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ORIGINAL ARTICLE

Impact of Topsoil Stockpiling Methods on the Viability of Seed Banks

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Abstract

The aim of this study was to determine the appropriate stockpiling methods for revegetation by comparing the germination status of seed banks before and after preservation for 2 years. Soil temperature in stockpiled topsoil was higher in open treatment and at 1.5 m, whereas soil water content was maintained at lower levels (14.06-19.08%), than those in the control group. The seed banks in stockpiled topsoil had 48 species and 1,559 individuals, among which perennials showed the highest number in terms of life forms, whereas Compositae and Gramineae were dominant in terms of families. Based on seed bank type, persistent seed banks had the highest number of species, while transient seed banks had the highest number of individuals. By stockpiling period, the number of species in the seed bank started to increase after 24 months, while the number of individuals began increasing after 12 months and exceeded that of the control group after 24 months. Regarding the treatment of stockpiling methods, the number of species and individuals in open treatment were closer to those of the control group. When analyzed by height, the number of species and individuals were higher at 0 m, but still lower than those of the control group. A multivariate analysis of variance (MANOVA) showed that the optimal combination was obtained in open treatment and the number of individuals increased with the lengthening of the stockpiling period.

Key words : Top soil recycling, Number of species, Number of individuals, Stockpiling period, Temporal storage

1. Introduction

When restoring forest vegetation, various ecological vegetation restoration methods that are appropriate for the target vegetation are required. These methods include planting multistoried and uneven-aged forests based on the analysis of actual vegetation, ecological planting using prototypes, duplicate transplantation, model planting, and the use of seeds (Korea Forest Service, 2015). Among restoration methods that use seeds, the method utilizing topsoil makes use of the seed bank's potential to restore vegetation, preventing gene disturbance and enabling resources recycling (Hosogi and Kameyama, 2006; Park et al., 2010). As topsoil has abundant organic matter and a variety of microorganisms, it can be reused for topsoil preservation, as well as planting soil or revegetation materials for slope surfaces in order to rehabilitate damaged ecosystems (Itou and Kishizuka, 2008). Moreover, the seed bank preserved within the topsoil does not have any genetic disturbance issues (Koh, 2007) as it possesses a genetic variety derived from the accumulation of different genotypes during the passage of numerous generations (Templeton and

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Levin, 1979). Furthermore, it also has the advantage of being relatively inexpensive and possesses high chances for the emergence of original vegetation, as well as diverse plant species. The method has undergone research as it received attention as a technique using plant materials from the actual sites. The stockpiling of topsoil can largely be divided into two categories, namely the topsoil recycling method that gathers topsoil from the restoration site and the nearby topsoil collection method that brings topsoil from areas in the vicinity of the site. The former is considered ideal as it does not create disturbance (Hosogi and Matsue, 2010). For the topsoil recycling method, it would be most desirable to immediately use the collected topsoil on the restoration site. However, in practice, topsoil can be used on-site only when it does not have issues, such as excessive soil water content due to the penetration of rainwater or high soil temperature due to the decomposition of organic matter (Park et al., 2010), or after tentatively storing it for a certain period of time in a place unlikely to produce disturbance (Itou and Kishizuka, 2008). Thus, for the topsoil recycling method, long-term storage of topsoil using an open-air storage yard is required.

In studies where topsoil was used for revegetation after mining, the number of species and individuals of the actual vegetation was higher when topsoil was used immediately after the collection than when topsoil had been stored at an open-air site for 2 years (Tacey and Glossop, 1980; Ward et al., 1996). On the contrary, after storing the topsoil in the forest for 1 year, the number of germinated species in the seed bank decreased, but the number of wood individuals increased (Ueda et al., 2004). Although there are no practical issues regarding the usage of topsoil that had been stored for a long time in revegetation, it is necessary to study the method, environment, and period applied for storing the topsoil in the field, as well as to accumulate data on its use for revegetation (Hosogi and Matsue, 2010).

In fact, water and oxygen are essential for seed germination while light also serves as an important factor (Bidlack et al., 2011). Thus, this study aimed to analyze the influence of the topsoil storage method, environment, and period over topsoil revegetation potential by blocking rainwater penetration and oxygen inflow, and differentiating the light intensity.

This study was conducted in a pine forest with the largest distribution range among single tree species in Korea, accounting for 16.3% of the land and 24.3% of the forested areas (Lee et al., 2006). Pine forest areas have gradually decreased in the country because of the damage created by increasing forest visitors, rising frequencies of diseases and insect pests, and the thriving of competitor species influenced by global warming (Lee and Hong, 2004). Studies on the preservation and restoration of pine forests mainly analyzed the succession stages of ground populations by looking into distribution patterns (Lee et al., 2006), vegetation types and species diversity (Cho and Lee, 2011), and planting models based on vegetation structure (Ahn, 2012). These studies set up appropriate ecological restoration stages while presenting a succession model that could best suit them. In terms of the topsoil of underground populations, a study investigated the planting site of a *Pinus densiflora*-*Pinus rigida* mixed forest in a secondary forest (Kang et al., 2014) while another explored six types of pine forests (Yi, 2016). However, both studies carried out the germination experiment immediately after the topsoil collection. Therefore, using the topsoil of a pine forest, this study aimed to seek appropriate stockpiling methods for the usage of topsoil as a revegetation material by stockpiling the topsoil for 2 years under different treatments and periods, and comparing the germination status of seed banks before and after storage.

2. Materials and Methods

2.1. Site

In this study, 900 L of topsoil (25 L mesh × 36 units) were collected from a pine forest located at Gyeongsan City, Gyeongbuk Province, on April 2, 2011. The pine forest where the topsoil was collected is a secondary forest with a 3 m wide trail while the study is dominated by *Pinus densiflora* that 5 m from the trail. The tree layer is dominated by *Pinus densiflora* of about 8 m high, the shrub layer is dominated by *Lindera obtusiloba*, and the herbaceous layer is dominated by *Oplismenus undulatifolius* (Table 1). To evaluate the light environment of the pine forest, a digital camera (Nikon D80s, Nikon Inc.) and a fish-eye lens with a view angle of 180° (4.5 mm F2.8 EX DC, SIGMA Inc.) were employed to take five rounds of photos toward the sky vertically from 2 m above the forest floor. The photos were analyzed with Gap Light Analyzer Version 2.0 and its results showed that the average canopy openness was 54.42%. The litter layer of the topsoil was less than 1 cm thick and in a dry state. Based on previous studies, which found that most of the seeds are concentrated 2-5 cm below the soil surface (Roberts, 1981; Young, 1985; Granström, 1988), topsoil was collected from the topsoil layer (Ao-A1 layers) after taking off the non-eroded litter layer and digging up to 5 cm deep into the soil, where the seed bank would likely exist. The procedure was performed manually with the aid of a shovel.

2.2. Methods

Among the topsoil collected in 25 L meshes, four were laid out at 0 m from the ground, without any of them overlapping, and a sandy soil was piled up above them. After that, four additional meshes were laid, in the same way, at 0.75 m above the ground and were once again covered up with sandy soil. At 1.5 m above the ground, four more meshes were placed in the same way and sandy soil was banked up above them up to 1.7 m high from the ground to form the stockpiling structure (Fig. 1). The stockpile was built in the shape of a trapezoid bank and the width of its upper side was created small so that rainwater penetration could be minimized in order to prevent drainage issues (Itou and Kishizuka, 2008). As the height of the 25 L topsoil meshes was approximately 15 cm, the ultimate height of the uppermost meshes situated at the highest point of 1.5 m above the ground was approximately 1.65 m. Thus, the height of the stockpile was ultimately set at around 1.7 m based on an existing studies (Roberts, 1981; Young, 1985; Granström, 1988). Three stockpiles were created and then set under three different environments: open treatment, shade treatment with a shade, and waterproofing treatment with PVC tarpaulin (Fig. 1). After the stockpiling, a storage experiment was conducted at a 300 m2 area of a farm located at Hayang-eup in Gyeongsan City, Gyeongbuk Province.

As mentioned before, the treatment of the stockpiles was differentiated into open treatment, shade treatment, and waterproofing treatment because water, oxygen, and light are important factors for the germination of seeds (Bidlack et al., 2011). Thus, shade treatment for differentiating light intensity and

Table 1. General description of the studied plot

	Canopy			Shrub			Herbaceous		
	Dominant species	Height (m)	Coverage (%)	Dominant species	Height (m)	Coverage (%)	Dominant species	Height (m)	Coverage (%)
Studied plot	*P. densiflora*	8 (6.5-9)	70	*L. obtusiloba*	2.5 (1.5-3.5)	25	*O. undulatifolius*	0.2 (0.1-0.2)	20

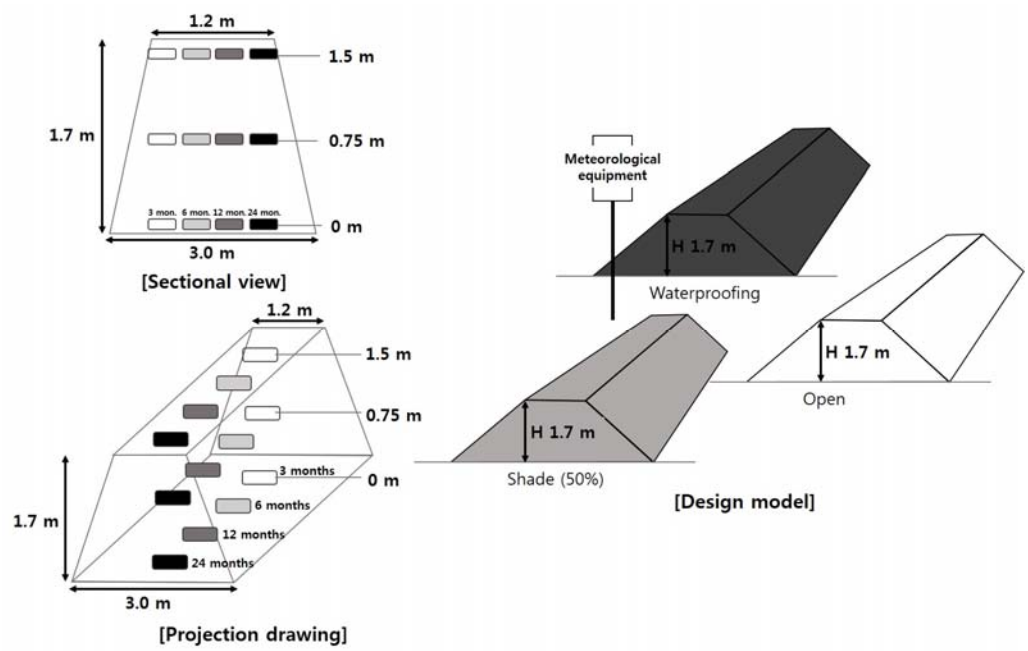

Fig. 1. Design of method of stockpiling seed bank of *Pinus densiflora* forests.

waterproofing treatment for blocking rainwater penetration and oxygen inflow were carried out. For shade treatment, a 50% shade made of polyethylene was used to create an environment similar to the canopy openness of the study site. For waterproofing treatment, a PVC tarpaulin with 100% waterproof capacity was used.

The height of the stockpile was set differently at 0 m, 0.75 m, and 1.5 m because the viability of different species emerging from the germination of seed banks depends on the thickness of the topsoil (Kim et al., 2015) and, at the same time, served to see the difference created by height when storing topsoil for long periods through stockpiling in the field.

To conduct the germination experiment after different stockpiling periods, the 25 L topsoil meshes at the uppermost area of each stockpile were taken out after 3, 6, 12, and 24 months using a mini excavator (PC 01, Komatsu Inc.) with soil piled up above them after each time. Topsoil meshes were sent to a greenhouse after being sealed in order to prevent the influx of external species. The germination experiment was conducted after spreading out the 25 L topsoil meshes three times in 2 cm height layers (9.9 L, 3.3 L × 3 times) over an artificial lightweight soil in order to supply water and nutrients. The artificial soil included gravel, coarse sandy soil, sandy soil, and a soil mix and was piled up 5 cm high (8.25 L, 50 cm × 33 cm × 5 cm) in a polystyrene box. The experiment was conducted for 1 year for each stockpiling period. The 3-months, 6-months, 12-months, and 24-months germination experiments lasted from July 2011 to June 2012, November 2011 to October 2012, April 2012 to March 2013, and April 2013 to March 2014, respectively. Monitoring was conducted in 2-weeks intervals as germination and growth began. The seedling emergence method, which counts the number of emerging species and individuals after species identification, was applied (Gross, 1990) and a digital camera (Nikon D80, Nikon Inc.) was used for taking pictures. After spreading the topsoil, watering was conducted (once a day during June-September, once every 2 days during October-May) and maintenance management, including temperature and pest control, was carried out in the greenhouse in order to prevent the inflow of external species. When temperature in the greenhouse rose up to 30℃ or above from June to September, an electric cooling device (KPE-400R, Kiturami Inc.) was used to bring down indoor temperature to 25℃.

As outlined above, the storage methods of the topsoil after stockpiling were categorized by the period, treatment, and height of the stockpiling. Also, a control group was brought to the greenhouse immediately after the collection to perform a germination experiment in order to check the germination status of seed banks before the storage. The control group also underwent three rounds of experimentation for a comparative analysis.

Moreover, to explore the weather environment of the site during the stockpiling experiment, devices measuring soil temperature (TMCx-HD series, Onset Inc.) and water content (S-SMD-M005, Onset Inc.) were set for the three studied treatments on three heights (0 m, 0.75 m, and 1.5 m) at the central part of the sandy soil where the 25 L topsoil meshes were laid. Additional data were collected through a temperature/water content data logger (HOBO U30-NRC, Onset Inc.) and a simple device measuring temperature/water content (U23-001, Onset Inc.), installed on its outer side, in order to observe the weather environment of the control group for comparative purposes.

2.3. Analysis

After identifying the plant species that emerged during the germination experiment of seed banks collected from each topsoil stockpile, the total numbers of species and individuals were counted. Based on the criteria of the Korea Biodiversity Information System (www.nature.go.kr), the life forms of plants were categorized into annual, annual-biennial, biennial, perennial, shrub, vine, and tree. Moreover, they were also divided in four types that categorize seed banks into transient seed bank and persistent seed bank depending on the duration of seed vigor (Thompson and Grime, 1979). Transient seed bank is classified into Type I, when composed of perennial Gramineae and annual plants that have seed vigor for less than 1 year, can be immediately germinated, and generally disperse and sprout in late spring and summer; and Type II, when composed of annual and annual-biennial plants that have seed vigor for less than 1 year and germinate in early spring after dormancy during the winter. Persistent seed bank is divided into Type III, composed of perennial herbaceous plants which have seed vigor for 1-5 years and generally germinate in autumn; and Type IV, composed of shrubs, trees, and perennial herbaceous plants that have seed vigor for more than 5 years (Thompson and Grime, 1979). For statistical analysis, the software SPSS 18.0 (IBM Inc.) was employed in order to study the variables recorded at the study site. To investigate differences in dependent variables, including the number of species and individuals, in accordance with independent variables including the stockpiling period (control group, 3-month, 6-month, 12-month, and 24-month), method (open treatment, shade treatment, and waterproofing treatment), and height (0 m, 0.75 m, and 1.5 m), a multivariate analysis of variance (MANOVA) was conducted.

3. Results and Discussion

3.1. Temperature and water content level of the stockpiled topsoil

The stockpiling methods were divided into open, shade and waterproof treatments while the height was set at 0, 0.75 and 1.5 m in order to measure the daily average temperature of the topsoil. The results showed that the highest daily temperature was 35.96℃ (August 5, 2011) at 1.5 m in open treatment, whereas the lowest daily temperature was -15.59℃ (December 23, 2011) at 0.75 m in waterproofing treatment (Table 2). However, in the latter case, the daily average temperature was measured at −6.48℃, daily maximum temperature at 8.01℃, and daily minimum temperature at −15.67℃, while the data of the Korea Meteorological Administration show the daily average temperature at −2.7℃, daily maximum temperature at 1.4℃, and daily minimum temperature at −6.8℃. Thus, given such 3-8℃ gaps in recorded temperatures, it seems that the temperature/water content data logger was probably malfunctioning at the time. The daily average soil temperatures by treatment of stockpiling were 15.54℃, 14.33℃, 14.11℃ and 12.74℃ for open, waterproofing, shade, and control group treatments, respectively; whereas by height, temperatures were 14.78℃, 14.73℃, 14.47℃ and 12.74℃ for 1.5 m, 0 m, 0.75 m and control group, respectively (Table 2). Soil temperature was higher at 1.5 m because the soil, which was a mixture of solid, liquid, and gaseous forms, presented lower heat conductivity near the surface than ordinary matter, while temperatures tended to fall near the bottom part compared to those at the soil surface in general (Wild, 1988). However, in the present study and contrary to what was expected, the 0.75 m point showed a lower temperature than the 0 m point.

Examining the daily average temperature of the soil by height, temperatures at 0 m were 15.68℃, 14.36℃, 14.16℃ and 12.74℃ for waterproofing, open, shade, and control group treatments, respectively. Temperatures at 0.75 m were 15.90℃, 14.28℃, 13.22℃ and 12.74℃ for open, shade, waterproofing and control group treatments, respectively. At 1.5 m temperatures were 16.35℃, 14.10℃, 13.90℃ and 12.74℃ for open, waterproofing, shade and control group treatments, respectively (Table. 2). Soil temperatures of stockpiled topsoil were higher than those of the control group due to the decomposition of organic matter that partially happened in the topsoil (Park et al., 2010). Overall, temperatures were slightly higher in open treatment, while the high temperature recorded at 0 m in waterproofing treatment was due to the characteristics of the PVC tarpaulin that made it more prone to heat conduction from solar radiation.

The monthly average water content level of the topsoil was 65.04% for the control group and 14.06%-19.08% for the open, shade, and waterproofing treatments (Table 2). The lower water content observed in the stockpiles was due to the fact that the soil piled up to create the stockpile was

Table 2. Soil temperatures (℃) and water content (%) of the stockpiling experiment

		Control	Open			Shade			Waterproofing		
			0 m	0.75 m	1.5 m	0 m	0.75 m	1.5 m	0 m	0.75 m	1.5 m
Daily temperature (℃)	Highest temperature	40.46	25.79	31.18	35.96	25.53	27.26	26.79	29.07	28.52	28.97
	Lowest temperature	-21.32	2.21	0.27	-2.07	2.88	0.93	1.07	2.53	-15.59	-1.44
	The mean temperature	12.74	14.36	15.90	16.35	14.16	14.28	13.90	15.68	13.22	14.10
				15.54			14.11			14.33	
Water content (%)	Highest content	78.02	26.32	27.53	33.35	30.58	29.79	29.73	18.53	28.03	25.79
	Lowest content	90.03	5.47	0.00	0.00	0.00	0.00	0.00	0.00	8.10	0.00
	The mean content	65.04	18.10	16.57	22.57	17.32	13.96	21.77	12.36	14.86	14.95
				19.08			17.68			14.06	

Table 3. Number of species and individuals by seed bank characteristics in stockpiling topsoil (percentage in parentheses)

Life form			Family			Type of seed bank		
Shrub	Spe.*	3(6.3%)	Compositae	Spe.*	12(25.0%)	I	Spe.*	8(16.7%)
	indiv.**	30(1.9%)		indiv.**	261(16.7%)		indiv.**	969(62.2%)
Vine	Spe.*	1(2.1%)	Gramineae	Spe.*	6(12.5%)	II	Spe.*	10(20.8%)
	indiv.**	1(0.1%)		indiv.**	946(60.7%)		indiv.**	150(9.6%)
Perennial	Spe.*	16(33.3%)	Solanaceae	Spe.*	3(6.3%)	III	Spe.*	13(27.1%)
	indiv.**	1,026(65.8%)		indiv.**	58(3.7%)		indiv.**	243(15.6%)
Biennial	Spe.*	9(18.8%)	Rosaceae	Spe.*	3(6.3%)	IV	Spe.*	17(35.4%)
	indiv.**	160(10.3%)		indiv.**	58(3.7%)		indiv.**	197(12.6%)
Annual ~biennial	Spe.*	5(10.4%)	Cyryophyllaceae	Spe.*	3(6.3%)			
	indiv.**	85(5.5%)		indiv.**	12(0.8%)			
Annual	Spe.*	14(29.2%)	Cyperaceae	Spe.*	2(4.2%)			
	indiv.**	257(16.5%)		indiv.**	23(1.5%)			
			Violaceae	Spe.*	2(4.2%)			
				indiv.**	14(0.9%)			
			Cruciferae	Spe.*	2(4.2%)			
				indiv.**	4(0.3%)			
			Rubiaceae	Spe.*	2(4.2%)			
				indiv.**	4(0.3%)			
			Commelinaceae	Spe.*	1(2.1%)			
				indiv.**	75(4.8%)			
			Others	Spe.*	12(25.0%)			
				indiv.**	104(6.7%)			
Total	Spe.*	48(100%)	Total	Spe.*	48(100%)	Total	Spe.*	48(100%)
	indiv.**	1,559(100%)		indiv.**	1,559(100%)		indiv.**	1,559(100%)

*: Number of species, **: Number of individuals

sandy, with few pores in total but mostly consisting of big-sized pores that facilitate drainage, leading to lower moisture levels (David et al., 2005). In addition, despite the fact that rainwater generally penetrates to bottom areas of soil due to gravity, capillary water underwent surface evaporation before penetrating to bottom areas, which further led to a decrease in soil moisture (Park et al., 2010). Low moisture levels of sandy soil lead to a lack of capillary water, which is the water used by plants (The Korean Association of Biological Science, 2004), likely affecting the germination of seeds.

3.2. Germination status of seed banks in stockpiling topsoil

Through the experiment on the germination of seed banks in stockpiled topsoil, a total of 48 species and 1,559 individuals were identified (Tables 3 and 4). When categorizing these species by life form, there were 3 species and 30 individuals of shrubs, 1 species and 1 individual of vines, 16 species and 1,026 individuals of perennials, 9 species and 160 individuals of biennials, 5 species and 85 individuals of annual-biennials, and 14 species and 257 individuals of annuals, showing perennials as dominant both in number of species and individuals

Table 4. Characteristic of plants germinated from seed bank in stockpiling topsoil

Scientific name	No. of individuals	Life form	Family	Type of seed bank
Oplismenus undulatifolius (Ard.) P.Beauv.	829	Perennial	Gramineae	I
Setaria viridis (L.) P.Beauv.	84	Annual	Gramineae	I
Commelina communis L.	75	Annual	Commelinaceae	II
Erigeron annuus (L.) Pers.	69	Biennial	Compositae	III
Conyza canadensis (L.) Cronquist	68	Annual~biennial	Compositae	III
Hemistepta lyrata Bunge	51	Biennial	Compositae	III
Solanum nigrum L.	41	Annual	Solanaceae	II
Oxalis corniculata L.	37	Perennial	Oxalidaceae	IV
Others	305	-	-	-

(Table 3). Meanwhile, when categorizing these species by family, there were 12 species and 261 individuals of Compositae and 6 species and 946 individuals of Gramineae (Table 3). In addition, when classifying these species by type of seed bank, there were 8 species and 969 individuals for Type I, 10 species and 150 individuals for Type II, 13 species and 243 individuals for Type III, and 17 species and 197 individuals for Type IV(Table 3). These results showed that persistent seed banks were more represented in terms of the number of species (62.5%), but transient seed banks were dominant in terms of the number of individuals (71.78%). In summary, the number of species germinated in the seed banks of stockpiled soil stood high for perennial herbaceous plants comprising of Compositae (25.0%) and Gramineae (12.5%), while the percentage of transient seed banks were high in terms of number of individuals (Table 3). This pattern is similar to the results obtained in a study conducted in a coniferous forest at a secondary forest area, which found that the representation of Compositae (13.8%) and Gramineae (10.3%) were high (Kang et al., 2014). The study site was a pine forest of a secondary forest, experiencing disturbances where various equipment vehicles for managing the forest circulated and the distance to the trail was short. Therefore, it was judged that the number of species increased because of the influx of transient seed banks that have the tendency to germinate using empty space within the soil created by disturbance and predation (Thompson and Grime, 1979).

3.3. Germination status of seed banks by the stockpiling period

In terms of the stockpiling period, the average number of germinated species per polystyrene box within seed banks (3.3 L, 50 cm × 33 cm × 2 cm) was 5.33 ± 0.58, 4.30 ± 1.56, 2.22 ± 0.70, 4.26 ± 4.01 and 5.48 ± 1.67 for the control group, 3-months, 6-months, 12-months and 24-months categories, respectively; while the average number of germinated individuals was 17.00 ± 1.00, 8.85 ± 4.19, 7.78 ± 3.75, 15.07 ± 11.25 and 26.04 ± 18.01 for the control group, 3-months, 6-months, 12-months and 24-months categories, respectively (Table 5). Although it may be difficult to make a simple comparison because of differences in the amount of accumulated topsoil, in previous results for Korea's pine forests, an average of 3.3 ± 2.5 species and 44.7 ± 8.1 individuals were identified in 13.2 L of topsoil (3.3 L × 4 sites) in tree layers dominated (80%—100%) by pine forest (Yi et al., 2010). Also, 29 species and 315 individuals in 100.8 L of topsoil (5.04 L × 40 sites) were identified from a *Pinus densiflora-Pinus rigida* mixed forest of balsa zone (Kang et al., 2014). In Japan, 43 species and a species density of 18.2 species/L were identified in 30 L of topsoil from pine forests (Hosogi et al., 2001). Unlike in these studies, the number of species and individuals in the present study was low. The difference probably resulted from the fact that the study site has low soil depth and almost no litter layer, being in a dry state and having a low number of emerging species among the ground population, which is 17 in total. In addition, another cause could be that, according to an analysis on species emergence by topsoil collection time (Kim et al., 2015), a higher number of species emerge in topsoil collected in autumn than in that collected in spring.

To explore the importance of the stockpiling period, an analysis of variance (ANOVA) was carried out with the number of species and individuals set as dependent variables and the stockpiling period as an independent variable. The ANOVA results showed that both the number of species and individuals were statistically significant (Table 5). In other words, the number of germinated species and individuals in the seed banks varied with the stockpiling period. The number of species and individuals that germinated immediately were lower after 6 months than those in the control group. In addition, the number of species started to increase after 24 months, while the number of individuals began to expand after 12 months and surpassed that of the control group after 24 months. Plants that were present after 6 months germinated after laying the topsoil in October 2011, which was an inadequate date for the germination of plants. After 12 and 24 months, one species (*Oplismenus undulatifolius*) had a drastic increase in its number of individuals, reaching 213 and then to 481 (Table 4). Nevertheless, the overall rise in the number of germinated species and individuals after 12 and 24 months was seen in plants that germinated after laying the topsoil in April 2012 and April 2013. Thus, the process is deemed to be affected by the relatively more favorable condition for seed germination during those times of the year. In terms of seed bank types, the increase of individuals in Type I after 12 and 24 months resulted from the increase of *Oplismenus undulatifolius*, while the relatively high species emergence of Type IV, a persistent seed bank, was caused by environmental conditions conducive to dormancy break.

The increase in the number of individuals with the increase of stockpiling period is relevant to the germination of seeds. Seed germination occurs when environmental conditions, including temperature and light, become adequate for germination. Such conditions as certain temperature, humidity, wavelengths of light, or mechanical stimulation vary for each plant (The Korean Association of Biological Science, 2004). Dormancy break relies on environmental conditions because germination is delayed under inadequate conditions in young individuals (The Korean Association of Biological Science, 2004). Moreover, even if environmental conditions worsen, persistent seed banks, composed of long-lived seeds, have the potential of sustaining plant species due to its storage capacity (Kalisz and McPeek, 1992). In other words, persistent seed banks and its storage effects could help to form plant communities although adequate temperature and humidity are not stably maintained (Kemp, 1989). In fact, 90 L of topsoil (30 L × 3 sites) from a *Quercus serrata-Pinus densiflora-Alnus japonica* mixed forest were collected in December and stockpiled for 1 year, and the results of its germination experiment showed that the number of species and individuals was within 88.2%-108.2% of the emergence observed in the control group (Hosogi and Matsue, 2010).

3.4. Germination status of seed banks by stockpiling method

Regarding the treatment of stockpiles, the average

Table 5. The number of species recorded from seed bank for stockpiling period(number of individuals in parentheses)

Life form		Control	3 months	6 months	12 months	24 months
Life form	Annual	3 (19)	7 (31)	4 (75)	8 (73)	9 (78)
	Annual~biennial	-	3 (8)	1 (7)	2 (18)	2 (52)
	Biennial	1 (2)	6 (83)	-	6 (30)	6 (47)
	Perennial	2 (28)	12 (116)	6 (128)	12 (265)	7 (517)
	Shrub	-	-	-	3 (21)	1 (9)
	Vine	1 (1)	1 (1)	-	-	-
	Tree	1 (1)	-	-	-	-
Type of seed bank	I	2 (30)	5 (78)	4 (101)	5 (248)	4 (542)
	II	2 (13)	5 (29)	2 (60)	6 (42)	6 (17)
	III	1 (2)	9 (91)	1 (7)	8 (48)	2 (52)
	IV	3 (6)	10 (41)	4 (42)	12 (69)	13 (92)
Total		8 (51)	29 (239)	11 (210)	31 (407)	25 (703)
	Open		11(58)	6(97)	13(145)	12(352)
	Shade		18(113)	4(45)	18(148)	13(279)
	Waterproofing		11(68)	5(74)	13(124)	8(72)
Average no. of species**		5.33 ± 0.58^{b}	4.30 ± 1.56^{ab}	2.22 ± 0.70^{a}	4.26 ± 4.01^{ab}	5.48 ± 1.67^{b}
Average no. of individuals**		17.0 ± 1.00^{ab}	8.85 ± 4.19^{a}	7.78 ± 3.75^{a}	15.07 ± 11.25^{a}	26.04 ± 18.01^{b}

**P<0.01, Duncan's multiple range test: a, b(P<0.05)

number of germinated species and individuals per polystyrene box within the seed banks (3.3 L, 50 cm × 33 cm × 2 cm) was statistically significant in an ANOVA. A total of 5.42 ± 2.92 species and 17.83 ± 15.09 individuals in open treatment, 3.83 ± 2.13 species and 15.31 ± 12.38 individuals in shade treatment, and 2.94 ± 2.04 species and 10.17 ± 10.42 individuals in waterproofing treatment were identified (Table 6).

In the case of open treatment, the number of species and individuals were closer to those observed in the control group. A similar result was obtained by Hosogi and Matsue (2010) when comparing stored and non-stored topsoils after storage for 1 year. In the case of topsoil storage, stockpiling in open treatment was considered similar to non-storage. Thus, it would be desirable to apply an open treatment to stockpiles when storing the topsoil. Furthermore, in the case of the 50% shade treatment, that was similar to the canopy openness of the study site, a similar number of individuals, but a lower number of species were found. These results suggest that more studies should be conducted by differentiating the light penetration ratio. Regarding the waterproofing treatment, that prevented the inflow of rainwater and oxygen, the number of species and individuals were lower than those observed in the control group. The water content of the control group was 65.04% while that of the waterproofing treatment was 14.06%. The observed lower value was presumably due to low moisture and lack of oxygen inflow of the soil, which undermined respiration and aerobic microorganism activities (David et al., 2005).

In terms of height of the stockpile, the average number of germinated species and individuals per polystyrene box of seed banks (3.3 L, 50 cm × 33 cm × 2 cm) were higher only at 0 m (5.92 ± 3.32 species, 20.83 ± 16.68 individuals) and 0.75 m (5.58 ± 2.91 species, 20.75 ± 18.26 individuals) in open treatment than in the control group (5.33 ± 0.58 species, 17.00

Table 6. The number of species recorded from the seed bank for the stockpiling method(number of individuals in parentheses)

Index		Control	Open				Shade (50%)				Waterproofing			
			0 m	0.75 m	1.5 m	**Total**	0 m	0.75 m	1.5 m	**Total**	0 m	0.75 m	1.5 m	**Total**
Life form	Annual	**3 (19)**	11 (85)	5 (36)	6 (22)	**12 (143)**	5 (18)	5 (33)	4 (18)	**8 (69)**	4 (16)	1 (2)	4 (27)	**5 (45)**
	Annual ~biennial	-	1 (7)	2 (13)	3 (23)	**3 (43)**	2 (9)	2 (6)	2 (9)	**4 (24)**	2 (4)	3 (8)	1 (6)	**3 (18)**
	Biennial	**1 (2)**	5 (28)	4 (33)	5 (22)	**7 (83)**	4 (11)	6 (22)	5 (26)	**7 (59)**	2 (8)	3 (8)	2 (2)	**3 (18)**
	Perennial	**2 (28)**	6 (124)	6 (147)	7 (85)	**10 (356)**	6 (207)	6 (132)	6 (84)	**10 (405)**	6 (81)	6 (105)	7 (61)	**11 (247)**
	Shrub	-	1 (6)	1 (11)	1 (4)	**1 (21)**	1 (2)	1 (3)	2 (4)	**3 (9)**	-	-	-	-
	Vine	**1 (1)**	-	-	-	-	1 (1)	-	-	**1 (1)**	-	-	-	-
	Tree	**1 (1)**	-	-	-	-	-	-	-	-	-	-	-	-
Type of seed bank	I	**2 (30)**	5 (129)	3 (133)	2 (60)	**6 (322)**	4 (206)	3 (126)	2 (84)	**5 (398)**	3 (83)	3 (95)	4 (53)	**4 (231)**
	II	**2 (13)**	7 (48)	4 (22)	5 (14)	**8 (84)**	3 (8)	3 (17)	3 (10)	**5 (35)**	3 (8)	-	2 (21)	**3 (29)**
	III	**1 (2)**	6 (35)	7 (47)	8 (45)	**10 (126)**	6 (20)	8 (28)	7 (35)	**11 (83)**	4 (12)	6 (16)	3 (8)	**6 (36)**
	IV	**3 (6)**	6 (38)	5 (39)	7 (37)	**9 (114)**	6 (14)	6 (25)	7 (12)	**12 (51)**	4 (6)	4 (12)	5 (14)	**9 (32)**
Total		**8 (51)**	24 (250)	18 (240)	22 (156)	**33 (646)**	19 (248)	20 (196)	19 (141)	**33 (585)**	14 (109)	13 (123)	14 (96)	**22 (328)**
Av. no. of species**		**5.33±0.58[b]**	5.92±3.32	5.58±2.91	4.75±2.63	**5.42±2.92[b]**	3.42±2.07	4.92±2.31	3.17±1.70	**3.83±2.13[ab]**	3.08±2.35	2.92±1.98	2.83±1.95	**2.94±2.04[b]**
Av. no. of individuals*		**17.00±1.00[a]**	20.83±16.68	20.75±18.26	11.92±7.44	**17.83±15.09[a]**	19.92±17.56	16.33±9.75	9.67±5.00	**15.31±12.38[a]**	10.17±12.33	13.45±10.98	8.00±7.66	**10.17±10.42[a]**

**$P<0.01$, *$P<0.1$, Duncan's multiple range test: a, b($P<0.05$)

± 1.00 individuals) (Table 6). The average number of germinations by height were 4.14 ± 2.86 species and 16.97 ± 16.03 individuals for 0 m, 4.47 ± 2.62 species and 16.47 ± 13.64 individuals for 0.75 m and 3.58 ± 2.23 species and 9.86 ± 6.80 individuals for 1.5 m, which were all lower than the germinated number in the control group (Table 7). Although the average number of germination at 0 m and 0.75 m in open treatment were higher than those of the control group, the average number of germinations at all heights were lower than those of the control group, which implies that treatment and height of stockpiling present big differences. Such results on the emergence of species were different from a study that claimed that the thicker the topsoil cover got, the more species emerged (Kim et al., 2015). As sandy soil was used to form the stockpile in the present study, this may have affected the results and produced differences with those of the study that only used topsoil.

Therefore, it would be advisable to apply an open treatment to stockpiles when storing topsoil and 0 m or 0.75 m points would be appropriate for stockpile. In other words, it would be advisable to store the topsoil in a low-height stockpile. However, caution should be taken regarding the possibility of seed

Table 7. The number of vascular plants recorded from the seed bank for the stockpiling height experiment

		Control	0 m	0.75 m	1.5 m
Total	No. of species	8	57	51	55
	No. of individuals	51	607	559	393
Mean	No. of species	5.33±0.58	4.13±2.86	4.47±2.62	3.58±2.23
	No. of individuals*	17.00±1.00[b]	16.97±16.03[b]	16.47±13.64[b]	9.86±6.80[a]

*P<0.05, Duncan's multiple range test: a, b(P<0.05)

Table 8. Correlation analysis between the number of species and the number of individuals

		No. of Individuals
No. of Species	Pearson Correlation Coefficient	.453
	Sig.	.006

banks germinating at the surface layer of stockpiles as the ratio the surface area to the volume would be high in a low-height stockpile (Hosogi and Matsue, 2010).

3.5. MANOVA

An analysis on the correlation between dependent variables was conducted and found that a significant positive correlation (correlation coefficient =0.453, P<0.01) existed between the number of species and the number of individuals (Table 8). Thus, a MANOVA was carried out by setting the number of species and individuals as dependent variables and treatment, height, and stockpiling period as independent variables serving as fixed factors. The analysis showed significant differences in the number

Table 9. MANOVA results considering the main effects for method, height, and period of the stockpiling experiment and the interaction effects of these variables

Dependent variable		F	Mean sq.	F	Sig.	Eta sq.
Method	No. of species	112.907	56.454	61.653	.000**	.598
	No. of individuals	1582.352	791.176	23.897	.000**	.365
Height	No. of species	14.519	7.259	7.928	.001**	.160
	No. of individuals	700.519	350.259	10.579	.000**	.203
Period	No. of species	169.065	56.355	61.546	.000**	.690
	No. of individuals	5683.657	1894.552	57.223	.000**	.674
Method*height	No. of species	16.037	4.009	4.379	.003**	.174
	No. of individuals	251.259	62.815	1.897	.119	.084
Method*period	No. of species	245.907	40.985	44.759	.000**	.764
	No. of individuals	5605.204	934.201	28.216	.000**	.671
Height*period	No. of species	12.519	2.086	2.279	.044*	.141
	No. of individuals	693.704	115.617	3.492	.004**	.202
Method*height*period	No. of species	48.259	4.022	4.392	.000**	.388
	No. of individuals	627.852	52.321	1.580	.113	.186

**P<0.01, *P<0.05

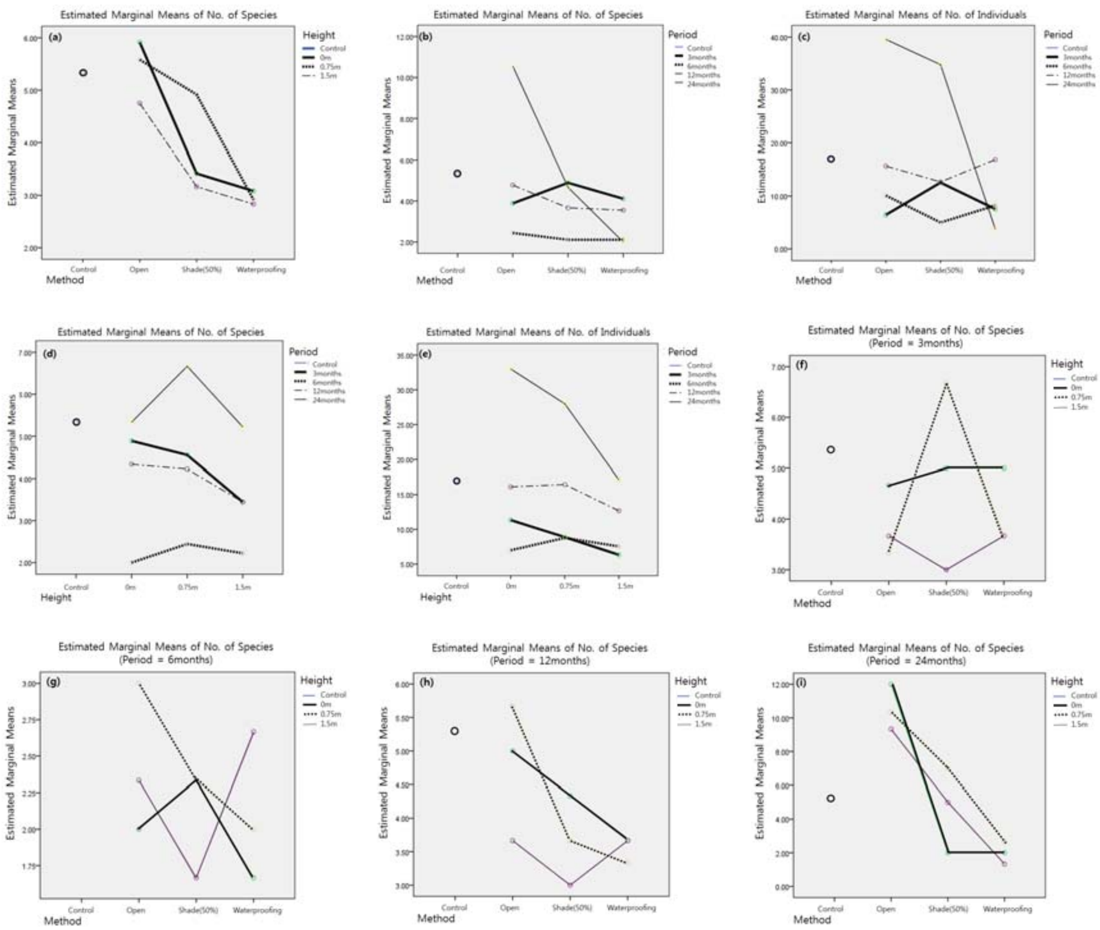

Fig. 2. Interaction effects by method, height, and period of stockpiling((a)Pattern of the no. of species for the method according to height, Pattern of the no. of species(b) and individuals(c) for the method according to period, Pattern of the no. of species(d) and individuals(e) for the height according to period, Pattern of the no. of species for the height according to period, (f)-(i) Pattern of the no. of species by period for the method according to height).

of species and individuals (P<0.01) depending on treatment, height and stockpiling period. Also, interactions between the two dependent variables and between the three dependent variables also found (Table 9).

Looking into a significant pattern identified in the interaction between the two dependent variables on the MANOVA, it was found that the number of species in each treatment differed by height higher in open treatment (Fig. 2-a). The number of species (Fig. 2-b) and individuals (Fig. 2-c) were higher in open treatment after 24 months while the total number of species and individuals that underwent germination after 24 months was higher than those of the control group at all height (Fig. 2-d, 2-e). When checking the interactions between treatment, height, and stockpiling period, it was found that the number of germinated species were higher in open treatment in all categories except for 3 months (Fig. 2-f), but no clear pattern was identified as they all differed by height (Fig. 2-g, 2-h, and 2-i).

In the case of open treatment in stockpiling, the number of species (5.41) was similar or slightly higher than that of the control group (5.33 species, 17.00 individuals) (Table 4), while the number of species (5.74) and individuals (26.03) increased after 24 months regardless of height. Moreover, the stockpiles showing a high number of species among stockpiling period categories were different depending on treatment method and height. Similar results were obtained in a study that conducted a comparative analysis on stored and non-stored topsoils after storing the topsoil for 1 year (Hosogi and Matsue, 2010), with similarities found in the number of species in the control group and open treatment, but the number of species decreased in the shade and waterproofing treatments. Regarding the stockpiling period, the number of species and individuals became higher (5.74 species, 26.03 individuals) than those of the control group (5.33 species, 17.00 individuals) after 24 months (Table 5), but as a result of variables interactions, such tendency was shown only in open treatment (Fig. 3-b, 3-c). In the present, the optimal combination of interactions was shown in open treatment, and the number of individuals increased with the stockpiling period. However, among the total number of individuals that emerged after 24 months (703) (Table 5), 352 were in open treatment, 279 in shade treatment and 72 in waterproofing treatment, showing a huge imbalance among treatments. Particularly, within the total number of individuals, there were 481 individuals of *Oplismenus undulatifolius*. Although the number of individuals increased with the extension of the stockpiling period, a significant change in the number of species could not be found.

3.6. Proper stockpiling methods for topsoil as a revegetation material

Seed banks present in topsoil are often utilized, for example, to revegetate slopes using the storage capacity of collapsed topsoil (Simpson et al., 1989). In practice, it is used at revegetation sites by storing the topsoil for short periods of time (Hosogi and Matsue, 2010). In fact, vegetation restoration was conducted by using topsoil in the slope of water-level-fluctuating areas near Minoogawa dam in Osaka, Japan. This was a successful case of vegetation restoration in which the collected topsoil was accumulated and stored for more than a year after facilitating ventilation and drainage, and then laid out for restoration (Itou and Kishizuka, 2008). To store topsoil for posterior use as revegetation material, the application of open treatment is recommended among the various types of stockpiling treatment methods, while storage in low-height stockpiles (~0.75 m) is likely to be more effective. In addition, more attention should be paid to the fact that higher number of species can be secured if the stockpiling period is prolonged.

Although wood species rarely appeared in the present study, the influx of wood species is highly important in actual revegetation works. Invasion strategies are generally divided into infiltration strategies, that maintain populations through seed production; and phalanx or guerrilla strategies, that maintain populations through vegetative growth using internode and ramet in plant communities (Kim and Lee, 2006). Because seed banks only make plant communities through infiltration strategies, plants using phalanx or guerrilla strategies do not appear. Therefore, plants using infiltration and phalanx or guerrilla strategies need to be considered when restoring vegetation. In addition, in order to restore vegetation, it would be desirable to form shrub forests dominated by pioneer wood plants (Hosogi and Matsue, 2010) and it would be advisable to actively induce the influx of woody plants that emerge within the vegetation in various ways instead of merely forming the seed bank of topsoil.

4. Conclusion

Soil temperature of the stockpiled topsoil was higher in open treatment and at 1.5 m, while soil water content was lower (14.06%-19.08%), that those in the control group. The seed banks in stockpiled topsoil had 48 species and 1,559 individuals, among which perennials showed the highest number in terms of life forms, while Compositae and Gramineae were dominant in terms of families. Based on seed bank types, the persistent seed banks had a higher number of species, while the transient seed banks had higher number of individuals. By stockpiling periods, the number of species in seed banks started to increase after 24 months, while the number of individuals began increasing after 12 months and then exceeded that of the control group after 24 months. In terms of the treatment applied to stockpiles, the number of species and individuals in seed banks in open treatment were closer to those of the control group, whereas by height, the number of species and individuals was higher at 0 m, but lower than the observed for the control group. The MANOVA showed that the optimal combination was in open treatment and that the number of individuals increased in line with the lengthening of the stockpiling period.

Based on these results, the following conclusions can be drawn. First, when stockpiling forest topsoil with sandy soils, which has lower water content levels than the topsoil, the level of capillary water necessary for plants was low and affected germination. Thus, in the case of stockpiling forest topsoils, the use of loamy soils, which has an adequate level of moisture, as well as materials with characteristics equivalent to clayey soils may give outstanding results. Second, the number of species and individuals in the seed banks of stockpiled topsoil was low due to the fact that the collection was done in spring and the study site did not have many emerging species due to disturbance. Therefore, if topsoil is collected from forests with diverse emerging species, without any disturbance, and in autumn, better results may be gained. Third, the number of species and individuals increased as the stockpiling period became longer. Seed banks begin germination when maintained in adequate germination environments. Therefore, as there are several tree species that can germinate only after going through a certain dormancy period, the use of dormancy breaks may be considered if the species exists in the ground population. Fourth, it was found that the number of species and individuals of seed banks in open treatment was higher than those of the control group, but the number of species and individuals at all height of the stockpile was lower than those of the control group. Therefore, when storing the stockpiled topsoil for long periods, open treatment will be the most effective. While the influence from different heights was minimal, creating low-height stockpiles may be an option. For such conditions, the use of open treatment is recommended and the number of individuals may be increased by extending the stockpiling period. Lastly, the present study has limitations as it was conducted solely on a pine forest experiencing disturbances and without many emerging species. Also, only sandy soil was used for stockpiling. Therefore, it would be necessary to carry out more studies on stockpiling with various soil such as actual soil, loamy soil, and clayey soil in high-quality vegetation with a large number of emerging species. In addition, it would be necessary to perform more studies on various methods of vegetation restoration such as the introduction of species that maintain populations through guerrilla strategies, as well as the introduction of pioneer wood plants.

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