Seed bank heterogeneity and its association with vegetation and micro-environmental variables in a secondary forest in Kyoto city, Japan

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Abstract: Buried seeds in forest soils have attracted attention as a natural greening material, making predictions of their number and spatial distribution important. We aim to assess the degree of variation in forest seed bank characteristics based on a nested sampling approach. The study was conducted in a secondary forest that consists of four patch types (conifer, canopy gap, deciduous broadleaf tree, and evergreen broadleaf tree) in a 100 m slope. Three 5 × 5 m plots containing three 20 × 20 cm quadrats were established under each patch type. We obtained soil samples from a depth of 0–5 cm, and seedling emergence was monitored over 5 months. In total, 116 seeds germinated (6.4 L), and twenty-five species were identified, with a dominance of the shrub Eurya japonica and the conifer Chamaecyparis obtusa. Variations in seed bank density were small between patches but were large between plots within the same patch type, and the latter accounted for approximately half of the total variances. The seed bank density tended to decrease in high-light environments (Indirect Site Factor = 0.12–0.16) and increase in plots with high densities of acorn-producing overstory trees, although there were also substantial within-plot variations in seed bank density, and further studies are necessary to confirm the results. Our simple method of nested sampling and statistical analysis for evaluating spatial heterogeneity of forest seed bank and for analyzing correlations with vegetation and micro-environmental factors are useful for considering effective sampling strategies of forest topsoil for revegetation practices.

Key word: forest topsoil, Nested ANOVA, revegetation, sampling strategy, Satoyama, seed bank

1. Introduction

Forest seed banks have attracted attention as a "greening material" due to their natural biodiversity and genetic background. Topsoil in a natural forest has been used in the creation of seminatural ecosystems (i.e., biotopes) in urban areas (Hosogi & Kameyama, 2004, 2006; Skrindo & Pedersen, 2004), as urban biotopes with similar flora and genetic backgrounds to natural forests are expected to have more ecological functions than artificial green areas. For example, ur-
ban biotopes function as an ecological corridor for wild animal movements (Hong et al., 2013). Thus, urban biotopes with natural vegetation contribute to the formation of ecological networks and biodiversity conservation in regional ecosystems. Furthermore, there have been several attempts to use forest topsoil for greening of bare land that has been created by artificial disturbances such as road construction (Koch, 2007; Fowler et al., 2015), enabling natural revegetation without any potential threat to indigenous ecosystems. Therefore, the ability to predict the number, species composition, and spatial distribution of forest seed banks is important for the successful application of forest topsoil as a greening material.

Understanding patterns in the number, species composition, and spatial distribution of forest seed banks is not straightforward because they are highly heterogeneous and affected by multiple environmental and biological factors. For example, the density, spatial distribution, and seed dispersal mode of the aboveground vegetation affect the density and composition of seed banks (e.g., Nakagoshi, 1985; Matlack & Good, 1990; Hyatt & Casper, 2000; Olano et al., 2002; Stark et al., 2008); and microenvironmental factors, such as soil moisture levels and the light environment, affect the preservation and dormancy status of buried seeds (e.g., Leckie et al., 2000; Wókiewicz & Kwiatkowska-Falińska, 2010). Furthermore, past vegetation may have historical effects on seed banks over long periods (e.g., Bossuyt & Hermy, 2001) and stochastic spatial variation in seed dispersal also exists.

Previous studies have quantified the degree and spatial scale of variation in forest seed bank characteristics, which is necessary for studying the factors that affect seed banks and provide useful information for the application of forest topsoil as a greening material. The species-area relationship has been used to explain the relationship between the numbers of species germinated from soil samples and the amounts of soil sampled (e.g., Csontons, 2007), and has led to a discussion of the size and number of soil samples that are necessary to obtain representative samples (e.g., Shen et al., 2014). Spatial autocorrelation analysis based on grid-sampling has also been adopted to estimate the patch size of seed bank distribution (e.g., Olano et al., 2002; Plue et al., 2010, 2011), which has led to a discussion on the appropriate distance between sampling points to make them statistically independent (e.g., Plue et al., 2011). Nested sampling design and statistical analysis was adopted by Lavorel et al. (1991) for estimating the degree and spatial scale of variation in forest seed bank characteristics. The factors that affect seed bank characteristics are distributed across a wide range of spatial scales, from microenvironmental factors such as soil moisture and light level changes in the forest understory, which occur at a small spatial scale (<1 m), to the density and composition of aboveground vegetation, which can change over a scale of a few meters, through to historical vegetation characteristics, which may differ over a few kilometers. Since these factors work at different spatial scales to have a combined effect on seed bank abundance and composition at a particular site, any assessment of their relative importance requires a nested sampling design and analysis.

We therefore employed a nested sampling approach to partition variation in forest seed bank data into components at different spatial scales and to assess the level of variation in each. Lavorel et al. (1991) conducted the study in a landscape consisting of a mosaic of successional stages from ploughed fields to coppices in subhumid Mediterranean region and demonstrated a large heterogeneity of seed bank composition between fields with different successional stages and relatively homogenous seed bank within each field. We conducted our study in a secondary forest in warm temperate region, where much more heterogeneity exists in microenvironment as well as vegetation in a field, which provided an opportunity to assess a finer-scale variability of seed bank composition in a natural environment and to search for microenvironmental and local vegetation parameters with significant associations with seed bank density and composition. Based on the results, we discuss (i) the mechanisms that cause forest seed bank variability at different spatial scales and (ii) an appropriate strategy for sampling forest topsoil for use as a natural greening material.

2. Materials and methods

2.1 Study site

The study was carried out in a secondary forest at the Kaminogawa Experimental Forest Station of Kyoto University, Japan (35°04’ N, 135°46’ E, 150 m altitude). The mean annual temperature at this site is 14.6°C, and the mean annual precipitation is 1,582 mm. The forest in this area primarily comprises Japanese red pine (Pinus densiflora Sieb. et Zucc.) and cypress (Chamaecyparis obtusa Endl.), which regenerated naturally at least 80 years ago. However, the red pine trees have been devastated by pine wilt disease since the end of the 1960s and the infected trees have been logged over the past 40 years. Consequently, most of the red pine trees have now been removed, and several broadleaf tree species have naturally regenerated.

The mixed stand with conifer and broadleaf trees distributed in a southeast-facing slope was chosen for the sampling area. Along the slope, four patch types can be readily recognized according to the canopy species and condition (Fig. 1): (C) The conifer C. obtusa dominated patch, mainly distrib-
uted in the ridge of the slope; (G) Canopy gap patch from the upper to the middle part of the slope; (D) Deciduous broadleaf tree, such as *Quercus serrata* Thunb. and *Lyonia ovalifolia* Drude var. *elliptica* dominated patch in the middle part of the slope; and (E) Evergreen broadleaf tree, such as *Q. glauca* Thunb., and *Ilex pedunculosa* Miq. dominated patch, in the lower part of the slope.

2.2 Sampling design

Three $5 \times 5$ m plots were randomly established in each of four patch types in the sampling area, and three $20 \times 20$ cm quadrats were established in the center of each plot (Fig. 1). Litter layer was removed, and soil was sampled at 0-5 cm depth from soil surface beneath the litter layer. Because litter layer contains many gap spaces between fallen leaves, branches, and their debris, accurate sampling of equal amounts of soils at equal depths cannot be done, so that we removed the litter layer. We then used a stainless sieve with 3-mm mesh to remove large stones and roots and to homogenize sampled soil. A total of 36 soil samples ($=4$ patch types $\times 3$ plots $\times 3$ quadrats) were collected in April 2015 before seedling emergence and kept in plastic bags in a dark and cool room (4°C) until germination experiment.

2.3 Vegetation survey and environmental measurements

The stem diameters of all overstory plants (>1.3 m tall) were measured at a height of 1.3 m, and the stem heights of all understory plants (<1.3 m tall) were measured in each plot. Hemispherical photographs were also taken at a height of 1.3 m in the center of each plot using a Nikon digital camera (COOLPIX 990) with a Nikon fisheye lens (FC-E8). These photographs were taken in October 2015 after the completion of overstory leaf expansion, and in March 2016 after leaf fall and before the beginning of leaf flush in deciduous trees. Hemiview software (DeltaT Devices, Burwell, Cambridge, UK) was then used to calculate the indirect site factor (ISF), which indicates the proportion of diffuse solar radiation reaching a given location relative to an open site. Slope inclination was also measured in the center of each plot. Seed dispersal modes were estimated based on the observation of fruit morphology.

2.4 Seed bank germination experiment

Plastic planters with a surface area of $50 \times 18$ cm were used for the seed bank germination experiment. First, a mixture of commercial soils (well-drained soil, [Akadama] and leaf mold; 3 : 5 v/v) was placed into each of 19 planters to a depth of 8 cm. The surface of each planter was then partitioned into two $25 \times 18$ cm areas, giving a total of 38 areas. Then, 0.5 L of each of the 36 forest soil samples was randomly placed in one of the partitioned areas and spread over the surface. No forest soil was added to the remaining two partitioned areas as a control. The planters were placed in a room maintained at 25°C and 40-W fluorescent lamps for plant cultivation (NEC FL 40 SBRHG) were set at 72 cm above the surface of each planter to give a photosynthetic photon flux density (PPFD) of 24 $\mu$mol $\cdot m^{-2} \cdot s^{-1}$ at the surface. The planters were watered every few days and the lamps were set on a 12 h : 12 h light-dark cycle. Seedling germination was recorded weekly from June to October 2015.

2.5 Data analysis

Two-level nested analysis of variance (Nested ANOVA, Sokal & Rohlf, 1995) was used to analyze variation in the density and species of seeds that germinated from the forest soils at different sampling levels. The uppermost level was

![Figure 1](https://example.com/figure1.png)

**Figure 1** Spatial distribution of the study plots and overview of the sampling design. Studied forest is located on a southeast-facing slope and contained four patch types along the slope: C, conifer, *Chamaecyparis obtusa* dominated patch at the ridge of the slope; G, canopy gap patch from the upper to the middle; D, deciduous broadleaf tree dominated patch at the middle; and E, evergreen broadleaf tree dominated patch at the lower part. Numbers above contour lines show altitudes. Three $5 \times 5$ m plots were randomly established in each of the four patch types (● = C, ○ = G, ✕ = D, △ = E), and three $20 \times 20$ cm quadrats were placed in each plot for soil sampling.
patch type, while the three replicate $5 \times 5$ m plots were nested below this (Fig. 1) and the three replicate $20 \times 20$ cm quadrats were nested within the plots. Variances (mean squares (MS)) in the seedling data among patch types (MS$_{vege}$), among plots within patch types (MS$_{plot}$), and among quadrats within plots (MS$_{quad}$) were calculated. The following equations were then used to decompose each of these variances into different components (Sokal and Rohlf 1995):

$$
MS_{vege} = \sigma^2 + n\sigma^2_{b,c,a} + nb\sigma^2_{A}
$$

$$
MS_{plot} = \sigma^2 + n\sigma^2_{b,c,a}
$$

$$
MS_{quad} = \sigma^2
$$

where $\sigma^2_{A}$ is the variation among patch types, $\sigma^2_{b,c,a}$ is the variation among plots within each patch type, and $\sigma^2$ is the variation among quadrats within each plot; and $n$ is the number of quadrats per plot and $b$ is the number of plots per patch type, both of which were three in this study. We computed MS$_{vege}$/MS$_{quad}$ to determine the significance of $\sigma^2_{b,c,a}$ and MS$_{vege}$/MS$_{plot}$ to determine the significance of $\sigma^2_{A}$. Any variance component that was found to be negative was assumed to be zero. For the seed bank variables that show significant plot-level variations ($p < 0.01$), we analyzed Pearson’s correlation coefficients between the seed bank variables and plot-level microenvironmental (light level, slope inclination) and vegetation characteristics (e.g., understory and overstory plant density).

3. Results

3.1 Vegetation, light environment, and topography

The vegetation survey identified a total of 33 species across all of the plots that mostly comprised woody perennial plants (Table 1). Vegetation characteristics in different patch types are summarized in Table 2. Briefly, the conifer patch (C; Fig. 1) was characterized by the dominance of C. obtusa and the presence of the evergreen broadleaf tall tree Castanopsis cuspidata. By contrast, overstory plants in the broadleaf tree dominated patch (D, E) comprised the deciduous tree L. ovalifolia var. elliptica and the evergreen trees I. pedunculosa and Q. glauca. The subcanopy layer in the evergreen broadleaf tree dominated patch (E) had a higher density of the evergreen shrubs C. japonica and E. japonica than occurred in the deciduous broadleaf tree dominated patch (D). Understory vegetation was the most diverse in the canopy gap patch (G), with seedlings of Rhododendron shrub species in particular being concentrated under canopy gaps. Understory vegetation in the evergreen broadleaf tree dominated patch (E) was characterized by the dominance of seedlings of the shade-tolerant tree Q. glauca.

There were clear differences in light environments between the patch types (Fig. 2). The three plots in the conifer patch were under closed canopy during all four seasons (ISF < 0.1), whereas those in the broadleaf tree patches showed large increases in light levels after leaf fall from the deciduous overstory trees, with this tendency being most pronounced in the deciduous broadleaf tree dominated patch. Slope inclination also differed between path types (Fig. 3). Conifer patch (C) was located at the ridge of slope with a flat topography, while the slope inclination increased from canopy gap patch (G) to the deciduous and evergreen broadleaf tree-dominated patches (D, E) located from upper to lower part of the slope.

Seed dispersal modes were estimated based on fruit morphology (Table 1). The conifer trees C. obtusa and Pinus densiflora with wing seeds (samara), the liana Trachelospermum asiaticum with hairy seeds (comose), the fern Dicranopteris linearis with sporocarps are categorized as wind-dispersed. Ericaceae plants (such as L. ovalifolia, R. reticulatum, Pieris japonica) and Clethra barbinervis have automatically-opened, dry fruits (capsule) and are considered as gravity-dispersed. In contrast, plants form fresh and succulent fruits, such Jex pedunculosa, E. japonica, Vaccinium spp. are all categorized as animal-dispersed (most probably by birds). Mallotus japonicas is also known as bird-dispersed (Sato & Sakai 2005). Oak trees, Quercus spp. and Castanopsis cuspidata with acorns are primarily dispersed by gravity and can be secondarily dispersed by wood mice (Iida 2006) and birds such as Garrulus glandarius and Nucigrage caryocatactes (Masaki 2009).

3.2 Seed bank density and composition

In total, 116 seeds germinated during the 5-month germination experiment, with an average of 6.4 germinants L$^{-1}$ of surface soil (0–5 cm depth). More than 90% of these germinations occurred during the first month of the experiment (Fig. 4). There were a few seedling deaths after germination (n = 14). A total of 25 species were identified (Table 1): Asteraceae spp. includes at least five species according to morphological differences, although their species names cannot be determined. Table 1 shows estimates of seed bank density (Number m$^{-2}$) at 0–5 cm depth by multiplying the number of germinations per 18 L topsoil (0–5 cm depth) × 50 ÷ 18. Table 3 shows the numbers of germinations from 4.5 L topsoil sampled in each of four patch types. The dominant species were the shrubs E. japonica (n = 41; Table 3, density = 114 per m$^2$; Table 1) and Rhododendron spp. (n = 8, density = 22 per m$^2$), and the conifer Chamaecyparis obtusa (n = 11, density = 31 per m$^2$), all of which were present in the vegetation in the study site (Table 1). There were also a few germinations of Morus australis, Rubus sp., and Poaceae sp., none of which were found during the vegetation survey (Table 1).

Seed bank composition and density tended to differ be-
Table 1  Density of plant species in vegetation and seed bank in the study site in a secondary forest in Kyoto city, Japan. The stem diameters of all overstory plants (>1.3 m tall) were measured at a height of 1.3 m and used to calculate total basal area (m² ha). Number of all understory plants (1.3 m tall) was counted.

| Name                                    | Family              | Growth habit | Fruit morphology (Seed dispersal) | Overstory basal area (m² ha) | Understory stem density (Number m⁻²) | Seed bank density (Number m⁻²) |
|------------------------------------------|---------------------|--------------|----------------------------------|-------------------------------|--------------------------------------|-------------------------------|
| Chamaecyparis obtusa Sieb. et Zucc.     | CUPRESSACEAE        | ET           | Samara (W)                       | 23.11                         | 0.92                                 | 31                            |
| Ilex pedunculosa Miq.                   | AQUIFOLIACEAE       | ET           | Berry (A)                        | 4.78                          | 3.52                                 | —                             |
| Quercus glauca Thunb.                   | FAGACEAE            | ET           | Acorn (G A)                      | 4.09                          | 9.80                                 | —                             |
| Lyonia ovalifolia Drude var. elliptica  | ERICACEAE           | DT           | Capsule (G)                      | 2.40                          | 0.04                                 | 17                            |
| Castanopsis cuspidata Schottky var. cuspidata | FAGACEAE       | ET           | Acorn (G A)                      | 1.88                          | 0.40                                 | —                             |
| Photinia glabra (Thunb.) Maxim.         | ROSACEAE            | ET           | Berry (A)                        | 1.62                          | 1.04                                 | —                             |
| Clearya japonica Thunb.                 | THEACEAE            | ET           | Berry (A)                        | 0.80                          | 2.08                                 | —                             |
| Eurya japonica Thunb.                   | THEACEAE            | ES           | Berry (A)                        | 0.28                          | 0.84                                 | 114                           |
| Rhododendron reticulatum D. Don         | ERICACEAE           | DS           | Capsule (G)                      | 0.28                          | 2.56                                 | 22                            |
| Pinus densiflora Sieb. et Zucc.         | PINACEAE            | ET           | Samara (W)                       | 0.24                          | 0.24                                 | —                             |
| Pieris japonica (Thunb.) D. Don         | ERICACEAE           | ES           | Capsule (G)                      | 0.22                          | 0.84                                 | —                             |
| Quercus serrata Thunb.                  | FAGACEAE            | DT           | Acorn (G A)                      | 0.16                          | 0.68                                 | —                             |
| Vaccinium bracteatum Thunb.             | ERICACEAE           | ES           | Berry (A)                        | 0.14                          | 0.32                                 | 3                             |
| Rhododendron macrosepalum Maxim.        | ERICACEAE           | SES          | Capsule (G)                      | 0.06                          | 1.84                                 | 22                            |
| Poutriacia siliosa var. laevis. (Thunb.) Stapf | ROSACEAE       | DT           | Berry (A)                        | 0.04                          | 0.04                                 | —                             |
| Symlocos prunifolia Sieb. et Zucc.      | SYMPLOCAEAE         | ET           | Berry (A)                        | 0.03                          | 0.28                                 | —                             |
| Vaccinium hirtum Thunb.                 | ERICACEAE           | DS           | Berry (A)                        | 0.01                          | 1.64                                 | 3                             |
| Evodia panas inoaxes (Sieb. et Zucc.) Nakai | ARAIACEAE        | ET           | Berry (A)                        | —                             | 2.12                                 | —                             |
| Tracheloperum asiaticum (Sieb. et Zucc.) Nakai | APOCYNACEAE   | L            | Comose (W)                       | —                             | 1.72                                 | —                             |
| Smilax china L.                         | LILACEAE            | L            | Berry (A)                        | —                             | 1.60                                 | —                             |
| Dicranopteris linearis (Burm.f.) Underw. | GLEICHENIACEAE     | F            | Sporangium (W)                   | —                             | 1.20                                 | —                             |
| Rhus trichocarpa Miq.                   | ANACARDIACEAE       | DT           | Berry (A)                        | —                             | 0.68                                 | 3                             |
| Ilex crenata Thunb.                     | AQUIFOLIACEAE       | ET           | Berry (A)                        | —                             | 0.60                                 | —                             |
| Acanthopanax scio phylloides. Fr. et Sav. | ARALIACEAE       | DT           | Berry (A)                        | —                             | 0.32                                 | —                             |
| Ardisia crenata Sims                    | MYRSINACEAE         | ES           | Berry (A)                        | —                             | 0.20                                 | —                             |
| Ardisia japonica (Thunb.) Bl.           | MYRSINACEAE         | ES           | Berry (A)                        | —                             | 0.16                                 | —                             |
| Commelina communis L.                   | COMMELINACEAE       | AH           | ND (U)                           | 0.08                          | 0.08                                 | 3                             |
| Juniperus rigida Sieb. et Zucc.         | CUPRESSACEAE        | ET           | ND (U)                           | 0.08                          | 0.08                                 | —                             |
| Clethera barbinervis Sieb. et Zucc.      | CLETHRACEAE         | DT           | Capsule (G)                      | 0.04                          | 0.04                                 | 3                             |
| Abelia spathulata Sieb. et Zucc.         | CAPRIFOLIACEAE      | DS           | Berry (A)                        | 0.04                          | 0.04                                 | —                             |
| Osmanthus heterophyllus (G.Don) P.S. Green | OLEACEAE        | ES           | Berry (A)                        | —                             | 0.04                                 | —                             |
| Sasa kurilensis (Rupr.) Makino et Shibata | POACEAE            | PG           | Caryopsis (U)                    | —                             | 0.04                                 | —                             |
| Viburnum erosum Thunb. var. punctatum Fr. et Sav. | CAPRIFOLIACEAE | DS           | Berry (A)                        | 0.04                          | 0.04                                 | —                             |
| Asteraceae spp.                          | ASTERACEAE          | AH           | PH (U)                           | —                             | —                                    | 22                            |
| Morus australis Poir.                   | MORACEAE            | DT           | Berry (A)                        | —                             | —                                    | 14                            |
| Amaranthus sp.                           | AMARANTHACEAE       | AH           | ND (U)                           | —                             | —                                    | 11                            |
| Poaceae sp.                             | POACEAE             | PG           | ND (U)                           | —                             | —                                    | 11                            |
| Phytolacca americana L.                 | PHYTOLACACEAE       | PH           | Berry (A)                        | —                             | 8                                     | —                             |
| Rubus sp.                               | ROSACEAE            | DS           | Berry (A)                        | —                             | 8                                     | —                             |
| Houttuynia cordata Thunb.               | SAURURACEAE         | PH           | ND (U)                           | —                             | 3                                     | —                             |
| Maclura cordata (Willd.) R.Br.          | PAPAVERACEAE        | PH           | ND (U)                           | —                             | 3                                     | —                             |
| Mai lotus japonicus (Thunb.) Mueller-Arg. | EUPHORBIACEAE     | DT           | Berry (A)                        | —                             | 3                                     | —                             |
| Rubiaceae sp.                           | RUBIACEAE           | AH           | ND (U)                           | —                             | 3                                     | —                             |
| Symlocos cinnamini (Lour.) Druce var. leucocarpa (Nakai) | SYMPLOCAEAE   | DS           | Berry (A)                        | —                             | 3                                     | —                             |

¹, ES = Evergreen Shrub; ET = Evergreen Tree; DS = Deciduous Shrub, SES = Semi Evergreen Shrub; DT = Deciduous Tree; PG = Perennial Grass; PH = Perennial Herb; AH = Annual Herb; L = Liana; F = Fern.

¹, ND = Not Determined.

¹, A = Animal-dispersed; W = Wind-dispersed; G = Gravity-dispersed; U = Unknown.

¹, Multiplying the number of germinations from 18 L soils (0–5 cm depth) × 50 = 18. Density of Rhododendron reticulatum and R. macrosepalum seedlings and that of Vaccinium bracteatum and V. hirtum are calculated by using total numbers of germinations of the two species as they cannot be distinguished at the seedling stage.
Table 2  Vegetation characteristics of three 5 × 5 m plots randomly established in each of four patch types in a secondary forest in Kyoto city, Japan. (a) Total basal area at a height of 1.3 m of all overstory trees per plot. (b) Total number of understory plants (<1.3 m tall) per plot. The dominant species are in bold, and characteristic species in each vegetation type are highlighted by underlines.

(a)  

| Vegetation type                          | Total Basal area | Conifer patch (C) | Canopy gap patch (G) | Deciduous broadleaf tree dominated patch (D) | Evergreen broadleaf tree dominated patch (E) | Total |
|-----------------------------------------|------------------|-------------------|----------------------|-----------------------------|---------------------------------------------|-------|
| Conifer patch (C) plot                  |                  |                   |                      |                             |                                             |       |
| plot C1                                 | 2,317            | 3                 | 178                  | 597                         | 718                                        | 49,602|
| plot C2                                 | 2,163            | 3                 | 136                  | 569                         | 715                                        | 47,143|
| plot C3                                 | 2,404            | 7                 | 90                   | 128                         | 177                                        | 64,498|
| Canopy gap patch (G) plot               |                  |                   |                      |                             |                                             |       |
| plot G1                                 |                  | 1                 | 26                   | 55                          | 81                                         | 436,330|
| plot G2                                 |                  | 3                 | 26                   | 55                          | 81                                         | 436,330|
| plot G3                                 |                  | 7                 | 106                  | 107                         | 296                                        | 1,226|
| Deciduous broadleaf tree dominated patch (D) plot | | | | | | |
| plot D1                                 |                  |                   |                      |                             |                                             |       |
| plot D2                                 |                  |                   |                      |                             |                                             |       |
| plot D3                                 |                  |                   |                      |                             |                                             |       |
| Evergreen broadleaf tree dominated patch (E) plot | | | | | | |
| plot E1                                 |                  |                   |                      |                             |                                             |       |
| plot E2                                 |                  |                   |                      |                             |                                             |       |
| plot E3                                 |                  |                   |                      |                             |                                             |       |
| Total number                            |                  |                   |                      |                             |                                             |       |

(b)  

| Vegetation type                          | Total number | Conifer patch (C) | Canopy gap patch (G) | Deciduous broadleaf tree dominated patch (D) | Evergreen broadleaf tree dominated patch (E) | Total |
|-----------------------------------------|--------------|-------------------|----------------------|-----------------------------|---------------------------------------------|-------|
| Conifer patch (C) plot                  |              |                   |                      |                             |                                             |       |
| plot C1                                 | 3           | 7                 | 90                   | 11                          | 26                                         | 48,88 |
| plot C2                                 | 2           | 5                 | 12                   | 25                          | 54                                         | 48,72 |
| plot C3                                 | 4           | 7                 | 4                    | 26                          | 52                                         | 48,72 |
| Canopy gap patch (G) plot               |              |                   |                      |                             |                                             |       |
| plot G1                                 | 6           | 1                 | 21                   | 16                          | 42                                         | 48,107|
| plot G2                                 | 4           | 1                 | 21                   | 16                          | 42                                         | 48,107|
| plot G3                                 | 11          | 5                 | 14                   | 10                          | 24                                         | 48,107|
| Deciduous broadleaf tree dominated patch (D) plot | | | | | | |
| plot D1                                 |              |                   |                      |                             |                                             |       |
| plot D2                                 |              |                   |                      |                             |                                             |       |
| plot D3                                 |              |                   |                      |                             |                                             |       |
| Evergreen broadleaf tree dominated patch (E) plot | | | | | | |
| plot E1                                 |              |                   |                      |                             |                                             |       |
| plot E2                                 |              |                   |                      |                             |                                             |       |
| plot E3                                 |              |                   |                      |                             |                                             |       |
| Total number                            |              |                   |                      |                             |                                             |       |

3.3 Seed bank heterogeneity at different spatial scales

Table 4 shows the results of the nested ANOVA. Although seed bank characteristics tended to differ between patch types (Table 3), in Nested ANOVA patch type had no significant effect on either density or species richness of seed bank (p > 0.1). This indicates substantial within-patch variations exist in these seed bank characteristics. In fact, there were significant differences in the number of species and seed germinants (abundance) among plots within each patch type (p < 0.001). In particular, there were significant variations among plots within patch types in the number of woody plant species (F = 3.9, p < 0.01) and berry-producing species (F = 5.3, p < 0.001), and the number of germinants of berry-producing species (F = 5.3, p < 0.001) and dominant species, such as E. japonica (F = 5.1, p < 0.001) and C. obtusa (F = 4.5, p < 0.01) (Table 4).

Variance decomposition analyses of the density and species richness of seed bank data indicated that there were large amounts of variance among the quadrats within each plot (Fig. 5), which explained more than 35% of the total variance. In the case of traits that had insignificant differences among plots (p > 0.05; Table 4), such as the number of herbaceous plant species, capsule-producing species, the numbers of L. ovalifolia var. elliptica and M. australis germinants, the variation among quadrats within plots explained 60–91% of the total variance, indicating an aggregated distribution of their seeds at a smaller spatial scale than the quadrat.

3.4 Microenvironmental and vegetation effects

We analyzed the effects of light level, slope inclination, and vegetation characteristics (e.g., understory and overstory plant density) on density and species richness of seed bank. We identified weak but significant associations between some of the current vegetation characteristics and the seed bank data (Table 5). For example, there was significant negative correlation between the light level and the number
of germinants \( (r = -0.37, p < 0.05) \), indicating that seed bank density decreases in high-light environments. As Figure 6a shows, canopy gap patches with 0.12–0.16 ISF tended to have lower densities of seed bank than the other patches.

Neither density of understory plants nor total basal area of overstory tree had significant associations with number of species or germinations of all species \( (p > 0.05) \). Number of seed bank germination of berry-producing species has also no significant correlation with density of berry-producing overstory trees (Fig. 6c). In contrast, total basal area of broadleaf overstory trees and that of acorn-producing overstory trees had significant correlations with seed bank density and species richness (Table 5). The strongest correlation \( (r = 0.54, p < 0.001) \) was found between number of seed bank germination and total basal area of acorn-producing overstory trees (Fig. 6b).

There were also a few significant correlations between number of germinations of dominant species \( (E. japonica \) and \( C. obtusa) \) and current environmental and vegetation characteristics. Plots with lower slope inclinations tended to contain lower densities of germinations of \( E. japonica \) \( (r = 0.35, p < 0.05) \), but higher densities of germinations of \( C. obtusa \) \( (r = -0.38, p < 0.05) \). This is mainly due to the association of topography and vegetation: conifer patch is located at the ridge of slope with low slope inclinations (Fig. 3), and in the patch high density of \( C. obtusa \) germinants and low density of \( E. japonica \) germinants are observed (Table 3). Conifer patch has also high density of overstory trees, especially \( C. obtusa \) trees (Table 2); Consequently, number of germinations of \( C. obtusa \) was significantly higher in plots with
Table 3  Seed bank composition, density, and species richness in four patch types in a secondary forest in Kyoto city, Japan. Dominant species are in bold. C = Conifer patch; G = Canopy Gap patch; D = Deciduous broadleaf tree dominated patch; E = Evergreen broadleaf tree dominated patch.

| Patch type                   | C | G | D | E | Total |
|------------------------------|---|---|---|---|-------|
| **Eurya japonica** Thunb.    | 4 | 2 | 19| 16| 41    |
| **Chamaecyparis obtusa** Sieb. et Zucc. | 10| 0 | 0 | 1 | 11    |
| **Rhododendron** sp.        | 2 | 0 | 5 | 1 | 8     |
| **Lyonia ovalifolia** Drude var. elliptica | 1 | 4 | 0 | 0 | 6     |
| **Morus australis** Poir.   | 1 | 2 | 0 | 2 | 5     |
| **Amaranthus** sp.          | 0 | 0 | 3 | 1 | 4     |
| **Poaceae** sp.             | 2 | 0 | 1 | 1 | 4     |
| **Rubus** sp.               | 0 | 1 | 2 | 0 | 3     |
| **Phytolacca americana** L. | 1 | 0 | 1 | 1 | 3     |
| **Asteraceae** spp.         | 1 | 1 | 2 | 4 | 8     |
| **Others**                  | 2 | 1 | 2 | 4 | 22    |

Total no. of germinants\(^1\) 26 12 38 40 116
Total Number of species 11 7 11 13 25

\(^1\) Number of germinants per 4.5 L topsoil (= 0.5 L soil \(\times 3\) quadrats \(\times 3\) plots) sampled in each patch type. Species, of which number of germinant is only 1 over four patch types, are all grouped into “Others”. Unidentified species are excluded from the list.

\(^2\) Total number of germinants per 4.5 L topsoil for all species, including unidentified species.

Table 4  Nested analysis of variance for seed bank characteristics. Variables are (a) seed bank species richness (number of species) in a 0.5-L soil, which are categorized by growth-habit or dispersal-mode, and (b) seed bank density (number of germination) in five dominant species. PT, Patch Type; Plot, 5 \(\times\) 5 m plot; Quadrat, 20 \(\times\) 20 cm quadrat. ***, \(p<0.001\); **, \(p<0.01\); *, \(p<0.05\); ns, \(p>0.05\). Highly significant effects (\(p<0.01\)) are highlighted in bold.

| Variables                              | Statistics (\(n=36\)) | Nested analysis of variance |
|----------------------------------------|------------------------|-----------------------------|
|                                        | Mean | SD  | Min | Max | MS (df) | F-value |
| (a) Number of species                  |      |     |     |     |         |         |
| Total number of species                | 1.9  | 1.6 | 0   | 5   | 3.3     | 0.72 ns |
| Number of woody plant species          | 1.3  | 1.0 | 0   | 4   | 0.52    | 0.29 ns |
| Number of herbaceous plant species     | 0.61 | 0.90| 0   | 3   | 1.7     | 2.1 ns |
| Number of berry-producing species      | 0.92 | 0.81| 0   | 3   | 0.47    | 0.38 ns |
| Number of capsule-producing species    | 0.36 | 0.89| 0   | 4   | 0.18    | 0.37 ns |
| (b) Number of germination              |      |     |     |     |         |         |
| Total number of germination            | 3.2  | 2.9 | 0   | 11  | 18.5    | 1.4 ns  |
| Number of berry-producing species      | 1.6  | 1.9 | 0   | 10  | 8.8     | 1.4 ns  |
| Number of capsule-producing species    | 0.42 | 0.69| 0   | 2   | 0.32    | 0.58 ns |
| Number of Eurya japonica               | 1.1  | 1.9 | 0   | 9   | 8.0     | 1.5 ns  |
| Number of Chamaecyparis obtusa         | 0.31 | 0.89| 0   | 4   | 2.6     | 2.5 ns  |
| Number of Rhododendron sp.             | 0.22 | 0.48| 0   | 2   | 0.52    | 2.1 ns  |
| Number of Lyonia ovalifolia            | 0.17 | 0.45| 0   | 2   | 0.33    | 2.0 ns  |
| Number of Morus australis              | 0.14 | 0.35| 0   | 1   | 0.10    | 1.2 ns  |

significantly reduced the density of seed bank (Table 5). In highlight environments such as at the forest edge and under large canopy gaps, seed germination is promoted, which may decrease the density of the seed bank. By contrast, previous studies have reported that seed bank densities increase in higher light environments in old-growth forests (Leckie et al., 2000; Wókiewicz & Kwiatkowska-Falinska, 2010), where the closed forest understory results in light being a limiting resource for plants. Thus, the effect of the light environment on the seed bank density in a forest depends on the forest floor.

4. Discussion

In forests, current vegetation characteristics are not always good indicators of seed bank abundance or diversity, as reviewed by Hopfensperger (2007) and Bossuyt & Honnay (2008). In our studied forest, patch type explained <25% of the total variance in the seed bank data (Fig. 5). Because the different patch types were colocalized on a forest slope (Fig. 1), it is possible that seed dispersal by animals and wind may have mixed the composition and density of seeds on the forest floor.

By contrast, there was evidence of local variations in seed bank characteristics among plots within the same patch type (Table 4), which explained approximately half of the total variance (Fig. 5). Increasing light levels in the understory significantly reduced the density of seed bank (Table 5). In highlight environments such as at the forest edge and under large canopy gaps, seed germination is promoted, which may decrease the density of the seed bank. By contrast, previous studies have reported that seed bank densities increase in higher light environments in old-growth forests (Leckie et al., 2000; Wókiewicz & Kwiatkowska-Falinska, 2010), where the closed forest understory results in light being a limiting resource for plants. Thus, the effect of the light environment on the seed bank density in a forest depends on the forest floor.
on the range of light levels tested: a 5-10% increase in light level under small canopy gaps may increase the seed bank density through its positive effects on seed production, as indicated by the positive correlation between seed bank density and Ellenberg indicator value L from 1 (deep shade) to 3 (shade, mostly <10%) in a deciduous forest (Wókiewicz & Kwiatkowska-Falińska, 2010). In contrast, an increase in light level of >10% in large canopy gaps and forest edges may decrease the seed bank density by promoting seed germination. As Figure 6a shows, seed bank density decreased in the plots with ISF 0.12-0.16 located under large canopy gaps, exposed to 12-16% diffuse sunlight. The significant negative effect of light level on seed bank density in our study can be also associated with topographic effect. The canopy gap patch is located on the upper part of the slope (Fig. 1), and previous studies have reported that seed bank density of upper part of slope tends to be lower than that of lower parts due to soil erosion (Ashton et al., 1998; Yamase & Sekioka 2006; Yamase et al., 2009).

Previous seed-trap surveys in Japanese forests have reported that density of bird-dispersed seeds significantly increased near the fruiting trees of berry-producing species (Manabe et al., 1993; Kominami et al., 1998; Abe et al., 2005), one of which (Abe et al., 2005) was conducted in the same secondary forest with this study. This is because frugivorous birds forage on fruiting trees, and bird-dispersed seeds are increased beneath their canopies by the birds’ droppings. However, our seed bank survey shows that there was not significant correlation between number of seed bank germination of berry-producing species and total basal area of berry-producing overstory trees (Fig. 6c). The number of seed bank germination of E. japonica was not correlated

![Table 5](image)

| Table 5 | Correlation analyses between seed bank and vegetation characteristics at the plot level. Seed bank variables with significant plot-level variations (p<0.01; Table 4) were analyzed. Pearson’s correlation coefficients (r) are shown: *** = p<0.001, ** = p<0.01, * = p<0.05, ** = p>0.05. / = unable to analyze, n = 36. Significant correlations are highlighted in bold. |}

Table 5: Correlation analyses between seed bank and vegetation characteristics at the plot level. Seed bank variables with significant plot-level variations (p<0.01; Table 4) were analyzed. Pearson’s correlation coefficients (r) are shown: *** = p<0.001, ** = p<0.01, * = p<0.05, ** = p>0.05. / = unable to analyze, n = 36. Significant correlations are highlighted in bold.

| Seed bank characteristics at the plot level | Number of species | Number of germination |
|--------------------------------------------|-------------------|----------------------|
|                                            | All plant species | Woody plant species  | Berry-producing species | All plant species | Berry-producing species | Eurya japonica | Chamaecyparis obtusa |
| Light level (ISF) in fall                  |                   |                      |                      |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Light level (ISF) in early spring          |                   |                      |                      |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Slope inclination (Degree)                 |                   |                      |                      |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Density of understory plants (Number)      |                   |                      |                      |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Total basal area of overstory trees (cm²)  |                   |                      |                      |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Total basal area of broadleaf overstory trees (cm²) | 0.34*             | 0.29*                | 0.36*                |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Total basal area of berry-producing overstory trees (cm²) | 0.15*             | 0.11*                | 0.23*                |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Total basal area of acorn-producing overstory trees (cm²) | 0.35*             | 0.34*                | 0.30*                |                   |                      | Eurya japonica | Chamaecyparis obtusa |
| Total basal area of conspecific plants (cm²) |                   |                      |                      |                   |                      | Eurya japonica | Chamaecyparis obtusa |
with overstory density of conspecific species (Table 5). This indicates that densities of berry-producing trees at the scale of 5×5 m plot cannot be reliable indicators of increased seed bank density of local sites (20×20 cm quadrat) in the plot. Spatial distribution of bird-dispersed seeds may aggregate at smaller spatial scales than the plot, reflecting the presence/absence of local individual fruiting trees.

In contrast, we found that seed bank density significantly increased in plots containing high densities of acorn-producing trees (Table 5; Fig. 6b). The underlying mechanism is unclear, but one possible explanation is that oak trees may be favored by birds for perches due to their large crowns with horizontal branches, and seed depositions via bird droppings may increase under their canopies. In fact, numbers of seed bank germination of berry-producing species increased in plots with high densities of acorn-producing trees (Table 5). Tanaka and Sano (2013) reported that dominant berry-eating birds Hypsipetes amaurotis and Sturnus cineraceus in secondary forests in Japan tended to stay longer time in tall tree canopies than shrub layers in spring (breeding season of bird) probably due to utilization of tall trees as song posts, and this might have resulted in the increases in bird-dispersed seeds beneath the tall trees. Although total basal area of overstory trees was not significantly correlated with seed bank density of berry-producing species ($r = -0.16, p > 0.05$; Table 5) in this study, total basal area of broadleaf overstory trees excluding C. obtusa, of which canopy consists of dense foliage and looks unsuitable for birds’ perches, was significantly correlated with number of seed bank germination of berry-producing species ($r = 0.36, p < 0.05$). Thus, the abundance of broadleaf overstory trees, especially acorn-producing trees at the scale of 5×5 m plot could be an indicator of increased seed bank density of local sites in the studied forest, although there were also substantial within-plot variations in seed bank density (Fig. 6b). Further studies, including observation of bird behavior and seed dispersal are necessary to clarify the underlying mechanism and to confirm the result.

Seed bank density and species richness at the plot level are poorly associated with vegetation parameters, such as density of understory plants and total basal area of overstory trees (Table 5). This is because there were large variations in seed bank characteristics between the quadrats within plots, which explained 35–91% of the total variance (Fig. 5).

The seed dispersal of berry-producing species (such as E. japonica and M. australis) inevitably aggregate in a localized point with a few square centimeters’ area via deposition in bird droppings. Seed dispersal distance of capsule-producing species is expected to be low (<3 m) for 1–3 m tall shrubs (e.g., Rhododendron spp.). These factors may result in a clus-
tered distribution of the forest seed bank at a scale of tens of centimeters. Spatial clumping of the seed bank at a scale of <1 m should be a common feature in forests. For example, Matlack & Good (1990) reported that soil seed bank in a mixed coastal pine forest in New Jersey (USA) was clustered at a scale of 100 cm². In a secondary forest in Spain, Olano et al. (2002) detected a clumping structure in the seed bank at a scale of 0-8 m. Plue et al. (2010) has also reported the presence of fine-scale (<2 m) spatial structures in forest seed banks, and Plue & Hermy (2011) indicated that the spatial scale of seed bank clumping was ca. 30 cm. The fine scale variability in forest seed banks requires that many small samples have to be collected over a wide area to obtain a representative sample (Csontos, 2007; Plue & Hermy, 2011; Shen et al., 2014).

4.1 Implications for revegetation practices using forest seed banks

So where in secondary forest is the best soil located for revegetating an area with natural vegetation? In our studied forest, the avoidance of soils in high-light environments, such as at the forest edge and in large canopy gaps, and in upper parts of slope with soil erosion, would be an effective strategy in the study forest. Moreover, the soils that lie beneath acorn-producing trees are expected to have an increased density and species richness of seed bank, although the underlying mechanism is unclear. At the same time, however, we must recognize that there are substantial heterogeneities in forest seed banks at a scale of tens of centimeters, i.e., samples that are collected from small areas (e.g., 20 × 20 cm quadrats) will contain large variations in density and species richness of seed bank among samples.

The seed bank density of our study (6.4 germinants L⁻¹ of 0-5 cm topsoil) was not so high, and no germinant was observed for dominant overstory Quercus species. This may be caused by our sampling method, the removal of litter layer at sampling time. Because in the litter layer, acorns were sometimes included (Kawamura personal obs.), and previous studies have reported equal to five-times more seeds contained in litter layer than soil layer in warm-temperate forests (e.g., Mizusaki et al., 2000). Therefore, it will be better to use litter layers together in the case of greening practices. The seed bank of our study site includes only a few alien species (Phytolacca Americana L. and Asteraceae sp.) and consists of many native woody perennial plants (Table 1), which are representative plants in secondary forest in warm-temperate Japan, so that the forest topsoil can be suitable for the creation of urban biotope with natural vegetation.

However, some dominant species, such as Ilex pedunculosa, Clevera japonica, and Pieris japonica had no germinant in our experiment. The same results were reported by a previous study (Abe et al., 2005), which sampled soils together with litter layers in the same secondary forest and conducted germination experiments in outdoor. These species may have low viabilities of seed bank and or requirements of specific conditions (e.g., high light, temperature) for germination. Fujii (1997) identified abundant I. pedunculosa seeds in soils of secondary forests in Hyogo, Japan, where average density was estimated as 2,300 m⁻²/5 cm (depth), but 96% were empty seeds, indicating a low viability of buried seeds of this species. Therefore, for these three species, direct collection of seeds from plants and sowing might be more promising methods for their restoration than the use of forest seed banks.

5. Conclusion

We presented a simple method of nested sampling and statistical analysis for evaluating spatial heterogeneity of forest seed bank data and for analyzing correlations with vegetation and micro-environmental variables. This method can provide useful information for considering effective sampling strategies of forest topsoil for revegetation practices, as discussed earlier. However, the large variations in seed bank data need to increase the sample size (i.e., 36 quadrats) in order to confirm the results. The variance decomposition analysis clarified that the observed variations in seed bank data were primarily due to between-plot variations and secondarily within-plot ones (Fig. 5). Therefore, firstly, increased repetition of the 5 × 5 m plots within each patch type and secondarily, increased repetition of the 20 × 20 cm quadrats within the plot will be effective strategy to estimate real averages of seed bank density and species richness in the secondary forest and to elucidate the vegetation and micro-environmental factors affecting the seed bank heterogeneity. Furthermore, sampling data at different years can be integrated in the nested ANOVA as a new level of hierarchy (‘year’), allowing a comparative analysis of spatiotemporal heterogeneity of seed bank data. This will also significantly increase the usefulness of the research data for practical uses.

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