1.5	7.6
4	Tai	Hisago-numa	A	2005	230	1685	6.7	137	0.3	−15.1	0.06	1.5	0.2
5	Tai	Kita-numa	A	2005	231	2005	1.8	18	0.7	−15.4	0.07	1.1	0.5
6	Tai	Ama-numa	A	2005	231	1845	0.1	5	0.4	−20.1	0.14	1.1	1.2
7	Tai	Kaundaira	A	2005	232	1865	0.01	15	0.7	−16.7	0.35	2.6	1.3
8	Tai	Numanohara-onuma	S	2005	232	1435	4.8	74	0.4	−18.1	0.24	1.8	0.5
9	Tai	Kara-numa	S	2006	229	1460	1.5	63	7.5	−8.8	0.85	1.5	0.6
10	Tai	Kogen-numa	S	2006	229	1470	0.5	17	0.4	−20.4	0.30	2.8	0.6
11	Tai	Shikibu-numa	S	2006	230	1450	0.2	39	0.4	−20.8	0.36	1.3	0.5
12	Tai	Midori-numa	S	2006	230	1360	0.3	101	0.5	−20.9	0.83	5.7	5.1
13	Tai	Piukenai-numa	A	2006	232	1580	0.3	17	1.1	−23.3	0.31	4.4	0.9
14	Tai	Rokuno-numa	A	2006	232	1450	0.5	25	1.5	−23.1	0.31	3.7	0.3
15	Tai	Kagami-ike	A	2006	234	1620	0.3	69	0.2	−19.6	0.22	2.7	0.7
16	Tai	Suribachi-ike	A	2006	234	1620	0.1	4	0.2	−18.7	0.24	2.5	0.8
17	Hak	Aka-numa	M	2005	209	685	6.0	133	0.7	−15.5	0.07	1.4	0.4
				2007	200				0.2	−16.7	0.21	4.0	0.1
18	Hak	Suge-numa	M	2005	209	465	1.2	32	6.4	−17.9	0.54	1.7	8.3
				2007	196				5.6	−15.6	0.60	1.0	4.7
19	Hak	Tashirotai	M	2005	211	575	0.2	115	2.9	−20.2	0.23	7.1	1.8
				2007	196				1.0	−19.1	0.21	7.3	0.9
20	Hak	Suiren-numa	S	2005	211	995	0.2	14	2.4	−22.3	0.50	7.0	0.9
				2007	197				1.5	−22.3	0.19	7.9	1.8

(continued)

Table 6.1 (continued)

No.	Region	Lake	Type	Year	Day of year	Elev. (m)	A_L (ha)	A_C (ha)	DIC (mg/L)	δ^{13}C- DIC (‰)	TP (μM)	DOC (mg/L)	Chl.*a* (μg/L)
21	Hak	Kagami-numa	A	2005	210	1525	0.02	1	0.5	−20.7	0.29	2.2	1.0
				2007	198				0.3	−21.8	0.23	1.6	0.8
22	Hak	Kamikenashitai	S	2005	212	1150	0.1	24	2.2	−24.8	0.41	15.7	0.4
				2007	199				1.2	−26.2	0.32	16.1	0.5
23	Hak	Shimokenashitai	S	2005	212	1055	0.02	11	2.5	−24.1	0.16	7.7	0.4
				2007	199				1.8	−23.6	n.a.	7.1	0.9
24	Hak	Sennin-numa	S	2005	210	1305	0.2	2	0.6	−22.6	0.22	6.1	0.2
25	Hac	Gozaisyo-numa	M	2005	200	890	1.0	12	1.4	−20.1	0.23	2.4	0.9
				2007	212				0.7	−23.5	0.31	0.1	0.7
26	Hac	Kuma-numa	S	2005	200	1330	2.2	50	2.4	−8.3	0.21	1.3	2.8
				2007	208				2.0	−9.9	0.27	1.6	1.2
27	Hac	Horai-numa	S	2005	201	1305	0.7	8	2.8	−15.6	0.15	1.6	2.4
				2007	209				1.7	−21.9	0.21	1.5	1.2
28	Hac	Hachiman-numa	S	2005	202	1560	6.1	47	0.3	−18.9	0.10	1.7	0.9
				2007	211				0.2	−26.8	0.08	1.1	0.5
29	Hac	Gama-numa	S	2005	202	1590	1.3	4	0.3	−16.0	0.07	0.9	0.3
				2007	210				0.1	−28.3	0.05	0.4	0.4
30	Hac	Naga-muma	M	2005	203	1109	3.7	23	2.5	−5.3	0.48	2.4	5.2
				2007	213				1.9	−7.2	0.59	2.6	15.9
31	Hac	Onuma	M	2005	203	944	5.0	57	8.3	−8.4	0.97	2.9	21.2
				2007	210				8.1	−15.0	0.41	2.2	11.4
32	Aki	Koma-ike	A	2006	211	1340	0.1	46	0.4	−22.7	0.32	1.5	3.2
33	Aki	Amida-ike	A	2006	211	1540	0.8	12	0.7	−13.0	0.23	1.1	1.3
34	Aki	Hiyagata	M	2006	212	820	0.4	5	0.5	−16.5	0.98	2.4	5.2
35	Cho	Tsuruma-ike	M	2006	208	815	2.5	29	1.8	−8.5	0.65	1.2	0.1

36	Cho	Chokai	A	2006	209	1575	3.7	30	0.4	−24.8	0.24	1.6	0.9
37	Yat	Kikko-ike	S	2006	201	2030	0.5	113	1.4	−16.6	0.32	3.4	0.05
38	Yat	O-ike	S	2006	201	2050	1.7	42	1.1	−10.0	0.51	1.6	0.3
				2007	247				1.3	−7.5	0.15	0.9	0.2
39	Yat	Matsubara	M	2006	202	1120	9.4	43	2.5	−18.4	0.96	3.3	8.8
				2007	232				1.5	−7.5	0.30	0.8	3.9
40	Yat	Nanatsu-ike	S	2006	203	2370	0.2	6	0.8	−26.3	0.29	4.8	0.1
				2007	240				0.5	−20.6	0.21	3.9	0.8
41	Yat	Me-ike	S	2007	251	2036	1.5	39	0.4	−19.1	0.28	1.6	0.6
42	Yat	Shirakoma-ike	S	2007	271	2113	7.7	230	0.4	−19.9	0.13	2.9	0.5
43	Nor	Tsuruga-ike	A	2006	198	2700	0.3	15	0.4	−12.4	0.36	14.5	0.3
44	Nor	Kamega-ike	A	2006	198	2660	0.1	14	0.3	−13.2	0.16	1.0	0.07
45	Nor	Gono-ike	A	2006	199	2690	0.4	56	0.6	−17.1	0.22	1.7	0.04
46	Fuj	Kawaguchi	M	2007	261	835	570	10,350	12.0	−7.1	0.25	1.3	2.1
47	Fuj	Sai	M	2007	265	896	213	3440	7.1	−7.6	0.16	1.3	2.5
48	Fuj	Motosu	M	2007	284	910	465	2460	3.0	−1.9	0.10	0.6	1.1
49	Fuj	Shibire	M	2007	289	887	6.8	44	4.0	−6.3	0.28	1.3	5.2

Elev. elevation, A_L lake area, A_C catchment area, *Aka* Akan, *Tai* Taisetsu, *Hak* Hakkoda, *Hac* Hachimantai, *Aki* Mt. Akita-Komagatake, *Cho* Mt. Chokai, *Yat* Yatsugatake, *Nor Mt. Norikura*, *Fuj* Mt. Fuji, *A* alpine lake, *S* subalpine lake, *M* montane lake

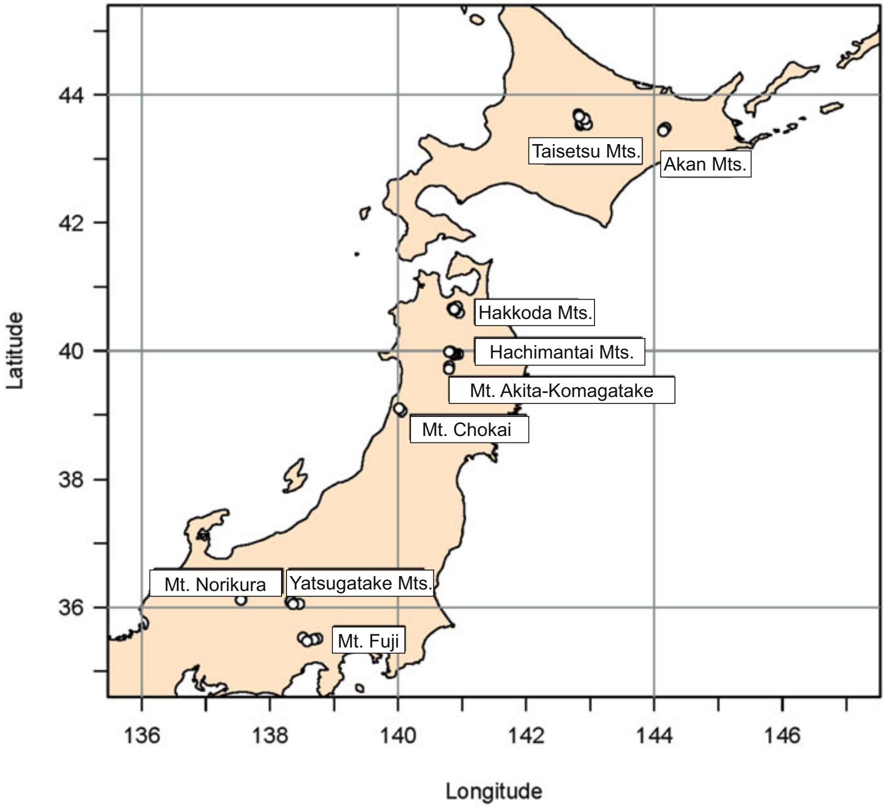


Fig. 6.1 Map of nine mountain regions showing locations of 49 study lakes in Japan

The $[CO_2]_{aq}$ was determined from the DIC concentration (TOC 500, Shimadzu Corp.), pH, and dissociation constants (K_1 and K_2) recommended by DOE (1994).

The δ^{13}C-DIC was determined using a vapor phase equilibration technique according to Miyajima et al. (1995). The sealed water samples were acidified with 0.2 mL of CO_2-free phosphoric acid (30 %) and 3-mL ultrapure He was then added to create a headspace. The samples were shaken vigorously and left at constant temperature for >24 h to equilibrate. A portion of the headspace gas was injected into a GC/C/IRMS (MAT-252, Finnigan MAT GmbH) for carbon isotope ratio determination. The δ^{13}C-DIC was calculated from δ^{13}C of headspace CO_2 using the Henry's law constant and isotopic fractionation between gaseous and aqueous CO_2 (Mook et al. 1974). δ^{13}C-DIC (‰) was expressed using delta notation as the parts per mil (‰) difference from the international standard (PDB) for the stable carbon isotope ratio.

We used the integrated, epilimnetic water samples for analyses of total phosphorus (TP), dissolved organic carbon (DOC), and chlorophyll *a* (Chl.*a*) concentrations. The TP samples were autoclaved with potassium persulfate and the resultant soluble reactive phosphorus was measured using spectrophotometric molybdenum blue method. The DOC concentration was measured using lake water passed through a pre-combusted GF/F glass fiber filter (TOC 500, Shimadzu Corp.). Chl.*a* collected onto a GF/F glass fiber filter was extracted using 90 % acetone and measured using a fluorometer (10AU, Turner Designs Inc.) according to Strickland and Parsons (1972).

6.3.3 Field Survey in 2007

Based on the limnological data collected from 2005 to 2006 field survey, we also revisited 17 mountain lakes (Table 6.1). Among them, we selected nine lakes to measure the ecosystem metabolism of epilimnetic lake waters. We also surveyed the additional six lakes as study sites. Consequently, the ecosystem metabolism was estimated in a total of 15 mountain lakes during July through early October in 2007 (Table 6.2). Planktivorous fish (e.g., salmonids, osmerids, and cyprinids) had been historically introduced or continued to be introduced for commercial or recreational use in the eight study lakes (Table 6.2). At the deepest point of each lake, we first measured the vertical temperature profile and then collected water from the upper, middle, and lower epilimnion. The collected water samples were evenly pooled into 10 L polyethylene containers and were used for both incubation and the analyses of the same chemical variables as those used for the 2005–2006 survey ($p\mathrm{CO_2}$, DIC, δ^{13}C-DIC, TP, DOC, and Chl.*a*). We also measured the δ^{13}C of DOC according to the method described by Bade et al. (2007). We acidified 1 L of GF/F filtrate water with 1N HCl and evaporated it at 70 °C on an electric hot plate. The dried residue after evaporation was scraped. Then, the ^{13}C contents were analyzed using a CF/IRMS (ANCA-GSL interfaced to a GEO20-20IRMS, Sercon Ltd.).

We also collected zooplankton by vertical hauling of the water column of epilimnion with a 100-μm mesh plankton net. The quantitative samples were preserved immediately in Lugol's solution for enumeration by microscopy. Additional zooplankton samples were collected, transported alive to the laboratory, and sieved through a 200-μm Nytal mesh. The macroplankton fraction (>200 μm) was sorted under a microscope. Each taxon was stored frozen for later analysis of enzymatic activity of the electron transport system (ETS).

6.3.4 Metabolic Measurements

We estimated the primary production and respiration rates in epilimnetic lake waters based on dissolved O_2 (DO) change in light and dark bottles. The integrated, epilimnetic water samples were sieved through a 200-μm Nytal mesh to remove the macroplankton fraction (F_{macro}). The filtrates ($F_{<200}$) were fractionated further through a 2-μm Nuclepore filter using a reverse filtration device (Dodson and

Table 6.2 Biological properties, volumetric metabolic rates, and photosynthetic parameters in the epilimnion of 15 study lakes surveyed in 2007

		Chl. *a*	ZP biomass	Metabolism (mgC m^{-3} day^{-1})					Photosynthesis		
No	Lake	(mg/m^3)	(mg wet weight/m^3)	GPP[a]	CR	R_{macro}	R_{micro}	R_{pico}	$P^B{}_{max}$	α	β
17	Aka-numa	0.11	6.0 ± 3.0	–	40	0.05 ± 0.02	26	14	–	–	–
20	Suiren-numa	1.77	4007 ± 4403	–	85	11 ± 11	12	62	–	–	–
21	Kagami-numa	0.84	678 ± 737	–	145	7 ± 7	35	103	–	–	–
27	Horai-numa	1.16	1584 ± 4762	–	61	4 ± 7	33	24	–	–	–
30	Naga-numa[b]	15.9	791 ± 594	41	167	2 ± 2	111	54	1.24	0.0019	0.0007
31	Onuma[b]	11.4	214 ± 177	435	333	0.3 ± 0.3	272	61	4.73	0.047	0
38	O-ike	0.22	4.3 ± 0.8	25	8	0.1 ± 0.004	1	6	9.14	0.542	0
39	Matsubara[b]	3.88	389 ± 354	173	99	1 ± 2	38	59	3.99	0.050	0
40	Nanatsu-ike	0.79	1825 ± 1442	76	52	7 ± 5	–	46	7.64	0.499	0
41	Me-ike[b]	0.55	183 ± 165	34	35	0.7 ± 0.6	11	23	11.0	0.155	0.045
42	Shirakoma-ike	0.46	1377 ± 915	20	27	4 ± 2	8	15	5.07	0.222	0
46	Kawaguchi[b]	2.05	260 ± 269	48	31	0.5 ± 0.5	13	18	4.53	0.049	0
47	Sai[b]	2.50	4.6 ± 2.6	81	49	0.01 ± 0.01	38	11	3.51	0.037	0
48	Motosu[b]	1.09	183 ± 152	25	19	0.4 ± 0.3	–	19	2.26	0.085	0.0004
49	Shibire[b]	5.16	260 ± 254	76	65	0.8 ± 0.7	24	39	3.14	0.059	0

GPP gross primary production, *CR* community respiration, R_{macro} macroplankton respiration, R_{micro} microplankton respiration, R_{pico} picoplankton respiration, $P^B{}_{max}$ maximum specific photosynthetic rate (mgC mg-chla^{-1} h^{-1}), α initial slope of the photosynthesis–irradiance (*P–I*) curve (mgC mg-chla^{-1} h^{-1}/μmol-quanta m^{-2} s^{-1}), β photoinhibition parameter of the *P–I* curve with the same units as α. Lake numbers (No.) correspond to those presented in Table 6.1

[a] Estimated rates of GPP shown were determined by assuming that the fraction of cloud-free sky is 0.6

[b] Planktivorous fish were introduced historically or continued to be introduced for commercial or recreational use

Thomas 1964) to obtain a picoplankton fraction (F_{pico}). The $F_{<200}$ and F_{pico} fractions of lake waters were incubated to determine their metabolic rates.

We carefully siphoned the $F_{<200}$ lake water into eight to ten light bottles (300-mL BOD bottles) through a Tygon tube. These light bottles were incubated for 4–6 h at various irradiance levels of photosynthetically active radiation (PAR, 400–700 nm; range =1–800 μmol quanta m^{-2} s^{-1}), as established using cheesecloths. To estimate the respiration rates, we filled 300-mL dark bottles with each of the $F_{<200}$ and F_{pico} lake waters ($n = 2$–3 for each fraction) and incubated them for 12–24 h. Additionally, we siphoned each of the $F_{<200}$ and F_{pico} lake waters into 300 mL initial bottles ($n = 2$–3 for each) and incubated them for 15–60 min. Incubations were performed either in situ using a drifting buoy (lake nos. 17, 20, 21, 27, 30, 31; see Table 6.2) or in the laboratory at in situ temperatures in each lake (lake nos. 38–42 and 46–49). The photon flux density inside the bottles was estimated by multiplying the ambient irradiance measured using an underwater spherical quantum sensor (LI-193SA, Li-Cor Inc.) with the attenuation coefficients of cheesecloth and borosilicate BOD bottles.

We determined the DO concentrations in the light, dark, and initial bottles using a high-precision Winkler method developed by Carignan et al. (1998). The precision of this method (up to 2 μg/L) is sufficient to estimate the planktonic metabolism of unproductive lakes. We titrated whole bottles after approx. 24 h of Winkler reagent additions using an automated precise titrator (798 MPT, Metrohm AG). The dark bottle DO consumption in the F_{pico} fraction from the initial bottles was determined as the picoplankton respiration rate (R_{pico}; $\mu\mathrm{gO}_2\ \mathrm{L}^{-1}\ \mathrm{h}^{-1}$). Similarly, we determined the DO consumption of $F_{<200}$ water ($R_{<200}$). The respiration of 2- to 200-μm microplankton fraction (R_{micro}) was then calculated as the difference between $R_{<200}$ and R_{pico}. The light bottles were used to determine the epilimnetic primary production ($\mu\mathrm{gO}_2\ \mathrm{L}^{-1}\ \mathrm{h}^{-1}$). The net production ($P_n$) was estimated from the DO evolution of light bottles relative to the initial bottle DO concentration. The gross production (P_g) was calculated as the sum of P_n and $R_{<200}$, with the assumption that macroplankton fraction ($F_{>200}$) does not contain autotrophic organisms. The chlorophyll-specific productivity, P^B ($\mu\mathrm{gO}_2$ μg-chl.a^{-1} h^{-1}), was then determined by dividing P_g ($\mu\mathrm{gO}_2\ \mathrm{L}^{-1}\ \mathrm{h}^{-1}$) by Chl.$a$ (μg-chl.a/L).

6.3.5 *ETS Activity of Macroplankton*

We estimated the respiration rate of macroplankton ($F_{>200}$ fraction) from their ETS activity (ETSA), as measured in vitro by quantifying the enzymatic reduction of an artificial electron acceptor (INT: 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride; Packard 1985; Arístegui and Montero 1995). Following the protocol presented by Båmstedt (2000), the samples of macrozooplankton species (3–30 mg wet weight) were homogenized in an ETS reagent (1–3 mL) and were incubated for 1 h at 40 °C. The resultant reduced INT (formazan) was quantified by measuring its absorbance at 475 nm using a spectrophotometer. Because 1 mol of formazan corresponds to 0.5 mol of O_2, the corresponding oxygen utilization per unit weight of organisms (μgO_2 used/mg wet weight) was calculated as

$$\mathrm{ETSA} = 16C\frac{V}{1000}\frac{1}{L}\frac{1}{W}, \tag{6.1}$$

where C is the concentration of formazan (μM), V is the final reagent volume (mL), L is the cell length of the cuvette (cm), and W is the wet weight of zooplankton samples. The estimates of ETSA obtained were converted to the respiration rates (R_{macro}) using a factor of 2 for R/ETSA ratio according to Båmstedt (2000). Then, they were multiplied by the epilimnetic biomass of each species to obtain the respiration rates of macroplankton (R_{macro}; μgO_2 L^{-1} h^{-1}).

6.3.6 Daily Metabolic Rates

We converted the metabolic rates (P_g, R_{pico}, R_{micro}, and R_{macro}) in oxygen units (μgO_2 L^{-1} h^{-1}) to carbon units (μgC L^{-1} h^{-1}) using a photosynthetic quotient of 1.25 and a respiratory quotient of 0.80. The community respiration (CR; mgC m^{-3} day^{-1}) was determined as the sum of R_{pico}, R_{micro}, and R_{macro}. We calculated the daily rates of epilimnetic water column gross primary production ($\mathrm{GPP}_{\mathrm{epi}}$; mgC m^{-2} day^{-1}) from the following model integrated over epilimnion depth (z_{epi}) and time as

$$\mathrm{GPP}_{\mathrm{epi}} = \int_{0}^{z_{\mathrm{epi}}}\int_{\mathrm{sunrise}}^{\mathrm{sunset}} P^B(z,t)\mathrm{Chl}.a\,dtdz, \tag{6.2}$$

where P^B (z,t) is the chlorophyll-specific productivity (mgC mg-chl.a^{-1} h^{-1}) at depth z at time t and Chl.a is the mean chlorophyll concentration in epilimnion (mg-chl.a/m^3; see Fig. 6.2). P^B (z,t) was estimated from the P–I curves and PAR irradiance (I) at depth z at time t. The P–I curve for each study lake was modeled using the following formula (Platt et al. 1980, Fig. 6.2a):

$$P^B = P_s\left(1 - e^{-\alpha I/P_s}\right)e^{-\beta I/P_s}, \tag{6.3}$$

where α is the initial slope of the P–I curve (mgC mg-chl.a^{-1} h^{-1} (μmol quanta m^{-2} $\mathrm{s}^{-1})^{-1}$), β is the photoinhibition parameter (same units as α), I denotes the PAR irradiance (μmol quanta m^{-2} s^{-1}), and P_s is a parameter with a relation to the maximum rate of chlorophyll-specific productivity (${P^B}_{\mathrm{max}}$) described as

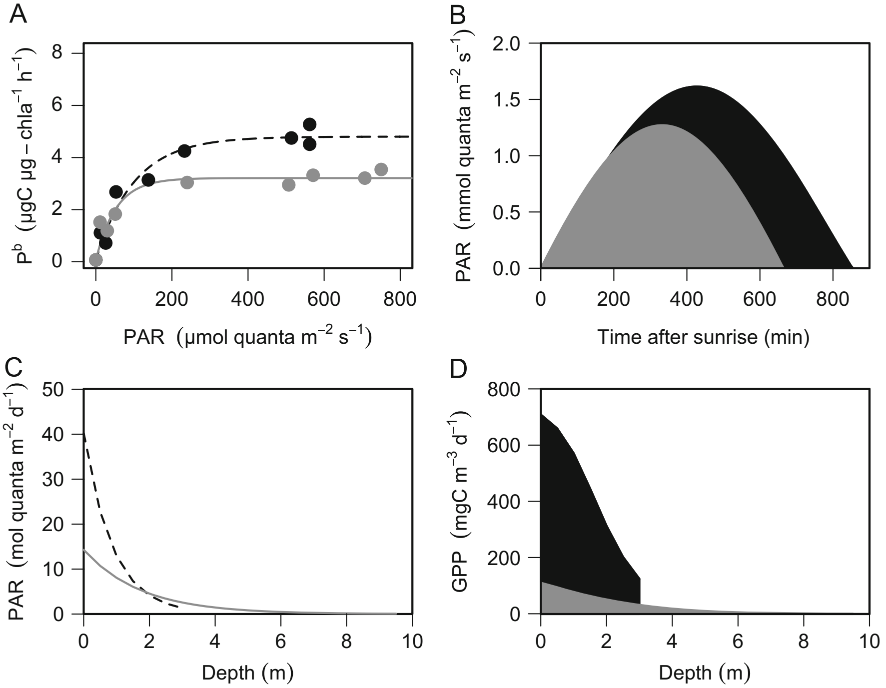


Fig. 6.2 Examples of modeled productivity and irradiance in two study lakes (*gray* and *solid line*, Lake Shibire; *black* and *dashed line*, Lake Onuma). (**a**) *P–I* curves showing the relationships between PAR irradiance and chlorophyll-specific productivity of epilimnetic plankton communities. (**b**) Diurnal change of PAR irradiance at the water surface of study lakes. The values are estimated using the FITSOLAR model (Fee 1990). (**c**) Estimated vertical profiles of PAR irradiance in water column environments. (**d**) Daily rates of volumetric gross primary production in water column environments

$$P^B_{\max} = P_s\left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{\beta}{\alpha+\beta}\right)^{\beta/\alpha}. \tag{6.4}$$

The diurnal change of PAR irradiance in the water column of epilimnion was calculated from the surface PAR irradiance $I(0)$ and the water column extinction coefficient of incident light (K_d). We applied the equation of FITSOLAR model (Fee 1990) to the sampling date and location of each study lake to estimate the $I(0)$ value (Fig. 6.2b). The extinction coefficient K_d was estimated from the vertical profile data of PAR taken at each sampling date for each lake as

$$I(z) = I(0)e^{-K_d z}, \tag{6.5}$$

where $I(z)$ is the irradiance at depth z (Fig. 6.2c). For a comparison of productivity among lakes with different epilimnion depths (z_{epi}), the mean volumetric rates of gross primary production rates (GPP; mgC m^{-3} day^{-1}) were calculated by dividing GPP$_{\text{epi}}$ by z_{epi} (Fig. 6.2d).

6.3.7 Models of Metabolic Rates

We develop the empirical models that describe epilimnetic metabolic rates (GPP and CR) of mountain lakes. Temperature, light, phosphorus, and DOC (i.e., a surrogate for water color) are known to influence planktonic GPP in mountain lakes (Hanson et al. 2003; Karlsson et al. 2009; Solomon et al. 2013). Therefore, we modeled GPP as the function of water temperature, TP, DOC, and PAR as follows:

$$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T_{20})/kTT_{20}} \mathrm{TP}^a\, \mathrm{DOC}^b\, \mathrm{dPAR}^c, \tag{6.6}$$

where $\mathrm{GPP}_{\max}$ is the maximum rate of GPP at a standardized temperature 20 °C (T_{20}, K), $\exp(E(T-T_{20})/kTT_{20})$ denotes the Boltzmann factor describing the temperature dependence of GPP (Gillooly et al. 2001), E represents the activation energy (eV), k is the Boltzmann's constant (8.62×10^{-5}, eV/K), T is the mean water temperature of epilimnion (K), and a, b, and c, respectively, denote the fitted constants for TP, DOC, and the daily mean of water column PAR (dPAR, mol quanta $\mathrm{m}^{-3}\ \mathrm{day}^{-1}$).

We constructed eight candidate models in which the combinations of TP, DOC, and PAR terms differed, although the temperature dependence term remained for all models (Table 6.3). The candidate models were first linearized by taking the natural logarithm of both sides of the equation. Then, the parameter values ($\mathrm{GPP}_{\max}$, E, a, b, and c) were adjusted using the nonlinear least squares method. We used the lowest AIC to choose the best-fitting model describing the GPP pattern.

We also constructed a model for CR using the Boltzmann factor and a power function of limiting resources as

$$\mathrm{CR} = \mathrm{CR}_{\max} e^{E(T-T_{20})/kTT_{20}} \mathrm{TP}^a \mathrm{DOC}^b, \tag{6.7}$$

where $\mathrm{CR}_{\max}$ is the maximum CR at 20 °C. We assumed that TP (a possible limiting element of aquatic metabolism) or DOC (a major substrate of heterotrophic respiration) is a critical limiting resource for respiratory activity of planktonic communities (Hanson et al. 2003; Karlsson et al. 2009; Solomon et al. 2013). As the GPP models, we constructed four candidate models for CR to find the parameter values ($\mathrm{CR}_{\max}$, E, a, and b) of the model best fitting the variation of epilimnetic CR (Table 6.3).

Table 6.3 Models of metabolic rates (GPP and CR) with water temperature (T), substrates (TP and DOC), and light (dPAR) in epilimnetic lake waters

		Parameters					
	Model	$\ln i_{\max}$[a]	E	a	b	c	AIC
1	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}$	4.2	1.3				30.3
2	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{TP}^{a}$	5.4	1.1	0.80			28.5
3	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{DOC}^{b}$	1.9	1.5		0.49		30.8
4	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{dPAR}^{c}$	3.0	1.5			0.49	30.0
5	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{TP}^{a}\mathrm{DOC}^{b}$	4.3	1.2	0.70	0.20		30.2
6	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{TP}^{a}\mathrm{dPAR}^{c}$	4.2	1.3	0.74		0.43	27.8
7	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{DOC}^{b}\mathrm{dPAR}^{c}$	0.004	1.8		0.58	0.55	29.1
8	$\mathrm{GPP} = \mathrm{GPP}_{\max} e^{E(T-T20)/kTT20}\mathrm{TP}^{a}\mathrm{DOC}^{b}\mathrm{dPAR}^{c}$	2.2	1.5	0.57	0.34	0.48	28.8
9	$\mathrm{CR} = \mathrm{CR}_{\max} e^{E(T-T20)/kTT20}$	4.1	0.62				44.2
10	$\mathrm{CR} = \mathrm{CR}_{\max} e^{E(T-T20)/kTT20}\mathrm{TP}^{a}$	6.3	0.31	1.47			35.0
11	$\mathrm{CR} = \mathrm{CR}_{\max} e^{E(T-T20)/kTT20}\mathrm{DOC}^{b}$	0.04	1.4		0.85		40.4
12	$\mathrm{CR} = \mathrm{CR}_{\max} e^{E(T-T20)/kTT20}\mathrm{TP}^{a}\mathrm{DOC}^{b}$	3.3	0.86	1.22	0.55		32.6

[a] $\mathrm{GPP}_{\max}$ and $\mathrm{CR}_{\max}$, respectively, denote GPP and CR model

6.4 Patterns of Organic and Inorganic Carbon Inputs to Mountain Lakes

The present results revealed that lake morphometry, rather than surrounding catchment vegetation, largely determines the source and concentration of organic carbon of mountain lakes. Geomorphic analyses for the study lakes showed the power relationship of the surface area with its catchment area (Fig. 6.3a). Moreover, the exponent of such a power relationship was less than 1 ($A_C \propto {A_L}^{0.67}$). As a result, the drainage ratio, the ratio of catchment area to lake surface area, decreased concomitantly with increasing lake area ($A_C/A_L \propto {A_L}^{-0.33}$; Fig. 6.3b). This result implies that small lakes have larger catchment area per unit lake surface area, from which we can predict that the influence of organic matter flux from terrestrial ecosystems on lake water chemistry and on aquatic communities is relatively strong in small mountain lakes.

In fact, high epilimnetic dissolved organic carbon (DOC) concentrations were found in small lakes regardless of lake types defined by the surrounding vegetation (i.e., alpine, subalpine, vs. montane lakes, Fig. 6.4a; one-way ANOVA, $F_{2,67} = 1.6$, $p = 0.22$). In addition, δ^{13}C-DOC showed almost constant values (about −27 ‰), which were consistent with terrestrial C3 plants (Fry 2006) across the wide range of lake sizes (Fig. 6.4b). Consequently, it can be considered that DOC in mountain lake ecosystems is predominantly terrestrial origin, and small lakes receive waters from terrestrial ecosystems with higher DOC concentration. The negative relationship between lake size and terrestrial DOC or the positive relationship between drainage ratio and terrestrial DOC is likely to be a general pattern often seen in the oligotrophic lakes of many regions (Rasmussen et al. 1989; Gergel et al. 1999; Sobek et al. 2003). These relationships might be attributed to the fact that smaller

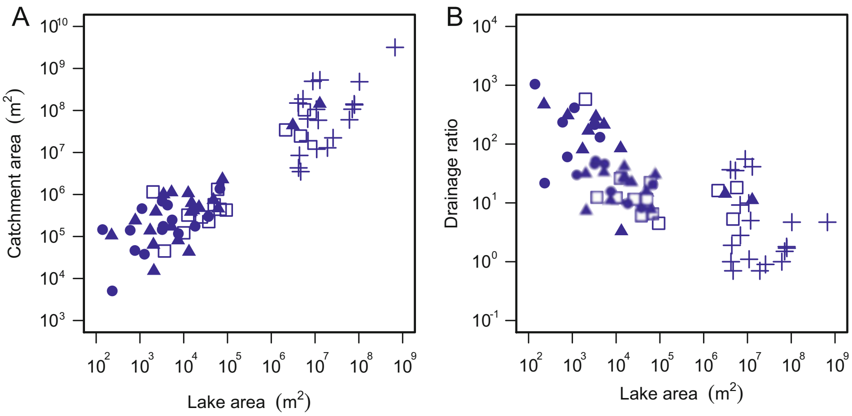


Fig. 6.3 Relationships between the lake surface area and catchment area (**a**) and between the lake surface area and drainage ratio (**b**) of alpine lakes (*circles*), subalpine lakes (*triangles*), and montane lakes (*squares*) in the study area (Data from other Japanese major lakes (*crosses*) are also shown)

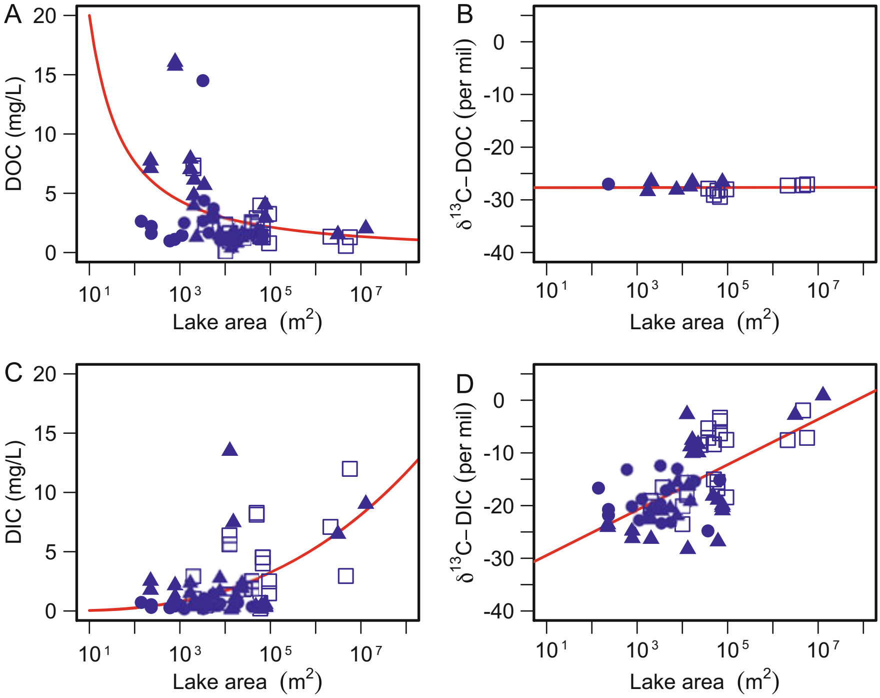


Fig. 6.4 Relationships between the lake surface area and the concentrations and stable carbon isotope ratios (δ^{13}C) of epilimnetic DOC (**a**, **b**) and surface water DIC (**c**, **d**) in alpine lakes (*circles*), subalpine lakes (*triangles*), and montane lakes (*squares*) in the study area. Linear regression or nonlinear power regression lines are shown

lakes with high drainage ratios tend to receive waters that have relatively greater contact with organic matter in terrestrial ecosystems during its path to a lake.

In contrast to the pattern of DOC, the dissolved inorganic carbon (DIC) concentration showed a positive relationship with lake size (Fig. 6.4c). The isotopic result also showed that δ^{13}C-DIC increased concomitantly with lake size (Fig. 6.4d). Particularly, the largest lakes (area = ~10^7 m^2) exhibited δ^{13}C-DIC values close to the atmospheric isotopic equilibrium value (i.e., ~0 per mil at pH 7–8). These patterns of δ^{13}C-DIC values are accord well with those reported from a previous study that showed the similar lake-size-dependent change in δ^{13}C-DIC values in lakes from diverse regions of the world (Bade et al. 2004). Several ecological and biogeochemical factors may explain these patterns. First, the gas exchange rate across the air–water interface may be higher in large lakes because of high wind speeds, which may account for the increase of δ^{13}C-DIC with lake surface area to the atmospheric equilibrium value (Bade et al. 2004). Second, high photosynthetic rates in large lakes are likely to contribute to the net uptake of atmospheric CO_2 by epilimnetic waters, although our metabolic measurements revealed no pattern of the lake-size-dependent increase in NEP (simple linear regression analyses between $\log_{10}$ (lake area) and NEP, $R^2 = 0.004$, $p = 0.86$, $n = 11$). Third, large lakes situated in lower catchments might receive greater inputs of groundwater with high amounts of bicarbonate (HCO_3^-) because HCO_3^-, the dominant carbonate species of DIC ($= CO_2 + HCO_3^- + CO_3^{2-}$) at around neutral pH (Stumm and Morgan 1996), is often generated by chemical weathering in catchments (Hartmann 2009; Iwata et al. 2013). Actually, the $\delta^{13}C$ value of bicarbonate weathered from carbonate rocks is usually higher than that of CO_2 (Bade et al. 2004). Therefore, $\delta^{13}C$-DIC might increase in response to the increase of HCO_3^- with lake size.

In small mountain lakes, however, the concentration and $\delta^{13}C$ value of DIC were generally low. Especially, $\delta^{13}C$-DIC of the smallest lakes (area = 10^2–10^3 m^2) showed isotopic signatures close to terrestrial DOC (about −27 ‰, Fig. 6.4d). This result suggests that the relative increase in ^{13}C-depleted inorganic carbon in small lakes originated mainly from the decomposition of terrigenous organic matter. Considering the facts that $\delta^{13}C$-DIC decreased quasi-exponentially with the increase of lake water pCO_2 (Fig. 6.5) and that more than half of the lakes were supersaturated in pCO_2 (38 out of the 64 measurements, median = 515 μatm; Fig. 6.5), the flux of terrestrial organic matter from the surrounding catchments generally increases the load of ^{13}C-depleted CO_2 through ecosystem respiration toward the level higher than photosynthetic rates in small mountain lakes. Previous studies also reported that terrestrial subsidies increase both heterotrophic respiration and CO_2 evasion in unproductive lakes (Lennon 2004; Karlsson et al. 2007, 2012; Jansson et al. 2008; Berggren et al. 2010; Scharnweber et al. 2014). We therefore infer that the carbon cycling (input, assimilation, trophic transfer, and respiration) in mountain lakes is dominated by terrestrially derived organic matter. The lake size largely determines the degree of trophic dependence of lake communities on such allochthonous supply from terrestrial catchments.

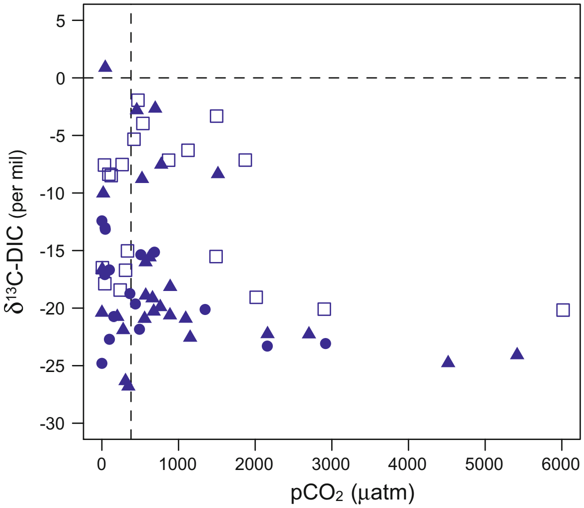


Fig. 6.5 Relationship between pCO_2 and the stable carbon isotope ratios ($\delta^{13}C$) of DIC in surface water of alpine lakes (*circles*), subalpine lakes (*triangles*), and montane lakes (*squares*) in the study area. The *vertical* and *horizontal dashed lines*, respectively, represent the partial pressure of atmospheric CO_2 (380 μatm) and the atmospheric isotopic equilibrium value of DIC (~0 per mil at pH 7–8)

6.5 Patterns of Metabolic Rates in Mountain Lakes

The subsidy concept describing the effects of allochthonous resource inputs on the structure and functioning of recipient ecosystems predicts that the flux of terrestrial organic matter often shifts the trophic balance of unproductive aquatic ecosystems to net heterotrophy by increasing the respiration of heterotrophic organisms (i.e., negative net ecosystem production, NEP = GPP – CR <0; Duarte and Agustí 1998; Cole et al. 2000; Hanson et al. 2003). However, the present study revealed that epilimnetic water of mountain lakes were net autotrophic or exhibited approximate balance between GPP and CR (Fig. 6.6). We used the high-precision Winkler titration method to measure the community metabolism in mountain lakes, which showed that the volumetric rates of GPP and CR were 20–435 mgC m^{-3} day^{-1} (median = 48 mgC m^{-3} day^{-1}) and 8–333 mgC m^{-3} day^{-1} (median = 52 mgC m^{-3} day^{-1}), respectively (Table 6.2). These rates are low, comparable to those of other high-elevation lakes (Sadro et al. 2011) or oligotrophic lakes (Carignan et al. 2000). We examined the relationships between volumetric GPP and CR at three light conditions because GPP varies with the cloud cover conditions (Fig. 6.6). The results showed that the volumetric GPP and CR were tightly coupled each other (Fig. 6.6), as observed in many unproductive lakes (Carignan et al. 2000; Hoellein et al. 2013; Solomon et al. 2013). Furthermore, the epilimnetic waters of most lakes were supersaturated in $p\mathrm{CO}_2$ with respect to the atmosphere (8 out of the 11 study lakes; see also Urabe et al. 2011). Despite these facts, however, epilimnetic GPP was generally greater than or almost equal to CR regardless of the cloud cover conditions (Fig. 6.6), implying that the respiratory release of CO_2 by epilimnetic communities did not exceed the CO_2 fixation by primary producers. These results argue against the prediction that terrestrially derived organic matter provides significant subsidies to heterotrophic plankton metabolism.

Recent studies have increasingly identified significant temporal and spatial variations in the planktonic metabolism (GPP, CR, and NEP) within a lake (Coloso

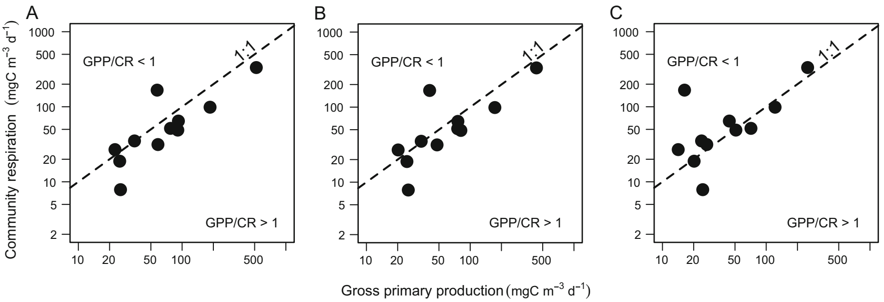


Fig. 6.6 Relationships between volumetric gross primary production (GPP) and community respiration rates in the epilimnetic water of study lakes. The relationships are determined for three light conditions where photosynthetic rates vary because of changing cloud cover (**a**–**c**). The fractions of cloud-free sky are assumed as 1.0 (cloudless, **a**), 0.6 (**b**), and 0.2 (**c**). *Dashed lines* represent GPP = CR

et al. 2008; Sadro et al. 2011; Staehr et al. 2012; Obrador et al. 2014). Particularly, the vertical gradients of metabolism are often observed in stratified lakes; net autotrophy (positive NEP) prevails in the epilimnion, whereas net heterotrophy (negative NEP) tends to occur in the hypolimnion below the thermocline if the photic zone does not extend below the mixed-layer depth. Coloso et al. (2008) reported that despite a positive water column NEP, the overall trophic balance of a clear-water lake is net heterotrophic (NEP), suggesting the dominance of hypolimnetic and benthic processes in the whole ecosystem metabolism. Urabe et al. (2011) also showed that sediment respiration is a major cause contributing to high CO_2 supersaturation and overall net heterotrophy in shallow lake ecosystems such as mountain lakes in this study (see also Kortelainen et al. 2006). These previous evidences correspond well with the present findings ($p CO_2$ supersaturation and positive epilimnetic NEP), suggesting that the input of terrestrial organic matter generally subsidizes heterotrophic metabolism of benthic and hypolimnetic communities, which may dominate carbon cycling in mountain lake ecosystems.

6.6 Size-Specific Metabolic Rates

We found the striking size-dependent changes in epilimnetic respiration along the productivity gradient of mountain lakes (Fig. 6.7). The fraction of picoplankton respiration (cell size <2 μm) in community respiration decreased strongly with increasing TP concentration (a surrogate measure of productivity). Particularly, picoplankton overwhelmingly dominated community respiration in oligotrophic lake waters (TP <0.32 μmol/L, Fig. 6.7c). Picoplankton primarily includes unicellular cyanobacteria and heterotrophic bacteria (Wetzel 2001). Therefore, these bacterioplankton assemblages might control organic matter breakdown processes, especially in ultra-oligotrophic mountain lakes (TP <0.16 μmol/L). Conversely, microplankton (body size = 2–200 μm; mainly eukaryotic algae, microflagellates,

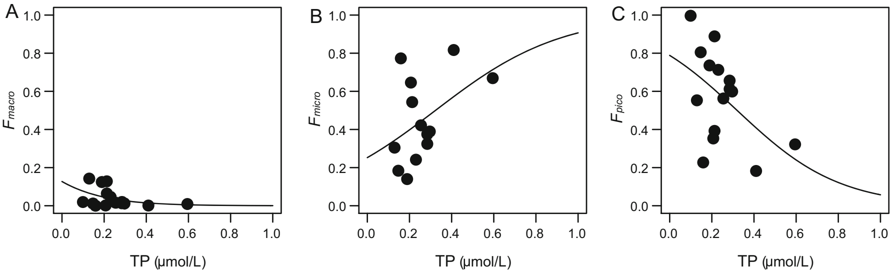


Fig. 6.7 Fractions of macroplankton (size <200 μm; **a**), microplankton (2–200 μm; **b**), and picoplankton (<2 μm; **c**) respiration rates to community respiration in the epilimnetic water along the productivity gradient of study lakes. Logistic regression curves are shown for each size-fractionated component of plankton communities. The total phosphorus (*TP*) concentration is used as a proxy measure of lake productivity

ciliates, and rotifers) showed a significant increase in their proportion of respiratory activity with increasing TP concentration (Fig. 6.7b). These opposite trends between picoplankton and microplankton metabolic activities suggest the existence of strong predator–prey or competitive interactions between bacteria and eukaryotic algae or small zooplankters (Wetzel 2001). Consequently, the increase in lake productivity is expected to promote the relative contribution of microplankton metabolic activities through bottom-up processes, thereby decreasing the fraction of smaller picoplankton metabolism.

Cascading effects of planktivorous fish may also regulate the aquatic metabolism. The fraction of macroplankton respiration was generally low but exhibited a slightly decreasing trend with increasing TP concentration (Fig. 6.7a). Moreover, when the study lakes were divided into those with and without planktivorous fish, the macroplankton metabolism (large cladocerans such as *Daphnia* and calanoid copepods such as *Acanthodiaptomus*) was much lower in the former lakes due to the lower biomass under fish predation pressure (Fig. 6.8a). Conversely, the metabolism of microplankton was higher in lakes with planktivorous fish, suggesting that

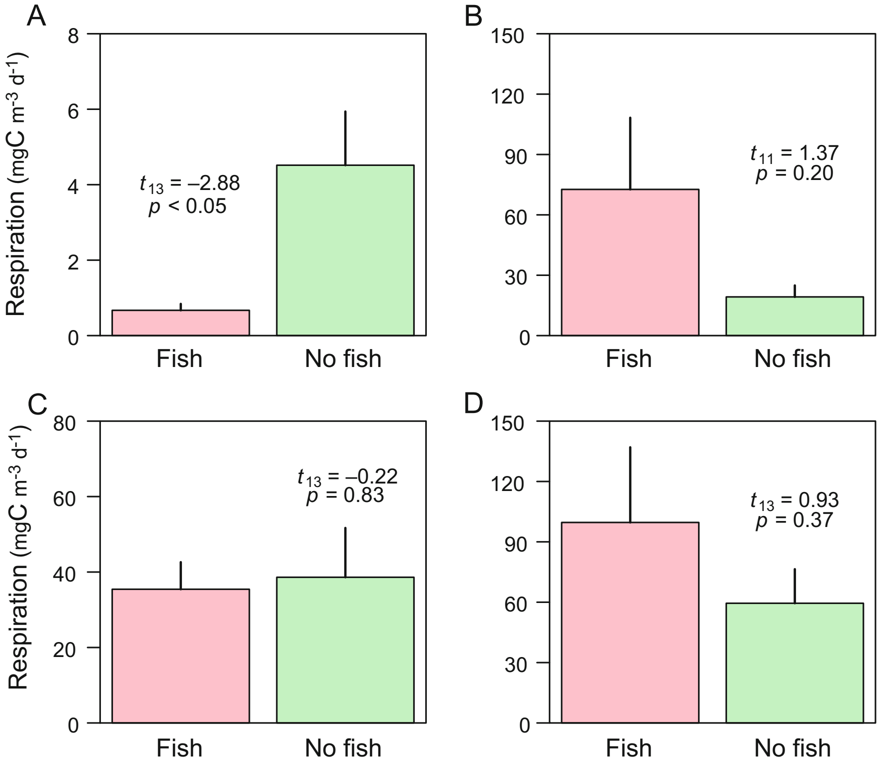


Fig. 6.8 Effects of the presence of planktivorous fish on the respiration rates of macroplankton (**a**), microplankton (**b**), and picoplankton (**c**) components and community respiration (**d**) in the epilimnetic water of study lakes. Results of *t*-test comparing means of respiration rates between fish and no fish lakes are shown

fish exerted strong cascading effects on size-dependent metabolic activities (Fig. 6.8b). The presence of planktivorous fish is known to increase the primary production rates of algae by depressing large-bodied grazers such as *Daphnia*, which in turn influence the CO_2 exchange between lakes and the atmosphere (Carpenter et al. 1985; Schindler et al. 1997; Cole et al. 2000; Parker and Schindler 2006). In this study, the effects of fish predation seemed not to extend down to the picoplankton size class (Fig. 6.8c). However, CR tended to be somewhat higher in fish lakes than no fish lakes, though no statistical difference was found (Fig. 6.8d). Therefore, the introduction of planktivorous fish is likely to have triggered the cascading trophic interactions, thereby strongly altering the food web structure and biogeochemical cycling in the epilimnetic water of study lakes.

6.7 Factors Affecting GPP and CR in Mountain Lakes

Environmental correlates of community metabolism revealed that water temperature, substrates, and light availabilities positively influenced the GPP and CR in the epilimnion of the mountain lakes (Table 6.3). The best-fitting model of nonlinear equation showed that epilimnetic GPP increased with increasing water temperature, TP, and PAR (Fig. 6.9a–c). Epilimnetic CR was greater in lakes with higher temperature, TP, and DOC (Fig. 6.9d–f). A number of studies have also described that TP often correlated with GPP and that DOC correlated with CR in lake ecosystems (e.g., Schindler 1997; Hanson et al. 2003; Solomon et al. 2013). In addition, temperature is known to regulate the rates of planktonic metabolism through the functional dependence of biochemical kinetics on environmental temperature (Yvon-Durocher et al. 2010). Moreover, a recent study has shown that light availability can limit the lake productivity of small, nutrient-poor lakes where the high concentration of colored terrestrial organic matter prevents vertical light attenuation (Karlsson et al. 2009). The present results support these previous findings, suggesting that the community metabolism of Japanese mountain lakes may follow the general pattern observed in many of world's unproductive lake ecosystems.

We obtained the predictive model of GPP/CR ratio in the epilimnion by dividing the best GPP model by the best CR model (Table 6.3) as shown below:

$$\mathrm{GPP/CR\ ratio} = 2.5e^{0.43(T-T_{20})/kTT_{20}}\mathrm{TP}^{-0.48}\ \mathrm{DOC}^{-0.55}\ \mathrm{dPAR}^{0.43}. \tag{6.8}$$

Based on this metabolic balance model, we obtained the predicted response curves of epilimnetic GPP/CR ratio along the environmental gradient of mountain lakes (Fig. 6.10). In the empirical models, temperature sensitivity is represented by the activation energy (E), which is a minimum chemical energy necessary for a chemical reaction to occur. Because E of GPP (1.3 eV) was estimated as higher than CR (0.86 eV; Table 6.3), GPP increases faster than CR with the elevation of

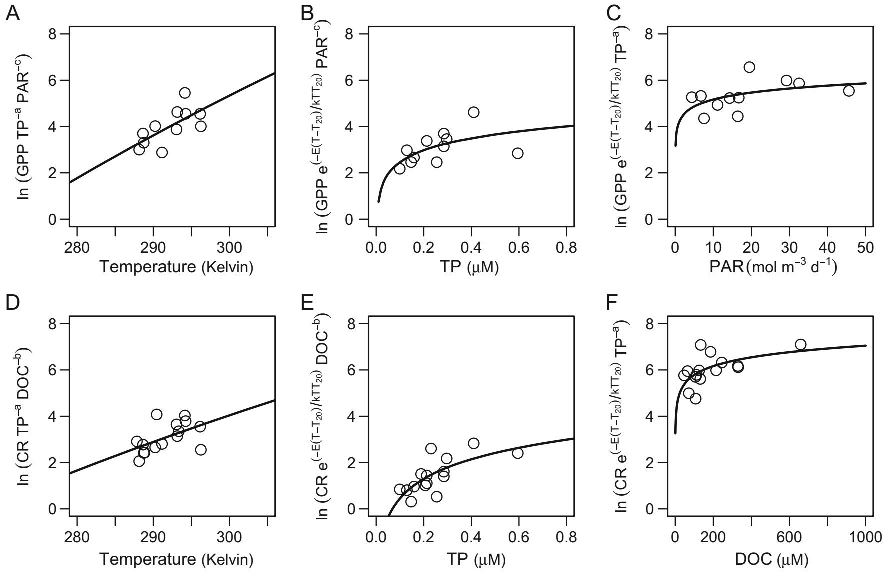


Fig. 6.9 Effects of environmental variables on epilimnetic metabolism of study lakes. Volumetric rates of GPP shown were determined by assuming that the fraction of the cloud-free sky is 0.6. (**a**) Effect of temperature on phosphorus-corrected and light-corrected GPP. (**b**) Effect of TP on temperature-corrected and light-corrected GPP. (**c**) Effect of PAR on temperature-corrected and phosphorus-corrected GPP. (**d**) Effect of temperature on phosphorus-corrected and DOC-corrected CR. (**e**) Effect of TP on temperature-corrected and DOC-corrected CR. (**f**) Effect of DOC on temperature-corrected and phosphorus-corrected CR. Response curves obtained from the best-fitting nonlinear models are shown

temperature (Fig. 6.9a, d). Consequently, the metabolic balance model yielded a prediction that the GPP/CR ratio increases with increasing water temperature (Fig. 6.10). Such a higher temperature dependence of primary productivity than that of respiration contradicts the patterns observed previously in lake and oceanic plankton communities. Yvon-Durocher et al. (2010) predicted theoretically that in lake ecosystems, the activation energy of CR should be greater than that of GPP under conditions where heterotrophic respiration exceeds in situ net primary productivity (NEP). In fact, they confirmed the higher activation energy of CR (~0.62 eV) than that of GPP (~0.45 eV) in a global warming mesocosm experiment. In addition, Lopez-Urrutia et al. (2006) showed differential temperature scaling of autotrophic and heterotrophic processes in a dataset of world's oceanic plankton communities, revealing weaker temperature dependence of photosynthetic rates than that of heterotrophic respiration. Consequently, they assert that if sea temperature rises, pelagic plankton communities would uptake less CO_2, although their predictive models are developed based on the global dataset of net primary production and heterotrophic respiration but not on measured GPP and CR (Lopez-Urrutia et al. 2006). We cannot ascertain why epilimnetic autotrophs of our mountain lake ecosystems showed high activation energy of GPP compared with

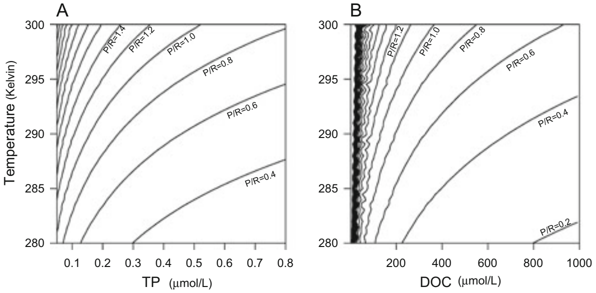
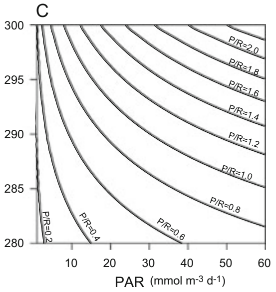


Fig. 6.10 Predicted responses of the ratio between epilimnetic GPP and CR (P/R) along the environmental gradients (temperature, TP, DOC, and PAR) of mountain lakes. Projections were obtained using the empirical model (see the text) while fixing the other variables at the average values of observed lakes

CR or with those observed in previous studies. Further ecological and physiological studies are necessary to identify the specific characteristics of the temperature dependence of planktonic photosynthesis and respiration of mountain lake communities.

The model also predicts that the metabolic balance of epilimnetic communities tends to be negative (GPP/CR <1) in higher DOC and lower light environments (Fig. 6.10). The input of terrestrial DOC often increases the secondary production of heterotrophic bacterioplankton and thereby promotes CO_2 efflux from lake ecosystems (Lennon 2004; Jansson et al. 2008). In addition, colored terrestrial DOM reduces light transmittance, thereby limiting the primary productivity of nutrient-poor lakes (Karlsson et al. 2009). Therefore, although allochthonous organic matter seems to support only a portion of the epilimnetic plankton respiration at current DOC level in this study (Fig. 6.6), the predicted future increase of DOC inputs accelerated by warmer climate (Weyhenmeyer and Karlsson 2009) is likely to shift epilimnetic communities from net autotrophic to net heterotrophic communities (i.e., excess respiration over photosynthesis). In fact, the DOC concentration in our study lakes is comparatively low (range = 0.6–7.9 mg/L, median = 1.6 mg/L; Table 6.1) as compared to those in lakes of other regions where strong net heterotrophy and high $p\mathrm{CO}_2$ supersaturation were observed (Hanson et al. 2003; Sobek et al. 2003; Lennon 2004; Jansson et al. 2008; Weyhenmeyer and Karlsson 2009; Karlsson et al. 2012). We therefore expect that the increased supply of terrestrial organic matter and resultant low light penetration may enhance the degree of allochthony of epilimnetic food webs, which in turn may decrease their reliance on planktonic and benthic primary productivity in mountain lakes. It is noteworthy that prediction of how much change will occur in DOC concentration of Japanese lakes with temperature rise is extremely difficult. In boreal lakes of Sweden, however, a rapid increase of DOC concentration associated with the increase of growing and runoff season (the number of days with air temperature exceeding 0 °C) was observed; lake DOC concentration increased by

~0.2 mg/L when the growing and runoff season was extended one day longer by the elevation of temperature (Weyhenmeyer and Karlsson 2009).

The strong effects of allochthonous dissolved organic matter on epilimnetic lake food webs, however, may be restricted to heterotrophic microbes in small mountain lakes with high DOC concentration. Pace et al. (2007) demonstrated that the energetic contribution of allochthonous organic carbon in a large clear-water lake is not substantial and that autochthonous organic carbon from in situ primary production largely supported the secondary production of many consumers such as bacterioplankton, copepods, cladocerans, and *Chaoborus* spp. Cole et al. (2002) also estimated that in a small humic lake, terrestrially derived dissolved organic carbon substantially subsidized the respiration of planktonic bacteria, but little of this organic matter was transferred to higher trophic-level consumers. This is because bacterial growth efficiency (BGE) is generally extremely low (del Giorgio and Cole 1998) and most organic carbon taken up by microbes is respired before being passed on to higher tropic levels. Consequently, bacterial biomass can meet only a small fraction of carbon demand of zooplankters (see also Cole et al. 2006; Karlsson et al. 2012), although low-molecular-weight dissolved organic compounds (organic acids, free amino acids, and simple carbohydrates) exported from forested watersheds can support a part of protozoan and metazoan secondary productions (Berggren et al. 2010). Moreover, even in nutrient-poor brown water lakes (dystrophic lakes), where primary production is limited under low nutrient and light availabilities, photosynthetic autotrophs can be the main basal resource of pelagic and benthic consumers (Lau et al. 2014). Therefore, increased inputs of terrestrial DOC by warmer climate will increase the allochthony of heterotrophic bacterioplankton, but most such organic matter will be respired to carbon dioxide. Instead, autochthonous carbon may dominate the organic matter transfer to higher trophic levels in warmer environments because higher trophic-level consumers may rely mainly on autotrophic resources from GPP, which will respond more rapidly than CR to a temperature rise (Figs. 6.9 and 6.10).

6.8 Conclusion

This study revealed that mountain lake ecosystems are linked strongly to their surrounding catchments through the flux of terrestrial organic matter. Particularly, the effects of such terrestrial subsidy tend to be significant in small lakes with high drainage ratios, where allochthonous organic matter predominates the carbon cycling in epilimnetic water. However, our results revealed that the metabolic balance of epilimnetic lake waters is net autotrophic or approximately in balance between GPP and CR, suggesting that allochthonous organic matter generally subsidizes benthic and hypolimnetic communities in small mountain lakes. We also developed an empirical model for the pattern of epilimnetic metabolism balance (GPP/CR ratio), which predicts that the GPP/CR ratio increases with temperature but decreases with DOC concentration. Mountain lakes are now expected to undergo a considerable increase of water temperature and DOC concentration associated with ongoing global warming (Parker et al. 2008; Weyhenmeyer and Karlsson 2009; Kissman et al. 2013). We expect, based on the present empirical model, that increased inputs of terrestrial DOC will increase the allochthony of planktonic, heterotrophic microorganisms, but warmer temperature will also stimulate in situ primary productivity, which will then increase the flux of autochthonous organic carbon to higher trophic levels. Consequently, it can be concluded that climate change may increase carbon flow from both autochthonous and allochthonous pathways in epilimnetic communities of unproductive small mountain lakes, directly through the functional dependence of community metabolism on the projected increase of temperature and indirectly through the increased supply of terrestrial organic matter from lands to lakes. The degree of future changes in both lake water temperature and DOC concentration may determine whether the metabolic balances of lake planktonic communities are driven to net autotrophy or net heterotrophy. If lake environments will change to the upper right corner along the curve of counter lines in the middle panel of Fig. 6.10, no significant change is expected to occur in the metabolic balance of epilimnetic communities, even in higher temperature and DOC conditions. However, to forecast future changes in the metabolic balance of mountain lake ecosystems, more detailed predictive models of lake water temperature and DOC concentrations are necessary. Finally, further quantitative studies of metabolism of deep-layer communities, such as benthic and hypolimnetic environments, are also necessary for the accurate prediction of the changes in the whole-scale carbon flux of mountain lake ecosystems in response to future climate change.

Acknowledgments This study was supported by Grants-in-Aid for Young Scientists (B) (16710016) and for Scientific Research (B) (15370010) and (A) (19207003) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and by the Global Environment Research Fund (F-052) of the Ministry of the Environment, Japan. The stable isotope analysis was conducted using Cooperative Research Facilities (Isotope Ratio Mass Spectrometer) at the Center for Ecological Research, Kyoto University.

References

Adrian R, O'Reilly CM, Zagarese H, Baines SB, Hessen DO, Keller W, Livingstone DM, Sommaruga R, Straile D, Van Donk E, Weyhenmeyer GA, Winder M (2009) Lakes as sentinels of climate change. Limnol Oceanogr 54:2283–2297

Algesten G, Sobek S, Bergström A-K, Ågren A, Tranvik LJ, Jansson M (2003) Role of lakes for organic carbon cycling in the boreal zone. Glob Chang Biol 10:141–147

Arístegui J, Montero MF (1995) The relationship between community respiration and ETS activity in the ocean. J Plankton Res 17:1563–1571

Bade DL, Carpenter SR, Cole JJ, Hanson PC, Hesslein RH (2004) Controls of δ^{13}C-DIC in lakes: geochemistry, lake metabolism, and morphometry. Limnol Oceanogr 49:1160–1172

Bade DL, Carpenter SR, Cole JJ, Pace ML, Kritzberg E, Van de Bogert MC, Cory RM, McKnight DM (2007) Sources and fates of dissolved organic carbon in lakes as determined by whole-lake carbon isotope additions. Biogeochemistry 84:115–129

Båmstedt U (2000) A new method to estimate respiration rate of biological material based on the reduction of tetrazolium violet. J Exp Mar Biol Ecol 251:239–263

Bastviken D, Tranvik LJ, Downing JA, Crill PM, Enrich-Prast A (2011) Freshwater methane emissions offset the continental carbon sink. Science 331:50

Berggren M, Ström L, Laudon H, Karlsson J, Jonsson A, Giesler R, Bergström A-K, Jansson M (2010) Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. Ecol Lett 13:870–880

Carignan R, Blais A-M, Vis C (1998) Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method. Can J Fish Aquat Sci 55:1078–1084

Carignan R, Planas D, Vis C (2000) Planktonic production and respiration in oligotrophic Shield lakes. Limnol Oceanogr 45:189–199

Carpenter SR, Kitchell JF, Hodgson JR (1985) Cascading trophic interactions and lake productivity. Bioscience 35:634–639

Carpenter SR, Cole JJ, Pace ML, Van de Bogert M, Bade DL, Bastviken D, Gille CM, Hodgson JR, Kitchell JF, Kritzberg ES (2005) Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. Ecology 86:2737–2750

Cole JJ, Caraco NF (2001) Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. Mar Freshwat Res 52:101–110

Cole JJ, Pace ML, Carpenter SR, Kitchell JF (2000) Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. Limnol Oceanogr 45:1718–1730

Cole JJ, Carpenter SR, Kitchell JF, Pace ML (2002) Pathways of organic carbon utilization in small lakes: results from a whole-lake ^{13}C addition and coupled model. Limnol Oceanogr 47:1664–1675

Cole JJ, Carpenter SR, Pace ML, Van de Bogert MC, Kitchell JL, Hodgson JR (2006) Differential support of lake food webs by three types of terrestrial organic carbon. Ecol Lett 9:558–568

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middleburg J, Melack J (2007) Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171–184

Cole JJ, Carpenter SR, Kitchell J, Pace ML, Solomon CT, Weidel B (2011) Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. PNAS 108:1975–1980

Coloso JJ, Cole JJ, Hanson PC, Pace ML (2008) Depth-integrated, continuous estimates of metabolism in a clear-water lake. Can J Fish Aquat Sci 65:712–722

del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. Annu Rev Ecol Syst 29:503–541

del Giorgio PA, Cole JJ, Cimbleris A (1997) Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature 385:148–151

Dodson AN, Thomas WH (1964) Concentrating plankton in a gentle fasion. Limnol Oceanogr 9:455–456

DOE (1994) Handbook of methods for the analysis of the various parameters of the carbon dioxide system in seawater; ver.2. ORNL/CDIAC-74, Oak Ridge

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegel RG, McDowell WH, Kortelainen P, Caraco NF, Melack JM, Middelburg JJ (2006) The global abundance and size distribution of lakes, ponds, and impoundments. Limnol Oceanogr 51:2388–2397

Duarte CM, Agustí S (1998) The CO_2 balance of unproductive aquatic ecosystems. Science 281:234–236

Fee EJ (1990) Computer programs for calculating in situ phytoplankton photosynthesis. Can Tech Rep Fish Aquat Sci 1740:1–27

Fry B (2006) Stable isotope ecology. Springer, New York

Fujii M, Kojima H, Iwata T, Urabe J, Fukui M (2012) Dissolved organic carbon as major environmental factor affecting bacterioplankton communities in mountain lakes of eastern Japan. Microb Ecol 63:496–508

Gergel SE, Turner MG, Kratz TK (1999) Dissolved organic carbon as an indicator of the scale of watershed influence on lakes and rivers. Ecol Appl 9:1377–1390

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248–2251

Gudasz C, Bastviken D, Steger K, Premke K, Sobek S, Tranvik LJ (2010) Temperature-controlled organic carbon mineralization in lake sediments. Nature 466:478–481

Hanson PC, Bade DL, Carpenter SR, Kratz TK (2003) Lake metabolism: relationships with dissolved organic carbon and phosphorus. Limnol Oceanogr 48:1112–1119

Hartmann J (2009) Bicarbonate-fluxes and CO_2-consumption by chemical weathering on the Japanese archipelago – application of a multi-lithological model framework. Chem Geol 265:237–271

Hoellein TJ, Bruesewitz DA, Richardson DC (2013) Revisiting Odum (1956): a synthesis of aquatic ecosystem metabolism. Limnol Oceanogr 58:2089–2100

Iwata T, Suzuki T, Togashi H, Koiwa N, Shibata H, Urabe J (2013) Fluvial transport of carbon along the river-to-ocean continuum and its potential impacts on a brackish water food web in the Iwaki River watershed, northern Japan. Ecol Res 28:703–716

Jansson M, Hickler T, Jonsson A, Karlsson J (2008) Links between terrestrial primary production and bacterial production and respiration in lakes in a climate gradient in subarctic Sweden. Ecosystems 11:367–376

Kankaala P, Huotari J, Tulonen T, Ojala A (2013) Lake-size dependent physical forcing drives carbon dioxide and methane effluxes from lakes in a boreal landscape. Limnol Oceanogr 58:1915–1930

Karlsson J, Jansson M, Jonsson A (2007) Respiration of allochthonous organic carbon in unproductive forest lakes determined by the Keeling plot method. Limnol Oceanogr 52:603–608

Karlsson J, Byström P, Ask J, Ask P, Persson L, Jansson M (2009) Light limitation of nutrient-poor lake ecosystems. Nature 460:506–509

Karlsson J, Berggren M, Ask J, Byström P, Jonsson A, Laudon H, Jansson M (2012) Terrestrial organic matter support of lake food webs: evidence from lake metabolism and stable hydrogen isotopes of consumers. Limnol Oceanogr 57:1042–1048

Kissman CEH, Williamson CE, Rose KC, Saros JE (2013) Response of phytoplankton in an alpine lake to inputs of dissolved organic matter through nutrient enrichment and trophic forcing. Limnol Oceanogr 58:867–880

Kortelainen P, Rantakari M, Huttunen JT, Mattsson T, Alm J, Juutinen S, Larmola T, Silvola J, Martikainen PJ (2006) Sediment respiration and lake trophic state are important predictors of large CO_2 evasion from small boreal lakes. Glob Chang Biol 12:1554–1567

Lau DCP, Sundh I, Vrede T, Pickova J, Goedkoop W (2014) Autochthonous resources are the main driver of consumer production in dystrophic boreal lakes. Ecology 95:1506–1519

Lennon JT (2004) Experimental evidence that terrestrial carbon subsidies increase CO_2 flux from lake ecosystems. Oecologia 138:584–591

Lopez-Urrutia A, San Martin E, Harris RP, Irigoien X (2006) Scaling the metabolic balance of the oceans. PNAS 103:8739–8744

Macias-Fauria M, Johnson EA (2013) Warming-induced upslope advance of subalpine forest is severely limited by geomorphic processes. PNAS 110:8117–8122

Miyajima T, Yamada Y, Hanba YT, Yoshii K, Koitabashi T, Wada E (1995) Determining the stable isotope ratio of total dissolved inorganic carbon in lake water by GC/C/IRMS. Limnol Oceanogr 40:994–1000

Mook WG, Bommerson JC, Staverman WH (1974) Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Planet Sci Lett 22:169–176

Obrador B, Staehr PA, Christensen JPC (2014) Vertical patterns of metabolism in three contrasting stratified lakes. Limnol Oceanogr 59:1228–1240

Pace ML, Cole JJ, Carpenter SR, Kitchell JF, Hodgson JR, Van de Bogert MC, Bade DL, Kritzberg ES, Bastviken D (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240–243

Pace ML, Carpenter SR, Cole JJ, Coloso JJ, Kitchell JF, Hodgson JR, Middelburg JJ, Preston ND, Solomon CT, Weidel BC (2007) Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? Limnol Oceanogr 52:2177–2189

Packard TT (1985) Measurement of electron transport activity in microplankton. Adv Aquat Microbiol 3:207–261

Parker BR, Schindler DW (2006) Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166

Parker BR, Vinebrooke RD, Schindler DW (2008) Recent climate extremes alter alpine lake ecosystems. PNAS 105:12927–12931

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38:687–701

Quinlan R, Douglas MSV, Smol JP (2005) Food web changes in arctic ecosystems related to climate warming. Glob Chang Biol 11:1381–1386

Rasmussen JB, Godbout L, Schallenberg M (1989) Thu humic content of lake water and its relationship to watershed and lake morphometry. Limnol Oceanogr 34:1336–1343

Rosén P, Cunningham L, Vonk J, Karlsson J (2009) Effects of climate on organic carbon and the ratio of planktonic to benthic primary producers in a subarctic lake during the past 45 years. Limnol Oceanogr 54:1723–1732

Sadro S, Melack JM, MacIntyre S (2011) Depth-integrated estimates of ecosystem metabolism in a high-elevation lake (Emerald Lake, Sierra Nevada, California). Limnol Oceanogr 56:1764–1780

Scharnweber K, Syväranta J, Hilt S, Brauns M, Vanni MJ, Brothers S, Köhler J, Knezevic-Jaric J, Mehner T (2014) Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes. Ecology 95:1496–1505

Schindler DW (1997) Widespread effects of climatic warming on freshwater ecosystems in North America. Hydrol Process 11:1043–1067

Schindler DW (2009) Lakes as sentinels and integrators for the effects of climate change on watersheds, airsheds, and landscapes. Limnol Oceanogr 54:2349–2358

Schindler DE, Carpenter SR, Cole JJ, Kitchell JF, Pace ML (1997) Influence of food web structure on carbon exchange between lakes and the atmosphere. Science 277:248–251

Smol JP, Douglas MS (2007) Crossing the final ecological threshold in high Arctic ponds. PNAS 104:12395–12397

Smol JP, Wolfe AP, Birks HJP, Douglas MSV, Jones VJ, Korhola A, Pienitz R, Rühland K, Sorvari S, Antoniades D, Brooks SJ, Fallu M-A, Hughes M, Keatley BE, Laing TE, Michelutti N, Nazarova L, Nyman M, Paterson AM, Perren B, Quinlan R, Rautio M, ÉmilieSaulnier-Talbot E, Siitonen S, Solovieva N, Weckström J (2005) Climate-driven regime shifts in the biological communities of arctic lakes. PNAS 102:4397–4402

Sobek S, Algesten G, Bergström A-K, Jansson M, Tranvik LJ (2003) The catchment and climate regulation of pCO_2 in boreal lakes. Glob Chang Biol 9:630–641

Sobek S, Tranvik LJ, Prairie YT, Kortelainen P, Cole JJ (2007) Patterns and regulation of dissolved organic carbon: an analysis of 7,500 widely distributed lakes. Limnol Oceanogr 52:1208–1219

Solomon CT, Carpenter SR, Clayton MK, Cole JJ, Coloso JJ, Pace ML, Vander Zanden MJ, Weidel BC (2011) Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. Ecology 92:1115–1125

Solomon CT, Bruesewitz DA, Richardson DC, Rose KC, Van de Bogert MC, Hanson PC, Kratz TK, Larget B, Adrian R, Babin BL, Chiu C, Hamilton DP, Gaiser EE, Hendricks S, Istvánovics V, Laas A, O'Donnell DM, Pace ML, Ryder E, Staehr PA, Torgersen T, Vanni MJ, Weathers KC, Zhu G (2013) Ecosystem respiration: drivers of daily variability and background respiration in lakes around the globe. Limnol Oceanogr 58:849–866

Staehr PA, Christensen JPA, Batt RD, Read JS (2012) Ecosystem metabolism in a stratified lake. Limnol Oceanogr 57:1317–1330

Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis, second ed. Bull Fish Res Board Can 167:1–310

Stumm W, Morgan JJ (1996) Aquatic chemistry: chemical equilibria and rates in natural waters, 3rd edn. Wiley, New York

Thompson R, Ventura M, Camarero L (2009) On the climate and weather of mountain and sub-arctic lakes in Europe and their susceptibility to future climate change. Freshwat Biol 54:2433–2451

Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ, Dillon P, Finlay K, Fortino K, Knoll LB, Kortelainen PL, Kutser T, Larsen S, Laurion I, Leech DM, McCallister SL, McKnight DM, Melack JM, Overholt E, Porter JA, Prairie Y, Renwick WH, Roland F, Sherman BS, Schindler DW, Sobek S, Tremblay A, Vanni MJ, Verschoor AM, von Wachenfeldt E, Weyhenmeyer GA (2009) Lakes and reservoirs as regulators of carbon cycling and climate. Limnol Oceanogr 54:2298–2314

Urabe J, Iwata T, Yagami Y, Kato E, Suzuki T, Hino S, Ban S (2011) Within-lake and watershed determinants of carbon dioxide in surface water: a comparative analysis of a variety of lakes in the Japanese Islands. Limnol Oceanogr 56:49–60

Weiss RF (1974) Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Mar Chem 2:203–215

Wetzel RG (2001) Limnology, 3rd edn. Academic, San Diego

Weyhenmeyer GA, Karlsson J (2009) Nonlinear response of dissolved organic carbon concentrations in boreal lakes to increasing temperatures. Limnol Oceanogr 54:2513–2519

Yvon-Durocher G, Jones JI, Trimmer M, Woodward G, Montoya JM (2010) Warming alters the metabolic balance of ecosystems. Philos Trans R Soc B 365:2117–2126

Chapter 7
Planktonic Bacterial Communities in Mountain Lake Ecosystems

Masanori Fujii, Toshihide Hirao, Hisaya Kojima, and Manabu Fukui

Abstract Mountain lakes are thought to be sensitive to environmental changes, and bacterioplankton in these lakes may be suitable for monitoring the effects of global climate change. In this chapter, we discuss characteristics of the environmental conditions and bacterioplankton communities in mountain lakes, by integrating the results of our survey conducted in Japanese mountain lakes with the knowledge from previous studies. Mountain lakes have some characteristics, which can affect bacterioplankton communities significantly. There are some taxonomic groups of bacteria dominant in mountain lakes, such as the genus *Polynucleobacter*. These groups are also abundant in freshwater environments in low altitudes and regarded as cosmopolitans widely distributed in global freshwater environments. For some of these bacteria, their dominance in mountain lakes can be partially explained by their physiological characteristics. In addition to the environmental filtering illustrated by the dominance of these bacteria, community assembly of bacterioplankton in mountain lakes may also be regulated by other processes, including dispersal limitation and evolutionary constraint.

Keywords Alpine lake • Bacterioplankton • Community assembly • Humic substance • *Polynucleobacter*

7.1 Introduction

Mountain lakes have been recognized as an ecosystem that is particularly sensitive to global climate change (Williamson et al. 2008; Adrian et al. 2009). The rise in the average temperature of the Earth shifts the timing of freezing and melting of surface water during the annual cycle and reduces the ice-covered period in high mountain lakes (Magnuson et al. 2000). The associated seasonal changes in the light climate

M. Fujii • T. Hirao
The University of Tokyo Chichibu Forest, 1-1-49 Hinoda-machi, Chichibu, Saitama 368-0034, Japan

H. Kojima • M. Fukui (✉)
Institute of Low Temperature Science, Hokkaido University, Kita-19, Nishi-8, Kita-ku, Sapporo, Hokkaido 060-0819, Japan
e-mail: my-fukui@pop.lowtem.hokudai.ac.jp

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2_7

may alter ecosystems in the lacustrine water column. The rapid change in average temperature has a strong indirect impact on the water chemistry of alpine lakes, through enhanced weathering and biological activity (Sommaruga-Wögrath et al. 1997). Vegetation shifts associated with changing temperature may also indirectly affect ecosystems in mountain lakes because lake water body environments are closely related to the environments within the catchments (Grabherr et al. 1994; Keller et al. 2000). Furthermore, global desertification and land use changes in lowland areas have increased airborne dust containing large quantities of nutrients, such as nitrogen and phosphorus (Prospero and Lamb 2003; Moulin and Chiapello 2006; Neff et al. 2008), thereby altering the trophic conditions in various inland water systems, especially oligotrophic mountain lakes (Baron et al. 2000; Morales-Baquero et al. 2006).

In mountain lakes, bacteria may be the most suitable organisms for monitoring the effects of global climate change. First, bacteria inhabit almost all environments on Earth, even high alpine lakes located more than 5000 m above sea level (Liu et al. 2006, 2009, 2011), where most other organisms cannot survive. Second, their diversity and activity are highly sensitive to changes in environmental conditions due to their short generation time and fine niche partitioning (e.g., Glöckner et al. 2000; Crump et al. 2003; Liu et al. 2013). These ecological features may allow for real-time monitoring of the effects of global climate change. In aquatic ecosystems including mountain lakes, a dominant functional group among the bacterial communities is heterotrophic bacterioplankton, which play two important roles. First, as a decomposer, this group consumes a variety of organic remains. Second, this group assimilates dissolved organic carbon (DOC) derived from the surrounding terrestrial regions. The latter is a key step in carbon flow referred to as the "microbial loop," which has been recognized as an important carbon pathway in various aquatic ecosystems (Azam et al. 1983; Tranvik 1992). This is especially true in oligotrophic aquatic systems, such as mountain lakes, where participants in the microbial loop play an important role as primary producers in place of phototrophic organisms (Karlsson et al. 2002). Therefore, elucidating the characteristics of bacterioplankton community composition (BCC) and function is important to understanding not only the effects of global climate change but also the role of bacterioplankton in mountain lake ecosystems. Unfortunately, little information regarding bacterioplankton in mountain lakes in the Japanese Archipelagos is available.

In this chapter, we focus on the characteristics of bacterioplankton in mountain lakes located in the mountainous regions of Eastern Japan. We investigated 41 mountain lakes in the summers of 2005, 2006, and 2007 (Fig. 7.1). By integrating the results of our survey of Japanese mountain lakes with the knowledge from previous studies, we discuss the following topics. (1) What are the characteristics of the environments in mountain lakes, and how do the environmental conditions influence bacterioplankton? (2) What taxonomic groups of bacterioplankton are found in mountain lakes, and what are they doing there? (3) What factors are important to determine diversity and community composition of bacterioplankton?

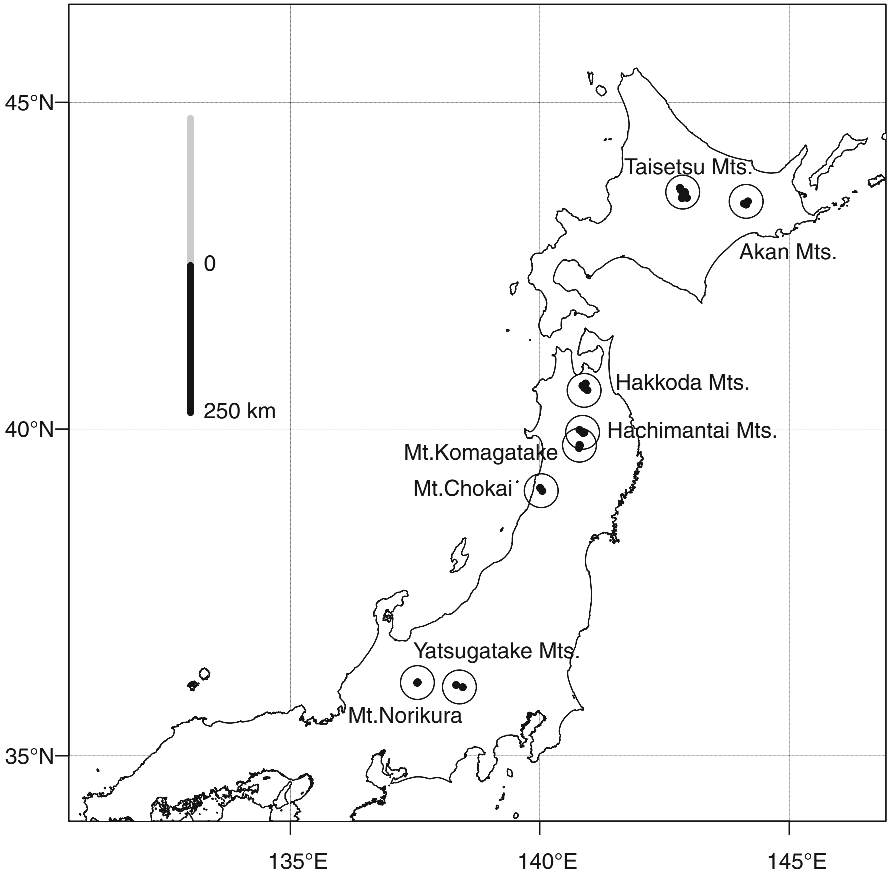


Fig. 7.1 Maps showing the locations of 41 mountain lakes of eastern Japan surveyed in the summer of 2005, 2006, and 2007

Finally, integrating the discussions of each topic, we discuss the future perspective of microbial ecology focusing on mountain lakes.

7.2 Physicochemical and Biological Environments of Mountain Lakes

7.2.1 Characteristics of Mountain Lakes

Most mountain lakes are characterized by their small size, with the exception of large artificial reservoirs (dam lakes) and crater lakes. This is due to the complex topography of mountainous regions. These small lakes tend to have short retention time, which can affect community structure of planktonic bacteria. In mountain lakes of Nepal, Sommaruga and Casamayor (2009) found a strong negative correlation between taxonomic richness of bacterioplankton and ratio of catchment to lake area. They regarded this ratio as an indicator of water retention time and suggested that many bacterial taxa would remain under the detection limit in lakes with short retention time (i.e., lakes with large ratio). Focusing on small mountain lakes has permitted modeling systems to understand the ecological functioning of this mountainous region and its vulnerability to global climate change (Williamson et al. 2008; Adrian et al. 2009). The main reason for this vulnerability is that small lakes with a poor buffering capacity are highly sensitive to the changes in external environment. This aspect may affect the community composition and functions of bacterioplankton of mountain lakes. For instance, inputs from terrestrial environments contain large amounts of humic substances, as well as microbial cells. These substances and organisms probably affect both the dominant metabolisms and the community compositions of bacterioplankton in lake ecosystems (Glaeser et al. 2010; Crump et al. 2012).

Oligotrophic conditions in the trophic status are also a distinctive characteristic of mountain lakes. In Japanese mountainous regions, more than half of the lakes investigated were oligotrophic (Fujii et al. 2012). The reason for oligotrophy in these lakes is probably due to an insufficient supply of phosphorus from outside of the lakes. The phosphorus supply is largely affected by the extent and land use (including vegetation type) of the catchment area, which change with elevation. In the Japanese mountain lakes, elevation negatively correlated with total phosphorus (TP) and chlorophyll *a* concentrations, both of which are indicators of trophic status (Fujii et al. 2012). Conversely, Karlsson et al. (2001) showed that elevation does not always determine the TP values. Furthermore, some lakes have been reported to exhibit eutrophic conditions despite their classification as high mountain lakes. For example, Lake Victoria in East Africa is located at an elevation of 1134 m above sea level but exhibits a eutrophic condition caused by land exploitation for agriculture and forest burning in the lake surroundings (Scheren et al. 2000). Certain parts of Lake Titicaca, where sewage flows into the lake, have also been eutrophic, despite the alpine lake's elevation of 3803 m (Vincent et al. 1984). As in the cases of these two mountain lakes, several Japanese mountain lakes were also directly exposed to anthropogenic impacts and consequently exhibited remarkably higher TP values compared with the other mountain lakes (Fujii et al. 2012). Therefore, the oligotrophic state in high-elevation lakes may be closely related to how isolated they are from human activity rather than the elevation itself.

In addition to the small size and oligotrophic conditions of alpine lakes, the solar radiation is an influential component. In general, the amount of solar radiation increases with altitude (Blumthaler et al. 1997); thus, its effects on ecosystems can be presumed to be more important in higher elevation regions. In lakes located above the tree line, solar radiation directly penetrates the water column because the presence of tall trees does not block the solar radiation. Furthermore, the high transparency of the water column in an oligotrophic state allows the solar radiation to penetrate deeper into alpine lakes. Karlsson et al. (2002) observed markedly higher values of ultraviolet-B (UV-B) penetration into lake water in high alpine lakes, in comparison to other lakes below tree line. In addition, they also observed that neither the effective light climate for photosynthetically active radiation nor UV-B radiation showed an association with elevation, based on an investigation of Swedish mountain lakes. Therefore, the effect of solar radiation on alpine lakes may be depending largely on relative position against tree line, rather than elevation itself.

Another characteristic shared by most mountain lakes is the absence of fish. In general, the distribution of fish is mainly determined by hydrographic history in inland water systems. The active migration of fish from a low to a high elevation without artificial transportation is generally difficult. Consequently, the absence of fish has often been observed in mountain lakes (Bahls 1992). Fish do not utilize bacterioplankton directly, but affect planktonic bacteria indirectly via trophic cascades involving protozoa and metazoan zooplankton. The primary predators of bacterioplankton in various aquatic environments are protozoa, such as ciliate and heterotrophic nanoflagellates (Callieri et al. 2006). They are known to prey on specific taxonomic groups of bacterioplankton (Jürgens et al. 1999; Jardillier et al. 2004; Posch et al. 2007). These protozoa are major diet of metazoan zooplankton (Sanders and Wickham 1993), which are often preyed by fish. Therefore, the presence/absence of fish may alter the taxonomic composition of bacterioplankton and the abundance of each taxon.

7.2.2 Temporal Variation of Environmental Conditions

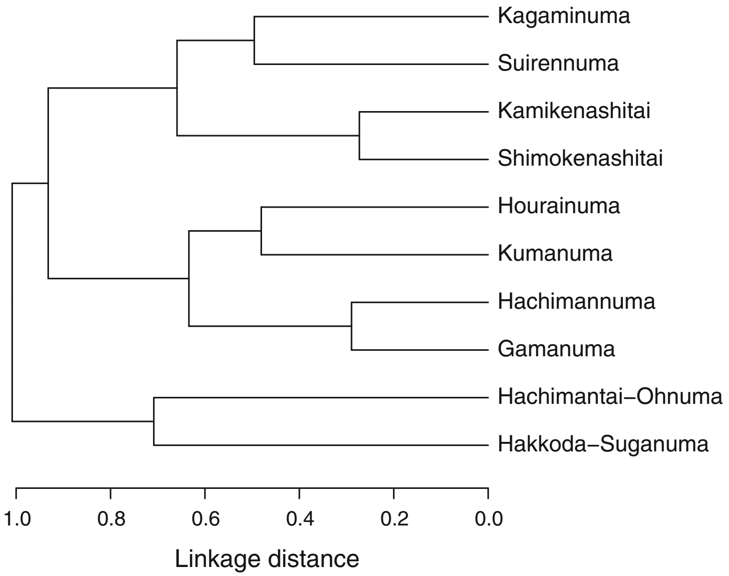
The seasonal dynamics of freshwater environments have a strong impact on bacterioplankton communities. In early spring, melting snow provides a large quantity of nutrients to lakes and consequently increases the primary production of photosynthetic organisms (Crump et al. 2003). The synthesized organic substances by primary producers are known to enhance the abundance of specific taxonomic groups, such as *Alphaproteobacteria* and *Bacteroidetes* (Eiler and Bertilsson 2004; Jardillier et al. 2004). The formation and collapse of thermal stratification are major seasonal events observed in many lakes. These events cause nutrient depletion at the surface of the lake during stratification and nutrient supply from the hypolimnion via mixing of lake water after the collapse of stratification, both of which influence the activity of certain specific bacterial taxa (Teresa Pérez and Sommaruga 2011; Liu et al. 2013). These large impacts of seasonal environmental dynamics on bacterioplankton communities have been reported not only in mountain lakes but also in other freshwater environments located at lower elevations.

On the other hand, some seasonal dynamics are prominent specifically in mountain lakes. The surface water of mountain lakes freezes during winter because of the small size and low temperature in high-elevation regions (Alfreider et al. 1996). This “ice cover” has a crucial role in community composition and the activity of bacterioplankton due to the prevention of solar radiation and atmospheric deposition entering the lake water (Panzenböeck 2007; Liu et al. 2013). Liu et al. (2013) reported that a lower abundance of bacterioplankton was observed during the ice-covered period, and the seasonal change in bacterioplankton abundance was associated more with water temperature and phytoplankton abundance than nutrients in Lake Namco in the central Tibetan Plateau. Llorens-Marés et al. (2012) also found a low abundance but high diversity of bacterioplankton during the ice-covered period in Lake Redon located in the Pyrenees, Spain. For Japanese mountain lakes, we found a large mass of ice floating at the surface of several lakes, despite the fact that the survey was conducted during the summer, implying that these lakes are influenced by ice cover during the winter.

Another event with a seasonal pattern is the aeolian dust deposition, which can transport various minerals and nutrients to isolated locations (Kellogg and Griffin 2006). Several studies have demonstrated that aeolian dust may serve as a nutrient source of phytoplankton in oceanic waters (e.g., Duarte et al. 2006). In mountain lakes, there is no supply of nutrients from outside of the lake except for rain- and snowfall because most of these lakes are isolated from anthropogenic impacts. Therefore, supply of nutrients from aeolian dust may have a large impact on mountain lakes (Mladenov et al. 2011; Nelson and Carlson 2011). In the Japanese Archipelagos, aeolian dust originating from the deserts in East Asia is transported by the Westerlies (Yamaguchi et al. 2012). Therefore, Asian dust may bring about seasonal changes in the nutrient conditions of Japanese mountain lakes and affect bacterioplankton communities.

Besides the seasonal changes, environmental conditions may vary from year to year. To understand the differences in environmental conditions (pH, electric conductivity, and chlorophyll *a*) and their effects on bacterioplankton community composition (BCC) from year to year, we conducted surveys in ten Japanese mountain lakes in the summer of 2005 and 2007. The BCCs were analyzed using the PCR-DGGE method, and community dissimilarity between the lakes was calculated using the Jaccard index based on the presence/absence of DGGE bands. As a result, similar patterns in BCC between 2005 and 2007 were demonstrated (Fig. 7.2), even though the environmental conditions were different for each year. However, a significant negative correlation was observed between the differences in dissolved organic carbon (DOC) concentrations and community dissimilarity of bacterioplankton (Table 7.1). This negative correlation means that large differences in DOC concentrations were associated with similar BCCs. The lakes, in which large changes in DOC were observed, contained higher concentrations of DOC compared to the other lakes. Hence, this result can be rephrased to state that BCCs were stable in the lakes with high DOC concentrations, even though the concentrations varied largely from year to year. Therefore, this result suggests that DOC concentrations greater than a certain threshold may determine the BCC strictly.

(a) 2005



(b) 2007

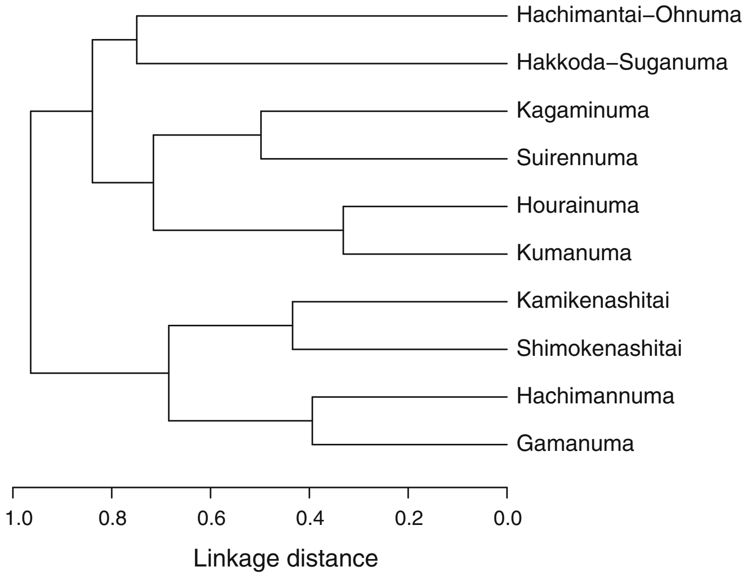


Fig. 7.2 Dendrograms from hierarchical clustering applied to the Jaccard dissimilarity matrix of presence/absence data, based on denaturing gradient gel electrophoresis profiles of bacterioplankton community compositions in ten Japanese mountain lakes surveyed in 2005 (**a**) and 2007 (**b**)

Table 7.1 Intercepts, unstandardized regression coefficients, and *P* values from multiple regression analyses on chronological changes of bacterioplankton community compositions in ten Japanese mountain lakes between 2005 and 2007, including relationships with environmental variables

	Estimate	Standard error	*t* value	*P* (> |*t*|)
Intercept	0.620	0.093	6.684	0.001**
pH	0.043	0.052	0.823	0.448
Con	0.004	0.008	0.574	0.591
DOC	−0.504	0.154	−3.286	0.022*
Chl	0.0267	0.011	2.567	0.050

Con electric conductivity, *DOC* dissolved organic carbon, *Chl* chlorophyll *a*
$*P < 0.05$; $**P < 0.01$

7.2.3 Spatial Heterogeneity of Environmental Conditions

In many aquatic ecosystems, formation of thermal stratification brings about vertical heterogeneity of environmental conditions in a water body. During the stratification, the water column is divided into three layers – epilimnion, thermocline, and hypolimnion – based on the differences in water density caused by the surface water temperature. In a stratified lake, dissolved oxygen in the hypolimnion is often depleted by the respiration of aerobic organisms. If additional oxygen is not supplied by mixing of the lake water, the water column is divided into oxic and anoxic environments (e.g., Casamayor et al. 2002). In oxic environments, aerobic respiration, in which oxygen is used as an electron acceptor, dominates due to its high-energy efficiency. In contrast, other types of respiration using different electron acceptors, such as nitrogen and sulfur oxides, dominate in anoxic environments devoid of oxygen. In most cases, these physiological differences in the type of respiration metabolism are closely related to phylogenetic clades of bacterioplankton. Therefore, the thermal stratification of the water column has a strong impact on the BCC within the water body. In many Japanese mountain lakes investigated, however, the dissolved oxygen was not depleted in the hypolimnion of the lakes with thermal stratification. In addition, the other environmental conditions were similar between the epilimnion and hypolimnion (unpublished data), and BCCs of different layers were almost identical (Fig. 7.3). As mentioned above, distinct environmental conditions in hypolimnion is largely attributed to biological activities represented by respiration. Therefore, in the lakes characterized by low biological activities, it takes longer time from the formation of thermal stratification until distinct differentiation of environmental conditions. The differences between conditions of water layers depend on duration of stratification and biological activities. Our investigation was performed in summer, but it is uncertain whether biological activities in these lakes can generate significantly different conditions until autumn mixing after short summer in mountain regions.

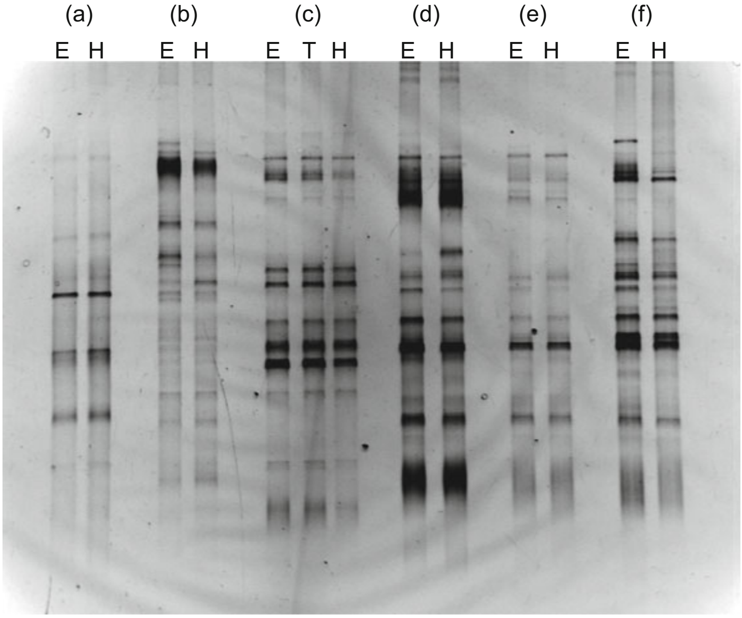


Fig. 7.3 Denaturing gradient gel electrophoresis profile of bacterioplankton community compositions in epilimnion (*E*), thermocline (*T*), and hypolimnion (*H*) of six Japanese mountain lakes in which thermal stratification was observed. Each lake name is as follows: Kikkou (*a*), Matsubara (*b*), Chokai (*c*), Kougennuma (*d*), Shikibunuma (*e*), Meotonuma-Suribachi (*f*)

Among lakes distributed far from one another, distinctive environments can be created by differences in geographical location and the surrounding environment. In mountain lakes, similar environmental conditions are predicted for lakes located in the adjacent catchments, due to the large impact of the terrestrial region. In Japan, we conducted surveys of mountain lakes in eight different areas that are geographically distinct from each other. However, there was no correlation between the BCCs and the sampling area (Fujii et al. 2012). This result implies that there is a weak correlation between lake environmental conditions, even in the adjacent catchment area. However, elevation correlated significantly with several environmental variables, including temperature, pH, electric conductivity, TP, and chlorophyll *a* concentration (Fujii et al. 2012), all of which are known to have a strong impact on bacterial community composition, diversity, and abundance (e.g., Karlsson et al. 2001; Fierer and Jackson 2006; Lozupone and Knight 2007). Therefore, the regional heterogeneity of environmental conditions of mountain lakes is largely affected by elevation.

7.3 Characteristics of Bacterial Community in Mountain Lakes

7.3.1 Abundance of Bacterioplankton

In general, bacterial cell densities in freshwater environments are known to be approximately 1.0×10^4 to 10^7 cells ml^{-1} (Whitman et al. 1998), although the density varies with the season and the environmental conditions (e.g., Lindström 1998; Crump et al. 2003; Vrede et al. 2003). In high-elevation regions, approximately 1.0×10^6 cells ml^{-1} of bacterioplankton have been reported for various European mountain lakes (Karlsson et al. 2001; Auguet and Casamayor 2008). This value is almost the same as that of oligotrophic lakes located in low-elevation regions. Similarly, slightly lower but similar values were also found in lakes on the Tibetan Plateau and Himalaya, despite being located at elevations greater than 5000 m (Liu et al. 2006, 2009, 2011). A similar bacterial cell density value was also observed in the Japanese mountain lakes, and the range and average of the values were 0.7×10^5 to 6.8×10^6 cells ml^{-1} and 1.6×10^6 cells ml^{-1}, respectively (unpublished data). These values did not exhibit a significant association with elevation, which suggests that there may be no difference in bacterial abundance among freshwater environments located at high and low elevations. As determinants of bacterial abundance, environmental factors associated with lake productivity, such as TP and chlorophyll *a* concentration, may be more important. Karlsson et al. (2001) reported that bacterial production and cell density were greatly restricted by TP in combination with DOC in the North Swedish Mountain lakes in summer. In the Japanese mountain lakes, significant positive correlations between bacterial cell densities and both TP and chlorophyll *a* were also observed (unpublished data). Therefore, Japanese mountain lakes generally have low densities of bacterial cells presumably because of their oligotrophy.

7.3.2 Dominant Taxa

In the Japanese mountain lakes, phylotypes belonging to the phyla *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Cyanobacteria* were detected as major components of bacterial communities. As discussed in detail below, these taxa have been repeatedly detected in many mountain lakes around the world, as well as other freshwater environments.

The class *Betaproteobacteria* (in the phylum *Proteobacteria*) is likely to be the most predominant taxonomic group at the class level in Japanese mountain lakes (Fujii et al. 2012). Currently, *Betaproteobacteria* in freshwater environments are known to be divided into seven clades, betI to betVII, based on the sequence of the 16S ribosomal RNA gene (Newton et al. 2011). Among them, the betII clade contains four subclades, PnecA, PnecB, PnecC, and PnecD, all of which are classified into the genus *Polynucleobacter* (Hahn 2003; Wu and Hahn 2006). In Japanese mountain lakes, the sequences affiliated with the PnecC were the most frequently detected from the DGGE profiles (Fig. 7.4). PnecC were also detected in alpine lakes at elevations greater than 5000 m on the Tibetan Plateau (Wu et al. 2006). In addition, PnecC has been found to be a dominant taxon globally

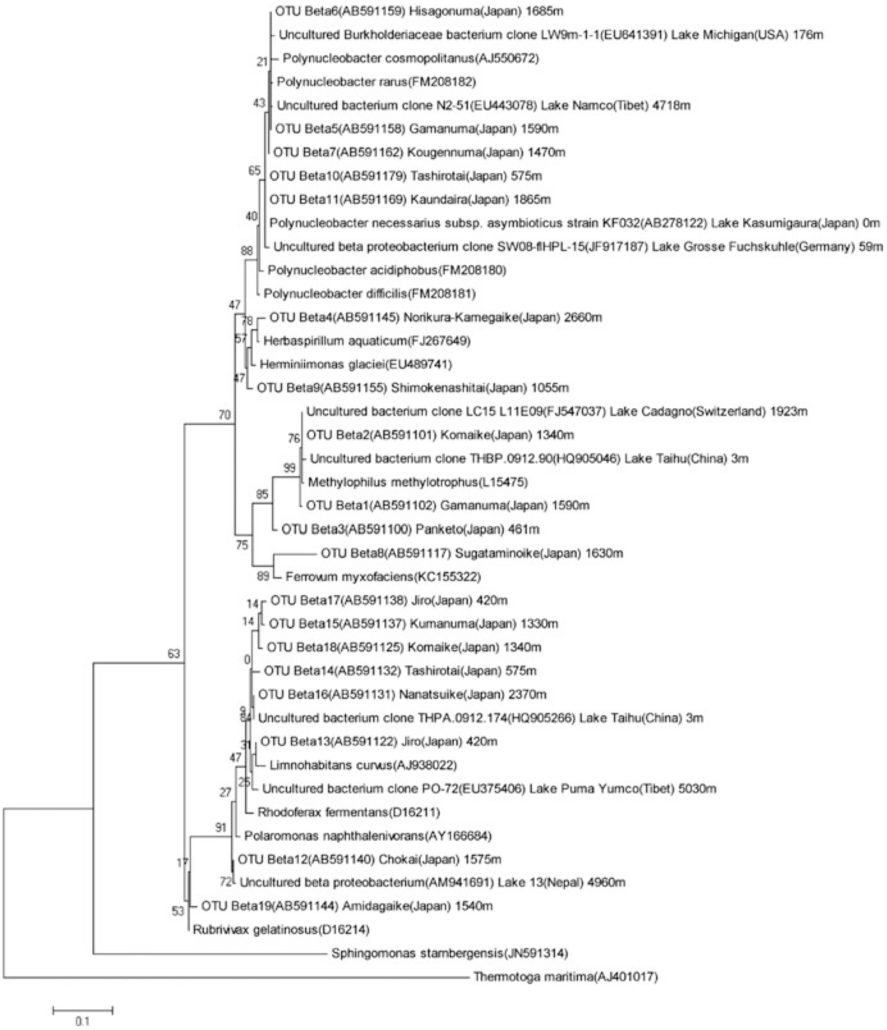


Fig. 7.4 Phylogenetic relationships of 16S rRNA gene sequences corresponding to the class *Betaproteobacteria*, obtained from denaturing gradient gel electrophoresis (DGGE) profiles of bacterioplankton community compositions in 41 Japanese mountain lakes. Each operational taxonomic unit (OTU) was defined based on the DGGE band position. Representative sequences closely related to the OTUs are shown. Lake names and altitude are shown after Genbank accession number

in highly humic mountain lakes (Jezberová et al. 2010; Jezbera et al. 2012). On the other hand, bacterioplankton affiliated with PnecC was also detected and isolated from a number of Japanese lakes in low-elevation regions (Watanabe et al. 2009). Therefore, PnecC may be a cosmopolitan rather than phylotype specific to mountain lakes. As another predominant taxonomic group within the class *Betaproteobacteria*, the genus *Methylophilus* (belonging to the betIV clade) was the second most frequently detected genus in Japanese mountain lakes. *Methylophilus* uses C1 compounds as growth substrates and has not been regarded as a cosmopolitan. The dominance of the genera *Polynucleobacter* and *Methylophilus* in mountain lakes may be explained by their physiological characteristics, as discussed in detail later. The family *Comamonadaceae*, which belongs to the betI clade, was also frequently detected in Japanese mountain lakes. The betI clade is a cosmopolitan phylotype that inhabits a wide range of freshwater environments, similar to the betII clade (Jezbera et al. 2012).

The phylum *Bacteroidetes* was the second most predominant taxonomic group at the phylum level in Japanese mountain lakes (Fujii et al. 2012). This phylum can be classified into six clades, bacI to bacVI (Newton et al. 2011). In Japanese mountain lakes, the obtained sequences were affiliated with the families *Chitinophagaceae* and *Flavobacteriaceae*, which belong to the bacI and bacII clades, respectively (Fig. 7.5). Most of the taxonomic groups within the phylum *Bacteroidetes* that are found in freshwater environments belong to these two clades. *Bacteroidetes* in freshwater environments are known to be a particle-associated bacterioplankton that may play an important role in the degradation of biopolymers, such as polysaccharides, which primarily constitute the cell walls of phytoplankton (Nold and Zwart 1998; Lemarchand et al. 2006). In addition, the population dynamics of *Bacteroidetes* shift in association with *Cyanobacteria* blooms (Eiler and Bertilsson 2007). Therefore, bacterioplankton of this phylum in freshwater environments may be strongly dependent on primary production of phototrophic organisms. Considering these characteristics, *Bacteroidetes* may favor eutrophic lake conditions in contrast to *Betaproteobacteria*. Although these conditions are rare in mountain regions, abundant *Bacteroidetes* were observed in some Japanese mountain lakes with eutrophic conditions, which were directly influenced by anthropogenic factors. On the other hand, many sequences of *Bacteroidetes* were also detected in Japanese mountain lakes with oligo- to mesotrophic conditions (Fujii et al. 2012). Therefore, the distributions of *Bacteroidetes* would be regulated by other factors in addition to the primary productivity of phytoplankton. Indeed, a high abundance of *Bacteroidetes* has also been found in alpine lakes at an elevation of more than 5000 m in the Himalaya and near Mt. Everest (Liu et al. 2011). These lakes were likely low in productivity because of low chlorophyll *a* concentrations.

The phylum *Actinobacteria* encompasses bacteria that have high GC content in their genomic DNA and gram-positive cell wall (Ventura et al. 2007). Although the group *Actinobacteria* has historically been considered typical soil bacteria (Goodfellow and Williams 1983), many studies applying molecular methods have demonstrated that bacteria of this phylum are one of the most abundant taxonomic groups of bacterioplankton in global freshwater environments (Zwart et al. 2002).

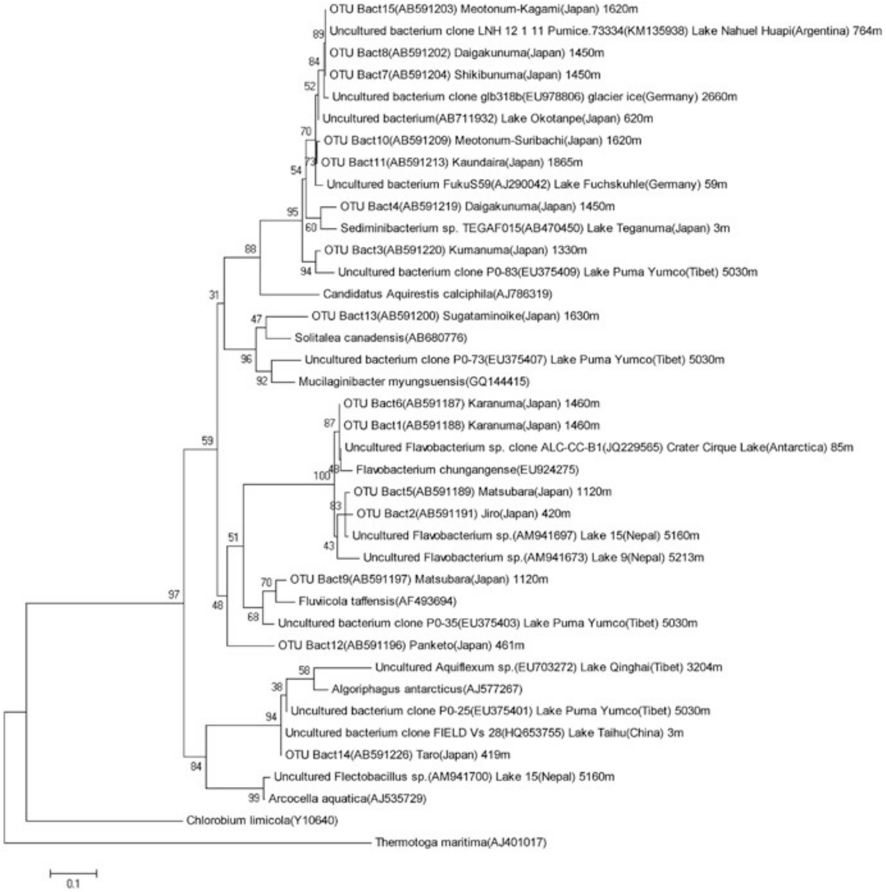


Fig. 7.5 Phylogenetic relationships of 16S rRNA gene sequences corresponding to the phylum *Bacteroidetes*, obtained from denaturing gradient gel electrophoresis (DGGE) profiles of bacterioplankton community compositions in 41 Japanese mountain lakes. Each operational taxonomic unit (OTU) was defined based on the DGGE band position. Representative sequences closely related to the OTUs are shown. Lake names and altitude are shown after Genbank accession number

Freshwater *Actinobacteria* can be classified into nine clades: acI, acIII, acIV, acV, acTH1, acTH2, acSTL, Luna1, and Luna3 (Newton et al. 2011). Among them, the acI clade is especially predominant in various freshwater environments. In previous studies, this clade accounted for a high proportion of total bacterioplankton sequences in lakes at low elevation (Humbert et al. 2009) and in mountain lakes (Warnecke et al. 2005). The prevalence and dominance of the acI clade may reflect the adaptive significance of some physiological characteristics, as discussed in detail later. Nevertheless, in our previous study, only a few sequences affiliated with *Actinobacteria* were obtained from Japanese mountain lakes (Fujii et al. 2012). This inconsistency may be partially due to the differences in the season when the surveys were conducted. In certain previous study that attempted to reveal the seasonal population dynamics of each bacterial taxon in freshwater environments, shift in the predominant taxon from *Actinobacteria* to *Betaproteobacteria* was found in summer, whereas a high abundance of *Actinobacteria* was observed during the other seasons (Glöckner et al. 2000).

The phylum *Cyanobacteria* plays a crucial role as a primary producer in freshwater ecosystems. Many groups of *Cyanobacteria* support the growth of various heterotrophic bacterioplankton by supplying organic matter through photosynthesis. However, certain taxonomic groups, including *Microcystis* and *Anabaena*, have been shown to occasionally have a strong impact on various organisms in different trophic levels by producing toxic compounds during blooms (Jöhnk et al. 2008). As planktonic cyanobacteria, the genus *Synechococcus* has often been detected in alpine lakes located at more than 4000 m above sea level (Sommaruga and Casamayor 2009; Liu et al. 2009, 2013). The genus *Synechococcus* in these lakes is considered to be UV tolerant (Xing et al. 2009) and may be well adapted to freshwater environments at high elevations. In Japanese mountain lakes, only a few cyanobacterial sequences were detected (Fujii et al. 2012). The low frequency observed may be partly because a large quantity of organic matter (e.g., humic materials) strongly affects the photoenvironment and thus phototrophic activities.

7.3.3 Physiological Characteristics of Bacterioplankton

As discussed above, some specific taxonomic groups of bacteria are frequently detected in mountain lakes. It seems reasonable to assume that these groups of bacteria have some physiological characteristics, which are advantageous to inhabit mountain lakes.

UV radiation is one of the constraining factors for the biological activity of most living things. It effects strongly on planktonic bacteria inhabiting pelagic environments because most of the taxonomic groups have no protection against UV radiation. On the basis of wavelength, UV radiation can be divided into three bands, UV-A, UV-B, and UV-C, and each has a different effect on planktonic bacteria. For instance, UV-B (<320 nm) directly induces DNA damage, whereas UV-A (320–400 nm) causes only indirect damage to various parts of the bacteria cell, such as DNA, proteins, and lipids, through the production of reactive oxygen species (Smith and Walker 1998; George et al. 2002). Certain reactive oxygen species, such as singlet oxygen and hydroxyl radicals, are also produced by photolysis of dissolved organic matter (DOM), which affects bacterioplankton communities and activities (Glaeser et al. 2010). As described above, the solar UV radiation level generally increases with elevation. Therefore, to adapt to mountain lakes located at high elevations, the bacterioplankton must have an ability to tolerate high UV radiation. Bacterioplankton have been shown to develop various UV tolerance mechanisms, such as the synthesis of photoprotective substances (Mueller et al. 2005) and efficient repair processes for damaged DNA (Boelen et al. 2001). As mentioned above, the acI clade in the phylum *Actinobacteria* is one of the bacterial groups typically detected in mountain lakes. Warnecke et al. (2005) revealed that the abundance of the acI clade was positively correlated with solar UV radiation in European mountain lakes by using fluorescence in situ hybridization. The potential ability of UV tolerance within acI may increase the abundance of this taxonomic group in freshwater environments at high elevations.

The oligotrophic state is one of the remarkable features of mountain lakes. Hence, the effective acquisition of energy may be a key ability for bacterioplankton inhabiting such environments. In previous studies, importance and diversity of heterotrophic bacteria utilizing solar energy have been demonstrated in pelagic marine environments (Béjá et al. 2000, 2002; Zubkov 2009). These photoheterotrophic organisms include two types of bacteria, which use different ways to convert solar energy to chemical energy. They are referred to as rhodopsin-containing bacteria and aerobic anoxygenic photosynthetic bacteria respectively, and they have also been detected from freshwater environments (Eiler et al. 2009; Sharma et al. 2009). The ability to utilize solar energy may be a key function of the predominant bacterioplankton in the surface of oligotrophic lakes. The key gene essential for this function has been found in the cosmopolitan species that are distributed in global freshwater environments (Martinez-Garcia et al. 2012). These species included rhodopsin-containing bacteria of the acI and Luna clades in the phylum *Actinobacteria*, as well as members of the genus *Polynucleobacter* representing the other type of photoheterotrophs. In addition, these genes are also distributed in diverse bacteria of separated phylogenetic positions, suggesting that they have been spread by horizontal gene transfer (Martinez-Garcia et al. 2012). Therefore, there might be more diverse photoheterotrophic bacterioplankton in mountain lakes, which have not been recognized as organisms utilizing solar energy.

In the oligotrophic lakes characterized by low productivity, the ability to use organic materials from external origin may also be significant. In mountainous regions, allochthonous DOC primarily consists of humic substances, which are biologically refractory and are not directly used by most heterotrophic organisms (Fenchel and Blackburn 1979; Geller 1983). The genus *Polynucleobacter* has been presumed to utilize allochthonous DOC, such as humic substances (Watanabe et al. 2009; Jezberová et al. 2010; Hahn et al. 2012). There are two known pathways transferring organic carbon from the humic substances to bacterioplankton. The first pathway is photochemical degradation of humic substances. Previous studies reported that certain taxonomic groups of bacterioplankton rapidly grow in humic substance-rich lake water after photolyzing (Wetzel et al. 1995; Bushaw et al. 1996; Vähätalo et al. 2003). Watanabe et al. (2009) demonstrated that the genus *Polynucleobacter* can utilize various organic acids that are typical products of DOM photochemical degradation, and this was not the case for isolates of *Alphaproteobacteria* and *Actinobacteria*. The other pathway is oxidation of humic substances by manganese oxides, generating low molecular weight C1 compounds (Sunda and Kieber 1994). *Methylophilus*, the second most predominant genus in Japanese mountain lakes, is known to be a utilizer of C1 compounds (Nercessian et al. 2005). Considering the prevalence of the these genera observed, acquiring carbon via these pathways may be important for bacterioplankton to thrive in lakes containing high quantities of humic substances, such as dystrophic lakes.

7.4 Community Assembly of Microbes in Mountain Lakes

7.4.1 Environmental Filtering

Because microbes play a crucial role in biogeochemical cycling through their diverse metabolic functions, understanding the mechanisms of how microbial community composition and diversity are regulated in nature is important. There is a prevailing hypothesis, namely, "everything is everywhere, but, the environment selects" (Baas-Becking 1934), which argues that microbial community assembly is regulated solely by environmental filtering due to microbes' extremely high dispersal abilities and large population size. In fact, the remarkable effects of environmental filtering on microbial community composition and diversity have been demonstrated in a number of previous studies (e.g., Fierer and Jackson 2006; Lozupone and Knight 2007).

To clarify which environmental factors determine the bacterial community assembly in Japanese mountain lakes, we investigated the importance of five environmental factors, including electric conductivity, pH, DOC, TP, and protozoan biomass (Fujii et al. 2012), all of which are generally recognized as influential factors for bacterial growth and activity. Only pH was found to be a significant variable that correlated with bacterioplankton community composition. In general, pH is a crucial factor associated with bacterial bioactivities, such as ATP synthesis. In fact, the large impact of pH on bacterial community composition and diversity has commonly been reported in various environments (Lindström et al. 2005; Fierer and Jackson 2006; Percent et al. 2008). Similarly, Jezbera et al. (2012) also showed the large impact of pH on the distribution of major bacterioplankton taxonomic groups in European mountain lakes.

In contrast to the community composition, the taxonomic richness of bacterioplankton in Japanese mountain lakes was negatively associated with DOC (Fujii et al. 2012). The DOC might include autochthonous and allochthonous ones in unknown proportions. However, it is likely that allochthonous DOC was the major component because total DOC was positively correlated with the catchment area per lake volume (Fujii et al. 2012). As described above, allochthonous DOC is biologically refractory, and these compounds are not used directly by most heterotrophic bacteria (Fenchel and Blackburn 1979; Geller 1983). Therefore, high concentrations of allochthonous DOC may cause a low taxonomic richness in the bacterioplankton communities of mountain lakes.

As the most frequently detected taxonomic group of bacterioplankton in Japanese mountain lakes, we identified major environmental factors affecting the presence of the genus *Polynucleobacter*. As a result, it was shown that the occurrence of *Polynucleobacter* was positively associated with DOC. On the other hand, the occurrence of *Polynucleobacter* was negatively associated with TP and effect of TP was stronger than that of DOC. As described above, the DOC concentration represents organic substrates supplied from the surrounding terrestrial region. In contrast, the TP concentration may represent autochthonous organic substrates associated with the primary production of phototrophic organisms, because the TP concentration was limiting factor for the abundance of phototrophs in these lakes (Fujii et al. 2012). Considering the stronger effect of TP than that of DOC, oligotrophy may be a more influential factor for the occurrence of *Polynucleobacter* in the Japanese mountain lakes than allochthonous DOC concentrations from the surrounding land.

7.4.2 Dispersal Limitation

For a long time, microbial community assembly was believed to be regulated solely by the process of environmental filtering. However, for a decade, a few studies report that microbial community assembly is also restricted by spatial factors, such as distance and area (dispersal limitation), as well as environmental factors (Horner-Devine et al. 2004; Reche et al. 2005; Yannarell and Triplett 2005). Consequently, there is a growing body of opinion that supports the idea that evaluation of the relative importance of environmental and spatial factors is essential for explaining the geographic distribution and diversity of microbes (Van der Gucht et al. 2007; Schiaffino et al. 2011; Fujii et al. 2015).

To determine the relative importance of spatial and environmental factors on the bacterial community assembly, variation partitioning was performed with canonical correspondence analysis (CCA) to determine the intensity data and redundancy analysis (RDA) for the presence or absence data, respectively, based on the results of PCR-DGGE analysis that quantified the bacterioplankton community composition in 41 Japanese mountain lakes. As a result, 8.1 % and 6.3 % of the total variation were explained by spatial and environmental variables in CCA, respectively, and 6.2 % and 6.4 % of the total variation were explained by spatial and environmental variables in RDA, respectively. These results suggest that both spatial and environmental factors contribute to the bacterioplankton community assembly in Japanese mountain lakes. A similar trend has also been reported in oligo- to eutrophic lakes in Antarctica and Patagonia (Schiaffino et al. 2011). However, Van der Gucht et al. (2007) showed that environmental variables explained a larger proportion of variation than spatial variables in a number of meso- to eutrophic lakes in Europe. Sommaruga and Casamayor (2009) also showed that local environmental constraints had a stronger influence than geographical distance, based on the dissimilarity matrices of bacterioplankton community composition and the Mantel test in high-elevation mountain lakes in Nepal. The large variations in the relative importance of environmental and spatial factors on planktonic bacteria among previous studies remain unexplained.

Recently, Fujii et al. (2015) proposed that inter-taxonomic differences in response to environmental filtering and dispersal limitation could be a potential explanation for inconsistency among previous studies on bacterioplankton community assembly in freshwater lakes. They found that community composition of *Betaproteobacteria* was mainly correlated with environmental variables, whereas *Bacteroidetes* was primarily associated with geospatial variables, by focusing on 27 oligotrophic mountain lakes with similar environments, located in eastern Japan. Furthermore, Fujii et al. (2015) suggested that biogeographic events such as geographic barriers could potentially play significant roles for the distribution and diversity of microorganisms, as is the case with macroorganisms. Therefore, the relative importance of environmental and spatial factors on bacterial community assembly may vary depending on taxonomic resolution and environmental conditions and geospatial configurations of study lakes. These perspectives should be considered in order to understand bacterial community assembly in mountain lakes.

7.4.3 Evolutionary Constraint

Recently, evolutionary processes have been recognized as significant drivers in community assembly (Cavender-Bares et al. 2009). Ecological traits are generally conserved on the evolutionary scale (Peterson et al. 1999; Prinzing et al. 2001; Webb et al. 2002). The trait conservatism means that similar ecological traits are observed between phylogenetically related species, and these traits determine community structures through environmental filtering and competitive interactions. This process had not previously been related to microbial community assembly due to lateral gene transfer that enables the exchange of functional genes between different species, regardless of the phylogenetic distance. However, Martiny et al. (2013) found that the vertical inheritance of functional traits was important even in microorganisms. In recent studies, phylogenetic information has been applied to understand the mechanisms of microbial community assembly (Barberán et al. 2014; Brown et al. 2014; Zimmerman et al. 2014).

In the context of the trait conservatism, we also examined the importance of evolutionary constraints on bacterioplankton community assembly in 41 Japanese mountain lakes. Based on the method of Helmus et al. (2007), we calculated the phylogenetic species variability (PSV), which is an index representing the degree of phylogenetic variability within a community and is independent of species richness. To better understand the mechanisms of the bacterial community assembly, we investigated the effects of environmental factors (temperature, pH, electric conductivity, chlorophyll *a*, and lake surface area) on PSV. A significant phylogenetic

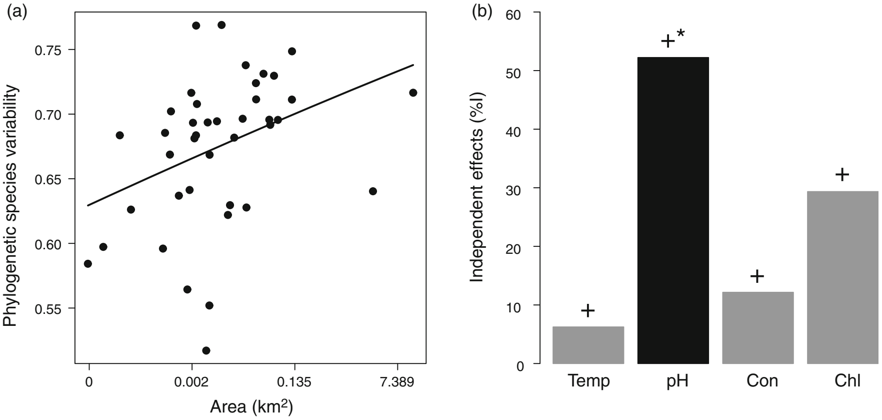


Fig. 7.6 Relationships between phylogenetic species variability and spatial (**a**) and environmental (**b**) variables. * means $P < 0.05$. *Area* lake surface area, *Temp* temperature, *Con* electric conductivity, *Chl* chlorophyll *a*

diversity-area relationship was found (Fig. 7.6a). Among the environmental variables, the pattern of PSV was positively correlated with pH (Fig. 7.6b), although the lake surface area was also positively correlated with pH. These results suggest that the harsh conditions (acidic pH) decrease the PSV. In small lakes, the BCC may be constrained to a narrow range of phylogenetic diversity based on the inflow of acidic compounds (e.g., humic substances) from the surrounding environment because the utilization of humic substances is restricted to a small minority of phylogenetic groups within the class *Betaproteobacteria*, such as the genera *Polynucleobacter* and *Methylophilus*. The small size and acidic nature are distinctive features of lakes in the Japanese mountainous regions. These features may indicate that the process of evolutionary constraint might have a nontrivial impact on bacterioplankton community assembly.

7.5 Conclusion and Perspectives

Although mountain lakes are exposed to distinctive environmental conditions, distinct and specific features of bacterioplankton community compositions in these lakes have not been identified. In other words, the dominant taxonomic groups in these lakes did not differ greatly from those of other freshwater environments. As a functional feature, bacterioplankton in mountain lakes were generally related to DOC derived from the surrounding terrestrial region. The utilization of allochthonous organic matter seems to be a key characteristic, because the primary productivity in mountain lakes is generally poor due to their oligotrophic nature. In addition, the community assembly of bacterioplankton was regulated by not only the environmental filtering process but also other processes, including dispersal limitation and evolutionary constraint.

The function of bacterioplankton in mountain lakes has been only partially determined, and knowledge with regard to the roles of the bacterioplankton community in mountain lakes is insufficient. Recently, the understanding of bacterial functions in nature has improved dramatically through the application of high-throughput molecular biology methods. For example, the analysis of massive oligonucleotide sequences using next-generation sequencing technologies (metagenomics) enables researchers to estimate the potential functions of bacteria at the community scale (e.g., Gill et al. 2006). In addition, metatranscriptomic and metaproteomic approaches enable the direct detection of bacterial functions, which are expressed onsite as mRNA and proteins, respectively (e.g., Morris et al. 2010; Radax et al. 2012). These methods enable the determination of each step of bacterial metabolism and the interactions of components. Such omics-based approaches can greatly improve the knowledge of bacterioplankton community compositions and functions in mountain lakes. Because the dynamics of bacterioplankton community composition and functions are sensitive to environmental changes, the results from omics-based approaches will contribute to an estimation of the effects of climate change on the ecosystems of mountainous regions.

References

Adrian R, O'Reilly CM, Zagarese H, Baines SB, Hessen DO, Keller W, Livingstone DM, Sommaruga R, Straile D, Van Donk E, Weyhenmeyer GA, Winder M (2009) Lakes as sentinels of climate change. Limnol Oceanogr 54:2283–2297

Alfreider A, Pernthaler J, Amann R, Sattler B, Glöckner FO, Wille A, Psenner R (1996) Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl Environ Microbiol 62:2138–2144

Auguet J-C, Casamayor EO (2008) A hotspot for cold crenarchaeota in the neuston of high mountain lakes. Environ Microbiol 10:1080–1086

Azam F, Fenchel T, Field JG, Gray JS, Meyerreil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Baas-Becking LGM (1934) Geobiologie of inleiding tot de milieukunde, Serie 18/19. Van Stockum's Gravenhange, The Hague (In Dutch)

Bahls P (1992) The status of fish populations and management of high mountain lakes in the western united-states. Northwest Sci 66:183–193

Barberán A, Casamayor EO, Fierer N (2014) The microbial contribution to macroecology. Front Microbiol 5:203. doi:10.3389/fmicb.2014.00203

Baron JS, Rueth HM, Wolfe AM, Nydick KR, Allstott EJ, Minear JT, Moraska B (2000) Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems 3:352–368

Béjá O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich S, Gates CM, Feldman RA, Spudich JL, Spudich EN, DeLong EF (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science 289:1902–1906

Béjá O, Suzuki MT, Heidelberg JF, Nelson WC, Preston CM, Hamada T, Eisen JA, Fraser CM, DeLong EF (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. Nature 415:630–633

Blumthaler M, Ambach W, Ellinger R (1997) Increase in solar UV radiation with altitude. J Photochem Photobiol B 39:130–134

Boelen P, Veldhuis MJW, Buma AGJ (2001) Accumulation and removal of UVBR-induced DNA damage in marine tropical plankton subjected to mixed and simulated non-mixed conditions. Aquat Microb Ecol 24:265–274

Brown MV, Ostrowski M, Grzymski JJ, Lauro FM (2014) A trait based perspective on the biogeography of common and abundant marine bacterioplankton clades. Mar Genom 15:17–28

Bushaw KL, Zepp RG, Tarr MA, SchulzJander D, Bourbonniere RA, Hodson RE, Miller WL, Bronk DA, Moran MA (1996) Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. Nature 381:404–407

Callieri C, Corno G, Bertoni R (2006) Bacterial grazing by mixotrophic flagellates and *Daphnia longispina*: a comparison in a fishless alpine lake. Aquat Microb Ecol 42:127–137

Casamayor EO, Pedròs-Aliò C, Muyzer G, Amann R (2002) Microheterogeneity in 16S ribosomal DNA-defined bacterial populations from a stratified planktonic environment is related to temporal changes and to ecological adaptations. Appl Environ Microbiol 68:1706–1714

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW (2009) The merging of community ecology and phylogenetic biology. Ecol Lett 12:693–715

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69:2253–2268

Crump BC, Amaral-Zettler LA, Kling GW (2012) Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. ISME J 6:1629–1639

Duarte CM, Dachs J, Llabrés M, Alonso-Laita P, Gasol JM, Tovar-Sánchez A, Sañudo-Wilhemy S, Agusti S (2006) Aerosol inputs enhance new production in the subtropical northeast Atlantic. J Geophys Res 111:G04006. doi:10.1029/2005JG000140

Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ Microbiol 6:1228–1243

Eiler A, Bertilsson S (2007) Flavobacteria blooms in four eutrophic lakes: linking population dynamics of freshwater bacterioplankton to resource availability. Appl Environ Microbiol 73:3511–3518

Eiler A, Beier S, Sawstrom C, Karlsson J, Bertilsson S (2009) High ratio of bacteriochlorophyll biosynthesis genes to chlorophyll biosynthesis genes in bacteria of humic lakes. Appl Environ Microbiol 75:7221–7228

Fenchel TM, Blackburn TH (1979) Bacteria and mineral cycling. Academic, New York

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103:626–631

Fujii M, Kojima H, Iwata T, Urabe J, Fukui M (2012) Dissolved organic carbon as major environmental factor affecting bacterioplankton communities in mountain lakes of eastern Japan. Microb Ecol 63:496–508

Fujii M, Hirao T, Kojima H, Fukui M (2015) Inter-taxonomic differences in bacterioplankton community assembly in oligotrophic mountain lakes of east Japan. Aquat Microb Ecol 75:251–257

Geller A (1983) Degradability of dissolved organic lake water compounds in cultures of natural bacterial communities. Arch Hydrobiol 99:60–79

George AL, Peat HJ, Buma AGJ (2002) Evaluation of DNA dosimetry to assess ozone-mediated variability of biologically harmful radiation in Antarctica. Photochem Photobiol 76:274–280

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. Science 312:1355–1359

Glaeser SP, Grossart H-P, Glaeser J (2010) Singlet oxygen, a neglected but important environmental factor: short-term and long-term effects on bacterioplankton composition in a humic lake. Environ Microbiol 12:3124–3136

Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. Appl Environ Microbiol 66:5053–5065

Goodfellow M, Williams ST (1983) Ecology of actinomycetes. Annu Rev Microbiol 37:189–216

Grabherr G, Gottfried M, Pauli H (1994) Climate effects on mountain plants. Nature 369:448

Hahn MW (2003) Isolation of strains belonging to the cosmopolitan *Polynucleobacter necessarius* cluster from freshwater habitats located in three climatic zones. Appl Environ Microbiol 69:5248–5254

Hahn MW, Scheuerl T, Jezberová J, Koll U, Jezbera J, Simek K, Vannini C, Petroni G, Wu QL (2012) The passive yet successful way of planktonic life: genomic and experimental analysis of the ecology of a free-living *Polynucleobacter* population. PLoS One 7:e32772. doi:10.1371/journal.pone.0032772

Helmus MR, Bland TJ, Williams CK, Ives AR (2007) Phylogenetic measures of biodiversity. Am Nat 169:E68–E83

Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM (2004) A taxa-area relationship for bacteria. Nature 432:750–753

Humbert J-F, Dorigo U, Cecchi P, Le Berre B, Debroas D, Bouvy M (2009) Comparison of the structure and composition of bacterial communities from temperate and tropical freshwater ecosystems. Environ Microbiol 11:2339–2350

Jardillier L, Basset M, Domaizon I, Belan A, Amblard C, Richardot M, Debroas D (2004) Bottom-up and top-down control of bacterial community composition in the euphotic zone of a reservoir. Aquat Microb Ecol 35:259–273

Jezbera J, Jezberová J, Koll U, Hornak K, Simek K, Hahn MW (2012) Contrasting trends in distribution of four major planktonic betaproteobacterial groups along a pH gradient of epilimnia of 72 freshwater habitats. FEMS Microbiol Ecol 81:467–479

Jezberová J, Jezbera J, Brandt U, Lindström ES, Langenheder S, Hahn MW (2010) Ubiquity of *Polynucleobacter necessarius* ssp. *asymbioticus* in lentic freshwater habitats of a heterogenous 2000 km^2 area. Environ Microbiol 12:658–669

Jöhnk KD, Huisman J, Sharples J, Sommeijer B, Visser PM, Stroom JM (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Glob ChangBiol 14:495–512

Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65:1241–1250

Karlsson J, Jonsson A, Jansson M (2001) Bacterioplankton production in lakes along an altitude gradient in the subarctic north of Sweden. Microb Ecol 42:372–382

Karlsson J, Jansson M, Jonsson A (2002) Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes. Ecology 83:2902–2910

Keller F, Kienast F, Beniston M (2000) Evidence of response of vegetation to environmental change on high-elevation sites in the Swiss Alps. Reg Environ Chang 1:70–77

Kellogg CA, Griffin DW (2006) Aerobiology and the global transport of desert dust. Trends Ecol Evol 21:638–644

Lemarchand C, Jardillier L, Carrias J-F, Richardot M, Debroas D, Sime-Ngando T, Amblard C (2006) Community composition and activity of prokaryotes associated to detrital particles in two contrasting lake ecosystems. FEMS Microbiol Ecol 57:442–451

Lindström ES (1998) Bacterioplankton community composition in a boreal forest lake. FEMS Microbiol Ecol 27:163–174

Lindström ES, Kamst-Van Agterveld MP, Zwart G (2005) Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Appl Environ Microbiol 71:8201–8206

Liu Y, Yao T, Jiao N, Kang S, Zeng Y, Huang S (2006) Microbial community structure in moraine lakes and glacial meltwaters, Mount Everest. FEMS Microbiol Lett 265:98–105

Liu Y, Yao T, Zhu L, Jiao N, Liu X, Zeng Y, Jiang H (2009) Bacterial diversity of freshwater alpine lake Puma Yumco on the Tibetan Plateau. Geomicrobiol J 26:131–145

Liu Y, Yao T, Jiao N, Tian L, Hu A, Yu W, Li S (2011) Microbial diversity in the snow, a moraine lake and a stream in Himalayan glacier. Extremophiles 15:411–421

Liu Y, Yao T, Jiao N, Liu X, Kang S, Luo T (2013) Seasonal dynamics of the bacterial community in Lake Namco, the largest Tibetan lake. Geomicrobiol J 30:17–28

Llorens-Marés T, Auguet J-C, Casamayor EO (2012) Winter to spring changes in the slush bacterial community composition of a high-mountain lake (Lake Redon, Pyrenees). Environ Microbiol Rep 4:50–56

Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. Proc Natl Acad Sci U S A 104:11436–11440

Magnuson JJ, Robertson DM, Benson BJ, Wynne RH, Livingstone DM, Arai T, Assel RA, Barry RG, Card V, Kuusisto E, Granin NG, Prowse TD, Stewart KM, Vuglinski VS (2000) Historical trends in lake and river ice cover in the Northern Hemisphere. Science 289:1743–1746

Martinez-Garcia M, Swan BK, Poulton NJ, Gomez ML, Masland D, Sieracki ME, Stepanauskas R (2012) High-throughput single-cell sequencing identifies photoheterotrophs and chemoautotrophs in freshwater bacterioplankton. ISME J 6:113–123

Martiny AC, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. ISME J 7:830–838

Mladenov N, Sommaruga R, Morales-Baquero R, Laurion I, Camarero L, Dieguez MC, Camacho A, Delgado A, Torres O, Chen Z, Felip M, Reche I (2011) Dust inputs and bacteria influence dissolved organic matter in clear alpine lakes. Nat Commun 2:405. doi:10.1038/ncomms1411

Morales-Baquero R, Pulido-Villena E, Reche I (2006) Atmospheric inputs of phosphorus and nitrogen to the southwest Mediterranean region: biogeochemical responses of high mountain lakes. Limnol Oceanogr 51:830–837

Morris RM, Nunn BL, Frazar C, Goodlett DR, Ting YS, Rocap G (2010) Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction. ISME J 4:673–685

Moulin C, Chiapello I (2006) Impact of human-induced desertification on the intensification of Sahel dust emission and export over the last decades. Geophys Res Lett 33:L18808. doi:10.1029/2006GL025923

Mueller DR, Vincent WF, Bonilla S, Laurion I (2005) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. FEMS Microbiol Ecol 53:73–87

Neff JC, Ballantyne AP, Farmer GL, Mahowald NM, Conroy JL, Landry CC, Overpeck JT, Painter TH, Lawrence CR, Reynolds RL (2008) Increasing eolian dust deposition in the western United States linked to human activity. Nat Geosci 1:189–195

Nelson CE, Carlson CA (2011) Differential response of high-elevation planktonic bacterial community structure and metabolism to experimental nutrient enrichment. PLoS One 6: e18320. doi:10.1371/journal.pone.0018320

Nercessian O, Noyes E, Kalyuzhnaya MG, Lidstrom ME, Chistoserdova L (2005) Bacterial populations active in metabolism of C-1 compounds in the sediment of Lake Washington, a freshwater lake. Appl Environ Microbiol 71:6885–6899

Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49

Nold SC, Zwart G (1998) Patterns and governing forces in aquatic microbial communities. Aquat Ecol 32:17–35

Panzenböeck M (2007) Effect of solar radiation on photosynthetic extracellular carbon release and its microbial utilization in alpine and Arctic lakes. Aquat Microb Ecol 48:155–168

Percent SF, Frischer ME, Vescio PA, Duffy EB, Milano V, McLellan M, Stevens BM, Boylen CW, Nierzwicki-Bauer SA (2008) Bacterial community structure of acid-impacted lakes: what controls diversity? Appl Environ Microbiol 74:1856–1868

Peterson AT, Soberòn J, Sánchez-Cordero V (1999) Conservatism of ecological niches in evolutionary time. Science 285:1265–1267

Posch T, Mindl B, Hornak K, Jezbera J, Salcher MM, Sattler B, Sonntag B, Vrba J, Simek K (2007) Biomass reallocation within freshwater bacterioplankton induced by manipulating phosphorus availability and grazing. Aquat Microb Ecol 49:223–232

Prinzing A, Durka W, Klotz S, Brandl R (2001) The niche of higher plants: evidence for phylogenetic conservatism. Proc R Soc Lond B 268:2383–2389

Prospero JM, Lamb PJ (2003) African droughts and dust transport to the Caribbean: climate change implications. Science 302:1024–1027

Radax R, Rattei T, Lanzen A, Bayer C, Rapp HT, Urich T, Schleper C (2012) Metatranscriptomics of the marine sponge *Geodia barretti*: tackling phylogeny and function of its microbial community. Environ Microbiol 14:1308–1324

Reche I, Pulido-Villena E, Morales-Baquero R, Casamayor EO (2005) Does ecosystem size determine aquatic bacterial richness? Ecology 86:1715–1722

Sanders RW, Wickham SA (1993) Planktonic protozoa and metazoa: predation, food quality and population control. Mar Microb Food Webs 7:197–223

Scheren PAGM, Zanting HA, Lemmens AMC (2000) Estimation of water pollution sources in Lake Victoria, East Africa: application and elaboration of the rapid assessment methodology. J Environ Manage 58:235–248

Schiaffino MR, Unrein F, Gasol JM, Massana R, Balagué V, Izaguirre I (2011) Bacterial community structure in a latitudinal gradient of lakes: the roles of spatial versus environmental factors. Freshw Biol 56:1973–1991

Sharma AK, Sommerfeld K, Bullerjahn GS, Matteson AR, Wilhelm SW, Jezbera J, Brandt U, Doolittle WF, Hahn MW (2009) Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater *Actinobacteria*. ISME J 3:726–737

Smith BT, Walker GC (1998) Mutagenesis and more: *umuDC* and the *Escherichia coli* SOS response. Genetics 148:1599–1610

Sommaruga R, Casamayor EO (2009) Bacterial 'cosmopolitanism' and importance of local environmental factors for community composition in remote high-altitude lakes. Freshw Biol 54:994–1005

Sommaruga-Wögrath S, Koinig KA, Schmidt R, Sommaruga R, Tessadri R, Psenner R (1997) Temperature effects on the acidity of remote alpine lakes. Nature 387:64–67

Sunda WG, Kieber DJ (1994) Oxidation of humic substances by manganese oxides yields low-molecular-weight organic substrates. Nature 367:62–64

Teresa Pérez M, Sommaruga R (2011) Temporal changes in the dominance of major planktonic bacterial groups in an alpine lake: discrepancy with their contribution to bacterial production. Aquat Microb Ecol 63:161–170

Tranvik LJ (1992) Allochthonous dissolved organic-matter as an energy-source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia 229:107–114

Vähätalo AV, Salonen K, Munster U, Jarvinen M, Wetzel RG (2003) Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Arch Hydrobiol 156:287–314

Van der Gucht K, Cottenie K, Muylaert K, Vloemans N, Cousin S, Declerck S, Jeppesen E, Conde-Porcuna J-M, Schwenk K, Zwart G, Degans H, Vyverman W, De Meester L (2007) The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. Proc Natl Acad Sci U S A 104:20404–20409

Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, van Sinderen D (2007) Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev 71:495–548

Vincent WF, Wurtsbaugh W, Vincent CL, Richerson PJ (1984) Seasonal dynamics of nutrient limitation in a tropical high-altitude lake (lake titicaca, peru-bolivia) – application of physiological bioassays. Limnol Oceanogr 29:540–552

Vrede K, Stensdotter U, Lindström ES (2003) Viral and bacterioplankton dynamics in two lakes with different humic contents. Microb Ecol 46:406–415

Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J (2005) Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. Appl Environ Microbiol 71:5551–5559

Watanabe K, Komatsu N, Ishii Y, Negishi M (2009) Effective isolation of bacterioplankton genus *Polynucleobacter* from freshwater environments grown on photochemically degraded dissolved organic matter. FEMS Microbiol Ecol 67:57–68

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annu Rev Ecol Syst 33:475–505

Wetzel RG, Hatcher PG, Bianchi TS (1995) Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. Limnol Oceanogr 40:1369–1380

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci U S A 95:6578–6583

Williamson CE, Dodds W, Kratz TK, Palmer MA (2008) Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Front Ecol Environ 6:247–254

Wu QL, Hahn MW (2006) Differences in structure and dynamics of *Polynucleobacter* communities in a temperate and a subtropical lake revealed at three phylogenetic levels. FEMS Microbiol Ecol 57:67–79

Wu QL, Zwart G, Schauer M, Kamst-van Agterveld MP, Hahn MW (2006) Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. Appl Environ Microbiol 72:5478–5485

Xing P, Hahn MW, Wu QL (2009) Low taxon richness of bacterioplankton in high-altitude lakes of the Eastern Tibetan Plateau, with a predominance of *Bacteroidetes* and *Synechococcus* spp. Appl Environ Microbiol 75:7017–7025

Yamaguchi N, Ichijo T, Sakotani A, Baba T, Nasu M (2012) Global dispersion of bacterial cells on Asian dust. Sci Rep 2:525. doi:10.1038/srep00525

Yannarell AC, Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. Appl Environ Microbiol 71:227–239

Zimmerman AE, Allison SD, Martiny AC (2014) Phylogenetic constraints on elemental stoichiometry and resource allocation in heterotrophic marine bacteria. Environ Microbiol 16:1398–1410

Zubkov MV (2009) Photoheterotrophy in marine prokaryotes. J Plankton Res 31:933–938

Zwart G, Crump BC, Agterveld MPKV, Hagen F, Han SK (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28:141–155

Index

A
Abies mariesii, 2, 5, 7–13, 96, 97, 99, 107
Activation energy, 128, 136, 137
Adaptation, 2, 13, 43, 44, 55, 56, 65, 67, 72–80
Aerobic anoxygenic photosynthetic bacteria, 159
Allochthonous organic matter, 117, 139, 163
Allochthony, 117, 138–140
Alpine ecotones, 2
Alpine meadows, 4
Alpine zone, 2, 73, 95–96
Altitudinal gradients, 37, 55, 63–83, 89–109
Altitudinal trait differentiation, 73–82
Anoxic environments, 152

B
Bacterial growth efficiency (BGE), 139
Bee–flower size match, 77
Bee pollination, 43
Bee proboscis length, 78
Biogeography, 94, 97, 106–109
Boreal forest, 97, 106, 108
Broadleaf forest, 90, 94–96, 98–102, 105–107
Bumblebees, 43, 46–49, 53–56, 73, 75, 78, 80

C
Campanula punctata, 64, 73–78, 82, 83
Chlorophyll *a* (Chl.*a*), 119, 120, 122–126, 148, 150, 152, 154, 156, 162, 163
Cimicifuga simplex, 65, 80–82
Climate change, 1–13, 21, 43, 44, 52, 53, 64, 67, 83, 116, 117, 140, 145, 146, 148, 164
Coexistence, 17–37, 106
Coldness index (CI), 89, 92, 93, 95, 97, 101
Community respiration (CR), 117, 124, 126, 128, 129, 133–139
Conifer dominance, 89–109
Conifer forest, 90, 93, 94, 96–101, 105–108
Conifer-mixed forest, 106–108

D
Decision-tree model, 10
Dissolved inorganic carbon (DIC), 118, 119, 122, 123, 131, 132
Dissolved organic carbon (DOC), 119, 120, 122, 123, 128–132, 136–140, 146, 150, 152, 154, 159–161, 163
Distribution shift, 10–12
Diversity, 17–37, 52, 63–83, 109, 146, 150, 153, 159–163
Drainage ratio, 130, 131, 139

E
Ecosystem metabolism, 117, 123, 134
Electron transport system (ETS), 123, 125–126
Epilimnion, 118, 123, 124, 126–128, 134, 136, 152, 153

F
Fellfield habitats, 42, 44, 50, 53, 56
Fitness, 52, 74, 75, 77, 78
Floral size, 64, 73, 75, 76, 78
Flowering overlaps, 44–46, 49, 50
Flowering phenology, 41–59
Fly pollination, 43

G. Kudo (ed.), *Structure and Function of Mountain Ecosystems in Japan*,
Ecological Research Monographs, DOI 10.1007/978-4-431-55954-2

Fly pollinators, 48, 49, 58
Forest–meadow ecotones, 10
Forest zones, 93, 97, 102, 104, 106–108
Functional diversity, 18, 34, 35, 37
Functional groups, 18, 19, 21, 27, 30, 32, 37, 146
Functional traits, 18, 23, 27, 29, 33, 36, 37, 162
Functional type, 19

G
Gene flow, 55, 56, 66, 68, 72, 82, 83
Genetic differentiation, 64, 68–73, 81, 82
Genetic diversity, 63–83
Genetic structure, 55, 66–68, 71
Genetic variation, 66, 67, 69, 70, 72, 83
Global warming, 2, 12, 20, 21, 29, 31, 52, 58, 116, 117, 137, 140
GPP/CR ratio, 136, 137, 139
Gross primary production (GPP), 117, 124, 126–129, 133, 136–139
Growing degree days, 44, 45

H
Hakkoda Mountains, 1–13, 21–36
High-resolution aerial photographs, 5
Humic substances, 148, 159, 160, 163
Hypolimnion, 134, 149, 152, 153

L
Landscape phenology, 43, 45, 58, 59
Light acquisition efficiency, 28, 29, 32
Local adaptations, 43, 55, 56, 72, 73
Lowland forest, 94, 95, 100, 103, 106

M
Mesotrophic, 20, 118, 156
Microbial loop, 146
Mixed conifer-broadleaf forest, 90, 100, 102, 106
Mixed forests, 104, 106, 109
Molecular markers, 66, 68
Montane forest, 94, 100–103, 105, 106, 108
Monthly mean temperature of the coldest month, 93, 95
Moorlands, 4, 5, 7, 13, 17–37

N
Nestedness, 33
Net ecosystem production (NEP), 117, 131, 133, 134, 137
Niche, 18–37, 109, 146

O
Oligotrophic, 20, 118, 130, 133, 134, 146, 148, 154, 159, 162, 163
Outcrossing rate, 56, 57

P
PCR-DGGE method, 150
Phenological isolation, 55–56
Phenological mismatch, 52–55, 58
Phenological shifts, 52, 53
Photoheterotrophic organisms, 159
Phylogenetic species variability (PSV), 162, 163
Phytoplankton, 150, 156
Picoplankton, 124, 125, 134–136
Planktivorous fish, 123, 124, 135, 136
Plant–pollinator interactions, 41–59
Pollination ecology, 42
Pollination success, 48, 56
Pollination syndrome, 43, 46–51
Pollinator assemblages, 64, 73, 75, 80
Pollinator mouthpart length, 74, 75, 78
Prunella vulgaris, 64, 73, 75–79, 82, 83

R
Rainforest, 95, 96, 98–104, 109
Refugia, 2, 8, 10, 13, 67
Resources, 18, 20, 23, 27, 28, 45, 48, 50–52, 55, 80, 117, 128, 133, 139
Rhodopsin-containing bacteria, 159

S
16S ribosomal RNA gene, 154
Size match between flowers and bumblebees, 75
Snowbed habitats, 42, 44, 46, 50
Snowdrifts, 9, 10
Snowfall, 4, 8, 10, 94, 150
Snowmelt gradient, 42, 45, 55
Species diversity, 20, 22–23, 32, 33, 37, 52, 65–66, 82
Stable carbon isotope ratio ($\delta^{13}C$), 118, 122, 131, 132
Subalpine coniferous forests, 1–13
Subalpine forest, 2, 4, 10–12, 94–102, 104, 105, 108

T
Terrestrial subsidies, 132
Total phosphorous (TP), 118, 122, 123, 128, 129, 134–138, 148, 153, 154, 160, 161

Trait-based approaches, 17–37
Treelines, 93–97, 100, 101, 104

U
Upward shifts, 2, 7
Ultraviolet (UV)
  radiation, 158, 159
  tolerant, 158

V
Vegetation zonation, 89–109
Vulnerability assessment, 32–36

W
Warmth index (WI), 3, 4, 10, 11, 92, 93, 95–98, 101, 106
Winter monsoons, 9, 10