F-052 **System construction of vulnerability assessment for alpine and subalpine ecosystems based on biological interactions（Abstract of the Final Report）**

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[Abstract]

Alpine/subalpine ecosystems are expected to be highly vulnerable to climate change such as warming. To make better strategies for conserving these ecosystems under putative warming, therefore, a series of studies was performed for 3 years from FY2005 at several typical habitats in alpine/subalpine ecosystems: high altitude plant communities at Taisetsu Mountains, plant communities at high moors in Hokkoda Mountains, subalpline forests at Akan Mountains and aquatic communities at various mountains.

The studies showed that the response of plant communities to warming is highly site- or habitat-specific. For example, a warming experiment at the Taisetsu revealed that fellfield communities were more sensitive to warming than snowbed communities. Similarly, in *Picea glehnii* forests, trees at higher elevation were more sensitive to changes in climate conditions. The studies also showed that warming affects on plant communities through its effect on phenology and competitive interactions. For example, detailed analyses at high moors revealed that deciduous species were superior to evergreen species under increased growing season that may occur under warming. These results imply that effects of warming on plant communities differ depending on temporal and spatial variations of habitat-related snowmelt events, which should be taken into account for conservation of alpine/subalpine ecosystems.

Another important features revealed is that alpine/subalpine lakes are oligotrophic but have high nitrogen input relative to phosphorus, suggesting that atmospheric N deposition is substantial even at mountains. Nonetheless, a unique aquatic community was developed in alpine/subalpine lakes due to absence of predatory fish and subsidy of organic matter from the surrounding vegetation. These results imply that warming has potential to affects indirectly alpine/subalpine lakes through changes in quality and quantity of terrigenous input and expansion of fish distribution. To minimize warming impacts on alpine/subalpine ecosystems, any artificial transfers of organisms that do not live in these ecosystems should be prohibited.

1. Introduction

Alpine and subalpine ecosystems are important landscape resources with unique biodiversities, and provide goods and services to >10% population in Japan. However, these are expected to be highly vulnerable to climate changes such as global warming. In ecosystems, distribution of each species and biodiversity are sustained by biological interactions under given environmental conditions. For assessing vulnerability of alpine and subalpine ecosystems to warming, therefore, we have to understand not only physiological suitability of each species in the present abiotic conditions but also dependency of biological interactions on the environmental conditions. Although knowledge on the ecological processes is limited, terrestrial fauna and flora are well described in the alpine and subalpine regions. However, few are known on characteristics of aquatic communities in mountain regions. Thus, for conserving alpine and subapline ecosystems under global environmental change, integrated studies including both terrestrial and aquatic organisms and focusing on ecological processes are essential.

2. Research Objective

The objects of the present research project are (1) to understand physiological and functional diversities of alpine and subalpine species in response to warming, (2) to examine if these responses amplify or mitigate changes at the population and community levels through biological interactions, (3) to clarify if these changes depends on given environments sustaining biological interactions such as site-specific properties and food web structures, and then (4) to assess vulnerability of the communities to environmental changes including warming in the alpine and subalpine regions in Japan based on knowledge gained from (1)-(3). For accomplishing these objectives, we selected several typical habitats in Japanese alpine/subalpine ecosystems: high altitude plant communities at Taisetsu Mountains, plant communities at mountain moors in Hakkoda Mountains, subalpine forests at Akan Mountains and aquatic communities in alpine/subalpine lakes at various mountains. The present study started in FY2005 to grasp key issues and general properties of the habitats and to assess vulnerability of the mountain ecosystems to putative warming. For the past two years, we have performed comparative investigations along environmental clines in the alpine and subalpin area in Japan with some field experiments. In the following section, we show briefly main results obtained by this research project.

3. Results and Discussion

(1) Studies on high altitude moor ecosystems in Hakkoda Mountains

1) Warming effect on plant resource competition in moor ecosystems

To predict effects of global warming on plant community in mountain moors, we conducted comparative study for moor stands established at different altitudes at Hakkoda. We focused on competition and utilization for resources (light and nitrogen) to know processes and mechanisms of warming effects. We established 4-5 quadrats at each of three altitudes. Compared with stands at low altitudes, stands at high altitudes had a greater number and biomass of evergreen species. We found that evergreen and deciduous species utilize at different seasons; evergreen species absorb light mainly at early spring where deciduous leaves do not develop. Lower altitudes may be advantageous for deciduous species because growth season is longer, and in turn less advantageous for evergreen species. We also predict that warming in these wetlands may be disadvantageous for evergreen species if it accelerates snow-melting and extends growing season.

2) Structure and response to the environmental changes of aquatic communities in mountain lakes

To uncover current futures of limnological and ecological states in alpine and subalpine lakes and to assess potential effects of warming on these lakes, a series of field research was made for total 80 lakes from low to high altitudes in central to east Japan including Hokkaido. Here, we focused mainly on plankton because of the short generation time and high vulnerability to environmental changes. Most alpine/subalpine lakes examined were oligotrophic where primary production was limited by low supply of phosphorus. However, ratio of total nitrogen to phosphorus (N:P ratio) in these lakes was in general higher than that in low altitude lakes. Since there is little direct effects of anthropogenic activities in the watersheds of high altitude lakes, high N:P ratio in the alpine/subalpine lakes may reflect increased N supply from atmospheric precipitations. If this is the case, it is very worthwhile to monitor these lakes continually for assessing ecological impacts of regional disturbance factors such as nutrient deliveries through atmospheric precipitations.

Statistical analysis using data of total 80 lakes revealed that the plankton communities in alpine/subalpaine lakes were characterized by low species richness and predominance of large zooplankton species such as *Daphnia dentifera* which functioned to reduce phytoplankton abundance and thus to maintain high water quality. Genetic analysis showed that *D. dentifera* were genetically unique and locally isolated. However, at lower end of the subalpine region in some areas, there were hybrid populations with *D. galeata* typical to low altitude lakes. Thus, warming may expand the hybrid zone. Using these data, statistical analyses was made to examine the relative importance of environmental factors affecting the plankton community. The analyses showed that the plankton community structure in alpine/subalpine lakes was relatively robust against slight increase in temperature but highly vulnerable to existence of fish species. Further analysis showed that probability of fish distribution in given lakes is highly related not only lake size but also water temperature. These results suggest that to make successful conservation to alpine/subalpine lakes against putative warming, it is at least essential to restrict any anthropogenic activities that may promote expansion of organisms such as fish species that are not naturally distributed at present in the alpine/subalpine lakes.

3) The effects of global warning on the range of species.

The range of species along latitudinal and altitudinal gradients were predicted by using individual-based models. The studies showed that a species failed to expand their range owing to stochastic extinctions of boundary populations though reducing population size by migration load.

When species might move to north by global warning, this species may contact with related species distributed in northern area, the northern species are likely to become extinct if there is little or no premating isolation but there is substantial postmating isolation between them. In addition, the introgression between two species reduce genetic diversity and species differentiation when premating isolation between them are incomplete. The homogenizing effects of the introgression are large especially when dispersal distance of individuals and gametes were not large. These results indicated that habitat managements especially for boundary areas of species ranges and contact areas between species are important to control the effect of global warning on species distribution.

(2) Studies on alpine and subalpine ecosystems in the Taisetsu and Akan Mountains

1) Structure and responses to the environmental changes of alpine ecosystem in the Taisetsu Mountains

Responses of alpine plant communities to artificial warming

Effects of environmental warming on growth and productivity of alpine plant communities were assessed using open-top-chambers (OTC: artificial warming device) between fellfield and snowbed communities in the Taisetsu Mountains during seven years (2001-2007). By setting the OTCs, daily mean temperature increased about 2ºC. Vegetation height and aboveground biomass were assessed nondestructively by the point-frame method. The warming effects highly varied among habitat types. The fellfield community at low elevation (1700m a.s.l.) showed most rapid responses in which maximum plant height in the OTC plots increased in the second year of the experiment, and total aboveground biomass increased to twice by the fifth year. Especially, dwarf shrubs increased the biomass by the OTC. In the fellfield community at high elevation (1900m a.s.l.), significant increase in plant height was detected in the third year, and the aboveground biomass increased to twice in the seventh year in which sedges and grasses became to dominant. In contrast, responses of snowbed communities were very snow. In the snowbed community with relatively early snowmelt time (mid-July), significant responses were detected at the seventh year of the experiment in which sedges increased biomass. However, plants in the late-snowmelt snowbed (late June) did not show significant responses to the warming treatment. These results indicate that responses of alpine plants to the warming highly depend on the species and habitat types.

Spatial genetic structures of alpine plants along snowmelt gradient

The flowering phenology of alpine-snowbed plants varies greatly depending on the time of snowmelt, which may cause the spatial and temporal variations of pollen dispersal. We analyzed how pollen-mediated gene flow influences the genetic structure of a snowbed herb, *Primula cuneifolia*, within a continuous snow-patch (100-m × 250-m) in the Taisetsu Mountains, Hokkaido. Within the snow-patch, the flowering season varied over 50 d from late June to mid-August. The effects of flowering phenology on the genetic structure were assessed using spatial autocorrelation analyses with reference to the phenological isolation among plants. The intensity of the spatial genetic structure based on the *Sp* statistics increased with degrees of phenological isolation. This indicates that pollen-mediated gene flow has a substantial effect on the fine-scale spatial genetic structure. Flowering segregation caused by the snowmelt regime is a critical factor to generate the spatial genetic structures of alpine plants. Warming should seriously disturb the present genetic structures of alpine-plant populations if snow-melting regime may change by global warming.

Pollination competition and reproductive success of alpine plants along snowmelt gradients

The timing of snowmelt fundamentally controls the flowering phenology of alpine plants; flowering of single plant species occurs sequentially as the snowmelt progresses. In the present study, we focused on pollination competition between *Phyllodoce aleutica* and its F_1 hybrid, *P. caerulea* f. *yesoensis*, along the snowmelt gradients. It is demonstrated that the dominance of *P. caerulea* f. *yesoensis* in early-snowmelt sites causes the extensive pollen limitation in *P. aleutica*. Autonomous autogamy (self-fertilization following intra-floral self pollination that occurs spontaneously without the activity of pollen vectors) ensures the sufficient quantities of seed production. However, strong inbreeding depression after seed germination almost completely prevents the establishment of selfed-seeds. The accelerated snowmelt timing via global warming would intensify the pollination competition between these taxa, and might strongly decrease the reproductive success of *P. aleutica*. Biological interaction through pollination might be an important example of vulnerability in alpine ecosystems.

2) Analysis of tree rings of *Picea glehnii* trees at the Akan Mountaions

Effects of climate changes on forest ecosystems may differ depending on the altitudes. Forest ecosystem in the subalpine zone, where short growing season, low temperature and large amount of snowfall may restrict plant growth, may be greatly influenced by climate changes. In this study, we examined altitudinal changes in the relationship between growth of trees and climate. We set three study plots at 500, 800 and 1100m elevation on Mt. Oakan, which is located in eastern part of Hokkaido, Japan. Tree rings of 300 *Picea glehnii* trees were measured. We estimated annual growth of diameter at breast height (DBH) in past 40 to 100 years. Annual growth of DBH fluctuated during the past 100 years, but they did not synchronize among elevations. Cumulative temperature during growing season was negatively correlated to annual growth, and winter precipitation of snow was positively correlated to annual growth. High-altitude population at 1100 m responded greatly with climatic variation. We conclude that coniferous forests in the subalpine zone are vulnerable to climate changes. There was possibility for forest limit not to climb up, but to descend with global warming.

3) Analysis of microbial community structures in mountain lakes

The accurate understanding of pristine ecosystems is indispensable to the estimation of the effects of global warming on the ecosystems. However, there are only a few studies on bacterial communities in alpine lakes. In this study, the bacterial community structures in alpine lakes were analyzed with molecular biological techniques. From the lake water samples, microbial cells were collected and then subjected to DNA extraction. The fragments of small-subunit rRNA gene were amplified from the extracted DNA, and subject to denaturing gradient gel electrophoresis (DGGE). The sequences of the respective DGGE bands were determined to deduce their phylogenetic affiliations, and similarity analysis of DGGE band patterns was also conducted. These analyses demonstrated the presence of community structure specific for alpine lakes, and specified representative bacteria, which characterize such community. These bacteria seemed to utilize organic substances supplied from terrestrial ecosystems. These results suggest that bacterial communities in alpine lakes act as a linkage between the terrestrial and aquatic carbon flows.

(3) Terrestrial subsidies to aquatic food webs in mountain lakes: implications for understanding the influences of global change

We studied the linkage between terrestrial and lake ecosystems in alpine and subalpine areas to predict the possible effects of terrestrial vegetation change associated with global change on aquatic biodiversity. Field survey was conducted in 49 mountain lakes of Japan during summer season, to quantify the input of terrestrially derived carbon into lake food webs. Watershed landscape analysis (using GIS) revealed that smaller lakes tend to have larger perimeters and larger basin areas per unit of lake area, implying that the terrestrial-aquatic interface and the source area of terrestrial organic carbon increase as lake size decreases. In fact, stable carbon isotope ratios ($\delta^{13}C$) of dissolved organic and inorganic carbon showed that smaller lakes receive greater inputs of terrestrial organic matter and respire that carbon within the lakes. Moreover, the mixing models using $\delta^{13}C$ values showed that in small lakes, terrestrial organic carbon mainly supported benthic invertebrates as an energetic base. These results suggest that lake size largely determines the strength of energetic linkage between mountain lakes and the surrounding vegetation. Although planktonic food webs may be fueled by autochthonous primary production, terrestrial subsidy was also suggested to be an important energy source that supports some microbial respiration. The present study predicts that small oligotrophic waters, such as small ponds in marsh, may be susceptible to the change in organic matter supply from the watersheds. In particular, the effects of change in terrestrial primary production would be more pronounced for benthic invertebrate predators that depend heavily on terrestrial organic carbon than for other aquatic organisms.

4. General Discussion

The present research showed that the response of plant communities in alpine/subalpine ecosystems to warming is not uniform but highly site- or habitat-specific. First, our studies demonstrated the importance of the variation in growth responses of plants to climate change. The warming experiment conducted in the Taisetsu Mountains revealed that fellfield communities are more sensitive to the warming treatment than snowbed communities. Furthermore, responses of fellfield communities differed between the elevations in which plants growing at lower elevation showed rapider responses. Our results predict that shrubs may become a dominant growth form at lower alpine nearby timberline, while graminoids may become a dominant growth form at higher alpine around ridges if global warming offers milder climate conditions for plant growth. Thus, habitat specific responses should be taken into account for the predictions of the intensity and directions of vegetation changes against global change. Vegetation dynamics of boundary zone around timberline are very important for both alpine and subalpine ecosystems. Our growth analyses of *Picea glehnii* demonstrated that trees growing at higher elevation are more sensitive to climate variations than trees at lower elevation. Because dynamics of timberline may be site specific, comparisons among mountain regions may be important for future works. Second, phenological modification by the global change may cause various impacts on alpine ecosystem. If global change modifies the snowmelt patterns in alpine ecosystem, current phenological patterns of plant communities should be disturbed. The plant community was characterized by fine-scale genetic structures of the composed species, which were maintained by subtle temporal and spatial variations of habitat-specific snowmelt events through phenology and interactions with pollinators. These results suggest that the high mountain plant community is highly vulnerable to changes in temporal and spatial variations of snowmelt events.

In mountain moors, plant communities were established upon competitive interactions for light and nitrogen among species with different physical and morphological characteristics. We found that evergreen species utilize light mainly at early spring while dominant deciduous species utilize light at other season. If warming extends growing season, it benefits light competition of deciduous species and evergreen species may be disadvantageous. It may then reduce species number and biomass of some evergreen species.

As stated previously, there were few studies systematically examining limnological and ecological conditions of alpine and subalpine lakes in Japan. Apparently, this is the first effort to clarify intensively and broadly the current ecosystem situation of the alpine and subalpine lakes. One of important features revealed in the present project is that the alpine/subalpine lakes are in general still oligotrophic due to low phosphorus input but have high nitrogen input relative to P compared with low altitude lakes. This high N to P ratio suggests that N input from atmospheric precipitation may be substantial in these lakes because there are few anthropogenic activities in the watershed. If this is the case, we need to pay more attention on these impacts to alpine/subalpine ecosystems by monitoring periodically limnological conditions of these lakes.

Another important features are that the bacterial and zooplankton communities have unique structures, serve to maintain the water quality and are sustained more or less by subsidy of terrestrial organic matter from the surrounding vegetation. In addition, organic matter flow from terrestrial ecosystems mainly supported benthic macroinvertebrates in small water bodies. Furthermore, an empirical analysis suggests that the zooplankton community structure typical to these lakes is highly vulnerable to existence of fish. These results imply that warming may indirectly alter alpine/subalpine lake ecosystems by changing subsidy input from the surrounding vegetation and expansion of fish distribution. In particular, benthic biodiversity in small ponds seems to be especially susceptible to the effects of terrestrial ecosystem change because of their strong dependence on terrestrial organic matter. Since the effects of global change or land development are expected to be more amplified in smaller lakes, “lake size“ can be used as a surrogate for the vulnerability of alpine and subalpine lake ecosystems. Thus, the knowledge gained in the present study would serve to make better strategies for conservation of these lakes against putative warming

Major Publications

1) Kudo, G. and Hirao, A.S. (2006) Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: implications for global-change impacts. Population Ecology, 48, 49-58

2) Kameyama, Y., Kasagi, T. and Kudo, G. (2006) Eight microsatellite markers for sympatric alpine shrubs. *Phyllodoce aleutica* and *P. caerulea* (Ericaceae). Molecular Ecology Notes, 6, 402-404

3) Kohyama, T. 2006. The effect of patch demography on the community structure of forest trees. Ecol. Res. 21, 346-355.

4) Kameyama, Y., Kasagi T. and Kudo, G. (2008) A hybrid zone dominated by fertile F1s of two alpine shrub species, *Phyllodoce caerulea* and *Phyllodoce aleutica*, along a snowmelt gradient. Journal of Evolutionary Biology, 21, 588-597

5) Kudo, G., Tani, T. and Ida, Y.T. (2008) Linkages between phenology, pollination, photosynthesis, and plant reproduction in deciduous forest understory plants. Ecology, 89, 321-331.

6) Hirao, A.S. and 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.

7) Kawai, Y. and Kudo G.(2008) Effectiveness of buzz pollination in Pedicularis chamissonis: significance of multiple visits by bumblebees. Ecological Research, 23, in press.

8) Kato, S., Urabe, J. and M. Kawata. (2007) Effects of temporal and spatial heterogeneities created by consumer-driven nutrient recycling on algal diversity. Journal of Theoretical Biology, 254,364-377

9) Miyagi, K.M., T. Kinugasa, K. Hikosaka, T. Hirose (2007) Elevated CO2 concentration, nitrogen use, and seed production in annual plants. *Global Change Biology*, 13, 2161-2170.

10) Ishikawa K., Y. Onoda, K. Hikosaka (2007) Intraspecific variation in temperature dependence of gas exchange characteristics of *Plantago asiatica* ecotypes from different temperature regimes. *New Phytologist*, 176, 356-364.

11) Shimizu, Y. & Urabe J.: Regulation of phosphorus stoichiometry and growth rate of consumers: theoretical and experimental analyses with Daphnia. *Oecologia*, 155: 21-31(2008).

12) Sterner,R. W., T. Andersen, J. J. Elser, D. O. Hessen, J. M. Hood, E. McCauley, J. Urabe (2008) Scale-dependent carbon:nitrogen:phosphorus seston stoichiometry in marine and freshwaters Limnology and Oceanography,53:1169-1180.