

# Chapter 12

## Vegetation Surveys, Environmental Measurement, and Analysis: Biodiversity Conservation in *Satoyama*



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### 12.1 Introduction

#### 12.1.1 *What is Satoyama?*

Satoyama includes various types of semi-natural grasslands, agricultural lands, forests, wetlands, and settlements where both biodiversity and agricultural and forestry productivities are maintained through moderate levels of human intervention. Satoyama supports the livelihoods of local peoples not only from the perspective of biodiversity conservation, but also by providing various ecosystem services. For this reason, Satoyama has been a major conservation target in Japan's national biodiversity strategy. Sustainable resource management and land use systems in Satoyama, or equivalent semi-natural landscapes, are recognized not only in Japan but also throughout the world. Such systems are referred to as socioecological production landscapes and seascapes (SEPLS).

The Satoyama concept has become widely known worldwide through the Satoyama Initiative, which was proposed by the Ministry of the Environment, Japan and the United Nations University Institute for the Advanced Study of Sustainability (UNU-IAS) at the 10th meeting of the Conference of the Parties to the Convention on Biological Diversity. The Satoyama Initiative is an effort to promote sustainable resource management and land use at the global scale by recognizing SEPLS throughout the world and by sharing techniques, wisdom, and lessons learned to balance biodiversity conservation and resource utilization. The initiative has further developed into an international network (IPSI; International Partnership for the

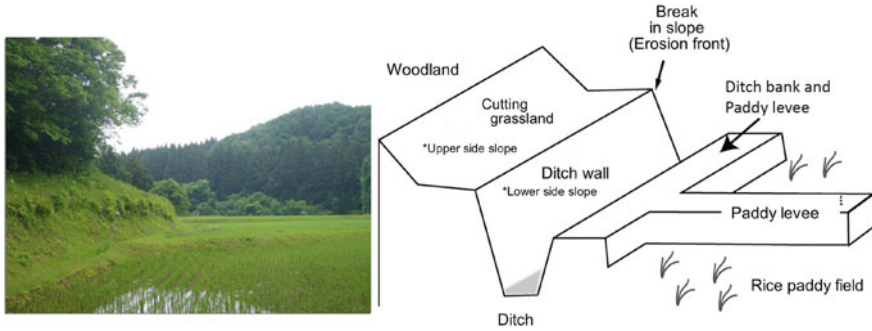
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**Fig. 12.1** Various field margin types in Japanese Satoyama. Modified from Ito and Katoh (2007)

Satoyama Initiative) of governments, academic institutions, and private organizations working toward sustainable conservation of secondary natural landscapes around the world.

Satoyama contains various types of semi-natural grasslands with high plant species diversity that are maintained through anthropogenic disturbance associated with agriculture and forestry management (Ito and Katoh 2007). In particular, linear or strip-shaped semi-natural grasslands such as paddy levees, stream embankments, ditch walls, and grassland along the forest edge are known as the field margin (Marshall and Moonen 2002) (Fig. 12.1). The field margin has various ecosystem functions including as a dispersal route for organisms, habitat for natural predators that consume agricultural pests as well as many other organisms, and a honey source for pollinator insects.

### 12.1.2 Challenges Facing Conservation of Biodiversity in Satoyama

*Satoyama* is currently on the brink of a major regime shift due to transformation of socioeconomic systems. In the past, grassland ecosystems such as field margins mentioned above were inhabited by a wide variety of organisms, and the dominance of competitively exclusive species was suppressed through continuous artificial management practices such as mowing, burning, and grazing associated with agricultural work. However, due to the depopulation of agricultural areas and mountain areas and the decrease in the number of farmers the management pressures on agricultural land have decreased, and vegetation succession is progressing in various manners in *Satoyama*. On abandoned farmlands, the invasion of exotic plant species distorts the succession process, creating a barrier to biodiversity conservation.

In field margin areas facing such difficulties, what research questions can you ask and what conservation guidelines can you submit based on scientific methods?

## 12.2 Setting a Research Hypothesis

### 12.2.1 *Defining the Scope of Your Research Project in a Summer School*

In short-term research programs such as summer schools, careful planning of study design is essential to drawing conclusions about the study hypothesis with limited resources (time and labor). Usually, preparation for hypothesis-testing research is difficult at a field site that you are visiting for the first time. In such cases, the first step is to clarify the scope of the project based on a preliminary survey of the target field, survey of related literature, and interviews with local farmers or conservationists.

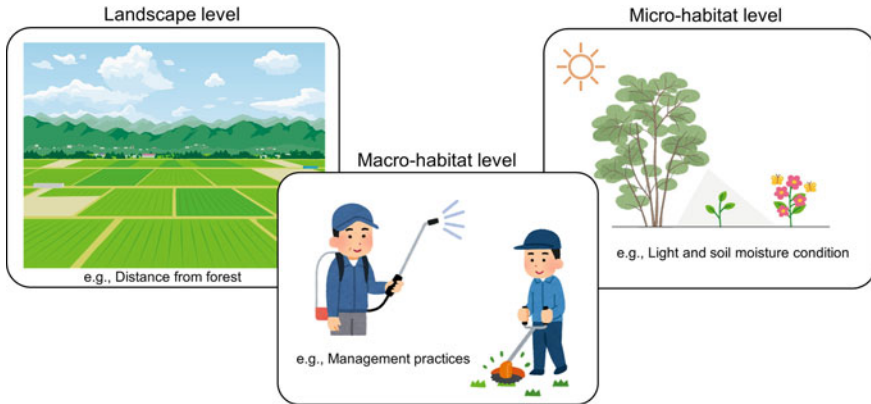
The research scope used in a summer school project should be as simple as possible. The research scope provides a framework for when, where, from what perspective, and how your research will be conducted. If the scope is too wide, timely investigation will be difficult, and therefore it should be established with consideration of the amount of effort that can be invested during the research period. For students at the beginning of their exploration of environmental studies and ecology, the first hurdle to field research will be the great deal of time required to identify species and learn how to use environmental measuring devices. Therefore, in a short research program, students should focus on acquiring these research skills while engaging in several research activities.

### 12.2.2 *Establishing the Research Questions and Hypothesis*

Once the research scope is determined, the next step is to establish the research question and hypothesis. In general, research questions can be proposed for a research project. For example, “Why are the herons (e.g., *Egretta garzetta*, *Ardea intermedia*, *A. alba*, and *A. cinerea*), medium-to-large waterfowl that inhabit Japanese *Satoyama*, preferentially forage in only certain paddy fields?” In addition to the feeding environment, research questions exploring the nesting environment are other options when studying the habitats of herons.

The research hypothesis is obtained through conversion of the research question into the format “If XX is true, then XX follows.” For the example given above, we could create a research hypothesis that “A higher density of the weather loach (*Misgurnus anguillicaudatus*) in paddy field is associated with more frequent visits of herons to such fields.” Thus, research hypotheses are more specific questions used to draw specific conclusions based on measurable data. In general, multiple research hypotheses may be needed to answer to a single research question.

Generally, a common research topic for understanding the ecology of a species and considering conservation measures is clarification of the relationships between organisms and environmental factors. Habitat suitability and distribution models for



**Fig. 12.2** Three hierarchical levels characterizing plant community composition at different spatial scales: landscape, macro-habitat, and micro-habitat

various species have been developed to aid understanding and prediction of species pattern and biodiversity (Guisan 2017).

To elucidate the relationships between the distribution of wildlife and various environmental factors, three hierarchical levels at different spatial and temporal scales must be analyzed, thus at the landscape, macro-habitat, and micro-habitat levels (Fig. 12.2). A plant habitat is an area wherein a plant species can obtain the resources required for growth and reproduction. The definition of a hierarchical level may vary depending on the ecology of the target species and the distribution of the local population. For example, the spatial dispersal of cherry trees, the seeds of which are spread by birds, is larger than that of violets, the seeds of which are dispersed by ants, and therefore, the spatial extent of the relevant landscape differs among target species.

The landscape level is characterized by the variety of habitats and the movements (dispersals) of a species within an area or region. The concept is used to learn and describe the ecological characteristics of an area, and how species are affected by their surroundings. The assumption is made that the environmental factors determining species distribution and diversity are represented by the spatial position of habitats within the landscape (e.g., distance from forest or an adjacent water body, habitat connectivity, elevation) and by the land-use composition surrounding the habitat (e.g., urbanization rate).

A macro-habitat is a series of contiguous, similar vegetation patches with environmental conditions favored by certain species. The relevant environmental factors include the degree of management (e.g., the intensity or frequency of mowing, the use of herbicide spray), the vegetation structures (e.g., canopy coverage, canopy height), and geomorphological and geological features (e.g., convex or concave topography, soil types).

At the micro-habitat level, various local areas with similar environmental resources or microtopographic locations, the environmental factors are light availability and soil conditions (e.g., soil moisture, soil nutrient, soil texture). These factors represent environmental conditions that can vary on the spatial scale of herbaceous plant survey quadrats, which are usually  $1 \times 1$ -m square sampling frames but may be rectangular depending on site conditions.

The appropriate hierarchical levels to assess depend on the research questions. In ecological research, statistical methods such as hierarchical linear model (also known as multilevel linear model, or mixed model), and hierarchical partitioning (e.g., Quinn and Keough 2002) can be used to evaluate the relative importance of environmental factors on species distributions across multiple hierarchical levels. However, when multiple environmental variables are incorporated into a statistical model, a larger number of quadrats is needed to estimate many parameters. Thus, focusing on a certain hierarchical level is most practical for short-term research projects.

### ***Exercise 1***

In your study field, what environmental factors are positively/negatively affecting the ecosystem and the biodiversity? List as many examples as you can think of.

Tips: Observe and interview local farmers about local issues such as habitat alterations (caused by civil engineering), changes in land use and agricultural management systems, the use of chemical pesticides and fertilizers, or the presence and prevalence of invasive species.

### ***Exercise 2***

Assume that you have set the research hypothesis “when the degree of human influence differs between paddy levees, the composition of the plant community established there also differs.” What kinds of response variables (those of target organisms or communities) and explanatory variables (e.g., environmental factors related to grassland managements) should be measured? List as many as you can.

Tips: Not only the abundance/frequency of occurrence of certain species but also the numbers of species or diversity indices (e.g., Shannon-Weiner index) are useful when seeking to understand a species ecology and a plant community. Environmental variables relevant to grassland management can be obtained by interviewing farmers or by surveying indicators (e.g., the average height of vegetation reflects the mowing management strategy in play).

## 12.3 Study Design and Methodology

### 12.3.1 Study Design for Vegetation Surveys

The first challenge in an investigation is determining how to arrange the quadrats spatially, known as sampling design. Sampling is conducted for the purpose of extracting representative samples from the whole set (e.g., vegetation, the population of a certain plant species, buried seeds) of an object to clarify the overall picture with a small amount of research effort. Therefore, the extent (e.g., area or number) and strategy of sampling (random or systematic) are critical issues that must be examined. When investigating the composition of plant communities, careful selection of plots that capture vegetation heterogeneity is essential.

If you are targeting a particular plant species (e.g., endangered species or indicator species) and the number of individuals within a study site is limited and easily detectable, all individuals should be surveyed rather than sampling with quadrats.

In addition, experimental operations such as mowing may be performed using field experimental plots. See other textbooks (Quinn and Keough 2002) for sampling designs of such field experiments.

Below, we introduce sampling methods that are commonly used in vegetation surveys.

#### 12.3.1.1 Simple Random Sampling

The term *random plots* is often misused when setting up quadrats. If sampling is to be truly random, a statistically correct randomization procedure is required, such as dividing the survey area into equal-area sections, numbering the sections with consecutive numbers, and then selecting survey locations using a random number function in a spreadsheet program (e.g., Excel). Outside of experimental settings, haphazard sampling is often adopted in lieu of random sampling for convenience.

In many cases, closer quadrats will exhibit habitat similarities, and individual plants may spread through cloning. Therefore, setting up quadrats requires consideration of the representativeness of sampling locations so that the range of vegetation variation across the entire study site is covered. To address that need, stratified random sampling, which is an improved method based on simple random sampling, was developed.

#### 12.3.1.2 Stratified Random Sampling

For stratified random sampling in vegetation surveys, the vegetation patches that are expected to have different species compositions within the study site must be identified in advance. Random sampling is then performed for each patch type (this is known as stratification). This method was devised to capture the variation in species

composition across the entire survey area better than methods that do not consider stratification.

### **12.3.1.3 Systematic Sampling and the Transect Method**

Systematic sampling is a method of sampling at regular intervals. For example, to evaluate the soil nutritional status of a square field, estimates are made based on sampling at the center and four corners.

The transect method is a systematic sampling method in which quadrats are set at regular intervals or continuously along a line or belt. This method is often used to investigate changes in species composition along an environmental gradient, such as distance from the water or forest edge.

### **12.3.1.4 Chrono-Sequence Method**

The chrono-sequence method is a sampling method that aims to capture time-series changes through simultaneous investigation and comparison of sites with different histories as an alternative to direct observation methods. This method requires that conditions affecting vegetation other than vegetation history do not differ significantly among study sites. This technique is used, for example, to simultaneously investigate community patches at different vegetation succession stages to estimate the recovery process after plant community disturbance.

## ***12.3.2 Precautions for Sampling Design***

### **12.3.2.1 How Many Quadrats Should You Have?**

When estimating the number of species, if a preliminary survey can be conducted, the required survey area is estimated using the species-area curve method. In this method, the species observed are recorded while gradually increasing the size of the square plot (quadrat), creating a species-area curve. In this manner, the area where the rate of increase in the number of species is nearly constant can be determined. If such preparations cannot be made, a rough method involves adding quadrats until new species scarcely appear in the vegetation survey. As a result, the number of quadrats may vary among vegetation study sites. In such a case, use of the rarefaction method described below allows for comparison of the number of species while considering research effort.

Even in surveys that do not aim to estimate the number of species at a site, the number of quadrats is an important decision in assessment of variation and detection of statistically significant differences of abundance. If the variation in the

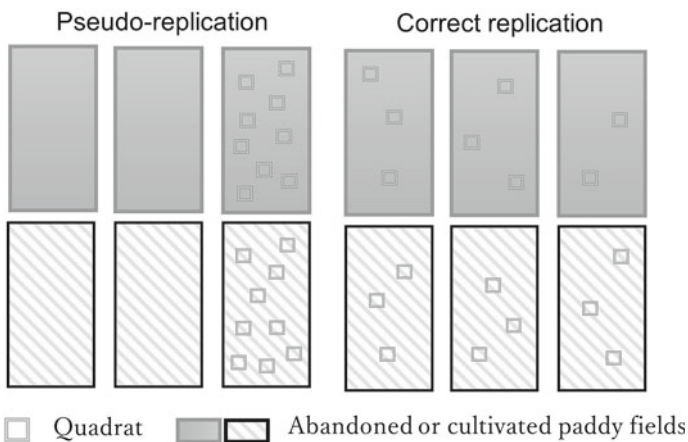
target organism cannot be evaluated in advance, a study plot is typically set with approximately 10 replications per target area.

### 12.3.2.2 Size and Shape of the Quadrat

When determining the size of a vegetation survey quadrat, canopy height is often used as a guideline for the length of each side of the quadrat. For example, in turf herbaceous communities, the length of one side is approximately 0.5 m to 1 m, while in tall herb communities, 1 m to 2 m is often used. A quadrat does not necessarily need to be square, and in field margin areas such as paddy levees, a rectangular quadrat matching the shape of the target habitat is best.

### 12.3.2.3 Avoiding Pseudo-Replication

If sample replication is done improperly, the results of hypothesis testing may be inaccurate. For example, such inaccuracy occurs when multiple quadrats are set between a pair of abandoned and cultivated paddy fields to investigate the impact of abandonment of cultivation on the weed community. Although differences in plant species composition and species numbers may be observed in this study, the effects of abandonment of cultivation are not strictly compared (Fig. 12.3). Such a situation is known as pseudo-replication (Hurlbert, 1984). For proper replication, multiple pairs of abandoned paddy fields and actively cultivated paddy fields should be selected to avoid pseudo-replication.



**Fig. 12.3** Pseudo-replication and correct replication diagrams



**Table 12.1** Braun-Blanquet scale for vegetation surveys

Braun-Blanquet scale	Vegetation cover rate (%)	Value for analysis (%)
5	75–100	87.5
4	50–75	62.5
3	25–50	37.5
2	10–25	17.5
1	1–10	5.5
+	1 or less	0.5

### 12.3.3 Measurement of Response Variables

#### 12.3.3.1 Field Measurement

In surveys of herbaceous plant communities, the response variable is selected mainly from the following parameters, taking into consideration the research hypothesis and costs (time and labor).

#### 12.3.3.2 Vegetation Cover Rate and Coverage

The percentage of the ground surface covered by plants in a quadrat is the vegetation cover rate (%) and determined visually. This is not often used as a response variable, rather as an explanatory variable, surrogate of animal habitat, or foraging intensity.

The area occupied by each plant species when the quadrat is viewed from directly above is known as coverage (%) and is usually recorded as an ordinal variable (on Braun-Blanquet scale) obtained by translating coverage (%) into a class (Table 12.1). Coverage can be easily visualized as the area of shadow (foliar cover) created when light is projected from directly above the quadrat. Therefore, when multiple plant species overlap and grow, the total vegetation cover rate may exceed 100%. Generally, the coverage is estimated visually.

A measurement that represents the degree of plant spread, such as solitary, crowded, or covering, is known as sociability. Sociability is required when conducting a phytosociology survey but is usually unnecessary if not explicitly included in the research objectives.

#### 12.3.3.3 Biomass

To accurately evaluate the amount of biomass, plants must be harvested by species from the quadrats to measure dry weight. This method is a destructive testing process and is not suitable for tracking communities in the same location over a long period. As a non-destructive method to determine biomass, the coverage or vegetation cover

rate and maximum plant height of each species may be measured, with biomass estimated by multiplying the percent cover by the plant height. However, this estimate is not a strict measure of biomass and should be interpreted with caution.

#### **12.3.3.4 Abundance (Number of Individuals)**

Abundance or the number of individuals is rarely used in vegetation surveys targeting communities but may be used in plant surveys targeting rare species and indicator species. Individuals of clonal plants may be difficult to distinguish, as they are connected by underground stolons and rhizomes, and in such cases the number of shoots may be used rather than the number of individuals.

If abundance is too high for efficient counting, it can be evaluated through grading on a logarithmic scale. For example, for a power of 5, the possible grades are 0, 1–5, 6–25, 26–125, 126–625, 626–3125, and higher.

#### **12.3.3.5 Presence–Absence**

Presence–absence is used when you are interested only in the occurrence of a particular plant species within a quadrat. No quantitative information on focal species such as conservation target can be obtained when presence–absence is used as a measure. However, the prevalence of each plant species in the study site can be evaluated based on its frequency of occurrence across multiple quadrats.

### ***12.3.4 Correcting Environmental Data***

Environmental variables used to analyze vegetation survey data include those that can be measured in the field and those that require samples be returned to the laboratory for measurement and analysis. In exploratory research, environmental variables, which are often costly to measure and analyze, may be impractical to use. Therefore, we describe a two-step approach for identifying trends using factors that can be readily measured in the field and then more fully evaluating the appropriate environmental variables in subsequent research. Here, assuming the survey is being conducted during summer school, we introduce variables that can be measured easily and quickly in the field.

### 12.3.4.1 Landscape Level

#### Geographic Coordinate Information

Geographic coordinate information can be obtained using a commercially available global navigation satellite system (GNSS) receiver. Alternatively, a smartphone with GNSS function can be used to take photographs with geotags. The positioning accuracy of smartphones is often approximately 2–3 m. However, in research, accuracy at the sub-meter level (i.e., error of 1 m or less) is often necessary. In such cases, a dedicated receiver can be used, although such devices are expensive.

As the altitude information measured with a GNSS receiver is often ellipsoid height, it must be corrected to obtain the altitude. In Japan, the altitude data acquired by GNSS receivers represents the true altitude + 30–40 m, but some receiver models automatically correct the displayed altitude. For details, please refer to the manual of each receiver model used.

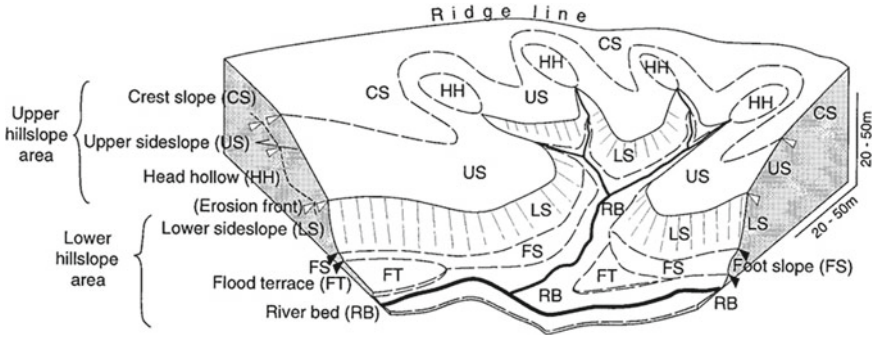
#### Land-Use Type in the Surrounding Area

Documenting land-use types around the study site can provide indicators of ecological processes and anthropogenic impacts at the landscape level. For example, if the forest coverage of the surrounding terrestrial area is high, a more natural environment can be maintained, and forest plant species from the surrounding area will more readily disperse into the target area. If the surrounding land use includes largely residential and commercial areas, the degree of human impact is assumed to be high. In addition, recording the cropping status of adjacent land (e.g., paddy fields, fields, abandoned land) is advisable, as the use and management practices of adjacent land affects the composition of plant species in the field margin (Biaix and Moonen 2020). If the location of the study site can be determined with high precision using GNSS, geographic information systems (GIS) and aerial photographs or satellite images may be used to assess land use around the study site.

### 12.3.4.2 Macro-Habitat Level

#### History of Agriculture and Forestry Management

To accurately determine the frequency of mowing and whether herbicides are applied at a study site, questionnaires and interviews with land managers must be conducted. When such surveys are difficult, canopy height may be used as a surrogate indicator of grassland management, as grassland height is suppressed where mowing is more frequent.



**Fig. 12.4** Micro-topographical classification of hilly landscape in Japan (Nagamatsu and Miura 1997)



**Fig. 12.5** Field survey tools for environmental data collection: gradometer (left) and the Yamanaka soil hardness meter (right)

### Microtopographic Classification

Geomorphological classifications may be used as a comprehensive indicator of multiple environmental conditions, including moisture, light, and soil development. Microtopographic classification (Tamura 1974) is often used as a classification method representing the small-scale topographic features of a study site in Japanese hilly landscapes (Fig. 12.4).

To determine the relative height from water surface levels such as rivers and lakes, a cross-sectional graph may be created using a total station (TS) or auto-level instrument. If no such equipment is available due to cost, relative height can be obtained easily and quickly using a simple slope surveying instrument (e.g., Gradometer, Geopacks Ltd., UK) (Fig. 12.5).

**Table 12.2** International classification of soil texture

Soil Texture Classification	Criterion
Sandy Soil (S)	Almost all sand, no stickiness
Sandy Loam (SL)	Feels very sandy with little stickiness
Loam (L)	Feels somewhat sandy and sticky Sand and clay in similar amounts
Silt Loam (SiL)	Little sandiness, feels smooth like flour
Clay Loam (CL)	Feels slightly sandy and quite sticky
Light Clay (LiC)	Scarce sand, adheres firmly
Heavy Clay (HC)	No sandiness, sticks very well

### 12.3.4.3 Micro-Habitat (Quadrat) Level

#### Soil Hardness

The Yamanaka-type soil hardness tester is used to measure soil hardness (Fig. 12.5). A metal pointed cone is thrust against the soil surface and the subduction depth (mm) is measured.

In soil with high hardness, water permeability is poor and plant growth may be hindered. If the activity of soil microorganisms is high, an aggregate structure may develop and soften the soil.

#### Soil Texture Classification

Soil texture classification is conducted based on the particle size composition of the soil. This classification reflects some soil properties that affect its role as a growth substrate for plants. For example, coarser particle size leads to better drainage, while finer particle size is associated with higher fertilizer retention capacity. As a simple method for soil texture classification, a small amount of surface soil can be collected with a finger moistened with water and evaluated based on the feel when rubbed between the thumb and index finger. Prior training using standard soil samples (Fuji-hira Industry Co., Ltd., Tokyo) as the judgment criteria is useful, but judgments can also be made based on Table 12.2 below.

#### Soil Moisture

Volumetric water content (%) and soil moisture potential (pF) are typical measured values representing soil moisture conditions. For multipoint measurement, the volumetric water content (VWC) is often measured using a time domain reflectometry

(TDR) or amplitude domain reflectometry (ADR) soil moisture meter (e.g., SM150-KIT, Environmental Measurement Japan, Co., Ltd., Fukuoka). Although these instruments are relatively expensive ( $\geq 110,000$  JPY;  $\sim 840$  USD as of December 2022), they have the advantage of taking measurements rapidly on-site.

As soil moisture varies over time, measurement should be completed in the shortest time possible when comparing multiple locations. In addition, due to the difficulty of detecting differences between measurements immediately after rainfall, measurement more than 2–3 days after rainfall is most suitable.

### Soil Electrical Conductivity and pH

Soil pH is often affected by surface geology and base rocks. It may also be affected by anthropogenic factors such as concrete and road-surface antifreeze. No significant effect on the growth of many plants is observed around neutral pH, but plant growth may be hindered at extremely low or high pH levels. However, acidophilic plants and alkaliphilic plants exist, and soil pH is an essential factor regulating their growth. Soil electrical conductivity (EC) reflects the concentration of nutrients in the soil and provides insight into the degree of salt accumulation.

These surveys begin with on-site soil sampling. If soil is collected only at a specific point, the sample may not be representative of the overall soil environment of the study site. Therefore, to account for variability, small amounts of surface soil should be collected from multiple locations and mixed well into a single sample prior to extraction.

For this analysis, prepare the soil by diluting it with water. Place the raw (not air dried) soil sample in a plastic bottle with a lid, dilute it with distilled water (soil:water volume ratio = 1:2), and shake. To achieve this dilution, add soil up to the 150 mL line of a graduated bottle pre-filled with 100 mL water, because the soil has many voids and its exact volume cannot be measured if soil is added to the bottle first. After allowing the mixture to stand for a period, the supernatant can be measured with a handheld pH and EC meter (e.g., LAQUA twin, HORIBA Ltd., Kyoto).

### Light Environment

The light environment is an indispensable environmental factor for the growth of many plants, and it greatly affects the success or failure of reproductive events such as flowering and fruiting. The method of taking hemispherical photographs using a circular fisheye lens is widely used for multipoint measurement of the light environment in the field. The light environment is evaluated by obtaining the open-air ratio (the ratio of area not covered by vegetation cover to the total celestial sphere area at a specific observation point) from the photograph.

Through analysis of these photographs in the laboratory with dedicated software such as Hemiphot.R (ter Steege 2018), the theoretical solar radiation time and estimated value of photosynthetic photon flux density (PPFD) can be calculated. Please refer to the manual of each software program for the specific methods.

Although this method is generally not suitable for multipoint measurement, the light environment can be evaluated using an illuminance meter or a quantum flux meter. Two units of the same model, one at the quadrat and the other in an open area adjacent to the quadrat, must be used simultaneously. Then the light environment of the site can be evaluated through comparison of the values measured with both instruments.

### ***Exercise 3***

A student research group set up a belt transect to record vegetation transitions across a riverside ecotone. For this study, what environmental conditions should they measure to investigate the relationship between vegetation and the environment?

Tips: In general, riparian vegetation is less disturbed by runoff during high flow or flood conditions as the distance from the river edge increases. The frequency and intensity of disturbances can affect plant community composition and structure. However, under normal flow conditions, the distance from the soil surface to the groundwater level is an important environmental factor that determines whether wet or dry plant communities can become established.

## **12.4 Case Study 1: Study Design to Compare the Effects of Human Management on Plant Communities in *Satoyama***

A typical field margin habitat in the *Satoyama* is paddy levee grasslands. The frequency and timing of mowing of the grasslands varies among farmers, and many areas have become poorly managed. Based on this background information, the student team set a research scope that can be assessed through a 1-day field survey. They decided to investigate how the composition of the plant community differs among three paddy levee grasslands with different mowing management practices.

### ***12.4.1 Research Question and Scope***

The research question was set as “Is the plant species diversity and composition of the paddy levee grassland affected by human management?” To answer this question, vegetation surveys using quadrats and measurements of environmental factors related to human impacts were conducted in three paddy levee grasslands with different

management levels (high, moderate, and low intensity) in the target area. The effects of environmental factors other than human management were also explored.

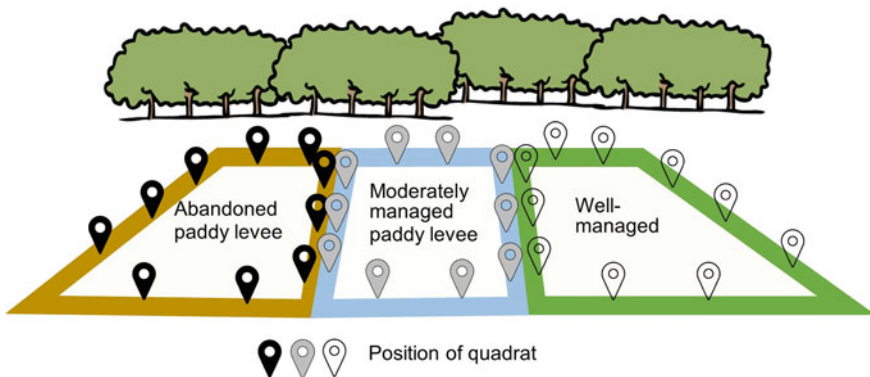
### 12.4.2 Research Hypothesis

Based on the research questions described above, the following three research hypotheses were formulated.

- If paddy levee grassland is mowed infrequently, less competitive plant species will be eliminated due to the dominance of certain more competitive plant species, and overall species diversity will decrease.
- Increasing the frequency of mowing in the paddy levee grassland enables new plant species to disperse from surrounding areas to sites where disturbance of the vegetation has occurred, increasing the diversity of plant species, and making species composition more similar across the area.
- The composition of the plant community in the paddy levee grassland is affected not only by the frequency of mowing but also by factors of the soil and light environments that fluctuate on the quadrat scale.

### 12.4.3 Study Design

The student team explored paddy levee grasslands in the study site and identified three plot types prior to the survey: relatively well-managed areas, mostly abandoned areas, and intermediate areas between the first two types. The team haphazardly set 10 quadrats in each of these three plots (Fig. 12.6).



**Fig. 12.6** Study design for examining comparative human influences on the plant communities of paddy levees



In the design shown in Fig. 12.6, each quadrat cannot be considered independent for assessing the impact of the mowing operation, as the mowing management operation is performed uniformly across the entire paddy levee grassland (i.e., pseudo-replication; see 12.3.2 Precautions for sampling design).

Therefore, the results obtained from this study design are simple comparisons of plant species diversity and community composition among the three selected paddy levees, and interpretation of the results must be careful and conservative. To interpret the results as representing the general response of the plant community to mowing, three or more study sites are needed for each treatment level of paddy levees with different mowing frequencies.

### ***12.4.4 Illustrating Results and Formulating Discussion***

In this section, we provide an example of how to summarize the results based on the survey design of Case 1 (see 12.4 Case Study 1) for comparison of plant species diversity among multiple habitats. We focus on the relationship between species ecology and the environment by summarizing the results using functional groups, which are species groups that respond similarly to certain environmental conditions (see 12.4.4.2 Comparing species diversity and similarity).

#### **12.4.4.1 Data Treatment**

The first step in summarizing the results is to input the survey data into a spreadsheet application such as Microsoft Excel. At that time, we strongly recommend first creating a list, as shown in Fig. 12.7, rather than directly inputting the matrix of site  $\times$  plant species into the Excel sheet.

Next, the pivot table function of Excel can be used to create the matrix data required for various analyses. In addition to the site  $\times$  species matrix, a separate site  $\times$  environmental variable matrix should be created.

#### **12.4.4.2 Comparing Species Diversity and Similarity**

When describing the characteristics of a plant community or considering conservation strategies, the most basic and first requirement is creation of a species list for each study site. However, as the amount of information is too large to include the entire list in a paper or report, the information should be reduced using an appropriate index. Typical results are evaluated using plant species richness, diversity indices, and the composition of plant functional groups.

	A	B	C	D	E	F
1	Site	Plot	Quadrat Name	Species Name	Braun-Blanquet scale	Converted Coverage %
2	A	1	A1_1	<i>Miscanthus sinensis</i>	5	87.5
3	A	1	A1_1	<i>Imperata cylindrica</i>	3	37.5
4	A	1	A1_1	<i>Platycodon grandiflorus</i>	+	0.5
5	A	1	A1_2	<i>Miscanthus sinensis</i>	2	17.5
6	A	1	A1_2	<i>Pueraria montana</i> var. <i>lobata</i>	2	17.5
7	A	1	A1_2	<i>Thalictrum minus</i> var. <i>hypoleucum</i>	1	5.5
8	A	1	A1_3	<i>Miscanthus sinensis</i>	4	62.5
9	A	1	A1_3	<i>Artemisia indica</i> var. <i>maximowiczii</i>	1	5.5
10	A	1	A1_3	<i>Picris hieracioides</i> subsp. <i>japonica</i>	1	5.5
11	A	2	A2_1	<i>Imperata cylindrica</i>	2	17.5
12	A	2	A2_1	<i>Gentiana zollingeri</i>	1	5.5
13	:	:	:	:	:	:

**Fig. 12.7** Example of Excel datasheet input format for vegetation survey results. The coverage (Column F) is obtained by transforming Braun-Blanquet scale (column E) according to Table 12.1

### Species Richness (Species Density)

Both species richness and species density are values related to the number of species; specifically, the former represents the number of species per individual number (or biomass), while the latter represents the number of species per fixed area (or volume or weight) (Gotelli and Colwell 2001).

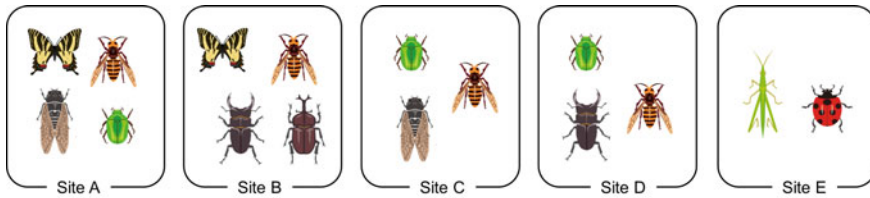
To assess the species density of a plant community, the number of species appearing in each quadrat is commonly calculated and then the average number of species appearing per unit area is determined for each study site (or treatment category). Such local diversity in the community is known as  $\alpha$ -diversity. On the other hand, the number of species appearing across the entire target area is designated  $\gamma$ -diversity.  $\beta$ -diversity is used as an index for evaluating the differences in species composition among sites within the target area and is obtained using one of the following two equations (Eqs. 12.1 and 12.2).

$$\beta = \gamma / \bar{\alpha} \tag{12.1}$$

$$\beta = \gamma - \bar{\alpha} \tag{12.2}$$

where  $\bar{\alpha}$  is the mean value of the number of species  $\alpha$  observed in each quadrat and  $\gamma$  is the number of species observed in the target area.

If circumstances allow only a limited number of habitats to be conserved,  $\alpha$ ,  $\beta$ , and  $\gamma$  diversities should be used as criteria for selecting locations for preferential



Site selection for biodiversity conservation	$\alpha$ diversity	$\beta$ diversity	$\gamma$ diversity
A+B+C+D	$(4+4+3+3)/4 = 3.5$	$6/3.5 = 1.71$	6
A+B+D+E	$(4+4+3+2)/4 = 3.25$	$8/3.25 = 2.46$	8

**Fig. 12.8** Examples of methods used to select target areas for habitat conservation based on  $\alpha$ ,  $\beta$ , and  $\gamma$  diversity

conservation. For example, rather than simply preferentially conserving areas with high  $\alpha$ -diversity, selecting multiple habitats with high  $\beta$ -diversity and  $\gamma$ -diversity will lead to better conservation of species diversity within the region (Fig. 12.8).

Furthermore, if the amount of research effort (number of quadrats) differs greatly among study sites or treatment levels being compared, especially if insufficient research effort is used in a survey area with high  $\beta$ -diversity,  $\gamma$ -diversity may be underestimated and the measured  $\alpha$ -diversity may not represent the situation throughout the area.

Under these conditions, a statistical method called rarefaction can be used to compare the number of species considering research effort. The freeware *Estimate S* (Colwell 2013) is widely used to calculate rarefaction. Please refer to the software manual for details of the analysis method.

### Evaluation Using Diversity Indices

A diversity index evaluates diversity based on the richness and evenness of species in a community. Shannon–Wiener  $H'$  (Eq. 12.3) and Simpson index of diversity  $D$  (Eq. 12.4) are commonly used. The  $H'$  index is influenced by species occurring at low frequency, while the  $D$  index is more susceptible to effects from the dominant species.

$$H' = - \sum_{i=1}^S p_i \ln p_i \tag{12.3}$$

$$D = 1 - \sum_{i=1}^S p_i^2 \tag{12.4}$$

$$D = 1 / \sum_{i=1}^S p_i^2 \quad (12.5)$$

where  $S$  is the total number of species in the community and  $p_i$  is the ratio of the abundance of species  $i$  to the total abundance of the community. When calculating Shannon–Wiener  $H'$ , the base of the logarithm is  $e$ , but 2 or 10 may be used. Therefore, you must carefully check what base has in fact been used in any comparative studies. Furthermore, reciprocal Simpson's index of diversity  $D$  could be calculated (Eq. 12.5). Therefore, you must also check which index has been used.

### Evaluation Based on Plant Functional Types

Plant species with similar morphological or ecological characteristics that do not necessarily align with phylogenetic relationships are called plant functional types (PFTs). Examples of PFTs include growth type, dormancy form, flowering time, seed size, disseminule form (seed dispersal type), native or nonnative species, and habitat type.

Comparing the composition of PFTs among study sites (or treatment levels) is useful for determining the characteristics of each community and the mechanism of the biological response to environmental changes. However, digitization of the database listing PFTs in Japan is incomplete. Therefore, each observed type should be validated using the literature, followed by creation of a database. For Japan, Miyawaki and Okuda (1991) and the Chiba historical Material Research Foundation (2003) are commonly used botanical pictorial resources that describe these types.

#### 12.4.4.3 Comparison of Environmental Conditions

Comparing the mean values of various environmental variables among study sites is the first step in examining the factors that explain differences in species composition. First, a boxplot for each study site (or the treatment level) should be made representing the measured values of the environmental conditions of interest. Close observation of the raw data for outliers and differences in the mean value are essential to such comparisons. For more details on how to detect outliers and what to do with them, see Benhadi-Marín (2018).

Then, a statistical test of the difference in mean values is performed. If environmental variables with significantly different mean values are found among the study sites being compared, these variables can be evaluated to identify possible relationships with the difference in species composition. However, as this method only demonstrates a correlation, experimental work is needed to support a causal relationship.

In addition, these data can be applied to advanced statistical analysis methods, such as evaluation of the relative importance of environmental factors using statistical

models. Please refer to other textbooks for details, such as Quinn and Keough (2002) and Gotelli and Ellison (2004).

#### **12.4.4.4 Items to Be Included in the Report**

The report summarizes the research scope and hypothesis, survey design and methods, summarizing and plotting of the results, and consideration of the results (interpretation of the data and conclusions drawn from multiple results).

##### ***Exercise 4***

Is a high-biodiversity community always considered to have high conservation value? In what cases can a community with low biodiversity have high conservation value? Please provide specific examples.

##### ***Exercise 5***

What plant functional types are needed to assess the habitat quality of flower-visiting insects (pollinators) through vegetation surveys?

##### ***Exercise 6***

What are the traits of plant species that are resilient to human disturbance? List as many functional types as you can think of.

#### ***Column 1 Tips for Plant Identification in Japan***

When conducting a vegetation survey, an illustrated botanical guide and list of regional flora are useful tools for identifying species names. Some plant guides (e.g., Numata and Yoshizawa 1975) describe the correspondence between Japanese names presented in Roman letters and scientific names, which is convenient for non-Japanese speakers. Lists of flora are often published at the prefectural level in Japan. Most of these lists are limited to description in Japanese, but scientific names are provided, providing useful clues for species identification. In addition, a Red Data Book containing a list of endangered species has been published at the prefectural level, which contains some information about species distributions.

For those who have begun to learn plant species identification, the first obstacle when using a specialized pictorial guide for identification is identifying the family or genus name of the plant you want to identify. For this purpose, a comprehensive judgment must be made based on the characteristics of plant organs such as flowers, stems, and leaves, which may be frustrating if the names of these parts are unfamiliar.

In such cases, an application can be used to identify species names from images using artificial intelligence technology. As a free application for smartphones and tablets, we recommend the “iNaturalist” app, which can be used in English. In many cases, iNaturalist cannot correctly identify a species from a photograph, but instead presents candidates of higher taxonomic levels such as the genus name and family name, providing clues that can be used to identify species further using pictorial guides.

## Column 2 Multivariate Analysis of Plant Communities

Statistical analysis in ecology has made remarkable progress due to the improvement of computer performance and has become an indispensable tool for students studying modern community ecology.

Multivariate statistical methods such as ordination and classification show great promise in exploratory research. Ecological knowledge about each species is undoubtedly essential to interpreting the results. However, identifying ecologically meaningful patterns from a large number of data and determining their relevance to environmental variables can be useful when constructing and verifying research hypotheses. These methods are collectively known as “data mining,” and as the name suggests, finding patterns can feel like a treasure hunt. For detailed explanation of these methods, please refer to another textbook (e.g., Quinn and Keough 2002; Šmilauer and Lepš 2014; Borcard et al. 2018).

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