

**Running Title:** Negative impact of protective management of trees on bryophyte  
diversity

**Title:** Protective management of trees against debarking by deer negatively impacts  
bryophyte diversity

**Author:** Yoshitaka Oishi

**Affiliation:** Laboratory of Applied Plant Ecology and Landscape Ecology, Department  
of Forest Science, Faculty of Agriculture, Shinshu University, 8304 Minami-minowa,  
Kami-ina, Nagano, 399-4598, Japan

**Corresponding author:** Yoshitaka Oishi

Laboratory of Applied Plant Ecology and Landscape Ecology, Department of Forest  
Science, Faculty of Agriculture, Shinshu University, 8304 Minami-minowa, Kami-ina,  
Nagano 399-4598, Japan

E-mail: oishiy@shinshu-u.ac.jp, Phone: +81-265-77-1529, Fax: +81-265-77-1505

## **Abstract**

When wildlife populations become too large, they impact other flora and fauna within the ecosystems that they inhabit. For example, the recent rise in population numbers of sika deer in Japan has led to the stripping of bark from tree overstories in forested areas. This has led to protective management actions, such as wrapping the trunks of trees in wire mesh. The present study investigates the impact of this management action on epiphytic diversity at Mt. Ohdaigahara, which is one of the hotspots for bryophyte diversity in Japan. The correlation between the diversity of epiphytic bryophytes and environmental variables was examined, including the presence/absence of wire mesh protection. A generalized linear model showed that species richness and bryophyte cover was significantly correlated with both tree diameter (at 1.5 m height) and tree density ( $p < 0.01$ ), but negatively correlated with wire mesh protection. Inductively coupled plasma-mass spectrometry analysis showed a significant 3- to 6-fold higher concentration of zinc in bryophytes occupying tree bark under wire mesh protection than for those without wire mesh. Hence, the high sensitivity of bryophytes to zinc accumulation, as a result of toxicity caused by galvanized iron mesh, has led to the loss of species richness and bryophyte cover on tree trunks. Furthermore, other heavy metals found in wire mesh may also contribute to the negative effect on bryophytes. Therefore,

to establish best practices for biodiversity conservation that include bryophytes,  
materials that are free of heavy metals should be preferentially used for tree protection.

### **Key words**

epiphytic bryophyte; deer; plant protection; galvanized iron; zinc toxicity

### **Abbreviations**

GLM = generalized linear model

AIC = Akaike information criterion

DBH = diameter at breast height

ICP-MS = inductively coupled plasma-mass spectrometry

ORS = octopole reaction system

PTFE = polytetrafluoroethylene

## **Introduction**

When deer populations exceed the carrying capacity of their habitat (i.e., overpopulation), environmental problems arise that may have a severe effect on ecosystems (Fuller and Gill 2001; Pellerin et al. 2006; Rooney 2001; Schütz et al. 2003; Stewart and Burrows 1989; Stockton et al. 2005; Takatsuki 2009; Webster et al. 2005).

While stripping bark, grazing on grasses, and browsing on tree understories are normal foraging behaviors in deer, damage is caused when these activities occur in excess. For example, the stripping of bark causes wood decay leading to a decline in forest cover (Akashi and Nakashizuka 1999; Miquelle and van Ballenberghe 1989; Yokoyama et al. 2001), while browsing and/or grazing may alter the structure and composition of vegetation on the forest floor (Kumar et al. 2006; Rooney and Waller 2003; Schütz et al. 2003; Stockton et al. 2005; Webster et al. 2005). Furthermore, these environmental changes may also have an indirect effect on other organisms in the forest ecosystem (Allombert et al. 2005; Feber et al. 2001; Flowerdew and Ellwood 2001; Rooney 2001).

Sika deer (*Cervus nippon* Temminck) are widespread in Japan, and have recently caused serious environmental problems to the forests of this country (Takatsuki 2009).

From 1979 to 2002, the range of this species expanded by as much as 70% (Nakajima

2007). As a result, a number of management actions were implemented to protect forest vegetation from further damage by sika deer. Examples include deer population control by culling, erection of deer-proof fences, and wrapping tree trunks or saplings with wire or plastic mesh (Ministry of the Environment-Kinki Regional Environment Office 2009; Takatsuki 2009).

At Mt. Ohdaigahara, which is located in the Central Honshu region of Japan, the population density of sika deer rapidly increased from about 12.0–22.2 individuals per square kilometer in the 1980s to 17.5–39.5 individuals per square kilometer in the 1990s (Ando and Goda 2009). This increase resulted in serious damage to the forest vegetation of the mountain due to bark stripping, which caused the dieback of damaged trees (Yokoyama et al. 2001). As a result, the Ministry of the Environment initiated a forest protection program in 1986 to conserve the forest ecosystem in this region. Within the framework of this management program, the trunks of about 32,500 trees in a 703-hectare section of forest were wrapped with wire mesh to protect against bark stripping by deer, in addition to other protective measures (Ministry of the Environment-Kinki Regional Environment Office 2009).

Aside from the rapidly increasing population of sika deer, Mt. Ohdaigahara is also recognized as one of the most important regions in Japan for the conservation of bryophyte diversity. In fact, around 30% of the bryophyte species in Japan (>620 species) have been found in this area (Doei 1988). These species include several nationally endangered species that are listed in the Red Data Book of Japan (e.g., *Iwatsukia jishibae* (Steph.) N. Kitag. Bryophyte flora is characterized by a rich diversity in epiphytic bryophytes, due to the high humidity in this region (Doei 1988). While the usefulness of wire mesh protection against bark stripping by sika deer has been emphasized (Ministry of the Environment-Kinki Regional Environment Office 2009), its effects on epiphytic bryophytes have not been examined. The wire mesh is attached directly to the bark of tree trunks using staples, without removing any epiphytes (personal communication with a management spokesperson). This study investigates the impact of this form of management protection on epiphytic bryophyte diversity. Differences in bryophyte cover and diversity on trees with and without wire mesh were evaluated, and values of zinc toxicity from representative bryophyte species were obtained. The results are applied to show the importance of evaluating the impact of protective management actions on non-target organisms.

## Methods

### *Study area*

Mt. Ohdaigahara (34°N, 136°E; ca. 1,500 m alt.) is located in the Yoshino Kumano National Park, which is in the southeastern part of the Nara prefecture in Japan (Figure 1). The climate of this region is relatively mild (annual mean temperature: 5.7 °C), with high levels of precipitation (annual mean precipitation: 4,500 mm; Nara Local Meteorological Observatory 1997). The vegetation of Mt. Ohdaigahara is classified into two main types: (1) the dominant tree species of the eastern part of the mountain is *Picea jezoensis* (Sieb. et Zucc.) Carriere var. *hondoensis* (Mayr) Rehder, and (2) the dominant tree species of the western part of the mountain are *Fagus crenata* Blume and *Abies homolepis* Sieb. et Zucc (Ide and Kameyama 1972). The coniferous forests, which include *P. jezoensis* var. *hondoensis*, are in decline on the eastern part of the mountain due to bark stripping by a dense population of sika deer (Ando et al. 2003; Yokoyama et al. 2001). As a result, wire mesh was attached to these coniferous trees to protect against further damage by the deer (Ministry of the Environment-Kinki Regional Environment Office 2009).

← Figure 1

### *Site selection*

A preliminary survey of the vegetation was conducted to identify patches of forest that were dominated by *P. jezoensis* var. *hondoensis* trees, including trees with and without protective wire mesh (Figure 1). In total, nine patches (each of 20 × 20 m in size) were selected (Figure 1 A- I) to examine the influence of wire mesh protection on epiphytic bryophyte diversity. The tree trunks were completely wrapped with wire mesh from the ground up to a height of 150–180 cm (Figure 2). The mesh was made of iron galvanized with zinc, which is a commonly used material for wire meshes (Japan Society of Corrosion Engineering 2000). In each plot, the tree density (m<sup>2</sup>/plot) was measured by calculating the total basal area of trunks. In addition, the percentage of trunk area of *P. jezoensis* var. *hondoensis* trees that had been debarked by sika deer was also recorded in each plot.

← Figure 2

### *Bryophyte sampling*

The epiphytic bryophyte flora on the trunks of *P. jezoensis* var. *hondoensis* trees was surveyed in the study plots from October to November 2008. The bryophyte species covering the tree trunks from ground level to a height of 1.5 m were recorded.

The nomenclature of bryophytes followed that reported by Iwatsuki (2001). The proportion of bryophyte cover, as a percentage of the total available bark area being



investigated, was divided into six categories: 1 (<1%), 2 ( $\geq 1\%$  to <10%), 3 ( $\geq 10\%$  to <25%), 4 ( $\geq 25\%$  to <50%), 5 ( $\geq 50\%$  to <75%), and 6 ( $\geq 75\%$ ).

### *Analysis*

Generalized linear models (GLMs) were used to identify correlations between species richness and bryophyte cover with respect to environmental variables. A simple GLM with linear terms was performed using R software for Windows 2.11.0 (R Development Core Team 2010). To find the most parsimonious model, we performed automated stepwise model selection using the Akaike information criterion (AIC), using the minimum AIC as the best-fit estimator. Bryophytes that had been identified only up to the genus level were not included in the calculation of species richness if any species in that genus was sampled. The environmental variables that were used in the GLMs were tree density, host tree diameter at breast height (DBH), percentage of debarked area, and percentage of wire mesh protection on tree trunks.

### *Zinc concentration*

The main material that is used for galvanizing iron is zinc. Therefore, to examine the influence of wire mesh on bryophytes, inductively coupled plasma-mass spectrometry

(ICP-MS) was used to compare the concentration of zinc in bryophyte samples that had been collected from tree trunks with and without wire mesh. For this evaluation, two species of bryophyte that are commonly found on the trunks of *P. jezoensis* var. *hondoensis* trees, both with and without wire mesh, in this region were used: *Hypnum tristo-viride* (Broth.) Paris and *Scapania ampliata* Steph. For each species, three sets of samples were collected from trees with and without wire mesh.

Dry samples (0.05–0.10 g) were weighed after being placed in polytetrafluoroethylene (PTFE) vessels. Subsequently, 5 mL of nitric acid was added to the samples, and then digested using a microwave system (MLS-1200 MEGA; Milestone General, Tokyo, Japan) before ICP-MS analysis. Then, the samples were analyzed using an Agilent Technologies 7500CX ICP-MS system (Agilent Technologies, Wilmington, DE, USA). Spectral interferences were minimized or eliminated using the octopole reaction system (ORS), with helium as the reaction gas flowing at a rate of 2.5 mL/min. ICP-MS analysis was repeated twice for each sample, and the mean values were used for one-sided student *t*-test comparisons of zinc concentration from bryophyte samples on tree trunks with and without wire mesh.

## Results

In the nine plots, a total of 110 *P. jezoensis* var. *hondoensis* trees were present (Table 1), all of which were investigated. On average,  $12.2 \pm 9.1$  trees (mean  $\pm$  SD) were in each plot, with about half of the trees that were investigated (58 trees in total) being wrapped with protective wire mesh. A total of 68 species were identified from the bryophyte flora survey of the 110 tree trunks in the sampling plots, comprising 29 mosses and 39 liverworts. The mean species richness on a single tree ranged from as low as no species to a maximum of 34 species (mean = 9.1, SD  $\pm$  9.0).

← Table 1

← Figure 3

### *Relationships between bryophyte diversity and environmental conditions*

The GLMs that were constructed using the environmental variables are presented in Table 2. Species richness showed a significant positive relationship with tree density and host tree DBH, and significant negative relationship with the presence of wire mesh. Bryophyte cover showed a similar trend to that of the species richness, being positively related to tree density and host tree DBH, and negatively related to the presence of wire mesh. The GLM for species richness and bryophyte cover explained 70.1% and 80.4% of the variance, respectively ( $p < 0.001$  for both models).

← Table 2

### *Zinc concentration*

The zinc concentrations that were obtained from the two bryophyte species in this study are shown in Figure 4. The average zinc concentration in *H. tristo-viride* and *S. ampliata* trees with wire mesh was  $388.23 \pm 154.28$  ppm and  $459.56 \pm 286.19$  ppm (mean  $\pm$  SD), respectively. In comparison, the zinc concentration in the same two species on trees without wire mesh was  $117.57 \pm 9.16$  ppm and  $70.70 \pm 4.24$  ppm (mean  $\pm$  SD), respectively. Hence, bryophytes growing on trees with wire mesh protection are subject to 3- to 6-fold higher levels of zinc concentration than bryophytes on tree trunks without wire mesh, and these differences were significant (*H. tristo-viride*:  $t$ -value = 3.03, d.f. = 4,  $p = 0.02$ ; *S. ampliata*:  $t$ -value = 2.35, d.f. = 4,  $p = 0.04$ ).

← Figure 4

## **Discussion**

### *Effect of the wire mesh protection on bryophytes*

Epiphytic bryophyte diversity was positively impacted by tree density and host tree DBH, but negatively impacted by the wire mesh protection of tree bark. A number of existing studies also found a positive correlation for bryophyte diversity with tree density and host tree DBH (Boudreault et al. 2008; Hazell et al. 1998; Ojala et al. 2000; Thomas et al. 2001). In fact, high tree density has been suggested to be beneficial to

bryophyte diversity by providing better microclimates, such as humid conditions (Ojala et al. 2000; Thomas et al. 2001). Furthermore, the species richness and cover of bryophytes may be positively correlated with increasing host tree DBH, due to the changes in bark features (e.g., an increase in thickness and roughness) (Boudreault et al. 2008; Ojala et al. 2000).

Previous studies have shown that a considerable amount of zinc is removed from the zinc coating of galvanized iron by rain and dew (Harris 1946; Seaward 1974). Tree bark is one of the major habitats for bryophytes, particularly in humid climates such as Mt. Ohdaigahara (Doei 1988), yet studies showing the effects of wire mesh on bryophytes remain limited (but see Harris 1946). Tyler (1990) showed that zinc is toxic to bryophytes, inhibiting growth, net photosynthesis, and the germination of spores. Based on the decrease in diversity and the increase in zinc concentration of bryophytes occupying tree bark under wire mesh protection, in parallel to existing research showing the high sensitivity of bryophytes to zinc (Tyler 1990), it is reasonable to conclude that the loss of species richness and bryophyte cover in the current study arose primarily from the zinc toxicity caused by the galvanized iron mesh. Basically, rain or dew dripping from the protective iron mesh probably contains high concentrations of zinc,

which causes a decline in bryophyte diversity on tree trunks. Furthermore, other heavy metals that are present in wire mesh (e.g., iron) may also contribute to the negative effect on bryophytes, with different heavy metals being harmful to varying extents on bryophytes (Tyler 1990).

#### *Implications for biodiversity conservation*

The decline in bryophyte abundance and diversity on the lower parts of the tree trunks may be a cause for concern for biodiversity conservation on Mt. Ohdaigahara. This is because bryophytes contribute significantly to the species richness and biomass of tree trunks (Fritz 2009; Hale 1952; Lyons et al. 2000), as well as for ecosystem functions such as rainfall interception and nutrient cycling (Coxson 1990; Nadkarni 1984; Pypker et al. 2006a, b).

Furthermore, in addition to bryophytes, tree bark also provides important habitats for lichens and vascular epiphytes (Williams and Sillett 2007). However, as heavy metals are toxic to these plants (Tyler et al. 1989), wire mesh protection may also contribute towards decreasing their levels of diversity and ecosystem functions. Unfortunately, considering that wire mesh protection is generally used against mammalian pests due to

its direct effectiveness (Salmon et al. 2006; Vercauteren et al. 2006), this negative impact on bryophyte diversity may be widespread.

Therefore, to establish best practices for biodiversity conservation that includes bryophytes, materials that are free of heavy metals should be preferentially used for tree protection, such as tree shelters using plastic tubes (Ward et al. 2000) and/or forest enclosures with plastic mesh fencing (Vercauteren et al. 2006). However, such alternative forms of plant protection (i.e., that do not use wire mesh) also have negative aspects with respect to biodiversity conservation. For example, tree shelters decrease light transmission (Ward et al. 2000), which may cause changes in the species composition on tree trunks. Furthermore, Shibata et al. (2008) reported that forest enclosures were found to unexpectedly cause the prevention of tree regeneration within fenced areas because of serious seed predation by increased mouse populations.

These cases show the difficulty of minimizing the impact of plant protection methods on ecosystems with complex interactions. To establish best practices for biodiversity conservation, adaptive management is necessary. Within such frameworks, we should examine and revise protective management practices based on data assimilated from the

regular monitoring of such ecosystems, while also preferentially using plant protection methods using with metal-free materials.

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### **Figure legends**

**Figure 1.** Location of Mt. Ohdaigahara and the study plot. This mountain is located on the Kii Peninsula in Kinki District, central Japan.

**Figure 2.** Examples of tree trunks with (right) and without (left) wire mesh. The middle part of the tree trunk that does not have wire mesh has been debarked by deer.

**Figure 3.** Comparison of species richness and epiphytic bryophyte cover on *P. jezoensis* var. *hondoensis* trees in each plot. The bars represent the mean value of species richness and epiphyte cover on a single tree, and the error bars represent the corresponding standard deviations.



**Figure 4.** Comparison of mean zinc concentrations (ppm) for (a) *H. tristo-viride* and (b) *S. ampliata* epiphytes with and without wire mesh protection, which are found growing on *P. jezoensis* var. *hondoensis* trees. The bars represent the mean value of species richness and epiphyte cover on a single tree, and the error bars represent corresponding standard deviations.

## Tables

**Table 1**

A summary of the characteristics of the study plots, including altitude, tree density, and number of trees surveyed

| Plot | Altitude<br>(m) | Tree density<br>(m <sup>2</sup> /plot) | No. of <i>Picea jezoensis</i> var. <i>hondoensis</i> trees |                |
|------|-----------------|--|--|----------------|
|      |                 |  | Total  | With wire mesh |
| A    | 1572            | 11.9                                   | 15   | 0              |
| B    | 1576            | 19.9                                   | 11   | 11             |
| C    | 1597            | 13.9                                   | 12   | 0              |
| D    | 1597            | 12.7                                   | 22   | 0              |
| E    | 1590            | 9.9                                    | 30   | 30             |
| F    | 1676            | 3.1                                    | 3  | 2              |
| G    | 1672            | 4.0                                    | 7  | 6              |
| H    | 1619            | 4.3                                    | 8  | 7              |
| I    | 1621            | 5.5                                    | 2  | 2              |

**Table 2**

Results of generalized liner models explaining species richness and cover of bryophytes with respect to environmental variables. The significance level of the coefficients and adjusted R squared are shown in this table.

| Variables          | Species richness       |                 |       | Cover                  |                 |       |
|--------------------|------------------------|-----------------|-------|------------------------|-----------------|-------|
|                    | coefficients           | <i>t</i> -value | p     | coefficients           | <i>t</i> -value | p     |
| Intercept          | 7.30                   | 3.81            | <0.01 | 2.26                   | 7.30            | <0.01 |
| Tree density       | $3.51 \times 10^{-4}$  | 3.10            | <0.01 | $6.30 \times 10^{-5}$  | 3.43            | <0.01 |
| Host tree DBH      | $2.01 \times 10^{-1}$  | 3.12            | <0.01 | $2.29 \times 10^{-2}$  | 2.18            | <0.05 |
| Wire mesh          | $-1.43 \times 10^{-1}$ | -14.2           | <0.01 | $-3.19 \times 10^{-2}$ | -19.5           | <0.01 |
| Adjusted R squared | 0.701                  |                 |       | 0.804                  |                 |       |

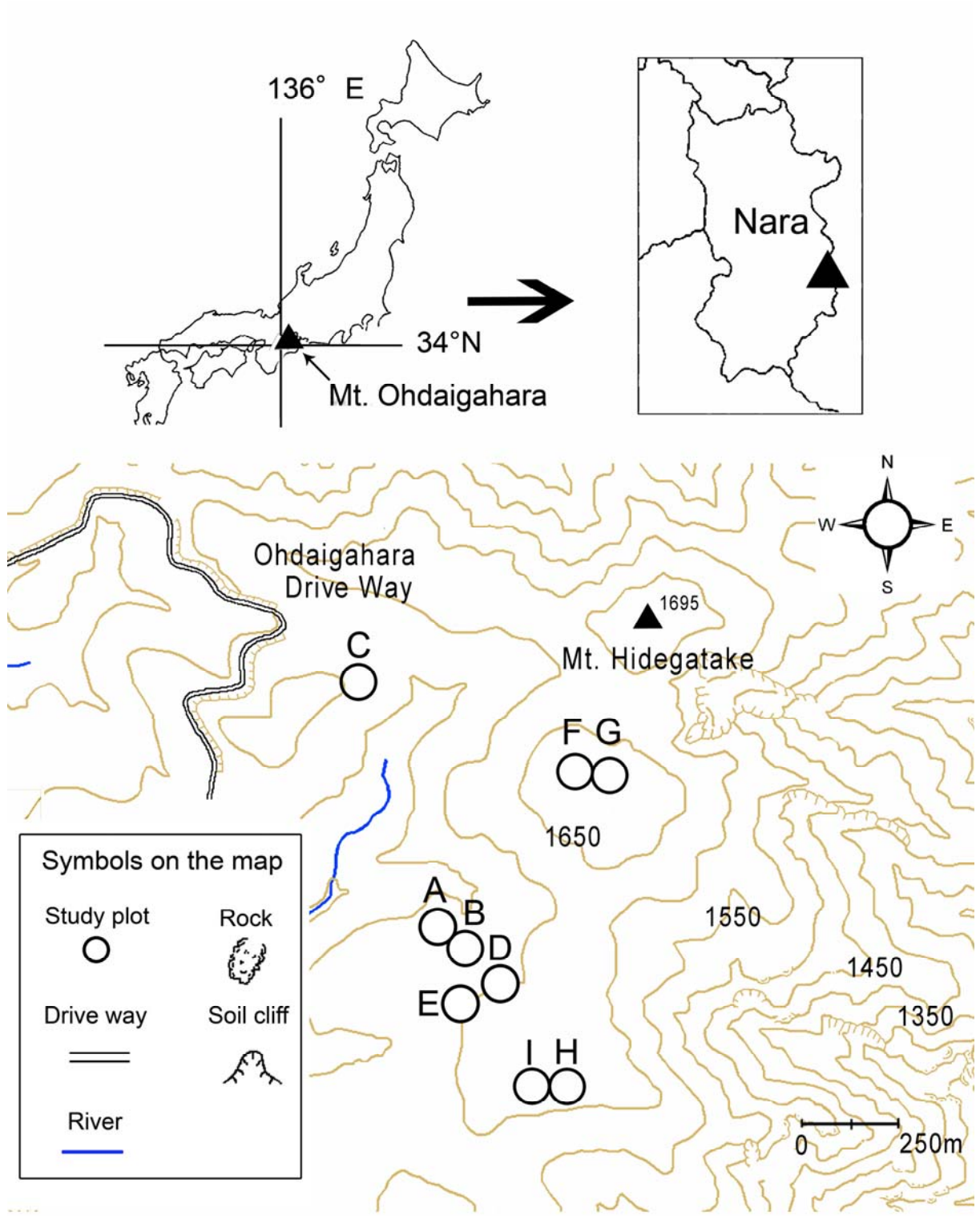


Figure 1



Figure 2

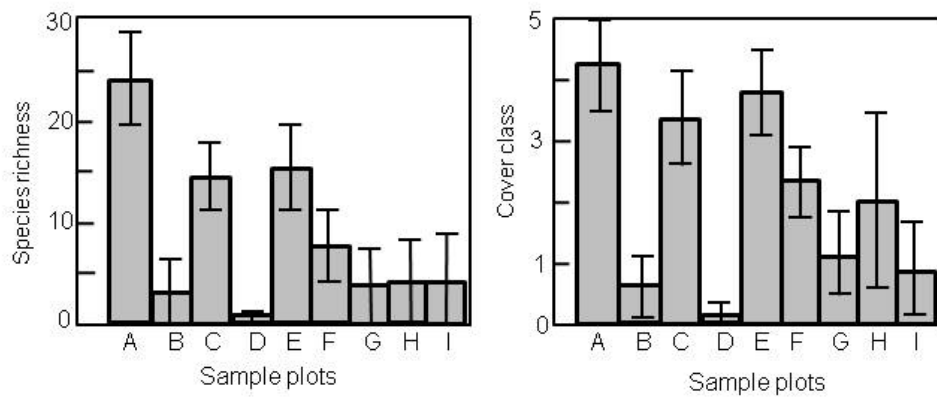


Figure 3

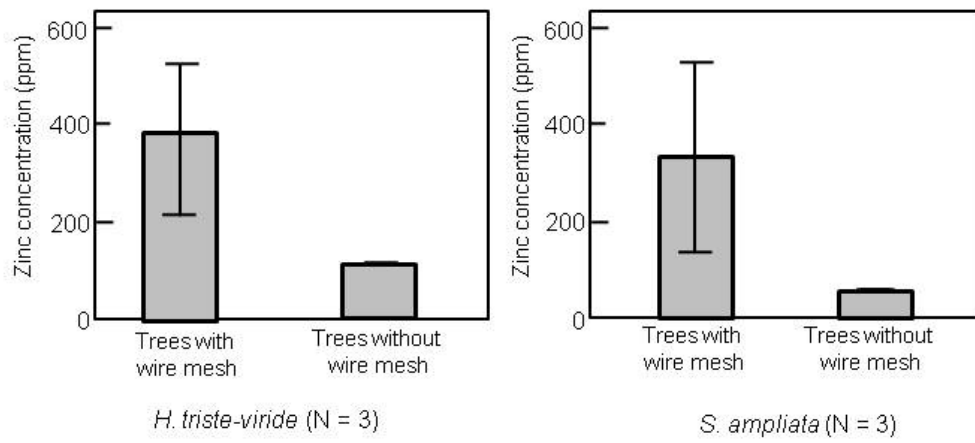


Figure 4