Doctoral Thesis

博士論文

Regeneration potential of secondary forests under herbivore pressure by wild ungulates in the Boso Peninsula

（房総半島の野生偶蹄類の影響下にある二次林の再生可能性）

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Abstract

Deer overpopulation is a prevalent issue reported in Japan associated with forest damage, vegetation cover loss, potential local extinction of important and rare plant species, and deforestation with altered ecosystem services. To resolve these problems caused by deer overpopulation, deer exclusion and culling have been explored. However, the effectiveness of deer exclusion is dependent on the environmental conditions such as light availability which can be enhanced through artificial gap creation. This has led to recent studies investigating the combined effects of “deer exclusion + gap creation” on restoring deer damaged forests. However, these studies were based on short-term observation and focused on aboveground systems while overlooking the effects on belowground systems. The current study assesses the regeneration potential of secondary forests with a long history of deer herbivory and the decadal changes of different treatments on the aboveground vegetation (chapter 3), belowground systems (chapter 4) and sapling recruitment of common tree species (chapter 5) to determine effective treatments towards restoring forest ecosystems.

Three study sites within the Boso peninsula, Kotsubosawa (KBS), Hiratsuka (HRT) and Hinokio (HNK) within the University of Tokyo Forests Chiba Forest (UTCBF) were selected for this study. The estimated deer density in 2018 was 10 – 13.5 deer km^{-2} (Hisamoto et al., 2019). Canopy gaps of about 20-by-20 m were created by logging once in 2008 followed by the complete removal of woody material on the forest floor at each site. The canopy gaps were further subdivided into four 10-by-10 m subplots, two of which were installed with deer exclosure fences (deer exclusion + gap creation: EG). The remaining two subplots were defined as gap creation plots (gap with deer: G). The deer-exclusion fences were also installed under closed canopy natural forests adjacent to the gap plots (closed canopy + deer exclusion: E) accompanied by unfenced subplots set as the control (closed canopy with deer: Ctrl). The set of experimental treatment was replicated at the three study sites, establishing a total of six replicates for each treatment (n = 24). From 2019 - 2021, foliar cover and species richness of plants of different growth forms (forbs, shrubs, graminoids, vines and trees, chapter 3), soil physical properties (soil bulk density, soil finer porosity and coarse porosity), soil nutrient concentrations (Total N, Soil P, K, Ca, Mg and Na) and litter invertebrate abundance (Araneae, Amphipoda, Collembola, Isopoda, Chilopoda and Diplopoda, chapter 4) were investigated and compared among the different treatments. In addition, six tree species (*Abies firma, Quercus acuta, Eurya japonica, Cinnamomum tenuifolium, Castanopsis sieboldii* and *Neolitsea sericea*)

that are common in the studied region were selected and their regeneration success within each treatment was assessed by comparing their sapling densities (chapter 5).

Deer exclusion facilitated increase in tree foliar cover (E > Ctrl and EG > G) while gap creation facilitated increase in non-tree species cover (EG and G > E and Ctrl). Compared to Ctrl, treatment E increased the foliar cover of non-tree species while maintaining the overall plant species composition similar to that of Ctrl. Treatment G plots were dominated by graminoids and shrubs due to constant selective herbivory by deer which suppressed regeneration of palatable species. In treatment EG, cover of all plant forms (Forbs, Graminoids, Shrubs, Herbs and Vines) were able to increase in the absence of deer. Despite the increase in foliar cover after clearcutting, the litter mass in treatment G and EG remained lower compared to Ctrl and treatment E plots even after a decade.

Soil samples from treatment G plots showed higher values of soil bulk density than those from other treatment, indicating increased soil compaction in treatment G plots. This compaction effect was mitigated in treatment EG plots by deer exclosures that inhibit physical disturbances and increased foliar covers. There seemed to be remnant effects of clearcutting on litter mass and concentrations of total N, K and Na in topsoil: they all tended to be lower in gap plots (EG and G), though total N concentration was mitigated by deer exclusion (EG). No remnant effects of clearcutting were observed for soil P, K and Mg concentrations. The abundance of litter invertebrates generally increased with treatment E compared to Ctrl irrespective of taxonomic groups, likely due to the reduction of deer disturbance on the litter substrate. Predatory groups (Chilopoda and Araneae) showed positive responses to deer exclusion in both E and EG suggesting their sensitivity to deer disturbance. Although the abundance of litter invertebrates in treatment G was lower than Ctrl in a short-term study (Suzuki and Ito, 2014), for Collembola, Isopoda and Chilopoda that difference was not found after a decade suggesting natural recovery over time. The positive effects from treatment EG compared to treatment G reported in the previous study (Suzuki and Ito, 2014) continued to be observed for Amphipoda, Araneae and Chilopoda even after a decade highlighting the positive effect of deer exclusion on both aboveground and belowground systems.

For tree recruitment, treatment EG increased the diversity of tree species and accelerated sapling growth, while in treatment G, recruitment of common tree species either failed or were suppressed below one meter height except for *A. firma* and *N. sericea*. Tree saplings tended to be less abundant in treatments with deer compared to their counterpart (Ctrl < E, G < EG), suggesting limited successful recruitment at the current deer density (10–13.5 deer km^{-2}). Although treatment EG facilitated recruitment of all six tree species, the recruitment success among treatments differed according to tree species. *Quercus acuta*, *C. tenuifolium* and *C. sieboldii* required deer exclusion for sapling recruitment while *A. firma*, required increased light availability with deer to successfully grow. It is likely that deer suppressed plant interspecies competition through herbivory on palatable plant species facilitating the recovery of non-palatable species such as *A. firma*. *Eurya japonica* saplings similarly required increased light availability but their sapling heights were suppressed by deer presence in G. *Neolitsea sericea* on the other hand varied responses with high density of small sapling density in E and high density of large saplings in G.

The current study examines decadal treatment effects with sufficient regeneration periods, which provide reliable and realistic information on treatment effects that are difficult to discern in short-term studies. Consequently, informed decisions can be made by identifying whether certain tree species are capable of naturally recruiting without human intervention and how best to ensure successful recruitment if necessary. One example is the current concern of decreasing *A. firma* saplings within the Boso peninsula. The current efforts of deer population control may not be a suitable approach to facilitate sapling regeneration of *A. firma* which appears to require gap creation with deer presence. In the case of belowground systems, remnant effects of clearcutting (treatment G) were observed in soil nutrient concentrations and litter invertebrate abundance even after a decade, partially mitigated by treatment EG. However, the plots with clearcutting treatment were not significantly different from the control for both soil nutrient concentrations and litter invertebrate abundance suggesting minimal negative impacts remaining after a decade.

In general, the restoration of forest ecosystems can be achieved by applying different treatments to achieve specific management goals, such as rapid regeneration of the forest canopy cover through treatment E and EG, increased cover of non-tree species in the understory layer through treatments G and EG, or a balanced increase in both the forest canopy cover and understory layer via treatment EG. Although treatment EG appears to elicit the most balanced effect by improving foliar cover of both tree and non-tree species, EG may not be suitable for light-demanding, non-competitive species such as *A. firma*. To increase the ecosystem heterogeneity, a multi-treatment approach with an array of different treatments across a region would be more suitable compared to a single treatment approach. Moreover, large-scale treatments would be too costly and infeasible to implement in reality. It would be far more realistic to establish multiple small-scale treatments of 100 m^2 across a region which would also minimize the impacts on aboveground and belowground systems. In addition, clearcutting (G and EG) on a small-scale setting would ensure viable seed sources from surrounding vegetation outside the treatment area and minimize impacts to belowground systems.

Chapter 1. General Introduction

1.1. The population expansion of deer

Deer population increase is a prevalent issue reported in North America, Europe and New Zealand (Gill, 1992; Côté et al., 2004; Shelton et al., 2014) and Japan (Takatsuki, 2009; Suzuki et al, 2013). The causes of deer overpopulation are numerous but usually an indirect result of anthropogenic interventions on the natural ecosystem creating ideal conditions for deer. In Japan, native population of sika deer (*Cervus nippon*) have exploded for past two decades. The explosion has been attributed mainly to the extinction of its natural predator, the Japanese wolf (*Canis lupus hodophilax*) and previous legislations against hunting of female deer. This problem is also exacerbated by the human population decline in Japan, as villages in remote areas become deserted, farmlands, orchards and previously managed forests in the countryside being abandoned (Oono et al., 2020). This abandonment of farmlands, orchards and managed forests would serve as ideal food reservoirs and safe refugia deer to migrate and reproduce, increasing their population density and habitat range. Another possible explanation for deer population increase is that effects of climate change increasing global temperatures have resulted in warmer winters which also reduced winter mortality rates of deer (Ueno et al., 2018). In addition, due to modern social lifestyles, the decreasing number of active hunters in Japan (Kaji et al, 2010) together with high deer fecundity (Koizumi et al., 2009) makes it difficult for deer population control.

According to the census by the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF, 2019), deer accounts for approximately 70% of total forest damage in Japan, though the total area of forests damaged by deer has been declining (Figure 1.1). This decline in damaged forest area is attributed to installation of deer fences and deer population management efforts to reduce the impact of deer. Meanwhile in Chiba prefecture, the population of sika deer and habitat range have increased over the years and are projected to further increase in the future (Chiba prefecture, 2017; Figure 1.2 and 1.3). Studies on Kinkazan and Nakanoshima islands (Nakajima 2007; Takatsuki 2009; Ueno et al., 2018) have shown that deer overpopulation results when deer densities exceed the ecosystem's carrying capacity causing irreversible damage to plants, resulting in deer population crash due to lack of food. However, such examples of deer population crash are mainly based on island observations and have yet to be reported for deer on the mainland.

1.2. Impacts of deer overpopulation

Selective browsing of seedlings and saplings by deer suppresses the regeneration of tree species (Gill 1992; Tremblay, Huot and Potvin, 2007; Koda et al., 2008) preventing the successful establishment of new generations (Coomes et al., 2003; Takatsuki and Hirabuki, 1997; Takatsuki, 2009). The lack of sapling recruitment results in generation turnover failure as mature trees gradually disappear leading to local extinction of important and rare plant species (Tamura, 2019). It also gives negative implications towards the regeneration potential of secondary forest ecosystems, highlighting the importance of effective forest restoration methods (Castro et al., 2021). As a consequence of long-term selective browsing by deer, vegetation cover is lost, plant species diversity is diminished (Côté et al., 2004; Miyashita et al., 2004; Thompson, 2011), non-palatable species become dominant (Takatsuki 2009; Tanentzap et al., 2012; Itô, 2015; Itô, 2016). It not only alters plant species composition but also cause significant losses of ecosystem services (Côté et al., 2004). In contrast, positive effects have also been reported where deer herbivory supressed fast-growing plant species, reducing interspecies competition among plants, increasing the species richness (Nishizawa et al., 2016). However, in facing food shortages, deer exhibit diet switching (Nugent et al., 2001; Coomes et al., 2003), consuming non-palatable plants that were previously avoided in the presence of other food sources (Suzuki, 2013; Agetsuma et al., 2011; Hashimoto and Fujiki, 2014; Tamura and Yamane, 2017). At higher population densities, sika deer consume most plant species and the litter layer (Takahashi and Kaji, 2001; Nakahama et al., 2020), causing further damage to the ecosystem.

The consumption of litter by deer reduces the litter layer resulting in habitat loss for litter invertebrates decreasing their abundance (Bressette et al., 2012; Lessard et al., 2012; Suzuki and Ito, 2014; Katagiri and Hijii, 2017). Even when not consuming the litter, deer directly and indirectly disturb the litter layer reducing litter invertebrate abundance. Reduction in litter invertebrates is suggested to reduce litter decomposition rates (Suzuki and Ito, 2014; Chollet et al., 2021). Furthermore, deer are known to alter nutrient cycling processes (Bardgett and Wardle, 2003; Mohr et al., 2005), increase soil bulk density through trampling (Gass and Binkley, 2011; Shelton et al., 2014; Suzuki and Ito, 2014) and reduce soil nutrient concentrations (Mohr et al., 2005; Gass and Binkley, 2011; Furusawa et al., 2016; Maillard et al., 2021) through reduction of vegetation cover exposing soil surfaces to environmental impacts. In general, the impacts of deer are not limited to vegetation in the aboveground systems (Chapter 3) but also cascade to belowground systems (Chapter 4), both of which may affect the recruitment of tree saplings (Chapter 5).

1.3. Deer management efforts in Japan

The current deer management plan in Chiba prefecture, Japan is to reduce deer population numbers through hunting and culling to reduce their damage to farmlands and the environment (Chiba prefecture, 2017). However, studies have suggested that forest regeneration does not occur immediately following deer population control (Coomes et al., 2003; Tanentzap et al., 2012). Despite population control efforts, deer impacts continue to be present even at low population densities, interrupting regeneration of palatable plant species and preventing recovery of the original vegetation (Coomes et al., 2003; Tanentzap et al., 2012; Harada et al.,2020). Therefore, deer population control does not guarantee the regeneration of palatable plant species as they could disappear before deer populations are effectively reduced. Furthermore, a reduction in deer population increases the availability of food resources which may result in population irruptions in consecutive years following deer culling programs, maintaining a high abundance of deer in the ecosystem (Kaji and Takeshita, 2022). Deer population control is further complicated by the decline in active hunters, making it increasingly difficult to effectively control deer. As a result, deer population control will be increasingly challenging and, in the future, may no longer be able to keep up with increasing deer numbers. Currently, despite population control efforts in Chiba prefecture, deer density and habitat range (Figure 1.2 and 1.3) have continued to increase (Chiba prefecture, 2017).

Deer exclusion fencing effectively eliminates herbivore browsing pressure, facilitating plant regeneration and maintaining species diversity within the ecosystem, as potential refugia and seed source for the ecosystem (Shinoda et al., 2022). However, deer exclusion fencing is costly, difficult to achieve in steep terrain and susceptible to damage from treefall. Studies on deer exclusion fencing towards forest regeneration remains limited in Japan with smaller fenced areas compared to the global average (Shinoda et al., 2022). Some of the deer exclusion fences have been associated with successful vegetation regeneration (Kumar et al., 2006; Suzuki, 2013). However, successful regeneration is not always guaranteed (Royo and Carson, 2006; Nuttle et al., 2014; Harada et al., 2020; chapter 3), dependent on local environmental conditions such as light availability (Tremblay, Huot and Potvin, 2007; Suzuki, 2013). There is a need to assess the effectiveness of deer exclusion fencing in promoting tree regeneration and identify suitable forest restoration methods for different situations (Castro et al., 2021).

1.4. Forest underuse and role of artificial canopy gaps

Negative impacts of deer overpopulation seen today, may partly be a result of abandonment of previously managed forests (Suzuki, 2013). In Japan, before the 1970s, many forests were regularly harvested through coppicing for firewood which maintained the cyclic occurrence of canopy gaps (Oono et al., 2020). However, with the industrial revolution, the demand for firewood decreased, resulting in the abandonment of previous coppicing practices and a subsequent maturation of secondary forests towards closed canopies.

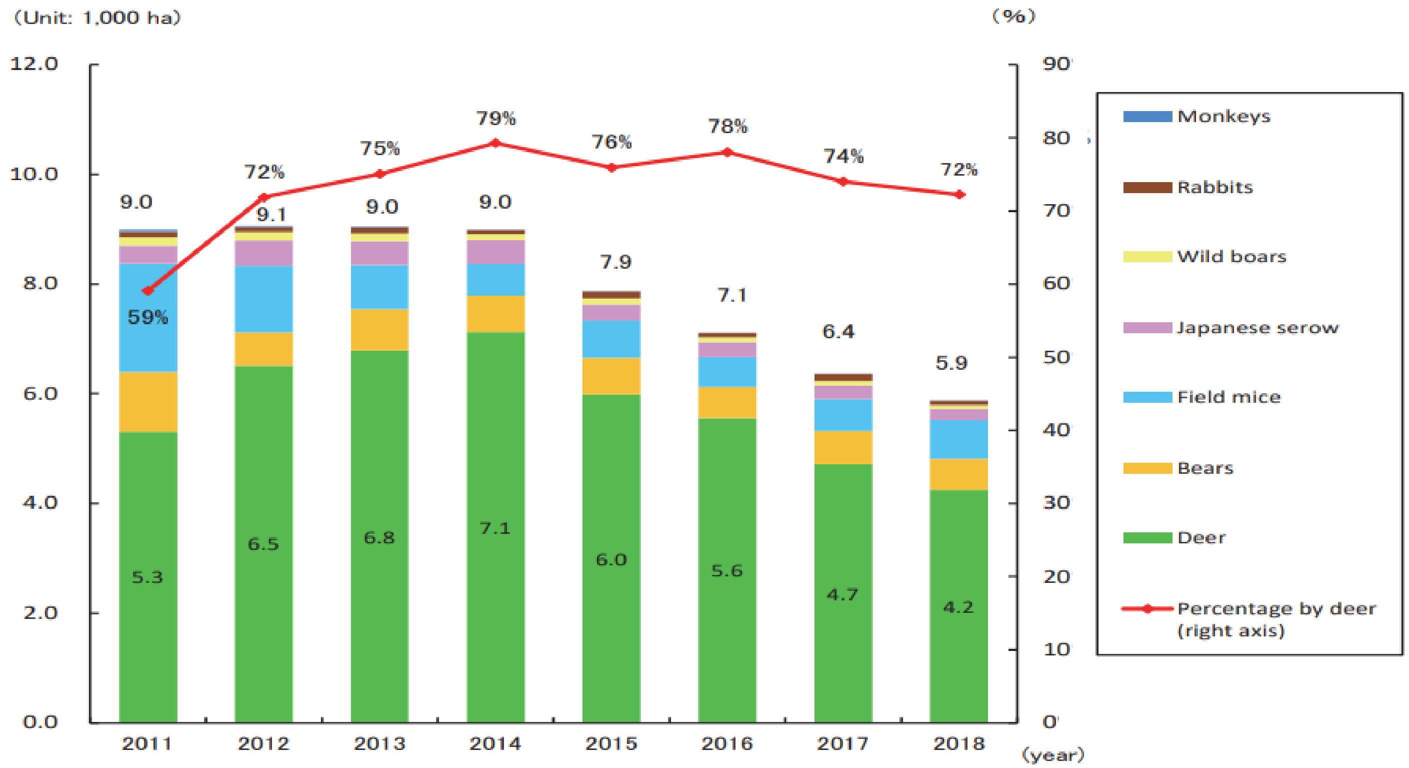
Under a closed canopy, the regeneration of tree seedling and saplings is usually limited by light availability (Ricard et al., 2003; Suzuki, 2013; Muscolo et al., 2014; Feldmann et al., 2020). The negative effects of light limitation can be mitigated through artificial gap creation (Shimoda et al., 1994; Tremblay, Huot and Potvin, 2007; Suzuki, 2013), enhancing the positive effects of deer exclusion fencing on plant species richness (Suzuki, 2013; Sabo et al., 2019; Chapter 3), survival and growth of tree saplings (Shimoda et al., 1994; Coates and Burton, 1997; Tremblay, Huot and Potvin, 2007; Kern et al., 2017; Tamura and Nakajima, 2017; Nagashima, Shimomura and Tanaka, 2019; Petersson et al., 2020; Chapter 5). Similarly, depending on the gap size, the spatial heterogeneity of vegetation (Feldmann et al., 2020) is increased through secondary succession. There is an increasing emphasis on incorporating gaps creation into silviculture practices in some areas of North America and Europe to mimic the natural environment to restore forests naturally (Muscolo et al., 2014). The topic of gap creation is still widely debated by ecologists, especially in the case of primeval forests, but in countries such as North America, Europe and Japan where coppicing for firewood used to be common, a recent concern of “forest underuse” has led to the welcome of artificial gap creation (Oono et.al., 2020).

Artificial gap creation increases plant species diversity and sapling recruitment, but in the presence of deer, it may result in opposite effects, including recruitment failure and partial deforestation (Barrette et al., 2014). Creating gaps with deer present has been reported to limit the height growth of regenerated trees (Horsley et al., 2003), dominance of fern and forb species due to legacy effects (Nuttle et al., 2014), resulting in different plant compositions from those in the uncut forests (Stromayer and Warren, 1997; Côté et al., 2004; Augustine et.al., 1998; Tremblay, Huot and Potvin, 2007; Koda et. al., 2008, Suzuki et al., 2021). The continuous browsing by deer on young leaves and shoots of regenerating trees supresses their growth and dominance, the space and resources being taken up by non-palatable plant species that are not supressed by deer. These non-palatable species (fern and forb species) become dominant, eventually supressing saplings of tree species, altering the plant species composition. Light-demanding species such as ferns (Takatsuki, 2009), disturbance related species such as *Eurya japonica* (Manabe and Yamamoto, 1997) or herbivory-tolerant ones (Gill, 1992; Côté et al., 2004; Royo and Carson, 2006) tend to dominate gaps, the ecosystem being different from that before gap creation. Furthermore, when fast-growing competitive plants are present, gap creation may result in increased competition (Kern et al., 2012) and the formation of "recalcitrant layers" that inhibit regeneration of tree species (Royo and Carson, 2006). Thus, artificial gap creation must be approached with caution as the results vary depending on the environment conditions.

1.5. Overall thesis objective and chapter descriptions

The objective of the current study is to assess the decadal treatment effects of deer exclusion, gap creation and a combination of both treatments (see chapter 2.3) on the aboveground and belowground systems as well as the forest regeneration potential within each treatment. The experiment was conducted in the warm temperate evergreen broad-leaved forests in the Boso peninsula with a long history of deer overpopulation. Early changes in the vegetation cover and species richness up to two years after treatments (2008 – 2009) and those up to 7.5 years (2008-2015) were described by other studies (Suzuki 2013, Suzuki et al., 2021) in the same study plots. According to these studies, ground vegetation was limited by light availability, mitigated by a combination of gap creation with deer exclusion fencing which increased species diversity and facilitated tree sapling recruitment. Conversely, clearcutting with deer resulted in ecosystems with reduced species diversity dominated by shrubs and non-palatable plants. Rather, previous studies examined the short-term (< 7.5 years) effects of the experimental treatments and derived their conclusions on the recovery of forest ecosystems based on the short-term responses. The current study examines the decadal effects of the experimental treatments on different plant forms, tree species regeneration and soil nutrient concentrations which were not addressed in the previous studies.

A description of the different experiments and results from this study is presented in the following chapters. Chapter 2 describes the study site history and the experimental design using a 2-by-2 factorial treatment of deer exclusion and canopy gap creation established simulating the ecosystem response to different levels of disturbance. Chapter 3 focuses on the changes to the plant species richness, plant composition, and regeneration of vegetation cover after a decade of exposure to the treatments. The results provide an insight to the relative rate of secondary succession and estimate the secondary succession trajectory under different levels of disturbance. Chapter 4 looks at the decadal impacts of the different treatments on belowground systems. The decadal treatment effects on soil physical property, soil nutrient concentrations and litter invertebrate community were assessed to determine suitable reforestation methods with minimal impacts to belowground systems. Chapter 5 examines the treatment effects on sapling recruitment of common tree species and whether these saplings can survive and grow within each treatment. Chapter 6 summarizes the key findings of this study and provides overall discussions including potential applications to forest management. The information from this study would increase the general understanding towards suitable forest management techniques to restore and conserve natural ecosystems.



Source: Survey by Forestry Agency.

Figure 1.1. Wildlife damage to forests in Japan. Image taken from “Annual report on Forest and Forestry in Japan, Fiscal year 2019, Ministry of Agriculture, Forestry and Fisheries (MAFF)”.

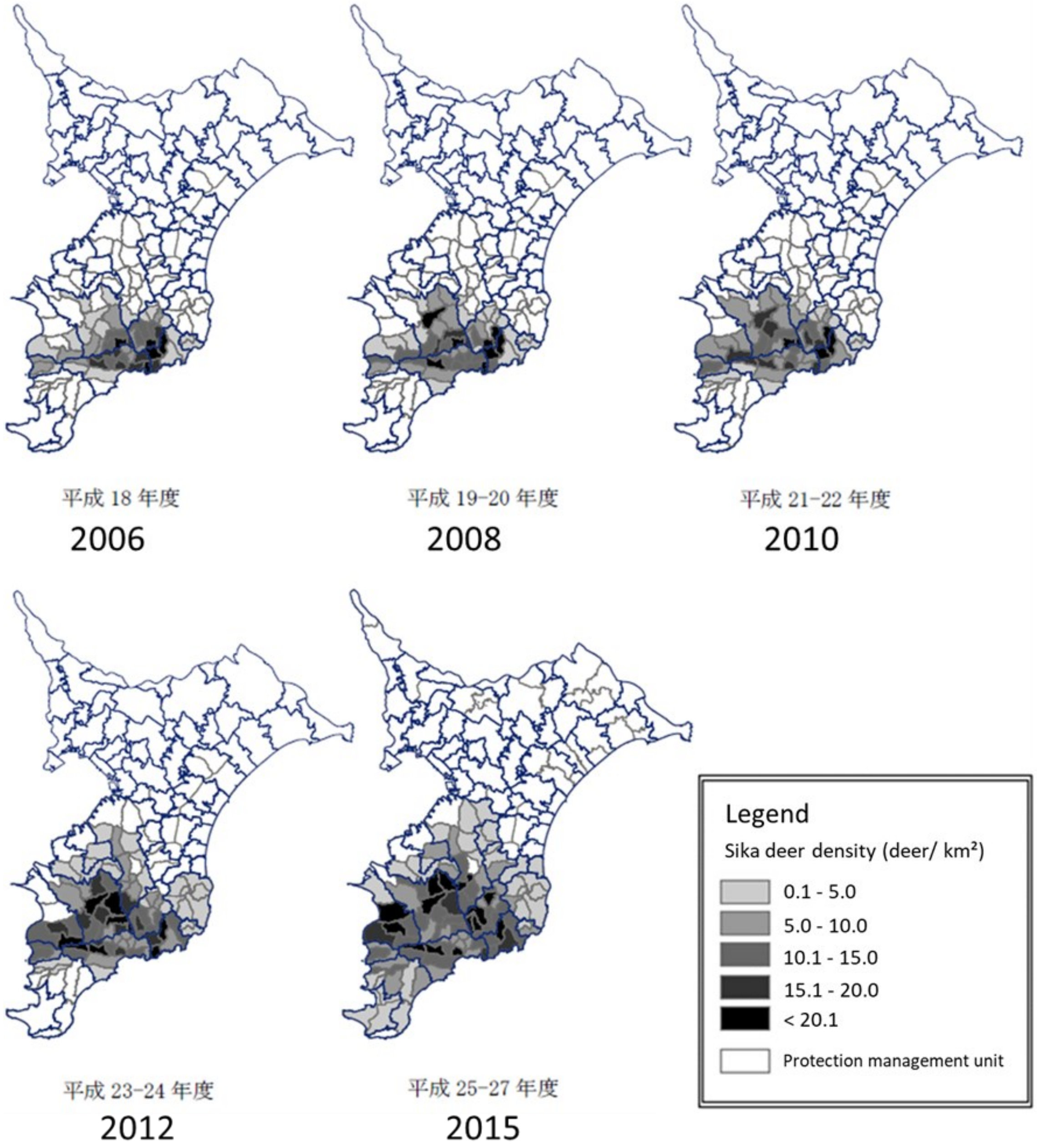


Figure 1.2. Population density and habitat range expansion of sika deer within the Boso peninsula. The image was modified and translated from figure 15 in "4th Chiba Prefecture Wildlife Management Plan for Sika deer (2017)".

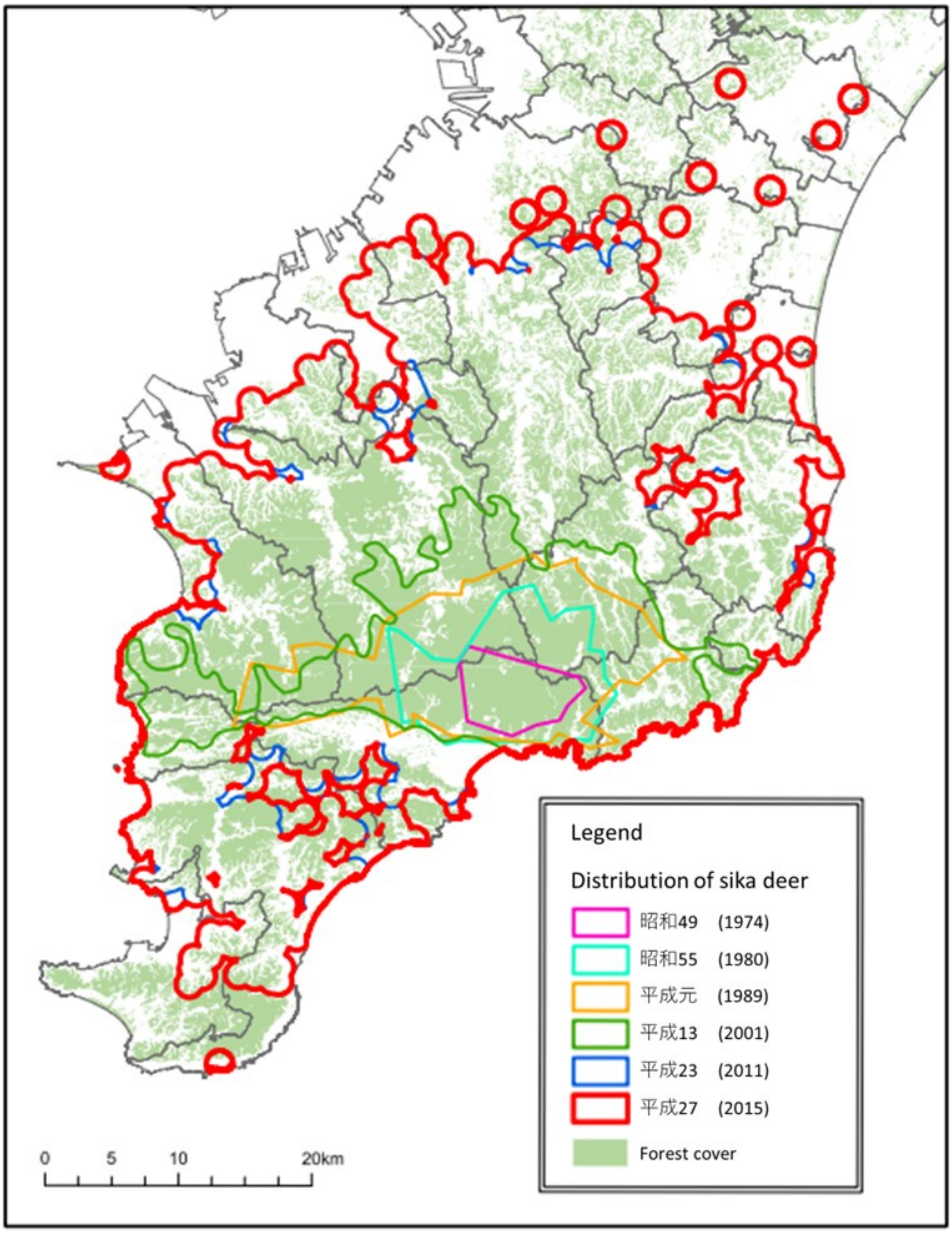


Figure 1.3. Sika deer habitat range expansion within the Boso peninsula. The image was modified and translated from figure 1 in "4th Chiba Prefecture Wildlife Management Plan for Sika deer (2017, in Japanese)". The legend depicts the distribution of deer from 1974 (pink) to 2015 (red).

Chapter 2. Study site

2.1. Study site history

This study was conducted in warm temperate evergreen secondary forests within the University of Tokyo Chiba Forest (UTCBF) established in 1894, located in the southern part of the Boso Peninsula, Japan. Before the 1970s, many forests were regularly harvested through coppicing for firewood which maintained the cyclic occurrence of canopy gaps (Oono et al., 2020). However, with the industrial revolution, the demand for firewood decreased, resulting in the abandonment of previous coppicing practices and a subsequent maturation of secondary forests towards closed canopies (Figure 2.1). The forests in this study were previously coppiced (mainly oak species) before abandonment in the 1960's (Shiraishi et al., 2004; Suzuki, 2013). Prior to the experiment in 2008 by Suzuki (2013), the vegetation at the ground layer under the closed canopy had disappeared due to deer herbivory, which continues today, except under canopy gaps. Currently within the Boso peninsula, there is an increasing concern for the future of current *A. firma* stands due to the declining number of *A. firma* saplings (Abrams et al., 2017). Both *Abies firma* and *Quercus* species made up the majority of the canopy layer in the Boso peninsula. According to personal communications with the local community, utilisation of *A. firma* was low whereas *Quercus* species provided excellent fuelwood and exhibited high regeneration potential after coppicing. Observations in the field, however, indicated that saplings from *Quercus* species were not detected, being supressed by deer herbivory.

Sika deer (*Cervus nippon*) are native to the Boso peninsula and their population density (Figure 1.2) and habitat range (Figure 1.3) has gradually increased since the ban on deer hunting in the 1970s (Figure 2.1). Historically, the deer population declined due to overhunting and habitat loss, reaching a bottleneck since the 1900's until laws were based to prohibit deer hunting in the 1960's (Figure 2.1). Since the prohibition of deer hunting was implemented, the deer populations rapidly increased and began to negatively affect plant regeneration. The estimated sika deer density in the studied region in 2018 was 10 – 13.5 deer km^{-2} (Hisamoto et al., 2019) which exceeds the target set by the Chiba prefecture government for forest conservation between 3 – 7 deer km^{-2} (Chiba Prefecture, 2017). This target set by the Chiba prefecture government was based on previous reports of negative effects on sapling regeneration of *Quercus* species under deer densities of 5–15 deer km^{-2} (Chiba Prefecture, 2004). Initial efforts to control deer populations were mainly focused on culling deer populations (Chiba Prefecture, 2017) which are still ongoing, though the efforts are becoming more challenging due to decreasing number of active hunters. Therefore, deer density in the study sites (10–13.5 deer km-2) was considered to be overpopulated with a negative influence towards tree sapling regeneration. Other ungulates in the region included wildboar (*Sus scrofa*) and the non-native, introduced muntjac species (*Muntiacus reevesi*) which also exert herbivory pressure on the vegetation. In terms of biomass, sika deer is five times greater than the muntjac suggesting relatively higher impacts to the ecosystem compared to muntjac. Among the ungulate species, sika deer accounts for greatest proportion of forest damage (Figure 1.1; Chiba prefecture, 2017). Thus, the current study assumes sika deer to be the main cause of vegetation cover loss compared to wildboar and muntjac.

2.2. Study site descriptions

Three study sites, Kotsubosawa (KBS), Hiratsuka (HRT) and Hinokio (HNK) were selected taking advantage of previously established experimental sites (Suzuki, 2013) and are located 2 – 5 km apart from one another (35° 8 – 11′ N, 140° 7 – 9′ E, Figure 2.2). HNK and HRT are located 300 m above sea level (a.s.l), with mean, minimum, and maximum annual air temperatures in 2019 of 14, - 4.5, and 33.8 °C, respectively, and annual precipitation of 3007 mm (The University of Tokyo Forests, 2021). The forest age at the three study sites were at least 52 to 59 years old prior to the experimental treatment in 2008.

KBS is located two kilometres away the nearest coastline with an altitude of 50 m a.s.l. (Figure 2.2). The mean, minimum, and maximum annual air temperatures for KBS in 2019 was 14.8, - 2.3, 33.2 °C, respectively, with an annual precipitation of 2617 mm (The University of Tokyo Forests, 2021). The study plots in KBS are located next to a forest road which is regularly used as an access road to other sites in the area and is located close to residential areas. The canopy layer in KBS is dominated by evergreen trees such as *Quercus glauca*, *Castanopsis sieboldii* and *Eurya japonica* together with a mix of other evergreen and deciduous woody species.

HNK is located seven kilometres away the nearest coastline with an altitude of 300 m a.s.l. (Figure 2.2). The mean, minimum, and maximum annual air temperatures for HRT in 2019 was 14, - 4.5, and 33.8 °C, respectively, and annual precipitation of 3007 mm (The University of Tokyo Forests, 2021). HRT is located approximately 1,200 metres from the main road away from residential areas. The canopy layer in HRT is dominated by *Quercus glauca*, *Cleyera japonica*, *Eurya japonica*, *Castanopsis sieboldii* and *Quercus salicina*.

HRT is located seven kilometres away the nearest coastline with an altitude of 300 m a.s.l. similar to that of HNK (Figure 2.2). The mean, minimum, and maximum annual air temperatures for HRT in 2019 was 14, - 4.5, and 33.8 °C, respectively, and annual precipitation of 3007 mm (The University of Tokyo Forests, 2021). HRT is located approximately 700 metres from the main road away from residential areas. The canopy layer in HRT is dominated by *Quercus acuta*, *Eurya japonica*, *Camellia japonica* and a mix of other evergreen and deciduous species.

Despite sika deer density being similar among study sites, there was a difference in the presence or activity period of deer across study sites, which was also observed for muntjacs and wild boars. This was verified using preliminary camera trap results between May 2019 to June 2020 (unpublished) to calculate the average photo capture rate [total number of pictures / camera trap days] for each ungulate species (Table 2.1). In both KBS and HNK, muntjac capture rates were higher than that of sika deer. However, different capture rates for different species do not necessarily correspond to their actual impact on the ecosystem. Based on camera trap data, wildboar and sika deer tended to avoid camera traps whereas muntjac either ignored or were curious of camera traps, resulting in inflated capture rates. Furthermore, due to body size differences and biomass between sika deer and muntjac, sika deer are generally believed to consume greater amount of vegetation and thus, have greater impacts compared to muntjac. In addition, the capture rates of ungulate species among the study sites was low (> 0.5 pictures per camera trap day, Table 2.1) and thus, judged to be similar.

2.3. Experimental treatment

A 2-by-2 factorial experiment was designed with two factors: canopy gap creation and deer exclusion were established in 2008 by UTCBF (Suzuki, 2013; Figure 2.3). Canopy gap plots of 20-by-20 m were created by logging in 2008 followed by the complete removal of woody material on the forest floor prior to the experiment at each site. The artificial canopy gap (400 m^2) was greater than the typical sizes of natural gaps in Japanese temperate forests (30-140 m^2; Yamamoto, 2000). The canopy gap plots were further subdivided into four 10-by-10 m subplots. Of these four subplots, two were installed with deer exclosure fences, polyethylene nets of 30 mm mesh up to 1.5 m height from the ground to exclude middle to large-sized mammals (deer exclusion + gap creation: EG). The remaining two subplots were defined as gap creation plots (gap with deer: G). The experimental setup was also repeated under closed canopy natural forests adjacent to the gap plots with the unfenced subplots set as the control (closed canopy with deer: Ctrl) while the fenced subplots defined as deer exclusion (closed canopy + deer exclusion: E). The experimental treatment was replicated at the three study sites, establishing a total of six replicates for each treatment (n = 24). The sampling locations for the vegetation cover (chapter 3), soil physical properties, soil nutrient analyses, litter mass and litter invertebrates (chapter 4) and tree sapling density of common tree species (chapter 5) are illustrated in Figure 2.4.

According to the experiment design, the vegetation in the clearcut plots reflect the recruited vegetation with/without deer after gap creation (G vs EG). Similarly, the difference in vegetation in the closed canopy plots reflect the changes from deer exclusion (Ctrl vs E). The gap plots (G and EG) and the closed-canopy plots (Ctrl and E) are separated more than 10 m apart in HNK and KBS, while the gap and closed canopy plots in HRT were adjacent to each other due to topographic limitations. The proximity between the gap and closed canopy plots allows for seed dispersal from the natural vegetation surrounding the study plots and those present in the closed canopy plots.

Table 2.1. Mean camera trap capture rate for ungulates among study sites.

Ungulates	Study site		
	KBS	HNK	HRT
Sika deer	0.04	0.08	0.25
Muntjac	0.38	0.43	0.25
Wild boar	0.03	0.09	0.05

The values represent the mean number of pictures captured per camera trap per day between May 2019 to June 2020.

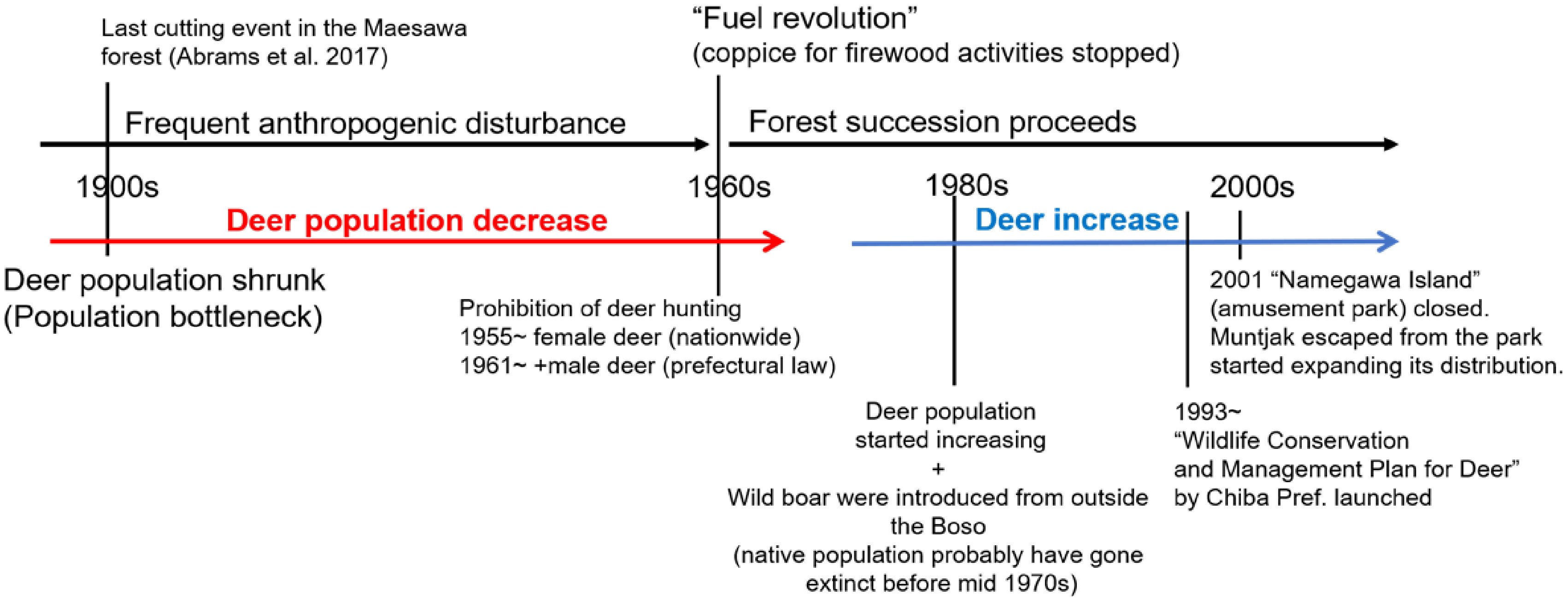


Figure 2.1. Study site history.

Figure 2.2. Map of study area within the UTCBF, Chiba prefecture, Japan. The locations on the map insert (right) refer to study sites Kotsubosawa (KBS), Hinokio (HNK) and Hiratsuka (HRT) respectively. The map of Japan was downloaded from an online free resource (https://d-maps.com/carte.php?num_car=365&lang=en) while the map of Kamogawa city was generated using shapefiles downloaded from GADM (https://gadm.org/download_country_v3.html) and modified in ArcGisPro (Ver 2.5.2).

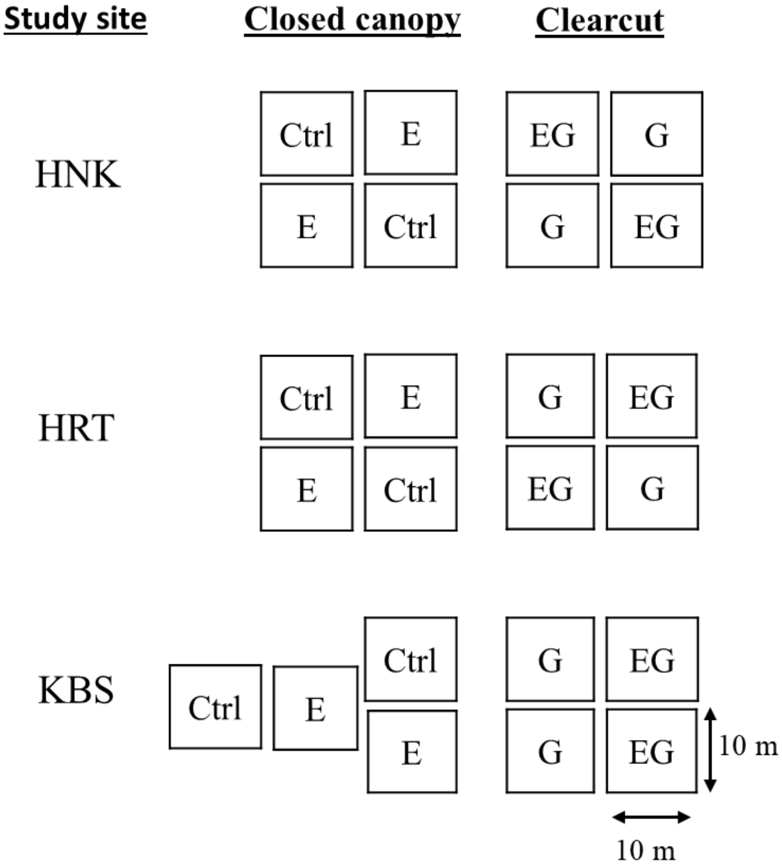


Figure 2.3. Experimental design with clearcut and deer exclusion fencing. The 20-by-20 m plots at each study site were separated according to two levels of canopy cover (closed canopy and clearcut) in 2008 (Suzuki, 2013), each further divided into four 10-by-10 m subplots subjected to one of four treatment groups: **Control (Ctrl)** = Closed canopy and open to herbivores, **E** = Closed canopy with herbivores excluded, **G** = Clearcut and open to herbivores and **EG** = Clearcut with herbivores excluded.

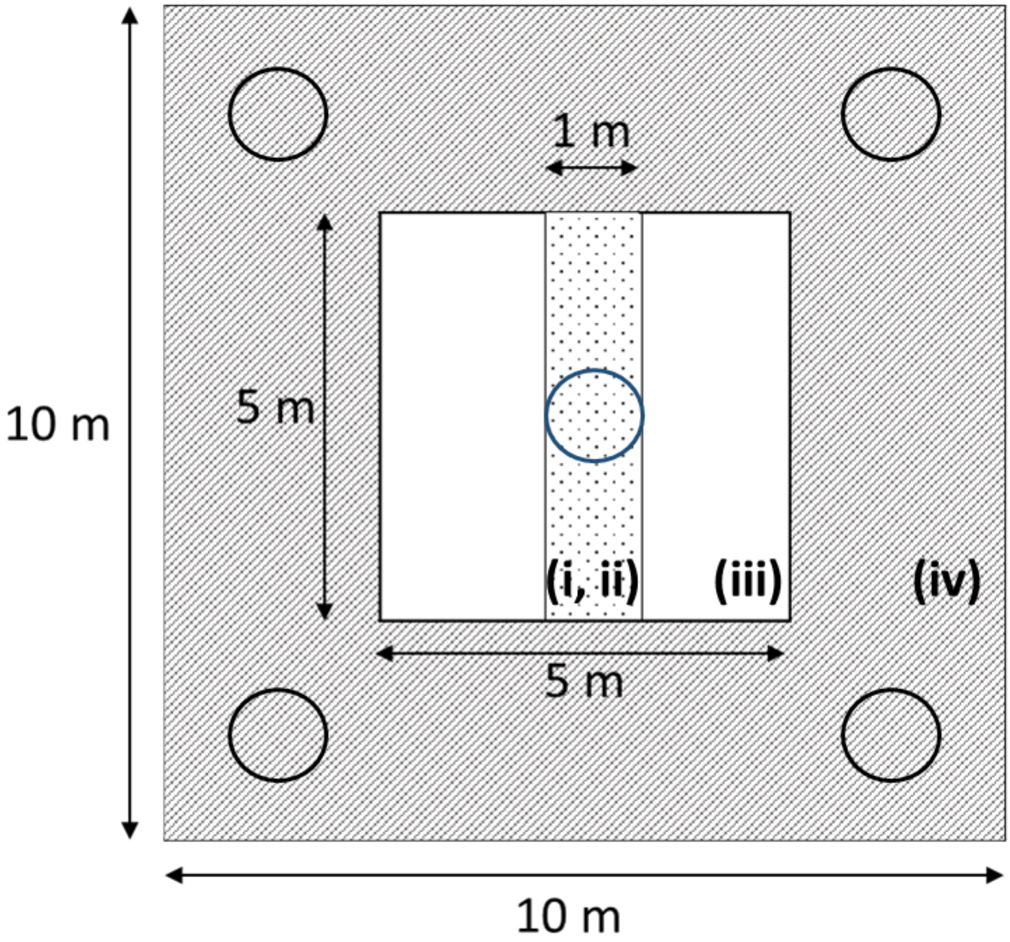


Figure 2.4. Sampling locations for different analyses in the current study.

Vegetation surveys were conducted within 5 m quadrats established at the centre of each 10 m subplot (chapter 3). The five circles indicate the sampling locations for soil physical analyses (centre circle only), soil nutrient analyses, litter mass and litter invertebrates (chapter 4). Sapling density of common tree species were sampled according to their specific height classes (i to iv, chapter 5); height class (i) and (ii) within 5 m by 1 m belt transects, height class (iii) within 5-by-5 m quadrats and height class (iv) saplings within 10-by-10 m subplots (refer to chapter 5 for detailed explanations).

Chapter 3. Deer exclusion and gap creation effects on aboveground systems

3.1. Introduction

This chapter focuses on the effects of the different treatments established in 2008 (Suzuki, 2013, see chapter 2.2) on the vegetation cover and regeneration of trees which make up the canopy layer as well as non-tree species plants in the understory layer of warm temperate forests and compares the decadal changes within each treatment from the control. The impacts of deer and artificial gap creation on the aboveground vegetation have been introduced in the earlier chapters (1.2 and 1.4 respectively) highlighting their influence on the succession trajectory of secondary forests.

Deer exclusion is a well-known method, traditionally believed to be a safer method as opposed to gap creation with regards to promoting forest regeneration, maintaining the current tree flora, though it may be more time-consuming. However, recent improvements on the understanding of light limitation on forest regeneration have rekindled research interests on the combined benefits of gap creation and deer exclusion (Suzuki, 2013; Suzuki and Ito, 2014; Katagiri and Hijii, 2017). Combinations of canopy gaps and deer exclusion have been reported to increase plant species richness (Suzuki, 2013, Katagiri and Hijii, 2017; Sabo et al., 2019), improve survival and growth in tree saplings and seedlings (Shimoda et al., 1994; Tamura and Nakajima, 2017; Nagashima et al., 2019; Petersson et al., 2020) and improve the diversity of ground-layer plant communities (Burton et al., 2020). However, deer exclusion could result in low species diversity and plant species homogenisation (Nishizawa et al., 2016) due to fast-growing competitive species such as *Sasamorpha borealis* monopolizing available space and resources. In such cases, indirect positive effects of deer herbivory on fast-growing competitive species can improve seedling emergence and survival of other plant species (Murata et al., 2009). So far, there have been a limited number of studies on the long-term effects of a combination of "gap and fence" treatments on ecosystem restoration due to the long period needed for forest succession.

Earlier studies on the same experiment have shown that light limitation was the primary factor for inhibition of ground-layer vegetation recovery from herbivory damage (Suzuki, 2013) and that ground vegetation significantly increased in gap plots compared to the closed canopy plots (Suzuki and Ito, 2014). The successful recruitment of plants after clearcutting suggests the forest's ability to recover for a while after the disturbance. However, it remains unclear whether the recovery proceeded for a decade and became close to the vegetation of similar species composition to the canopy layer in the control plots. We set the following hypotheses: 1) The positive effects of clearcutting, which stimulated the regeneration and growth of vegetation at the start of the experiment, continued for 11 years and maintained high plant species richness. 2) Clearcutting and deer exclusion improved the foliar cover of trees and non-tree species which had been limited by deer herbivory and light availability. 3) When limitations of light availability and deer herbivory were overcome, tree species were able to successfully regenerate and were not supressed by non-tree species. 4) The tree species composition after clearcutting was closer to those in the uncut plots in the absence of deer. These hypotheses were tested based on the monitoring data to determine the treatment effects on the regeneration of trees and understorey species.

3.2. Method

3.2.1. Vegetation survey

Vegetation surveys were conducted within 5 m quadrats established at the centre of each 10 m subplot to avoid boundary effects, between October and early November in 2019 (Figure 2.4). The percentage of foliar cover of all plants (including mature trees and saplings) within the 5 m quadrat were estimated and classified according to their growth forms: trees and non-tree species (shrubs, vines, graminoids and forbs).

The foliar cover of each plant species was visually estimated by assessing their percent cover within the 5 m quadrat, accounting for those in the ground and canopy layer. All plant species that corresponded to each category of the plant growth form were summed for each respective category. In situations where high overlap of foliar cover occurs, such as in trees, the total foliar cover could exceed 100%. The plant species with a foliar cover of less than 1% was noted as '+' and assigned a value of 0.1% while those with cover exceeding 100% was assigned a value of 99.9% during statistical analyses. For this study, non-tree species cover in each subplot (within the 5 m quadrat) was defined as the sum of foliar cover of shrub, vine, graminoid and forb species.

For each treatment, the mean plant species richness, mean tree species richness, mean percentage cover (*MC*) of evergreen trees and *MC* of trees palatable to deer was calculated for each treatment. The mean species richness was calculated as the sum of different species per 25 m^2 = [5 x 5 m^2] of each treatment divided by six replicates per treatment. The total tree species in each treatment was the number of different tree species per 150 m^2 = [5 x 5 m^2 x 6 replicates per treatment]. The *MC* was calculated as the sum of the estimated foliar cover (%) of relevant species per 25 m^2 of each treatment divided by six (the number of replicates of each treatment).

The palatability of tree species to deer were assessed based on records of deer herbivory under varying conditions in Japan (Suzuki et.al., 2008; Agetsuma, Agetsuma-Yanagihara and Takafumi, 2011; Suzuki, 2013; Hashimoto and Fujiki, 2014). In situations where the records were inconsistent, the palatability of plant species to deer was determined according to the past experiences of the study site.

3.2.2. Statistical analyses

Treatment effects on plant species richness and foliar cover

The effects of the experimental treatments on plant species richness were estimated using Poisson regression with a log link taking treatment as the fixed variable while accounting for the effects of study site and the study plots of 20 x 20m as nested random factors. This analysis was repeated to determine treatment effects on tree species richness. To assess treatment effects on vegetation cover, given the binomial distribution nature (non-discrete) of the data, beta regression with logit link was applied, setting 99.9% as the maximum foliar cover. This analysis was again repeated for foliar covers of evergreen tree species and palatable tree species. The *glmmTMB* package for *R* was used for above Poisson and beta regressions. If the individual treatment effect was statistically significant in the regression analyses (*Wald* test, p-value < 0.05), a *post hoc* pairwise comparison of estimated marginal means (*emmeans* command of package *emmeans* in *R*) using the Tukey-Kramer method was applied.

Relationship between Tree foliar and non-tree species cover in clearcut plots

To estimate the negative effects of the non-tree species in the clearcut plots (EG and G) onto the foliar cover of tree recruitments, beta regression following a beta distribution with logit link was applied accounting for the effects of study site and the 20 x 20m study plots as nested random factors in the regression model. Because the foliar cover of trees and non-tree species were both affected by deer (see results), deer presence was added as a fixed variable in the model to statistically remove the effect.

Multicollinearity between the explanatory variables in the model was assessed using the *performance* package in *R*, revealing low correlation between them (VIF < 1.1). The goodness-of-fit of each regression model (M_0 to M_3, supplementary file A1) to the actual data was assessed by generating *Q-Q* and residual plots using *simulateResiduals* function in *DHARMa* package of *R*. The model for tree species cover explained by deer and non-tree species cover showed significant heteroscedasticity, and thus the issue was solved by removing the random effect of subplots which resulted in the best fit model based on AIC and BIC values. The best fit model was then plotted to estimate how tree foliar cover changed with increasing non-tree species cover, with and without deer.

Treatment effect on plant species composition

Non-metric multidimensional scaling (NMDS) was applied to the plant species data within the 5-m quadrats based on their incidence (presence/ absence) in the treatment plots. The NMDS graph was generated depicting the plant species composition among different treatments with each data point representing a different plant species.

In the case of tree species composition among the experimental treatments, dissimilarity indices (*vegdist* command of *vegan* package, Jaccard distance) were first generated based on the presence/absence data and then used to calculate the mean dissimilarity among treatment groups (*meandist* command of package *vegan* in *R*). These processes were performed using the vegan package (Oksanen et al., 2020) of R (R Core Team, 2021).

3.3. Results

3.3.1. Treatment effects on plant species richness and foliar cover

A total of 144 plant species were identified during the vegetation survey with the highest mean in treatment EG and the lowest mean value in Ctrl (Table 3.6.1). Of these 144 plant species, 36.1% consisted of tree species with an average of 24 tree species in Ctrl, treatment E and G while tree species richness was approximately 1.9 times greater in treatment EG. Deer exclusion showed a positive effect on the mean plant species richness under closed-canopy and gap conditions. In addition, the clearcut plots generally had significantly higher mean plant species richness compared to closed-canopy plots. This significant increase in plant species richness was likely attributed to clearcut effects rather than deer exclusion, both of effects appearing to be additive. However, in the case of tree species, the positive effect of deer exclusion was only detected under gap conditions.

After 11 years of treatment, the non-tree species cover remained depauperate in the closed canopy plots (Ctrl and E) while it increased in the clearcut plots (G and EG, Figure 3.7.1). Deer exclusion increased the foliar cover of trees, forbs and vine species (EG > G and E > Ctrl) while graminoid and shrub cover increased in the clearcut plots (G >EG, Figure 3.7.2). Treatment EG plots were dominated by tree species, similar to closed canopy plots whereas treatment G plots were dominated by non-tree species.

The foliar cover of trees was significantly higher in treatment EG than G, while both treatments remained significantly lower than the closed canopy plots. Tree foliar cover in closed canopy plots consisted mainly of palatable evergreen tree species, making up more than 90% of the total tree foliar cover (Table 3.6.1). The mean evergreen tree foliar cover in treatment G was less than a quarter compared to Ctrl and treatment E and Ctrl. Meanwhile, in treatment EG, foliar cover of evergreen tree species increased to approximately half that of Ctrl and treatment E. In the case of palatable tree species, the foliar cover was 17 times greater in treatment EG compared to that in treatment G indicating their recovery following deer exclusion after clearcutting. Foliar cover of non-tree species was highest in treatment G, significantly higher than the closed canopy plots (Figure 3.7.1). The foliar cover of non-tree species in the treatment G plots showed high spatial variation between and within study sites. For example, the non-tree species cover in both treatment G plots in KBS were > 60% and approximately 30% respectively while in HNK, one plot was > 85% and the other was approximately 21%. In addition, the dominant plant form for the non-tree species in treatment G plots varied greatly, with either graminoid, shrub or both forms being dominant.

3.3.2. Relationship between tree and non-tree species after clearcutting

The foliar cover of the trees recruited in the clearcut plots showed negative responses to the foliar cover of non-tree species and deer presence (Figure 3.7.3). The highest estimated tree foliar cover (86.5%) was achieved in the absence of deer and non-tree species while the least tree cover (9%) was obtained in the presence of both deer and dense (100%) non-tree species cover. According to the beta regression model M_2

(supplementary material A1), as the non-tree species cover increases, the suppression effect on the tree foliar cover also increases. The non-tree species cover was greater in treatment G (21–87%) compared to EG (6–55%). At 50% non-tree species cover, the estimated loss of tree foliar cover was 19.4% ((86.5-69.7)/ 86.5) in treatment EG while the loss in treatment G was approximately 50% ((43.5-21.7)/ 43.5). The current analysis successfully shows the presence of both direct and indirect negative effects of deer on tree regeneration. Overall, the combined effects of deer and dense non-tree species cover in treatment G severely limited regeneration of tree foliar cover.

3.3.3. Treatment effects on plant composition

The NMDS results (Figure 3.7.4) indicated that the plant species composition in treatment G was very different compared to the other treatments. In contrast to treatment G, treatment EG showed a small portion of similar plant species with treatment E. Based on the distribution of plant species represented by each data point in figure 3.7.4, comparisons between the closed canopy plots (Ctrl and E) and clearcut plots (G and EG) showed a greater proportion of plant species (forbs, graminoids, shrubs and vines) being found in the clearcut plots. Between treatments EG and G, tree and vine species were more abundant in treatment EG compared to G (see supplementary material A5). In general, plant species composition in treatment G diverged from those of Ctrl, E and EG while plant species composition in EG still retained similarities with treatment E.

The differences in tree species composition between treatments was assessed and quantified, showing a difference of at least 76% (0.76 / 1 x 100%) between closed canopy and clearcut plots (Table 3.6.2). Even between the clearcut plots, the difference in tree species composition was rather high, suggesting deer presence after clearcutting facilitated the recruitment of different tree species from the other treatments.

3.4. Discussion

3.4.1. Decadal treatment effects on vegetation cover and species richness.

This study assessed the successional trends in each treatment after a decade, using long-term data and sampling sizes five-times greater (25 m^2) than those of previous studies (Suzuki, 2013, Suzuki et al., 2021). In 2009, one year after the clearcutting event, treatment EG had the highest understory cover among the treatments (Suzuki, 2013). However, after a decade in 2019, the understory cover in treatment EG had decreased, becoming lower than that in treatment G. In the closed canopy plots, the vegetation cover (< 2 m height) remained low even after a decade.

A similar pattern in decadal change was also observed in the plant species richness. Between 2009 and 2019, plant species richness increased in treatments E and Ctrl, possibly due to differences in study plot size as well as the recruitment of species beyond the first year following experimental treatment which may not be detected by short-term studies. The possibility of plant species richness increasing under a closed canopy and deer browsing pressure has not been reported in previous studies, emphasizing the need for long-term observations.

The pattern of plant species richness among the different treatments in 2019 did not differ from those in 2009, indicating that plant species richness in the gap plots remained higher compared to closed canopy plots for over a decade supporting the hypothesis (1). In reality, plant species richness for treatments G and EG in 2019 was lower than that in 2009, which is remarkable considering that in this study, the plot size was five times greater than that used in 2009. The decrease in understory cover and species richness in treatment EG from 2009 to 2019 could be attributed to the regenerated overstory cover that limited availability of light and space for understory vegetation.

3.4.2. Competition between tree and non-tree species after clearcutting

The foliar cover of tree and non-tree species increased after clearcutting in both G and EG plots. However, foliar cover increase in tree and all non-tree species (forbs, graminoids, shrubs and vines) occurred only in the absence of deer supporting hypothesis (2). In contrast, deer presence in G plots facilitated foliar cover increase in graminoid and shrubs.

During the early phase of regeneration after clearcutting, recruited tree saplings would have been in competition with non-tree species and deer (Kern et al., 2012). The results suggest that the negative effects of non-tree species and deer to be additive, resulting in stronger suppression effects on tree species recruitment when both variables are present in G plots. The lone effect of non-tree species in EG plots did not appear to strongly inhibit tree regeneration compared to G plots, supporting hypothesis (3). Based on the regenerated tree cover in treatments G and EG, the suppression effect by deer on tree species appears to be greater than that of non-tree species. Deer herbivory on tree saplings (particularly palatable species) would negatively impact growth and survival of saplings; freeing up nutrients and space (or light) for non-palatable plants, such as ferns, to become dominant. Furthermore, the higher non-tree species cover in treatment G compared to EG suggests a greater chance of formation of "recalcitrant layers" that inhibit regeneration of tree species (Royo and Carson, 2006) appearing in treatment G. We caution the interpretation of the regression model outside of the actual data, due to the small sample size; nonetheless, the results indicate a higher rate of tree regeneration and species richness in treatment EG relative to treatment G.

3.4.3. Treatment effects on plant species composition

Deer herbivory and subsequent increased competition from non-tree species in G plots decreased the foliar cover of tree species, contributing to the large difference in tree species composition from other treatment plots. These results are corresponding to the second part of research question (1). In addition, mean foliar covers of evergreen trees and palatable tree species were both significantly lower in G plots than in EG plots, indicating dominance of non-palatable species in G plots.

Although EG plots also showed large differences in tree species composition from Ctrl plots, they included some common tree species of closed canopy plots, being closer to closed canopy plots compared to treatment G plots, supporting hypothesis (4). Considering these characteristics of species composition in EG plots together with the high species richness, if the management goal was to increase overall plant diversity, treatment EG may be a better option compared to treatments G, E and Ctrl. In contrast, if the management goal was to increase tree foliar cover with the least impact to the current forest composition, treatment E may be a safer option.

However, the current results only reflect the changes after 11 years, which is still a brief period in forest succession. Longer observations are necessary to confirm whether the current tree species composition persists, or changes as secondary succession progresses.

3.5. Conclusion

The results of the 11-year experiment indicates that (1) Clearcutting stimulated the regeneration and growth of vegetation which continued for 11 years, maintaining high plant species richness compared to the uncut plots. (2) Deer presence after clearcut delayed tree regeneration while increasing abundance of non-tree species. (3) Non-tree species exhibited negative relationship with tree cover in clearcut plots, but strong inhibitory effects were only detected in the presence of deer. (4) Deer presence after clearcutting delayed tree regeneration, altering the composition of tree species from those of other treatments. This difference was partially mitigated by deer exclusion after clearcutting. Overall, clearcutting can promote heterogeneity of vegetation in a forest ecosystem but only in the absence of deer.

3.6. Tables

Table 3.6.1. Plant species richness, tree species richness (including mature trees in closed canopy plots) and mean percent cover (*MC*, %) in treatment plots in 2019.

Treatments	Plant species richness (per 25 m^2)		Tree Species richness			Tree Foliar cover (%) (per 25 m^2)			
	All plants		Total			Evergreen		Palatable	
	Mean	SD	(150 m^2)	Mean	SD	*MC*	SD	*MC*	SD
Ctrl	18.5^{a}	6.6	24	8.8^{a}	2.9	96.9^{a}	13.7	95.7^{a}	14.7
E	21.5^{a}	8.4	24	9.7^{a}	2.7	109.3^{b}	17.7	102.5^{a}	26.8
G	31.5^{b}	6.0	23	8.7^{a}	3.0	20.0^{c}	12.6	2.7^{b}	2.9
EG	36.5^{b}	9.4	45	17.3^{b}	4.5	48.6^{d}	18.6	45.9^{c}	17.2

The total tree species richness (150 m^2) refers to the number of different trees recorded in all six replicates of each treatment. The tree foliar cover (%) of evergreen and palatable tree species was calculated by summing the estimated foliar cover of tree species according to their respective classifications within each subplot and taking the mean for each treatment. Significant differences following *post hoc* analysis of pairwise treatments are displayed as different letters (a-d) in superscript.

Table 3.6.2. Mean dissimilarity matrix of tree species composition (presence/absence) among experimental treatments.

Treatment	Ctrl	E	G
E	0.67		
G	0.82	0.81	
EG	0.81	0.76	0.78

3.7. Figures

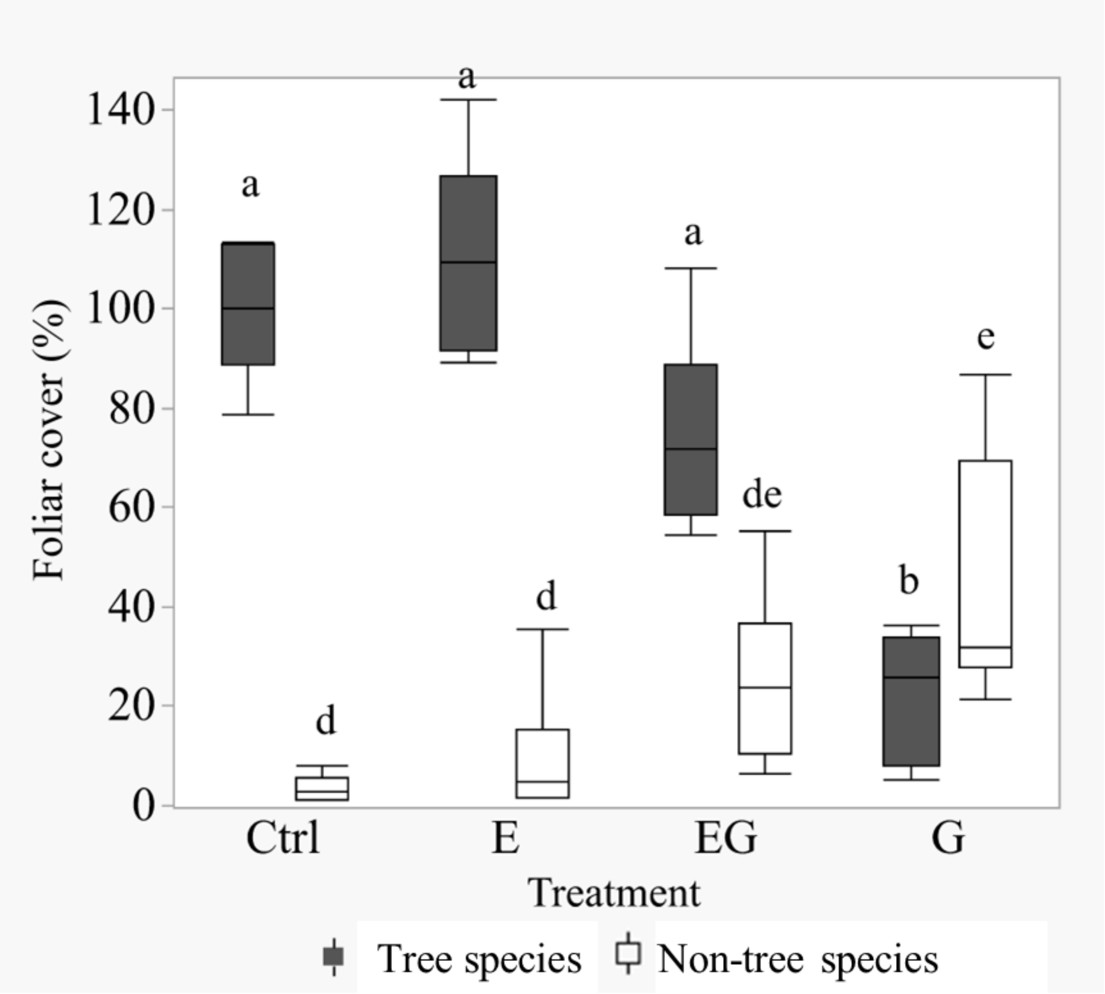


Figure 3.7.1. Boxplot of tree and non-tree species cover in the 5 m quadrats in 2019.

Tree cover in the Ctrl and treatment E represents changes after deer exclusion while tree cover in treatment G and EG represents the regenerated tree cover 11 years after clearcut. The non-tree species cover was calculated as the sum of graminoid, forb, shrub and vine species within the 5-by-5 m quadrats of each study plot. Significant differences following *post hoc* analysis of pairwise treatments are displayed by different letters; 'a-b' for tree cover and 'd-e' for non-tree species cover.

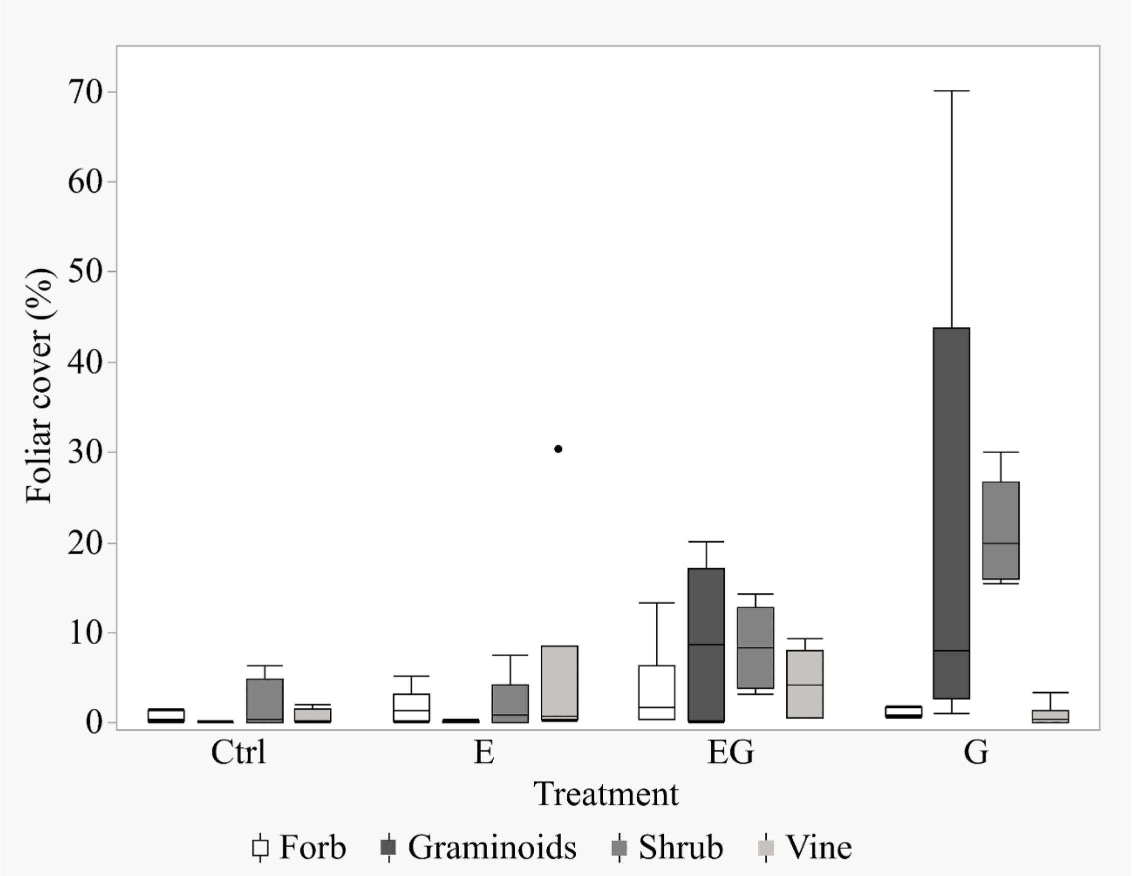


Figure 3.7.2. Boxplot of non-tree species foliar cover (%) in the treatment plots. The graph represents the non-tree species cover within the 5-by-5 m quadrats of each study plot, 11 years since the experiment began. The non-tree species were categorized according to plant forms: Forms, Graminoids, Shrubs and Vines.

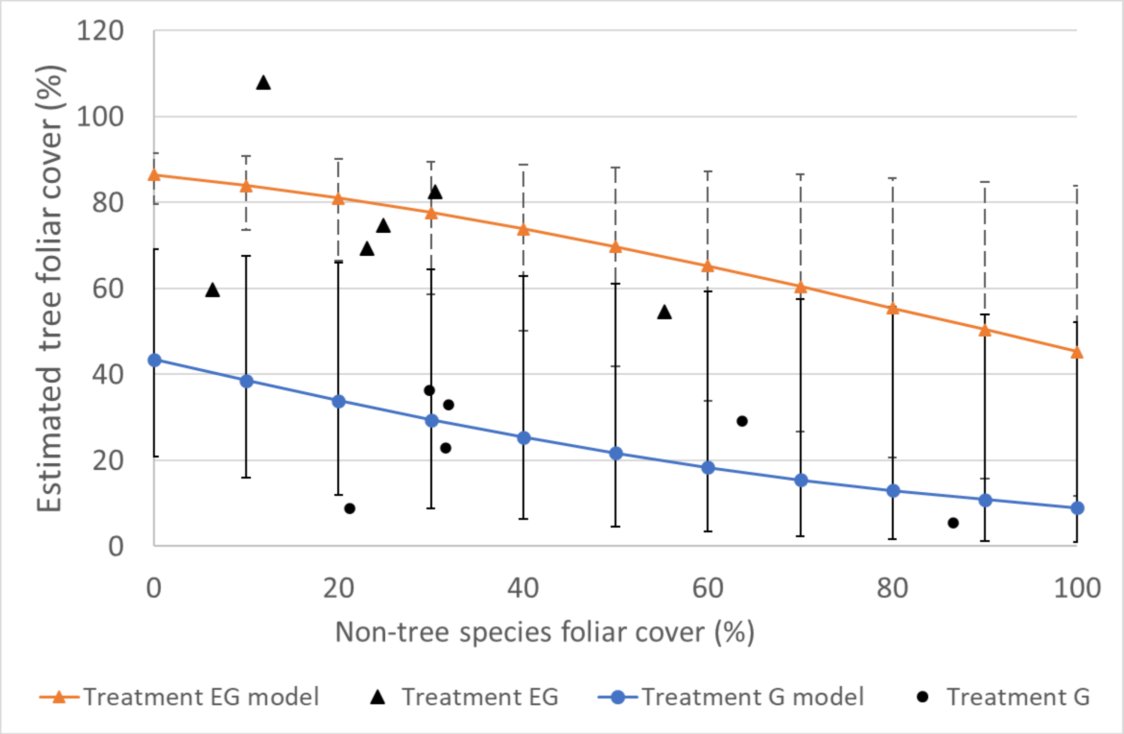


Figure 3.7.3. Plot of estimated tree foliar cover (%) against non-tree species cover (%). The data was generated based the regression model M2 (supplementary material A1). The individual data in the study plots are displayed as single data points. The error bars represent the standard error of the estimated tree foliar cover.

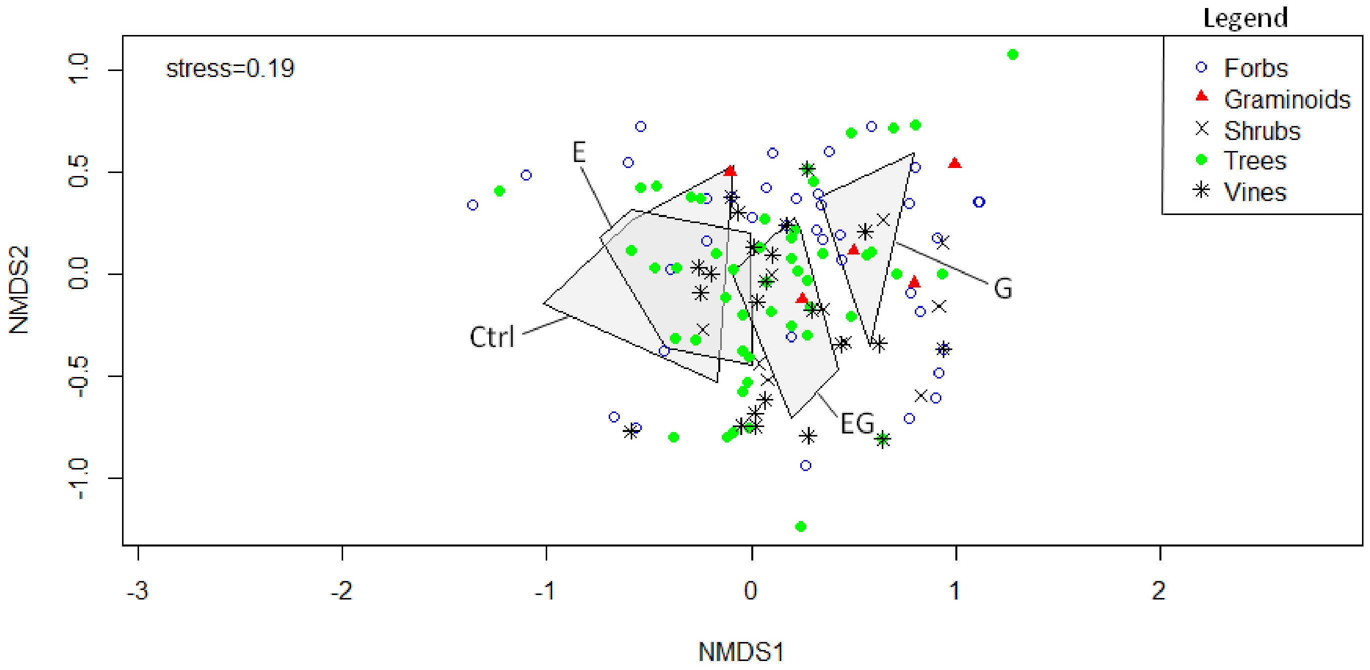


Figure 3.7.4. NMDS of plant species composition subjected to decadal treatment effects. The presence/ absence data of plant species within the 5 m quadrats were utilized in the NMDS to observe changes to the overall species composition. The abbreviations "Ctrl", "E", "G" and "EG" represent "Closed canopy with deer", "Deer exclusion fencing under closed canopy", "Clearcut with deer" and "Clearcut with deer exclusion fencing" respectively. Each data point in the figure indicates different plant species, classified into different plant forms in the legend.

Chapter 4. Treatment effects on belowground systems

4.1. Introduction

In this chapter, the decadal effects of the different treatments (deer exclusion, clearcutting, and deer exclusion combined with clearcutting) on belowground systems and their effects on the abundance of invertebrates are investigated. As discussed in Chapter 1.2, deer overabundance causes reduction of belowground systems as well. The loss of vegetation cover in aboveground systems through deer herbivory or gap creation reduces the input of litter material to the soil resulting in either thin litter layer or exposed soil surfaces. The litter layer provides a habitat for litter invertebrates, being directly proportional to invertebrate abundance (Suzuki and Ito, 2014). The reduction in invertebrate abundance in turn, reduces the rate of litter breakdown and decomposition, disrupting nutrient cycling processes (Bardgett and Wardle, 2003). In addition, the exposed soil surfaces due to vegetation cover loss is susceptible to leaching by rain reducing soil nutrient concentrations (Furusawa et al., 2016). The self-recovery of ecosystems, especially that of belowground systems would be a major issue in developed countries suffering from deer overpopulation and decline in hunters and predators of deer (Côté et al., 2004; Kaji et al., 2010).

Deer exclusion fencing (introduced in chapters 1.3 and 3) can mitigate negative effects of deer on aboveground and belowground systems, increasing vegetation cover (Shimoda et al., 1994; Bressette et al., 2012, Lessard et al., 2012; Suzuki and Ito, 2014) and reduces disturbance to the litter layer, increasing litter mass (Takahashi and Kaji, 2001; Suzuki and Ito, 2014; Nakahama et al., 2020). The indirect positive effects of deer exclusion on litter mass, increases the abundance of litter- and soil-dwelling invertebrates (Miyashita et al., 2004; Bressette et al., 2012, Lessard et al., 2012; Suzuki and Ito, 2014; Katagiri and Hijii, 2017), though the positive effects have been suggested to decline in significance over time (Ramirez et al., 2021).

Soil compaction, indicated by soil bulk density increases due to deer trampling and mitigated by deer exclusion (Suzuki and Ito, 2014) may also influence invertebrate abundance through changes in their physical environment (Cassagne et al., 2003; Lagendiijk et al., 2022) such as reduction of soil bulk density, or increased soil porosity (Gass and Binkley, 2011; Shelton et al., 2014; Suzuki and Ito, 2014). An increase of invertebrates is expected to speed up litter decomposition and nutrient release (Meyer et al., 2011), supporting vegetation growth. However, so far, such positive feedback has yet to be supported by field measurements. In addition, the effects of deer exclusion on the nutrient content of forest soil has not been generalised for most ecosystems and forest types. For one thing, it is because the effects of deer exclusion fencing are not easily detected by short-term studies. In a preceding study, significant changes in soil compaction have been reported after long periods of deer exclusion (Maillard et al., 2021). Moreover, an increase in soil total nitrogen (N) has been detected after 16 years of deer exclusion (Gass and Binkley, 2011). However, such trends were not found in deer exclusion experiments of less than 8 years (Mohr et al., 2005; Furusawa et al., 2016; Maillard et al., 2021). To understand the true effects of deer exclusion on forest belowground systems, fenced systems need to be monitored for more than a decade, especially focusing on the soil's physical and nutrient conditions.

Recent studies have examined the complex results of deer exclusion combined with canopy gaps (Suzuki and Ito, 2014; Katagiri and Hijii, 2017) with positive effects to aboveground vegetation (see chapter 3). Canopy gaps, both natural and artificial ones, facilitate plant regeneration by increasing light availability even in the presence of deer (Shimoda et al., 1994; Suzuki, 2013) with significant negative effects on the soil porosity (Suzuki and Ito, 2014). When deer exclusion is combined with gap creation, the abundance and species richness of ground vegetation are further increased (Suzuki and Ito, 2014; Katagiri and Hijii, 2017, see chapter 3). Meanwhile, canopy gaps expose the litter layer and soil, increasing soil temperatures and moisture loss from the litter layer (Katagiri and Hijii, 2017). If deer are not present in gaps, as when exclosure fences are installed, these negative effects might be mitigated by the rapid increase of vegetation and litter material after gap creation, resulting in a positive cascade on soil fauna and decomposition processes. In fact, deer exclusion from gaps is reported to reduce soil bulk density, increase litter mass, and resultantly increase the abundance of litter invertebrates (Suzuki and Ito, 2014). Consistent effects are also reported for small gaps caused by 60% thinning intensity (Katagiri and Hijii, 2017). In canopy gaps where deer are present, however, litter accumulation and the recovery of vegetation tend to be inhibited, causing a temporal decrease of invertebrate decomposers (Suzuki and Ito, 2014). From these results, installation of deer exclusion fences seems to be a good management option for forests where natural or anthropogenic gap creation occurs.

Despite this, the combined effects of deer exclusion and canopy gaps have yet to be clarified on soil nutrient conditions and invertebrate decomposers. Invertebrate taxa are reported to show different responses to gap creation: Chilopoda abundance is generally reduced (Richardson et al., 2010; Suzuki and Ito, 2014; Perry et al., 2018) whereas Collembola abundance either increased or decreased displaying mixed responses (Richardson et al., 2010; Suzuki and Ito, 2014; Perry et al., 2018). Meanwhile, canopy gaps enhance leaching by rain, and are expected to decrease the soil mineral content, though they gradually recover as vegetation increases (Bormann and Likens, 1979; Prescott et al., 2003). Such changes in soil nutrient concentrations may cause further responses in some invertebrate taxa, considering the established associations between invertebrate abundance and soil nutrient concentrations (Collembola with soil K and P, Geissen et al., 1997; Amphipoda and Isopoda with soil Ca, Kaspari and Yanoviak, 2009; Ohta et al., 2014). In particular, considering the dominant effect of soil Na content on the invertebrate community and abundance (Kaspari et al., 2009), leaching of Na after clearcutting may cause long-term population declines in invertebrates. So far, the effects of gap creation on various soil nutrient properties and invertebrate taxa are mostly unknown, especially when deer exclusion is combined with gap creation.

Previous studies (Suzuki and Ito, 2014; Katagiri and Hijii, 2017) only compared the short-term responses to different treatments lasting five to six years on belowground systems, while information on long-term responses is scarce and yet to be confirmed. Furthermore, the response of soil nutrient concentrations to the combined treatment effects are yet to be clarified, this study would be the first report on the subject. Based on the previous studies, it is predicted that deer exclusion in canopy gaps would successfully mitigate the negative impact of clearcutting even in the long run. Specifically, the decadal exclusion of deer after clearcutting is predicted to: 1) continue to show positive effects on the litter mass; 2) continue to offset the negative effects of clearcutting on the abundance of litter invertebrates, and 3) increase soil nutrient concentrations by indirectly promoting vegetation growth and nutrient cycling processes. Meanwhile, it is also predicted that 4) deer exclusion under closed canopies continuously mitigates the negative impacts of deer for a decade, by increasing litter mass and litter invertebrates. In addition to testing the above hypotheses, the specific responses of litter invertebrate taxa to each treatment are described. In addition, the potential effects of soil properties (physical and nutrient concentrations), vegetation cover and treatment conditions (deer and clearcutting) on abundances of invertebrate taxa were verified through model selection. By testing the hypotheses (1) – (4) and by revealing the responses of litter invertebrate fauna, the decadal success of deer exclusion in canopy gaps and other treatments can be assessed to suggest suitable effective management options for aboveground and belowground systems of forests.

4.2. Method

4.2.1. Sampling of Litter Invertebrates

Invertebrates in the litter layer (hereafter referred as litter invertebrates) were sampled during late October 2020 using 20-by-20 cm wooden frames within each 10-by-10 m subplot. Five samples were collected from each subplot, one at the centre and one at each corner located one metre away from the edge of the subplot (Figure 2.4). The litter materials within the frames were gathered into paper bags and hand sorted within 24 hours. Litter invertebrates were collected using aspirators and stored in 70% ethanol. Litter invertebrates were identified under a stereo microscope according to their respective major taxonomic groups. After sorting, the litter materials were oven-dried at 70 °C for 48 hours to obtain their dry weights. The litter mass (kg m^{-2}) within each 10-by-10 m subplot was pooled and calculated as [total dry weight of litter material in each subplot (Kg) / (0.2 m x 0.2 m x 5 replicates)]. Due to a typhoon event in 2020, one of two Ctrl study plots in KBS was damaged and could not be sampled for litter invertebrates. Thus, only five replicates of the Ctrl were available for litter invertebrate investigations.

4.2.2. Soil physical properties

Soil samples from each subplot (10-by-10 m) were collected for measurement of water retention capacity and soil bulk density. Soil samples of 0-5 cm depth were taken from the centre of each subplot using 400 ml corers in May 2019 and repeated in August 2020 using 100 ml corers (Figure 2.4). The 100 ml corer was used in the sampling in 2020 to avoid repeated sampling of the same locations while efficiently avoiding large roots. For measurement of soil fine porosity and soil bulk density, the soil samples were submerged underwater for 24 hours and then placed onto a porous plate for another 24 hours. The dry weight was obtained through oven-drying at 70 °C for 48 hours. The soil fine porosity (capillary) was determined using the porous plate method while the soil bulk density was calculated as the oven dry weight of fine soil material per unit volume (Editorial Committee of Methods for Forest Environment Study, 2010). The analyses in this paper utilise the averaged values of soil bulk density and soil fine porosity for each subplot between 2019 and 2020.

4.2.3. Soil nutrient properties

Soil samples at the centre and one metre from each corner within each 10-by-10 m subplot were collected in August 2019 using a 100 ml soil corer to sample up to 5 cm of the topsoil layer (Figure 2.4). The collected soil samples (500 ml subplot^{-1}) from each subplot were sieved through a 2 mm mesh and mixed thoroughly to obtain a composite sample per subplot for soil nutrient analyses. 0.05 M Ammonium acetate — 0.0114 M Strontium chloride solution (Japanese Society of Soil Science and Plant Nutrition, 1997) was utilized for exchangeable soil Potassium (K), Magnesium (Mg) and Calcium (Ca). Their concentrations (K, Mg and Ca) were determined using an Atomic Absorption Spectrophotometer (Shimadzu, AA-6800). The exchangeable soil Phosphorus (P) was determined using the *Truog* method with molybdenum blue colorimetry (Japanese Society of Soil Science and Plant Nutrition, 1997) while the total soil nitrogen (N) was determined with an NC analyser (Thermo Fisher, Flash Ea 1112 Series). In this chapter, we only considered the treatment effects on soil total N which includes both organic and inorganic forms of N as an overall indicator of soil fertility and quality (Al-Kaisi et al., 2005; Li et al., 2022). As such, the soil total N simply reflects the N content in the soil and not the available N for plant uptake, though N mineralization through symbiotic relationships with microbes such as mycorrhizal fungi are possible (Hestrin et al., 2019).

4.2.4. Statistical analyses

GLMM regression was performed using *glmmTMB* package for *R* to examine the experimental treatment effect on the litter dry mass (kg m^{-2}), soil physical properties (soil bulk density and soil fine porosity) and soil nutrient properties (concentration of Total N, P, K, Mg, Ca and Na) following Gamma distribution with log link. The data matrix for litter dry mass, soil nutrient properties and soil physical properties were based on their individual pooled data for each subplot. Treatment effects on litter invertebrate abundance was determined using Poisson regression with log link. Treatment (E, G, and EG) was used as the fixed variable while setting the study site (KBS, HRT or HNK) and 20 x 20 m subplots as nested random variables. If the treatment effect was judged as statistically significant by *Wald* test, a *post hoc* pairwise comparison of estimated marginal means (*emmeans* function of *emmeans* package for *R*) was applied using the Tukey-Kramer method.

Additionally, environmental parameters other than experimental treatments that could have influenced the abundance of litter invertebrates in the study plots was also considered. Given that litter invertebrates spend a major portion of their lifecycles in the soil, the relationships between litter invertebrate abundance and various soil parameters (soil bulk density, soil fine porosity, soil Ca, Mg, Na, K, P, and total N) were assessed by applying Spearman's correlation test with Bonferroni adjustment (*corr.test* function of *psych* package in *R*).

Based on the correlation test results, potential predictor parameters that explained the abundance of each invertebrate taxon were selected. Using all the potential predictors, a global linear model following a negative binomial distribution was generated to predict the abundance of the invertebrate taxon. Automated model selection based on the corrected Akaike information criterion (AICc) was then applied to the global model to determine suitable predictor parameters for each taxon (*dredge* function of *MuMln* package). For the automated model selection process, I limited the number of predictor parameters for each generated model to three or less due to the small sample size of the study. The AICc values indicates the relative goodness of fit of the model and the lower value suggests a better model fit to the data. Three variables for each invertebrate taxon were selected based on the frequency and order of appearance of up to three variables in top five models (ranked from lowest AICc); variables that appeared repeatedly in the top five models were judged to have stronger predictive powers than those that appeared just once.

In cases where several variables appeared only once, the variable from the model with the lowest AICc was selected. However, the model with the smallest AICc value may not always appropriately reflect the data. To avoid this issue, the residual distribution of the selected models were verified using the *simulateResiduals* and *plotResiduals* functions in *DHARMa* package for *R*. If the model with the lowest AICc value included less than three predictor parameters, parameters from the second-best model (based on the AICc value) were also considered. In the case for Collembola, either soil Ca or Na was selected for the top two models with the lowest AICc value. Given that only soil Na and Ca were selected separately, their combined effects on Collembola abundance were considered. If significant deviations in residuals were detected, predictor parameters with the lowest frequencies of occurrence were removed to simplify the model, and then the verification was repeated. The process was repeated until a true best model, or a model with the lowest AICc value among the models without significant deviation, was selected. These processes were performed using the statistical software *R* (ver 4.2.1, *R* Core Team, 2021).

4.3. Results

4.3.1. Treatment effects on vegetation cover and leaf litter mass

Vegetation cover in clearcut plots (G and EG) had regenerated after the clearcut 11 years ago according to the vegetation data explained in chapter 3 but remained less abundant than uncut plots that were dominated by evergreen tree species (Figure 4.7.1). As a result, uncut, closed canopy plots (Ctrl and E) had more litter mass than in gap plots (G and EG), the difference in litter mass being consistent with a preceding record in 2012 (Suzuki and Ito 2014). Unlike the preceding study, however, the difference in litter mass was not significant this time. Non-tree species were still abundant in treatment G, whereas trees, especially evergreens, became abundant in treatment EG. Despite the different vegetation, the mean litter mass of G and EG plots was similar. Overall, the effect of deer exclusion on the litter mass was not obvious, whereas the effect of reduced litter mass from clearcutting seemed to remain.

4.3.2. Treatment effects to soil physical properties

The mean soil bulk density in the study plots ranged between 0.72 and 0.85 g cm^{-3}, with the lowest value in EG and the highest value in G (Figure 4.7.2). The soil bulk densities were lower inside deer exclosures of both closed canopy and clearcut plots (Ctrl > E, G > EG) maintaining similar trends among treatments from an earlier study in 2011 (Suzuki and Ito, 2014) though the soil bulk densities in all treatments had increased since then (Figure 4.7.2). The effect of deer exclusion on soil bulk density was significant in the clearcut plots, but the bulk densities in the closed canopy plots were not significantly different.

The mean soil fine porosity was between 0.39 and 0.43 ml cm^{-3}, with the highest values in E and EG and the lowest in G, similarly to the earlier report (Suzuki and Ito 2014). Coarse porosity ranged between 0.19 and 0.22 ml cm^{-3}, slightly lower values than in the earlier report, with the highest value in G and the lowest in Ctrl. The differences in fine and coarse porosities among treatments were not significant this time.

4.3.3. Treatment effects to soil nutrient properties

Among the soil nutrient properties investigated, only soil total N showed a significant difference among treatments, with lower concentrations in treatment G compared to the other treatments (Figure 4.7.3). As a general trend within closed canopy plots, deer exclusion (treatment E) increased soil Ca, Mg and K concentrations compared to the Ctrl. In the clearcut plots, deer exclusion (treatment EG) reduced soil Ca, Mg, P concentrations compared to treatment G, while soil K and Na concentrations were similarly low in both the EG and G plots. Even a decade after clearcutting, the concentrations of soil K and Na remain lower in clearcut plots compared to closed canopy plots. However, most of these trends were not statistically significant, probably due to high data variability of soil nutrient concentrations within each treatment.

4.3.4. Response of litter invertebrate taxa to treatments

The responses of the invertebrate taxa to the different treatments varied according to taxon but generally shared similar responses to deer exclusion (Figure 4.7.4). Under a closed canopy, the invertebrate abundance tended to be higher within fenced plots (E) compared to their unfenced counterparts (Ctrl). Similarly, in the clearcut plots (E and EG), deer exclusion fencing significantly increased the abundance of Amphipoda and Araneae. Meanwhile, a slight decrease in abundance was observed for Collembola and Diplopoda by fencing in clearcut plots. In contrast to the preceding study (Suzuki and Ito 2014), the abundances of all investigated invertebrate taxa in clearcut plots (G and EG) were no longer significantly lower than those in Ctrl plots, suggesting recovery from the initial effects of clearcutting.

Among the investigated taxa of invertebrates, Collembola was the most abundant taxon within each treatment, followed by Aranea, Amphipoda and Isopoda, while only a few individuals of Chilopoda and Diplopoda were sampled (Figure 4.7.4). No significant differences among treatments were detected for Diplopoda and Chilopoda, probably due to a limitation in their sample size, and in the case of Collembola, due to the large spatial variations among plots of each treatment.

4.3.5. Relationship between litter invertebrate and soil properties

All investigated taxa of invertebrates showed positive relationships with litter mass and total vegetation cover (Figure 4.7.5). Araneae showed high correlations with both litter mass and total vegetation cover. These correlations were both weak in the case of Collembola. All invertebrate taxa showed negative relationships with soil bulk density, and thus they showed positive relationships with fine (Araneae and Collembola) or coarse (Amphipoda, Isopoda and Diplopoda) porosities of soil. Only Chilopoda did not reveal clear relationships with any the soil's physical properties, possibly because of its low sampling rate and its free-roaming predatory lifestyle. Meanwhile, concentrations of mineral species in soil generally revealed positive relationships with the abundance of invertebrate taxa, with different taxons responding to different mineral species (Figure 4.8.5). Strong positive relationships were detected for: Ca with Amphipoda, Araneae and Isopoda; Mg with Amphipoda and Isopoda; Na with Amphipoda, Araneae, Isopoda and Diplopoda, and total N with Araneae.

Based on these relationships, a global model for each invertebrate taxon was constructed, and likely models that best explained invertebrate abundance were generated through automated model selection (Supplementary material A3). According to the models that best predicted invertebrate taxon abundance (Table 4.6.1), litter mass positively contributed to the abundance of all invertebrate taxa except for Isopoda and Collembola. Soil coarse porosity positively affected the abundance of Amphipoda and Diplopoda, and the soil bulk density negatively affected Araneae abundance. Among the soil nutrient components, Na and Mg tended to be selected as the predictor parameters of the models. Soil Na was predicted to positively affect the abundances of Amphipoda, Collembola, Isopoda and Diplopoda. Soil Mg was predicted to positively affect Isopoda abundance. Soil Ca was also predicted to positively affect Collembola abundance and given the strong correlation between soil Ca and Mg, the positive effect may also apply to Isopoda.

4.4. Discussion

4.4.1. Litter mass

Although the litter mass in the clearcut plots increased compared to the preceding study (Suzuki and Ito, 2014), litter mass continued to be lower than uncut plots, indicating remnant effects of clearcutting. The remnant effect on litter mass was more remarkable in the presence of deer (plot G), where deer partly removed regrown vegetation (Suzuki and Ito, 2014). However, after a decade the litter mass between G and EG were similar negating hypothesis (1). This difference may be attributed to different sampling methods where the preceding study only focused on the centre of the study plots.

Meanwhile, under closed canopies, the litter mass was not increased by deer exclusion, concurrent with results of Ramirez et al. (2021) where the effects of deer exclusion on litter depth are reported to be non-significant over time. The positive effects of deer exclusion on litter mass were short-lived, disproving the hypothesis (4). One explanation why deer exclusion did not increase litter mass was that litter decomposition rate was also increased by deer exclusion that otherwise caused direct disturbance of topsoil and reduced invertebrates (Suzuki and Ito, 2014; Chollet et al., 2021). In summary, decadal effects of deer exclusion on litter mass were apparent only in canopy gaps where herbivory prevents regeneration tree cover after clearcutting (see chapter 3).

4.4.2. The soil's physical properties

Deer exclusion allows regeneration of ground vegetation, which generally protects the soil surface from physical impacts (tramping and raindrops) and prevents soil compaction (Gass and Binkley, 2011; Shelton et al., 2014; Suzuki and Ito, 2014; Ramirez et al., 2021). This might be the case with the clearcut plots, where deer exclusion significantly supported the development of ground vegetation, which probably mitigated soil compaction. In the closed canopy plots, however, the recovery of ground vegetation was limited (Chapter 3), and thus soil bulk density was not affected by deer exclusion. This might be why reduction of soil bulk density was significant only in clearcut plots. Although the differences between clearcut plots were not significant in a previous study (Suzuki and Ito, 2014), deer exclusion might have continuously affected soil bulk density after clearcutting.

In the earlier study, clearcutting was shown to significantly reduce fine porosity of the soil (40% to 35.4 and 38%, Suzuki and Ito, 2014). However, this significant reduction was not detected in this study and might have been mitigated in an earlier timeframe. The mitigation may be attributed to the increase in ground vegetation and a possible increase in plant root biomass which loosened soil particles. Meanwhile, coarse porosity of the soil was similar among the treatment groups in this study, being consistent with those in the fifth year of the experiment (Suzuki and Ito, 2014). It remains unclear whether the coarse porosity was unaffected by treatment or had been mitigated during the first five years. In any case, the cascade effects of deer exclusion on the soil's physical properties seem to be time-dependent; the changes of bulk density and fine porosity were only evident after five years whereas changes of coarse porosity may require shorter timeframes.

4.4.3. Soil nutrient property

Despite being not significant due to high spatial variation, deer exclusion in uncut plots tended to increase concentrations of Ca, Mg, and K in the topsoil. Deer exclusion promotes vegetation recovery (Shimoda et al., 1994; Suzuki, 2013), limits direct disturbance to the litter layer (Takahashi and Kaji, 2001; Nakahama et al., 2020), and maintains a suitable habitat conditions for litter invertebrates, thus increasing their abundance (Bressette et al., 2012, Lessard et al., 2012; Suzuki and Ito, 2014; Katagiri and Hijii, 2017). Increased invertebrate abundance in turn, likely enhances litter decomposition and nutrient cycling processes (Meyer et al., 2011; Suzuki and Ito, 2014), increasing concentrations of the above mineral species. The increased soil Ca, Mg and K concentrations in the closed canopy plots (E > Ctrl) may be attributed to increased vegetation cover of forest canopy condensing nutrients carried by fog and rain into water droplets, enriching the soil (Ewing et al., 2012). However, this trend was not observed for soil Na, likely due to its high solubility though actual mechanisms remain unclear. In addition, nutrient deposition from fog may have a limited impact towards the results in this study as its effects tend to be confined to ecosystems near coastal areas such as site KBS and may have negligible effects on distant ecosystems (from coastal areas) such as sites HNK and HRT.

Meanwhile, the remnant effect of clearcutting was suggested for soil K and Na concentrations, though not statistically significant. Potassium easily leaches from the soil to stream water right after clearcutting, but its content in stream water gradually decreases as vegetation develops (Bormann and Likens, 1979). The low K concentration in the soil of clearcut plots may reflect past leaching. Although little data is available for the movement of soil Na after clearcutting, it's movement pattern would likely not be very different from that of soil K.

Similarly, the concentration of total N was lower in treatment G plots than in plots of the other treatments (Ctrl, E and EG) and total N concentrations reported in Japanese temperate forests (10.1 mg g^{-1} soil, Furusawa et al., 2006). According to these results, the residual effects of clearcutting on soil total N seem to persist even after a decade, as they did on soil K and Na. The negative impacts on belowground systems arising from these decreased concentrations remain unclear and require future investigation. Notably, unlike K and Na, the soil total N in EG plots was significantly richer compared to that in G plots. This might be due to the indirect effect of deer exclusion that supported vegetation recovery and probably, promoted N cycling.

4.4.4. Variables affecting invertebrate abundance

Deer exclusion showed positive effects on invertebrate abundance, supporting past literature (Suzuki and Ito, 2014; Katagiri and Hijii, 2017; Ramirez et al., 2021). However, deer presence was only selected as a predictor parameter in the Araneae model. Deer physically alter the habitat structure resulting in non-trophic effects that reduce Araneae abundance (Miyashita et al., 2004). This effect might be more pronounced in the case of Araneae than other taxa.

Soil Na was a common predicting variable in the models among Amphipoda, Collembola, Isopoda and Diplopoda, indicating positive effects on their abundance. According to Kaspari et al. (2009), soil Na limits the abundance of invertebrates. The resulting effect of clearcutting and leaching of Na from the soil would limit the abundance of invertebrates, including soil fauna. Other than soil Na, Ca was also selected as an explanatory parameter for Collembola, contrasting with previous results (Geissen et al., 1997; Ohta et al., 2014). This result was also contradictory to the predictions of the Structural Elements Hypothesis, which proposes that abundance of calcareous species (e.g. Amphipoda) are limited by soil Ca availability, needed for their calcium-rich exoskeletons (Kaspari and Yanoviak 2009). One explanation for this discrepancy may be the ubiquitous abundance of Collembola and sampling bias resulting in an inaccurate representation of actual abundances. In addition, the reliability of the models for Chilopoda and Diplopoda should be treated with caution as the small number of individuals sampled may be insufficient to elicit reliable relationships. Nevertheless, the results indicated that soil nutrients such as Na, Mg and Ca may play a role towards increased invertebrate diversity and abundance.

4.5. Conclusion

The remnant effects of clearcutting on belowground systems were observed in soil total N, Na, K and soil bulk density even after a decade, while deer exclusion fencing was able to mitigate clearcut effects on soil total N and soil bulk density. No effects were detected on the litter mass, negating our hypothesis (1). Amphipoda and Araneae were sensitive to clearcutting effects and their abundance was increased by deer exclusion; However, the continued impacts of clearcutting were not detected for the remaining invertebrate taxa. These results partly supported but partly negated our hypothesis (2). Similarly, hypothesis (3) was supported only for soil total N, which was increased by deer exclusion in gaps but was rejected in the case of the other soil nutrients. Nevertheless, the effects of deer exclusion on belowground systems of clearcuts to a subset of invertebrate taxa, the soil's physical properties and nutrients should not be ignored. Therefore, deer exclusion should be paired with gap creation to support and speed up the recovery of affected belowground systems.

Meanwhile, deer exclusion under closed canopies increased the abundance of most invertebrate taxa and increased concentrations of K, Mg and Ca within the topsoil, supporting our hypothesis (4); however, the hypothesis was rejected for litter mass. In hindsight, the overall benefits of deer exclusion on the soil's physical properties and soil nutrient concentrations were not significant under closed canopies. Compared to those under gaps, the effectiveness of deer exclusion fencing under closed canopies might be lower and may not sufficiently justify its high costs.

4.6. Tables

Table 4.6.1. Predicted models for each invertebrate taxon.

Taxonomic group	Predicted models from automated model selection (*MuMln* package)	AICc	DHARMa residual test*
Amphipoda	Amphipoda~ (+) Coarse porosity + (+) Litter Mass	119.1	N.S.
	Amphipoda~ (+) Coarse porosity + (+) Litter mass + (+) Na	119.7	N.S.
Araneae	Araneae~ (-) Bulk density + (-) Deer $^{-}$ + (+) Litter mass	139.0	N.S.
Collembola	Collembola ~ (+) Na	162.2	N.S.
	Collembola ~ (+) Ca	164.4	N.S.
	Collembola ~ (+) Na + (+) Ca	164.9	N.S.
Isopoda	Isopoda ~ (-) Bulk density + (+) Mg + (+) Na	109.1	N.S.
Diplopoda	Diplopoda~ (+) Coarse porosity + (+) Litter Mass + (+) Total N	93.9	Sig.
	Diplopoda~ (+) Coarse porosity + (+) Litter Mass + (+) Na	94.1	N.S.
Chilopoda	Chilopoda~ (-) Deer + (+) Litter Mass	71.6	Sig.
	Chilopoda~ (-) Deer + (+) Litter Mass + (-) Mg	72.6	Sig.
	Chilopoda~ (-) Deer + (+) Litter Mass + (-) Ca	72.7	Sig.
	Chilopoda~ (+) Litter Mass	77.3	N.S.

* Models with Significant deviation (Sig.) between the observed and predicted residuals were rejected and only models showing no significant deviation (N.S.) were accepted. The "+" and "–" signs in parentheses in front of each variable indicates direction of the effect on the abundance.

4.7. Figures

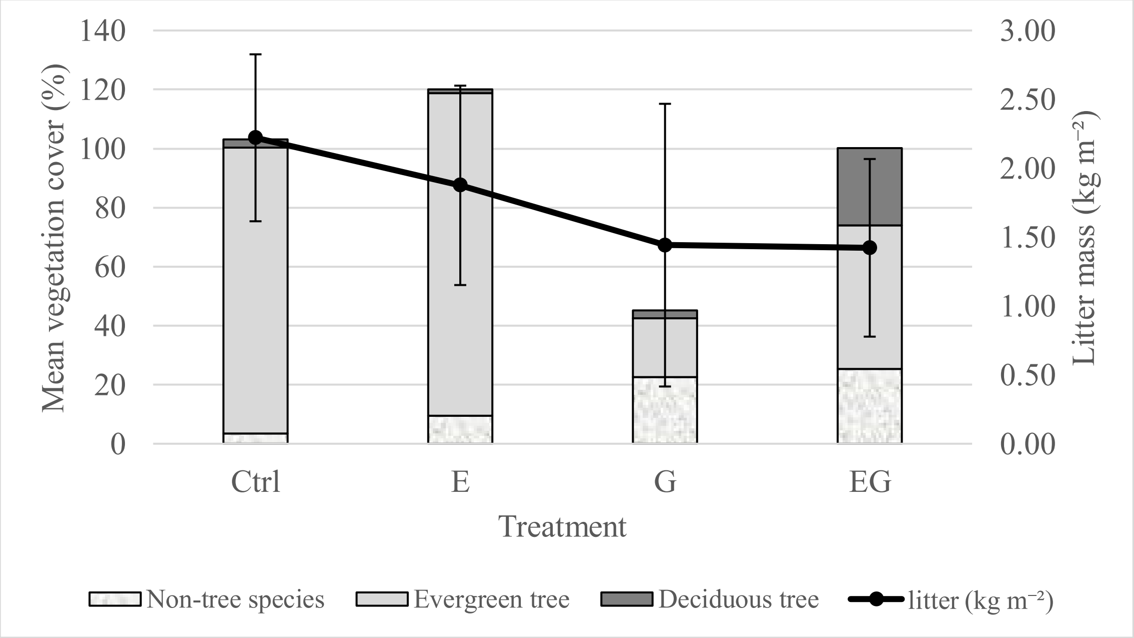


Figure 4.7.1. Mean vegetation cover (left) and litter mass (right) among experimental treatment plots. The error bars represent the standard deviation of the mean. One Ctrl plot was damaged during a typhoon event. The litter mass data in the figure represents the mean of 5 study plots for Ctrl ($n = 5$) as compared to 6 study plots in other treatments ($n = 6$).

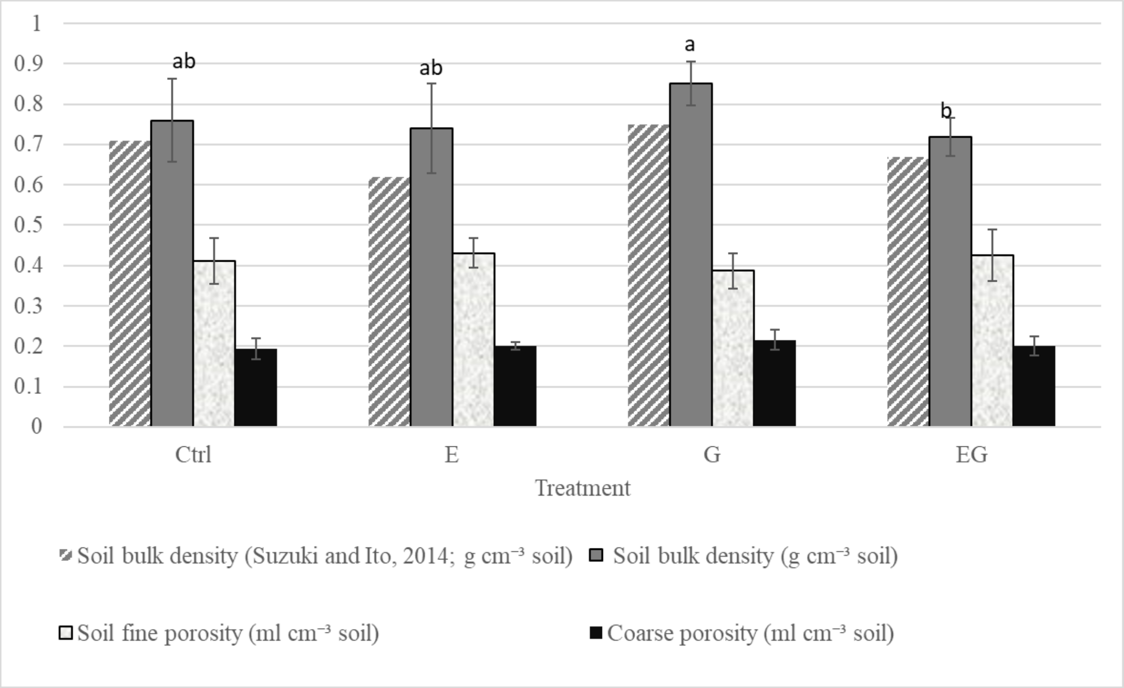


Figure 4.7.2. Soil physical properties among treatment plots, based on the averaged data between 2019 and 2020. The error bars represent the standard deviation from the mean. Significant differences from statistical comparison of pairwise treatments are denoted by different letters for each soil physical property. The units for the soil bulk density data from Suzuki and Ito (2014) was calculated as g cm^{-3} based on similar calculation methods in this chapter.

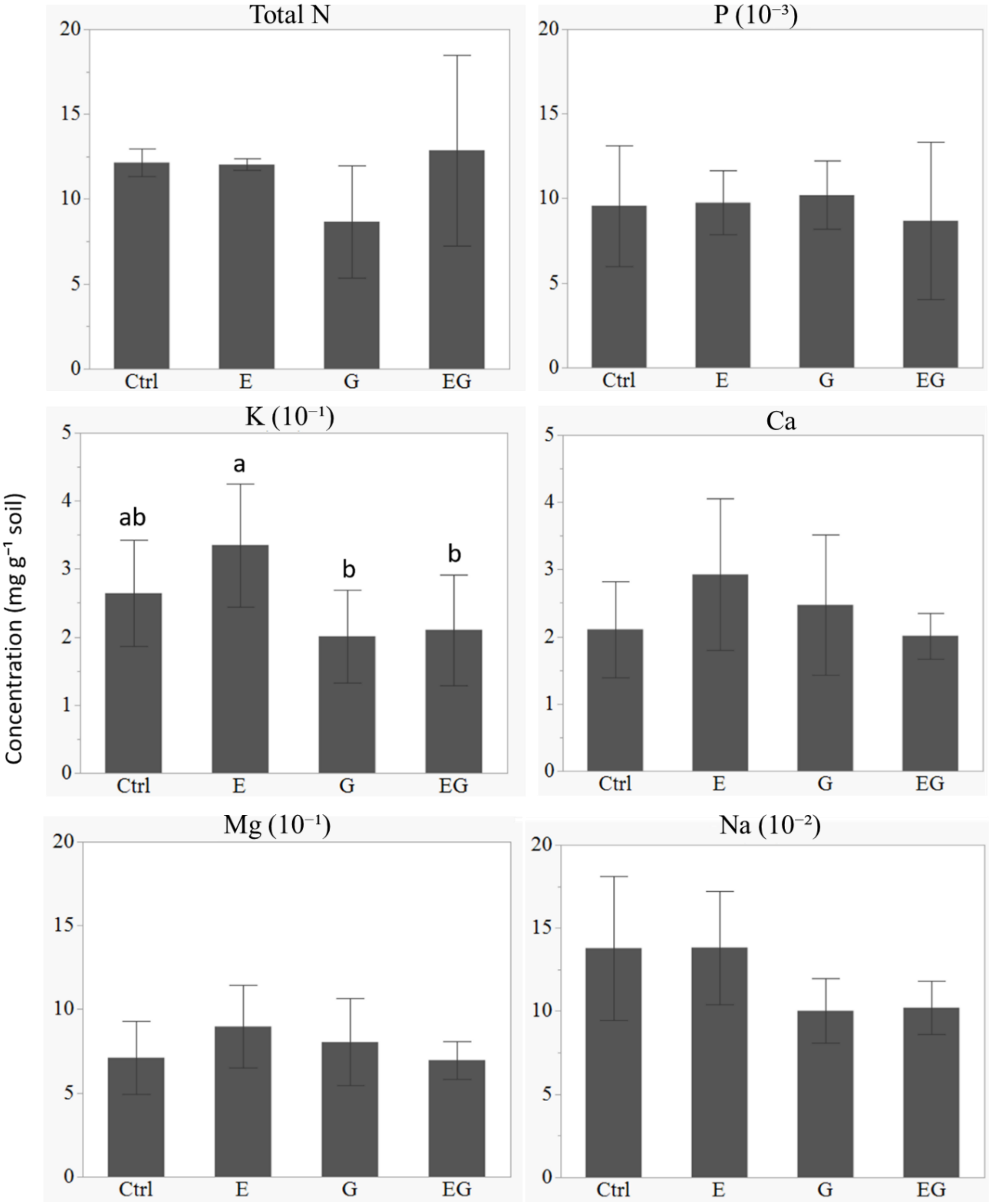


Figure 4.7.3. Soil nutrient property in different treatments in 2019. The error bars represent the standard deviation from the mean. Significant differences from statistical pairwise comparison among treatments are denoted by different letters for each soil nutrient property.

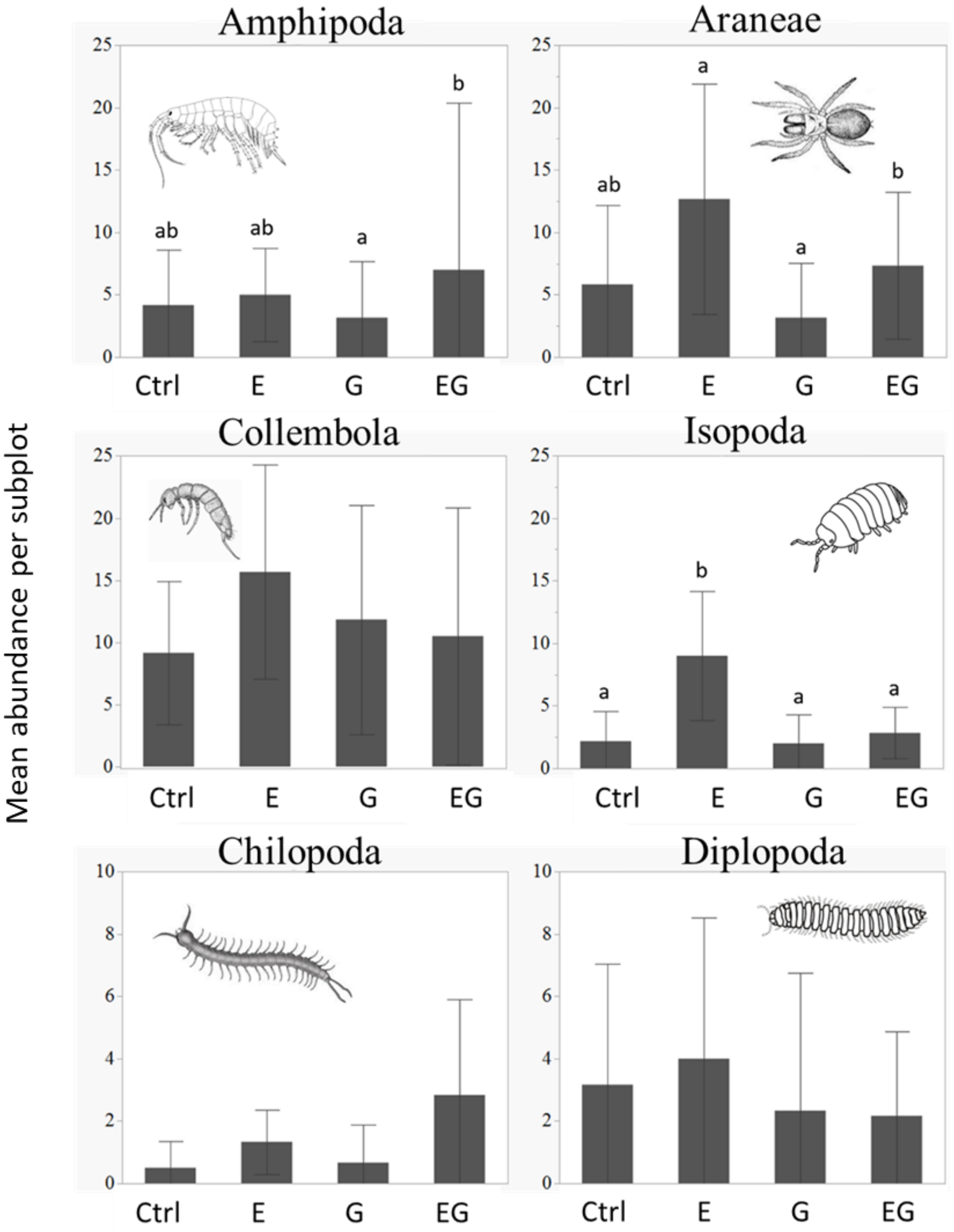
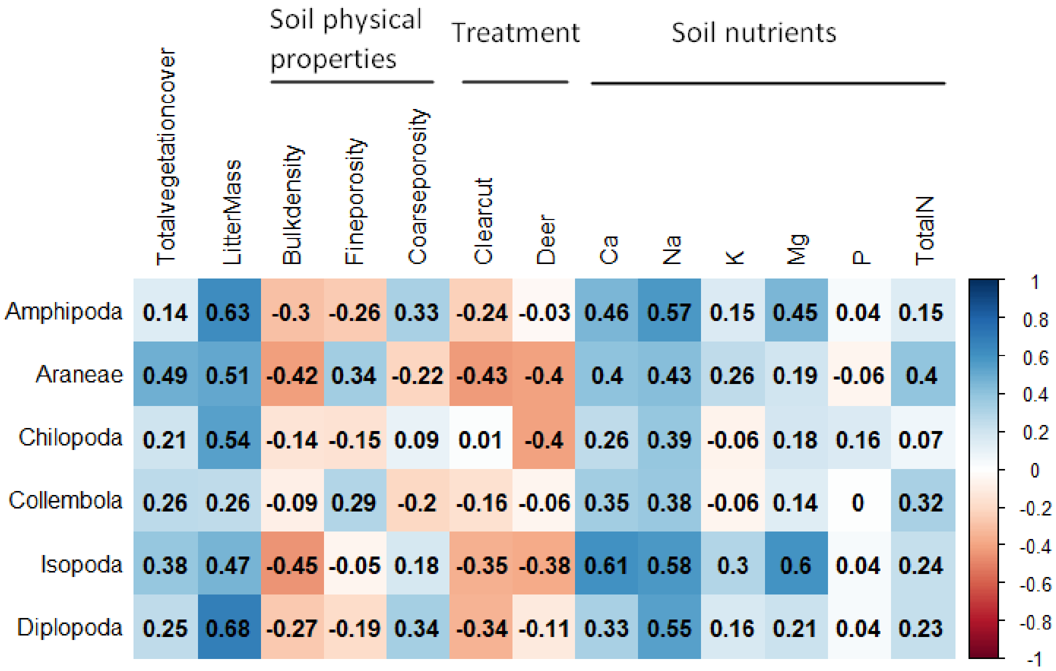


Figure 4.7.4. Mean litter invertebrate abundance in treatment study plots in 2020. The abundance for each taxon within each subplot was pooled and the mean calculated for each treatment. The error bars represent the standard deviation from the mean. Significant differences following statistical pairwise comparison between treatments are denoted by different letters ‘a-c’ for each invertebrate taxon.



	Totalvegetationcover	LitterMass	Bulkdensity	Fineporosity	Coarseporosity	Clearcut	Deer	Ca	Na	K	Mg	P	TotalN
Amphipoda	0.14	0.63	-0.3	-0.26	0.33	-0.24	-0.03	0.46	0.57	0.15	0.45	0.04	0.15
Araneae	0.49	0.51	-0.42	0.34	-0.22	-0.43	-0.4	0.4	0.43	0.26	0.19	-0.06	0.4
Chilopoda	0.21	0.54	-0.14	-0.15	0.09	0.01	-0.4	0.26	0.39	-0.06	0.18	0.16	0.07
Collembola	0.26	0.26	-0.09	0.29	-0.2	-0.16	-0.06	0.35	0.38	-0.06	0.14	0	0.32
Isopoda	0.38	0.47	-0.45	-0.05	0.18	-0.35	-0.38	0.61	0.58	0.3	0.6	0.04	0.24
Diplopoda	0.25	0.68	-0.27	-0.19	0.34	-0.34	-0.11	0.33	0.55	0.16	0.21	0.04	0.23

Figure 4.7.5. *Spearman's* correlation test with *Bonferroni* adjustment between litter invertebrate abundance and various soil parameters (soil bulk density, soil fine porosity, soil Ca, Mg, Na, K, P, and total N). The units are: Total vegetation cover (%), Litter mass (kg m^{-2}), soil bulk density (g cm^{-3} soil), Fine and coarse porosity (ml cm^{-3} soil) and soil nutrients (mg g^{-1} soil).

Chapter 5. Treatment effects on regeneration of common tree species

5.1. Introduction

This chapter focuses on the different treatments effect on the sapling recruitment potential of common tree species in the study sites (chapter 2.2). As seen in chapter 3, artificial gap creation in the presence of deer tends to increase mortality of tree saplings while the combined treatment (deer exclusion + gap creation) benefits forest regeneration, also reported in a previous study (Tamura and Nakajima, 2017). However, the benefits of the combined treatment may not be evenly shared by all tree species. For example, *Abies firma* (Tamura and Nakajima, 2017) and evergreen *Quercus* species (Tsujino and Yumoto, 2004), both shade-tolerant and reported to be browsed by deer, showed higher sapling abundance under closed canopy conditions compared to gap plots (Tamura and Nakajima, 2017; Petersson et al., 2020). The combined treatments may not be optimal for these species, possibly due to their slow growth rates and high inter-specific competition within gaps. Other tree species such as *Eurya japonica* and *Neolitsea sericea* showed positive response to deer exclusion (Tsujino and Yumoto, 2004), but considering their relatively high tolerance to moderate deer densities (Suda, Araki and Maruyama, 2001), these species might show higher survival rates and competitive advantage outside the deer fences. These specie-specific response to light conditions and herbivory by deer would affect the forest succession and species composition resulted by each treatment. So far, monitoring records of more than a decade are too limited to clarify the pros and cons of each treatment.

This chapter evaluates the 11-years effects of the combined treatments on the regeneration of evergreen tree species common in warm-temperate broadleaved forests, which forms the canopy and subcanopy layers of mature forests. Instead of the research scheme of preceding studies in the study site for ground vegetation sampling (five replications of 1 × 1 m quadrats), this study used sampling areas larger by a magnitude of five (5 × 5 m quadrats) and successfully evaluated the treatment effects on tree regeneration which was not addressed in previous studies. The current chapter describes how common tree species respond to experimental treatments in the study sites. In addition to improving general understanding of forest regeneration and succession, understanding these responses would also provide forest managers with new options for restoring forest ecosystems and increasing sapling density of targeted tree species.

5.2. Methods

5.2.1. Tree sapling recruitment and growth

Six common evergreen tree species were selected to assess the response of their saplings to the treatments: *Abies firma, Quercus acuta, Eurya japonica, Cinnamomum tenuifolium, Castanopsis sieboldii* and *Neolitsea sericea*. *Abies firma* is a slow-growing tall evergreen conifer, dominant in most warm-temperate forests in Japan, but their sapling regeneration is limited in Boso (Abrams 2017). *Quercus acuta* is a shade-tolerant evergreen tree species common in warm-temperate forests of Japan. Saplings of *Quercus* species are negatively impacted by current deer densities in Chiba prefecture (Chiba prefecture, 2004; chapter 2.5). *Cinnamomum tenuifolium* and *Castanopsis sieboldii* are emergent layer trees common in warm-temperate forests. *Eurya japonica* is a shade-tolerant subcanopy species, commonly found in forest gaps with strong regenerative capabilities (Manabe and Yamamoto, 1997). *Neolitsea sericea* is a shade-tolerant evergreen tree known to be unpalatable to deer (Tsujino and Yumoto, 2004).

All the six species were judged as fairly common based on their frequency in subplots of each treatment (*f*): they appeared in more than 3/6 plots of at least one experimental treatment, and their mean percent cover (*MC*) exceeded 10%, or they showed highest *MC* values in at least one treatment. The tree species found in less than 3/6 plots in any of the experimental treatments were avoided, because their biased occurrence might be accidental and not due to the treatment effect. Among the six tree species, only the *MC* of *Q. acuta*, *E. japonica*, *C, tenuifolium* and *C. sieboldii* exceeded 10% in at least one experimental treatment. Meanwhile in treatment G, the highest *MC* recorded was of *N. sericea* and *A. firma* exceeding 1.5%. Thus, both species were considered common species for treatment G.

The saplings of the above six species were assessed in October 2020 and categorized according to the following height classes: (i) between 0.1 m to less than 0.3 m, (ii) between 0.3 m to less than 1 m, (iii) between 1 m to 2 m and (iv) higher than 2 m. The sapling densities were calculated based on the total sapling count per unit area sampled (m^{-2}). The saplings of height classes (i) and (ii) were sampled within a 5-by-1 m belt transect (5 m^2 x 2 subplots x 3 study site = 30 m^2) in the centre of each study plot (Figure 2.4). Class (iii) and class (iv) saplings were sampled within 5-by-5 m (150 m^2) and 10-by-10 m quadrats (600 m^2) respectively.

Based on field observations in the closed canopy plots, there was a clear distinction between mature canopy trees that had stems with DBH > 10 cm and saplings below the canopy with DBH < 10 cm of the target species. Therefore, only stems with DBH < 10 cm were counted as saplings. Because the ground vegetation was mostly devoid due to long-term deer herbivory before the experiment started, all the stems found in the clearcut plots indicated new recruitments after the cutting event (all remaining vegetation were removed during clearcutting). Unlike the clearcut plots, saplings of the closed canopy plots include those recruited before the experiment started. The differences between Ctrl and treatment E depict the effectiveness of deer exclusion fencing on the recruitment and survival of those saplings. In this chapter, sprouts from clear-cut trees were also assessed as saplings and based on field observations, almost all tree saplings assessed in this chapter had regenerated from clear-cut stumps.

5.2.2. Statistical analyses

To examine the treatment effects on sapling height of target species, non-parametric *Kruskal-Wallis* rank sum test (*Kruskal.test* function in *R*) was performed on the treatment effect on sapling height class (i-iv) for each species, given the non-normal distribution of the data. If the treatment effect was significant (*p*-value < 0.05) a *post hoc* test was applied to determine significant differences between pairwise treatments. For the *post hoc* test, *Dunn's* test (*dunn.test* function in *dunn.test* package in *R*) with *Bonferroni* adjustment was applied.

The total sapling density of each target species within each treatment was calculated as the sum of height class i to iv sapling densities. *Dunn's* test with *Bonferroni* adjustment was again applied to determine significant treatment effects (pairwise p-value < 0.05) on total sapling density for each target species. These processes were implemented using the statistical software *R* (R Core Team, 2021).

5.3. Results

5.3.1. Sapling abundance of common tree species

All six common tree species were able to survive and regenerate in treatment EG, whereas in treatment G, only *A. firma*, *E. japonica* and *N. sericea* regenerated (Figure 5.7.1). The saplings of *Q. acuta*, *C. tenuifolium* and *C. sieboldii* were only observed in treatments with deer exclusion fencing (E and EG, Figure 5.7.1). The densities of small saplings (height classes i – iii) of these three species (except for *Q. acuta*) tended to be slightly higher in treatment E compared to EG while large saplings (height class iv) of all three species were significantly higher in treatment EG compared to the other treatments. The following section describes the species-specific characters of common tree species.

Quercus acuta

The cut stumps of *Q. acuta* tends to sprout many adventitious buds. However, no sprouts were observed in the presence of deer, although mature trees higher than two meters that exceeded the reach of deer were observed in Ctrl plots outside the 5 x 5 m quadrats (data not shown). The total sapling density of *Q. acuta* was significantly higher in treatment EG compared to the other treatments.

Cinnamomum tenuifolium

The sapling recruitment of *C. tenuifolium* showed similar trends to *Q. acuta*, though the overall sapling density was relatively lower than *Q. acuta* (Figure 5.7.1). The highest total sapling density was observed in treatment E compared to unfenced plots (Ctrl and G). Saplings of *C. tenuifolium* less than one meter height were not detected within treatment EG while saplings greater than two meters height were absent from treatment E, suggesting the absence of younger saplings in EG.

Castanopsis sieboldii

The sapling densities of *C. sieboldii* were relatively higher in treatment E compared to EG and unfenced plots (Figure 5.7.1). Like *C. tenuifolium*, saplings of all height class i to iii were observed in treatment E while young saplings below thirty centimeters height were absent from treatment EG.

Abies firma

The saplings of *A. firma* were mainly recorded in the clearcut plots (G and EG), showing an L-shaped population size structure with increasing height class (Figure 5.7.1). Between the gap plots, sapling densities were higher in treatment G compared to EG, though no significant differences were detected.

Neolitsea sericea

Interestingly, *N. sericea* showed regeneration under three different treatment conditions (Figure 5.7.1). Saplings of less than one meter in height were only recorded in treatment E, showing an L-shaped population size structure which greatly differed from saplings in treatment EG and G. In the gap plots, saplings under one meter height were not detected, though trees greater than one meter height were present. The highest sapling density for this species was observed in treatment G but no significant differences were detected among treatments.

Eurya japonica

The highest sapling density in this study was observed for *E. japonica* where the saplings increased greatly in the gap plots, significantly higher in treatment EG compared to the other treatments (Figure 5.7.1). Saplings of *E. japonica* showed an L-shaped population size structure even in the presence of deer, reflecting a high establishment rate from plentiful seeds. In the presence of deer, the regeneration of *E. japonica* saplings decreased, and the sapling heights were suppressed to less than one meter.

5.4. Discussion

Treatment EG generally supported regeneration and recruitment of tree saplings. There was, however, a difference in recruitment effects between tree species at the study site; each of the six common species in the study site behaved differently according to treatment conditions. Three common species of warm-temperate forests, *Q. acuta*, *C. siebioldii* and *C. tenuifolium*, showed similar responses to deer exclusion indicating regeneration failure under the current deer density. The current results support those of Petersson et al. (2020) where *Quercus* saplings were more abundant in deer-excluded clearcut plots compared to deer-excluded closed canopy plots. Our results show that saplings of these three species remained below one meter height in treatment E but were able to exceed two meters height in treatment EG, suggesting rapid growth with increased light availability. Thus, for these species, deer exclusion is necessary for their sapling recruitment. If the management goal was to maintain the current ecosystem (mature trees) while ensuring a sapling bank of these species under the closed canopy that can rapidly grow once conditions become favourable, deer exclusion alone may be sufficient.

On the other hand, *A. firma* and *E. japonica*, showed higher sapling densities under increased light conditions (G and EG) compared to under closed canopies even in the presence of deer. This suggested that for both these species, light was the limiting factor rather than deer. Tamura and Nakajima (2017) recommended the combined treatments (EG) to support regeneration of *A. firma* under deer densities of 10–17 deer km^{-2}. However, the results suggest the opposite with higher sapling densities in treatment G compared to EG. *A. firma* does not appear to be browsed by deer in the study region and regeneration is supported in gaps under the current deer density (10–13.5 deer km^{-2}). Despite the similar deer density in both studies, the reason for the discrepancy remains unclear and may be an effect of diet preference between local deer populations. Nevertheless, the results indicate the possibility that treatment G could solve the declining *A. firma* sapling recruitment rates within the Boso peninsula (Abrams et al., 2017). On the other hand, *E. japonica* saplings showed higher recruitment and growth under increased light availability (Manabe and Yamamoto, 1997) and in the absence of deer (Tsujino and Yumoto, 2004). However, the increased sapling recruitment in the absence of deer in this study was only observed in the clearcut plots, different from Tsujino and Yumoto (2004). The reason for this discrepancy remains unclear but could be attributed to increased competition and limited light availability under a closed canopy. Also, *E. japonica* saplings in treatment G were limited to less than 30 cm height while such limitations were not observed in treatment EG, which was expected given their palatability to deer (Agetsuma, Agetsuma-Yanagihara and Takafumi, 2011; Hashimoto and Fujiki, 2014). Thus, although *E. japonica* saplings are present in clearcut plots with deer present, successful regeneration to mature trees was suppressed under the current deer density.

Interestingly, *N. sericea* responded to deer differently under closed canopy and clearcut conditions, showing tolerance to low light intensity. The species is relatively unpalatable to deer (Tsujino and Yumoto, 2004) and can tolerate moderate deer densities under a closed canopy (22.8 deer km^{-2}, Suda, Araki and Maruyama, 2001). The results are consistent with those of Tsujino and Yumoto (2004) with higher sapling densities in treatment E compared to Ctrl but contradicts those of Suda, Araki and Maruyama (2001), as saplings were not detected in Ctrl plots. In contrast, under clearcut conditions, *N. sericea* sapling densities was greater with deer presence (G > EG), probably due to reduced competition by deer herbivory on other fast growing palatable tree species. The absence of this species in Ctrl plots suggests a lack of tolerance to current deer density under low light intensity, but sapling recruitment can be achieved through deer exclusion and improving light availability.

5.5. Conclusion

The recruitment of tree saplings (*Q. acuta*, *C. sieboldii* and *C. tenuifolium*) was inhibited at the current deer density. Deer exclusion fencing generally facilitated the recruitment of tree saplings, but this effect was not equal as the recruitment success depended on the species identity. The exclusion of deer generally improved recruitment and growth of palatable tree saplings, while the presence of deer after clearcutting improved recruitment of non-palatable tree species. In accordance with the need for more effective forest restoration (Castro et al., 2021), there is a need to consider how current deer densities and closed canopy forests influence the recruitment success of each tree species. Knowledge on various responses of tree species to herbivory, gap creation and their combined effects may influence the decision of different implementation strategies needed to assist in forest regeneration. On the other hand, this information could help forest managers set clear management goals and select appropriate strategies to achieve them.

5.6. Table

Table 5.6.1. Mean percentage cover (*MC*, %) of the target evergreen tree species and their frequency in the plots of each treatment (*f*).

Species name	Status*	Ctrl		E		G		EG	
	PA	*MC*	*f*	*MC*	*f*	*MC*	*f*	*MC*	*f*
Abies firma	N	+	3	+	3	1.8	5	+	4
Neolitsea sericea	N	+	3	+	3	2.9	3	+	3
Castanopsis sieboldii	P	25.2	4	33.7	5			2.7	1
Eurya japonica	P	17.8	6	9.3	6	+	6	16.7	6
Cinnamomum tenuifolium	P	11.8	4	3.4	6	1.3	1	2.5	4
Quercus acuta	P	25.5	5	30.5	6	+	1	3.8	4

*: Palatability to deer (PA: Palatable or Non-palatable) were based on Suzuki et.al., 2008; Agetsuma et.al., 2011; Suzuki, 2013; Hashimoto and Fujiki, 2014. Species with *MC* less than 1% are displayed as '+'.

5.7. Figures

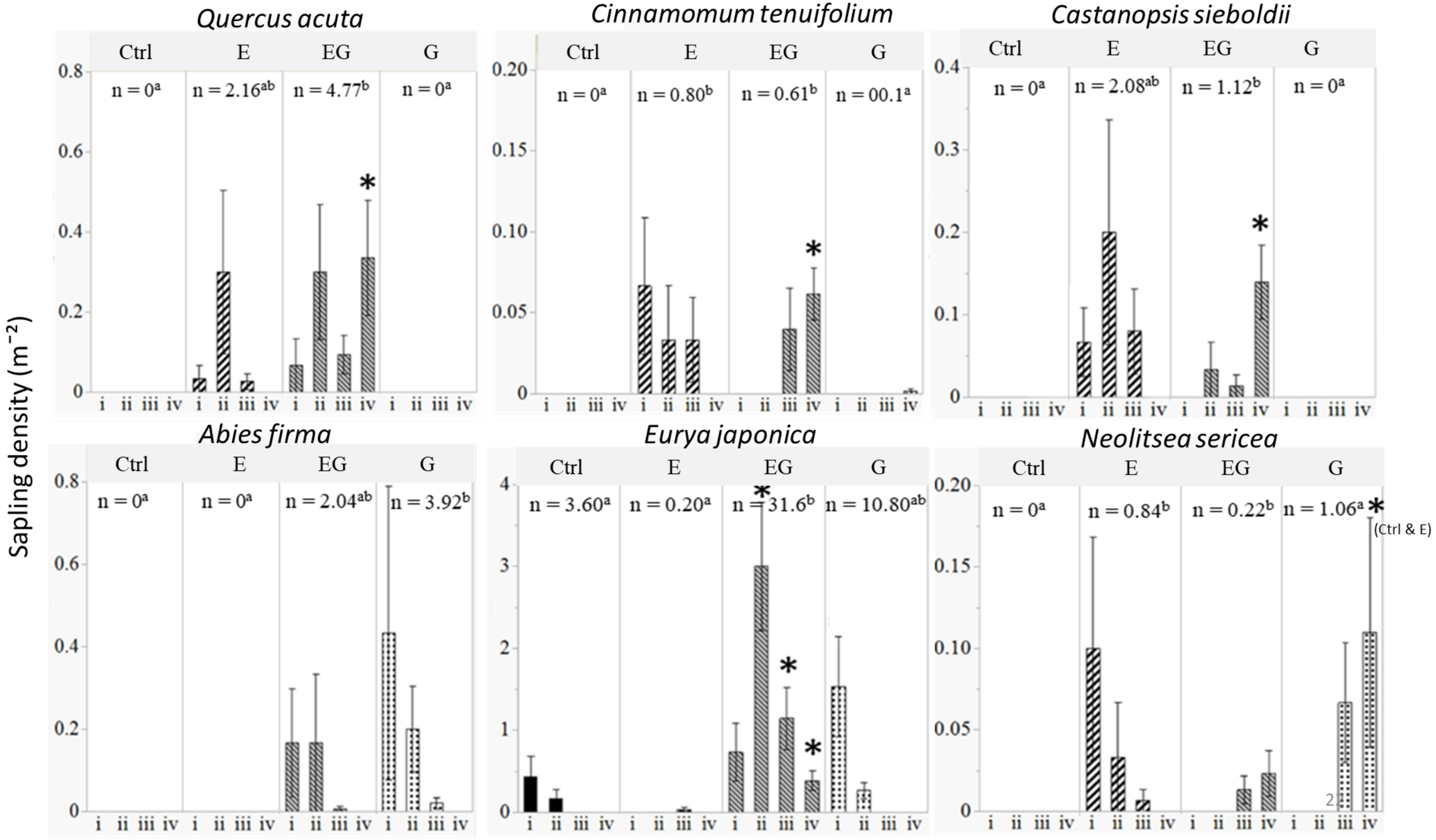


Figure 5.1. Comparison of sapling density (m^{-2}) of common tree species according to height class among experimental treatments.

Figure 5.1. (cont.) The total sapling density (n) within each treatment was calculated as the sum of densities of height class i to iv saplings: **(i)** $0.1 \text{ m} \leq x < 0.3 \text{ m}$, **(ii)** $0.3 \text{ m} \leq x < 1 \text{ m}$, **(iii)** $1 \text{ m} \leq x \leq 2 \text{ m}$ and **(iv)** > 2 m. The error bars represent the standard error of the mean sapling density per sapling height class. The significant differences between total sapling densities (n) are displayed by different letters in superscript. Significant differences between sapling counts for each sapling height class among treatments are displayed by an asterisk. The exception was for *N. sericea* class iv saplings where sapling counts in G were significantly higher compared to Ctrl and E but not for EG.

Chapter 6. General discussion and conclusion

6.1. Decadal vs short-term studies

The result of this decadal study reflects realistic changes and progression of secondary succession within each treatment as compared to short-term studies <5 years. Slow changes within the aboveground and belowground systems tend to be overlooked in short-term studies resulting in erroneous future predictions. For example, the significantly lower abundance of litter invertebrates in treatment G compared to Ctrl, 4 years after clearcutting (Suzuki and Ito, 2014) was no longer significant after a decade. In addition, the positive effects of deer exclusion on litter invertebrate abundance continued even after a decade suggesting long-term deer exclusion benefits to invertebrate abundance. In the case of soil physical property (Figure 4.7.2), the significance of deer exclusion after clearcutting, on the soil bulk density was only detected after a decade. Also, in a decadal study by Tamura and Nakajima (2017), a significant increase in seedling emergence of their targeted tree species occurred four years after the experiment began. This annual variation of seedling emergence would otherwise remain undetected by short-term studies less than 4 years, demonstrating the greater accuracy of long-term studies in representing actual field situations.

Long-term studies with repeated sampling designs are rare and limited, especially in the field of forest regeneration, due to the difficulty of maintaining the experimental setting and field observations for an extended period of time. Long-term studies entail inherent risks from natural disasters such as typhoons, landslides and earthquakes that can irreversibly damage the experiments, making long-term observations difficult. As such, the current study attempts to improve understanding of decadal treatment effects which are relatively few in literature compared to short-term studies.

6.2. Forest composition predictions within each treatment

Deer exclusion after clearcutting (EG) increases plant species richness, speeds up forest regeneration while mitigating the negative impacts of deer on aboveground and belowground systems observed in treatment G. Based on the current trend of deer herbivory suppressing palatable tree saplings and dominance of non-palatable species in Chapter 3 and 5, it is projected that the dominant vegetation cover in treatment G would be graminoids and shrubs with saplings of non-palatable trees such as *A. firma* and *N. sericea* and herbivoretolerant tree species such as *E. japonica* . If the current trend were to persist uninhibited by deer, *A. firma* and *N. sericea* trees would eventually survive and develop into new stands of *A. firma* and *N. sericea*. *E. japonica* saplings would either remain in the understory layer or disappear from the new stands as their vertical growth continue to be suppressed by deer.

Treatment EG, on the other hand would eventually consist of the common tree species (*Q. acuta*, *C. tenuifolium* and *C. sieboldii*) in the canopy layer with *E. japonica* and other evergreen broad-leaved species in the sub-canopy layer. The understory layer would consist of a combination of different plant forms (forbs, graminoids, shrubs and vines) with small tree saplings waiting for suitable conditions (e.g. natural gaps) to grow. It is likely that treatment EG supports forest regeneration towards those that were created through coppicing and later abandoned, similar to past forest conditions with low deer densities and low herbivory pressure. Moreover, a species rich ecosystem, like the one in EG, possesses ecosystem functions similar to developed forests, offering habitat and food resources to a broader array of plant and animal species, including rare ones.

Despite similar deer exclusion in treatment EG, the understory layer of treatment E remains dark due to the closed canopy cover. Under the closed canopy, the regeneration of forbs and vines would be limited to shade-tolerant species, with little change to the species richness and composition from Ctrl. It is likely that treatment E would maintain the current forest ecosystem structure similar to the Ctrl, major tree species and plant composition remain relatively unchanged progressing towards an old-growth forest ecosystem. In general, the ecosystem would not drastically change maintaining its current forest structure but with increased ground vegetation cover compared to Ctrl.

In general, treatment E increases the foliar cover of understory vegetation slightly without altering the dominant tree species. Treatment EG would recover towards a forest ecosystem with trees as the dominant plant form but with greater plant diversity in both the canopy and understory layer whereas treatment G would result in a completely different ecosystem with very different dominant plant forms (grassland or shrubland) from the other treatments.

6.3. Feedback between aboveground and belowground systems

With respect to the belowground systems, the small increase in soil bulk density observed in treatment G compared to the other treatments does not appear to negatively affect plant recruitment. At the current stage, decadal exposure to deer in G results in a small increase in soil bulk density compared to Ctrl. A prolonged exposure to deer trampling may further increase soil bulk density, inhibiting sapling recruitment in the future, but it remains unknown at what point in time, the increased soil bulk density would become detrimental to sapling recruitment.

According the three feedback mechanisms between aboveground and belowground systems mediated by deer suggested by Bardgett and Wardle (2003): (1) the changes to the aboveground systems (e.g. vegetation cover) by deer affects the quantity of organic matter returned to the soil, (2) herbivores alter the resource quality to the ecosystem through fecal material and urine, (3) changes to the vegetation composition may alter litter quality and decomposition. The result of this study only supports the third mechanism as deer exclusion in either closed canopy or gaps did not significantly alter the litter mass. In the current study site of steep slope inclination, the deer exclusion fences might have restricted movement of litter while in unfenced plots, litter either increased or decreased according to the topography. Thus, the effect of deer exclusion increasing litter mass and quantity of organic material to the soil as in (1) was not detected; rather the effect of clearcutting was stronger than deer herbivory according to the data. With regards to the mechanism (2), deer exclusion increased soil nutrient concentrations, suggesting that fecal material input from deer had minimal impact on soil nutrient concentrations. The mechanism (3) which is attributed to deer effects, may play a more significant role on future soil nutrient quality in clearcut plots, where the ratio of evergreen to deciduous tree foliar cover was extremely different in the presence and absence of deer (2:1 in treatment EG and 7:1 in G; Figure 4.7.1). Deer presence after clearcutting significantly reduced tree foliar regeneration while facilitating graminoid regeneration, changing the plant composition which alters the litter quality and nutrients released during decomposition. Moreover, the decreased soil nutrient concentrations observed in treatment G were mitigated by treatment EG, but only for soil total N. This may be partly due to the regenerated tree foliar cover in EG compared to G.

Similarly, clearcutting showed remnant negative effects on the litter mass, soil nutrient concentrations and litter invertebrate abundance compared to the uncut plots which remained even after a decade. The compaction of soil (increased soil bulk density) increased after clearcutting but only in the presence of deer. As a result, clearcutting in the presence of deer has long-lasting negative effects and can be partially mitigated by excluding deer after clearcutting. Over the course of a decade, the negative effects of clearcutting on the belowground systems were not significant compared to those in the uncut plots and future changes require further verification.

6.4. Deer presence facilitating *A. firma* sapling recruitment within the Boso peninsula

The declining number of *A. firma* saplings within the Boso peninsula reported by Abrams et al. (2017) suggests the possibility of generation turnover failure. As the once mature trees disappear, the lack of young saplings would result in a period where other plants are able to invade and colonize available resources, resulting in subsequent loss of current *A. firma* stands. The results in chapter 5 suggested that density and height of *A. firma* saplings was highest in clearcut conditions with deer. The current *A. firma* stands may have been supported by past coppicing practices in the presence of deer which facilitated their regeneration long ago. It is possible the current lack of saplings are a result of abandonment of past coppicing practices and secondary succession progressing towards evergreen broad-leaved forests with closed canopies. This may be attributed to the species' slow growth rate, being outcompeted by other faster growing plant species in EG. Plant competition was probably suppressed by deer herbivory in G delaying canopy layer formation, resulting in suitable conditions for *A. firma* sapling recruitment and growth. In general, under the current deer density treatment G may support recruitment of *A. firma* saplings within the Boso peninsula. The current results are in contrast to Tamura and Nakajima (2017) in an old-growth fir forest which may be attributed to a higher deer density and browsing of *A. firma* by deer in their study. Therefore, it is possible that regional differences may affect the effectiveness of treatment results and that treatment G facilitating *A. firma* recruitment may be limited only within the Boso peninsula.

6.5. Future management implications

The restoration of forest ecosystems can be improved by applying different treatments to target specific management goals, such as rapid regeneration of the forest canopy cover through treatment E and EG, increased cover of non-tree species in the understory layer through treatments G and EG), or a balanced increase in both the forest canopy cover and understory layer via treatment EG. To improve plant species diversity, clearcutting can be a solution, but risks altering the ecosystem in the presence of deer. Furthermore, treatment EG may not be suitable for light-demanding, non-competitive species such as *A. firma*. To facilitate regeneration of all plant species, increasing the overall ecosystem heterogeneity, a multi-treatment approach would be more suitable compared to a single treatment approach.

In reality, large-scale treatments are too costly and implementation would not be feasible. One solution is to establish small manageable areas (100 m^2) of treatments E, G and EG; as an array of different treatments across a region to maximize diversity from among the different treatments. This would facilitate the maintenance of current species using treatment E as refugia from herbivores, treatment EG to recruit a variety of different tree species (evergreen and deciduous) and increase understory vegetation cover, while treatment G facilitates the regeneration of non-palatable species such as *A. firma*. In addition, establishing multiple small-scale treatments would minimize the impacts to aboveground and belowground systems as compared large-scale treatments, especially in the case of clearcutting (G and EG), the small-scale setting ensuring input of viable seed sources from surrounding vegetation outside the treatments.

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Supplementary materials

Supplementary material AA. Photos of treatment plots after a decade taken in 2019.

The photos indicate the current status of study plots within each treatment plot, **Ctrl:** Closed canopy with deer, **E:** Closed canopy without deer, **G:** Clearcutting with deer and **EG:** Clearcutting without deer.

Supplementary material A1. Comparison of regression models in predicting non-tree cover and deer effects on tree foliar cover after clearcut.

Model	Description	AIC	BIC	DHARMa residual diagnostics
M_0	non-tree cover + Deer + non-tree cover*Deer + (1| location/plot)	-2.6	0.8	NS
M_1	non-tree cover + Deer + (1| location/plot)	-3.0	-0.05	**
M_2	non-tree cover + Deer + (1| plot)	-5.0	-2.5	NS
M_3	non-tree cover + Deer + non-tree cover*Deer + (1| plot)	-4.6	-1.7	**

** indicates a significant deviation between the expected and observed values. NS indicates no significant deviation detected.

Based on DHARMa residual diagnostics, only models M_0 and M_2 indicated that predicted values from the model did not deviate significantly from observed values. Based on the AIC and BIC values, M_2 was determined as the best-fit model.

Supplementary material A2. Effects of non-tree species cover (%) and deer presence on tree foliar cover (%) in clearcut plots (G and EG).

Fixed variable	Estimate	SE	p
Intercept (Tree cover)	1.86	0.50	
Non-tree species cover (%)	-2.05	1.33	0.12
Deer presence (0 or 1)	-2.12	0.56	<0.01*

The beta regression model based on the best fit model (M_2, see supplementary material A1).

Supplementary material A3. Multi-model inference (MuMln) analysis based on parameters that showed high correlation with each invertebrate taxonomic group.

Taxon	Model		AICc	Weight
Amphipoda	Global	Amphipoda~ Litter Mass+ Coarse porosity + Ca + Na + Mg + Clearcut + Deer	135.8	
	Predicted	Amphipoda~ Coarse porosity + Litter Mass	119.1	0.202
		Amphipoda~ Coarse porosity + Litter Mass + Na	119.7	0.147
		Amphipoda~ Coarse porosity + Deer + Litter Mass	120.2	0.117
		Amphipoda~ Clearcut + Coarse porosity + Litter Mass	121.2	0.070
		Amphipoda~ Ca + Coarse porosity + Litter Mass	121.4	0.064
Araneae	Global	Araneae ~ Litter Mass + Total vegetation cover + Bulk density + Fine porosity + Coarse porosity + Na + K + Total N + Clearcut + Deer	178.1	
	Predicted	Araneae~ Bulk density + Deer + Litter Mass	139.0	0.149
		Araneae~ Bulk density + Litter Mass	140.1	0.086
		Araneae~ Deer + Litter Mass	140.9	0.059
		Araneae~ Bulk density + Coarse porosity + Litter Mass	141.6	0.040
		Araneae~ Bulk density + Litter Mass + Total N	142.0	0.033
Collembola	Global	Collembola ~ Litter Mass + Total vegetation cover + Fine porosity + Coarse porosity + Ca + Na + Total N + Mg + K + Deer + Clearcut	216.6	
	Predicted	Collembola ~Na	162.2	0.07
		Collembola ~Ca	162.6	0.025
		Collembola ~Litter Mass	164.5	0.024
		Collembola ~ K + Na	164.6	0.023
		Collembola ~ Fine porosity + Na	164.7	0.021

Supplementary material A3 (cont.)

Isopoda	Global	Isopoda ~ Litter Mass + Bulk density + Ca + Na + Mg	115.7	
	Predicted	Isopoda ~ Bulk density +Mg + Na	109.1	0.250
		Isopoda ~ Mg + Na	109.6	0.190
		Isopoda ~ Bulk density +Ca + Na	111.1	0.090
		Isopoda ~ Bulk density +Mg	111.2	0.085
		Isopoda ~ Bulk density + Litter Mass + Mg	111.3	0.083
Diplopoda	Global	Diplopoda ~ Litter Mass + Total vegetation cover + Bulk density + Coarse porosity + Na + Ca + Total N + Clearcut	112.6	
	Predicted	Diplopoda~ Coarse porosity + Litter Mass + Total N	93.9	0.200
		Diplopoda~ Coarse porosity + Litter Mass + Na	94.1	0.184
		Diplopoda~ Clearcut + Coarse porosity + Litter Mass	95.2	0.107
		Diplopoda~ Coarse porosity + Litter Mass	95.6	0.085
		Diplopoda~ Bulk density + Coarse porosity + Litter Mass	95.9	0.076
Chilopoda	Global	Chilopoda ~ Litter Mass + Total vegetation cover + Ca + Mg + Deer + Na + Mg	83.6	
	Predicted	Chilopoda~ Deer + Litter Mass	71.6	0.331
		Chilopoda~ Deer + Litter Mass + Mg	72.6	0.205
		Chilopoda~ Ca + Deer + Litter Mass	72.7	0.193
		Chilopoda~ Deer + Litter Mass + Na	74.2	0.092
		Chilopoda~ Deer + Litter Mass + Total N	74.3	0.085

The table shows the global and the predicted models (best 5 models) with the lowest AICc value for each taxonomic group. The predicted models are arranged from the lowest AICc value (best model) in ascending order.

Supplementary material A4. Total sapling density (m^{-2}) of target tree species in 2020.

Species name	Ctrl	E	G	EG	Total
	n = 6	n = 6	n = 6	n = 6	
Abies firma	0	0	3.92	2.04	5.96
Neolitsea sericea	0	0.84	1.06	0.22	2.16
Eurya japonica	3.60^{a}	0.20^{a}	10.80^{a}	31.60^{b}	46.20
Castanopsis sieboldii	0^{a}	2.08^{b}	0^{a}	1.12^{ab}	3.20
Cinnamomum tenuifolium	0^{a}	0.80^{b}	0.01^{a}	0.61^{ab}	1.42
Quercus acuta	0^{a}	2.16^{a}	0^{a}	4.77^{b}	6.93

The sapling density per height class (I to iv) of each species was calculated as (sapling count / sampled area m^{-2}) for each subplot. These density values for each height class were then summed according to species and treatment to obtain the sapling density of each species within the 10-by-10 m subplots per treatment group (n= 6). The total represents the sum of each species density among the treatment groups, indicating the sapling dominance among the different tree species in the overall study. Significant differences in sapling density of each species among treatment groups following *post hoc* tests are displayed as different letters in superscript.

Supplementary material A5. Vegetation presence frequency data within each treatment.

Plant species	Plant form	Frequency in treatment plots			
		Ctrl	E	G	EG
Arachniodes rhomboidea	F	5	5	4	5
Deparia japonica	F	0	0	0	1
Deparia lancea	F	0	0	1	0
Deparia okuboana	F	0	0	2	0
Dicranopteris linearis	F	0	0	0	1
Dryopteris bissetiana	F	1	0	0	0
Dryopteris erythrosora	F	0	1	2	1
Gleichenia japonica	F	0	0	0	1
Huperzia serrata	F	0	0	0	1
Lemmaphyllum microphyllum	F	1	1	0	0
Lygodium japonicum	F	0	0	2	0
Microlepia marginata	F	0	0	1	0
Onychium japonicum	F	0	0	3	0
Polypodiophyta (Unknown spp.)	F	1	2	3	2
Pteris cretica	F	1	1	3	0
Stegnogramma pozoi	F	1	1	0	1
Carex lenta	G	2	2	4	6
Carex multifolia	G	1	1	0	1
Miscanthus sinensis	G	0	0	4	1
Oplismenus undulatifolius	G	1	2	6	3
Poaceae (Unknown spp.)	G	0	0	2	0
Alpinia japonica	H	0	0	0	5
Arisaema spp.	H	0	2	0	1
Asarum nipponicum	H	2	3	3	3
Calanthe discolor	H	0	1	0	0
Carpesium rosulatum	H	0	1	1	0
Conyza sumatrensis	H	0	0	1	0
Erechtites hieraciifolius	H	2	0	2	0
Gentiana zollingeri	H	1	0	0	1
Gnaphalium affine	H	0	0	2	0
Houttuynia cordata	H	0	0	2	0
Hydrocotyle sibthorpioides	H	0	0	2	0
Hypericum erectum	H	0	0	3	0
Lespedeza pilosa	H	0	0	3	0
Liparis nervosa	H	0	1	0	0
Lysimachia japonica	H	0	0	1	0
Ophiopogon japonicus	H	2	0	1	0
Oxalis corniculata	H	0	0	1	0

Supplementary material A5 (cont.)

Plant species	Plant form	Ctrl	E	G	EG
Perilla spp.	H	1	0	0	0
Phytolacca decandra	H	0	0	1	0
Platanthera sigeyosii	H	0	1	0	0
Salvia japonica	H	0	0	1	0
Scutellaria indica	H	0	0	1	0
Stellaria spp.	H	0	0	1	0
Viola grypoceras	H	3	2	6	3
Ardisia crenata	S	3	2	2	1
Asteraceae spp.	S	3	0	2	1
Broussonetia kazinoki	S	0	0	1	3
Callicarpa japonica	S	1	2	5	6
Callicarpa mollis	S	0	0	0	2
Damnacanthus indicus	S	2	3	3	3
Daphne kiusiana	S	0	0	0	1
Debregeasia orientalis	S	0	0	1	0
Deutzia crenata	S	0	0	4	2
Deutzia scabra	S	0	0	4	0
Edgeworthia chrysantha	S	0	0	1	1
Euchresta japonica	S	1	0	0	0
Ligustrum obtusifolium	S	1	1	0	0
Maesa japonica	S	5	4	6	6
Pertya scandens	S	0	0	0	1
Pittosporum tobira	S	0	1	0	2
Pourthiaea villosa	S	0	0	0	1
Rosa luciae	S	0	0	2	1
Rubus microphyllus	S	0	0	3	1
Rubus minisculus	S	0	0	2	0
Rubus palmatus	S	0	0	1	1
Viburnum erosum	S	0	0	1	0
Weigela coraeensis	S	0	0	0	2
Zanthoxylum piperitum	S	0	2	4	0
Abies firma	T	3	3	5	4
Acacia mearnsii	T	0	0	0	1
Acer mono	T	0	0	0	1
Albizia julibrissin	T	1	0	1	3
Aralia elata	T	0	0	4	1
Camellia japonica	T	1	3	0	4
Castanopsis sieboldii	T	4	5	0	1
Cerasus jamasakura	T	0	0	0	2
Cinnamomum camphora	T	0	0	0	2

Supplementary material A5 (cont.)

Plant species	Plant form	Ctrl	E	G	EG
Cinnamomum tenuifolium	T	4	6	1	4
Clerodendrum trichotomum	T	0	0	0	3
Cleyera japonica	T	2	2	0	2
Cornus macrophylla	T	0	0	1	1
Cryptomeria japonica	T	0	1	4	3
Daphniphyllum teijsmannii	T	1	2	1	1
Dendropanax trifidus	T	0	3	0	4
Diospyros japonica	T	0	1	0	1
Eurya japonica	T	6	6	6	6
Euscaphis japonica	T	0	0	0	2
Ficus erecta	T	1	1	0	1
Ilex integra	T	3	0	0	1
Illicium anisatum	T	0	0	1	1
Kalopanax septemlobus	T	0	1	0	4
Lindera umbellata	T	1	2	2	5
Lithocarpus edulis	T	0	0	0	1
Litsea coreana	T	0	0	0	1
Machilus thunbergii	T	1	1	0	0
Magnolia hypoleuca	T	0	0	0	3
Mallotus japonicus	T	2	2	3	4
Melia azedarach	T	2	1	0	0
Myrica rubra	T	0	0	0	1
Myrsine seguinii	T	1	0	0	1
Neolitsea sericea	T	3	3	3	2
Osmanthus heterophyllus	T	2	3	2	1
Padus buergeriana	T	0	1	0	1
Pinus densiflora	T	0	0	2	1
Pinus taeda	T	0	1	0	0
Podocarpus macrophyllus	T	2	1	0	1
Prunus spp.	T	1	0	0	1
Quercus acuta	T	5	6	1	4
Quercus glauca	T	3	1	0	2
Quercus myrsinifolia	T	0	0	1	0
Quercus salicina	T	2	0	1	1
Quercus serrata	T	0	0	0	1
Rhus javanica	T	0	0	3	4
Stachyurus praecox	T	0	0	1	6
Styrax japonica	T	1	2	4	3
Swida controversa	T	0	0	0	1
Symplocos prunifolia	T	0	0	1	0

Supplementary material A5 (cont.)

Plant species	Plant form	Ctrl	E	G	EG
Toxicodendron trichocarpum	T	0	0	1	0
Zanthoxylum ailanthoides	T	1	0	3	6
Actinidia arguta	V	1	2	0	2
Actinidia polygama	V	0	0	0	1
Akebia trifoliata	V	0	3	0	4
Ampelopsis glandulosa	V	1	2	3	4
Anodendron affine	V	2	2	2	1
Berchemia racemosa	V	0	0	1	2
Celastrus orbiculatus	V	0	0	0	1
Cucurbitaceae sp.	V	0	0	1	0
Dioscorea tokoro	V	0	2	1	2
Ficus nipponica	V	1	1	1	1
Hedera rhombea	V	1	2	1	1
Jasminanthes mucronata	V	2	0	1	0
Kadsura japonica	V	1	1	0	0
Parthenocissus tricuspidata	V	0	0	0	1
Piper kadsura	V	0	1	0	1
Pueraria lobata	V	0	0	0	1
Rubus buergeri	V	0	0	0	2
Rubus hakonensis	V	0	1	0	0
Smilax china	V	1	0	3	3
Stauntonia hexaphylla	V	0	2	1	3
Toxicodendron orientale	V	0	1	3	1
Trachelospermum asiaticum	V	5	6	4	6
Vitis flexuosa	V	0	0	0	1
Wisteria floribunda	V	0	2	2	3

The plant species recorded in the study plots are assigned according to their plant forms (**F**- Ferns, **G**- Graminoids, **H**- Herbs, **S**- Shrubs, **T**-Trees and **V**- Vines). The frequency of each species within each treatment is the sum of their presence (1 or 0) among the six replicates of each treatment. The six common tree species in the current study are highlighted within the table.

Supplementary material A6. Estimated vegetation cover (%) of all plants (mature and saplings) within 5-by-5 m subplots in 2019. Vegetation cover less than 1% were recorded as '+'. Plant form denoted by **F:** Ferns, **G:** Grasses, **H:** Herbs, **S:** Shrubs, **T:** Trees and **V:** Vines.

	Plant form	**Study sites / Treatment**																									
		KBS									**HNK**									**HRT**							
		Ctrl		**E**		**G**		**EG**			**Ctrl**		**E**		**G**		**EG**			**Ctrl**		**E**		**G**		**EG**	
		1	**2**	**1**	**2**	**1**	**2**	**1**	**2**		**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**		**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Arachniodes rhomboidea	F		+		+		+	1			1	+	+	5	+	+	2	+		+	+	1	1		+	+	2
Deparia japonica	F							+																			
Deparia lancea	F																								+		
Deparia okuboana	F														+	+											
Dicranopteris linearis	F																										1
Dryopteris bissetiana	F		+																								
Dryopteris erythrosora	F						1										+						+		+		
Gleichenia japonica	F																										6
Huperzia serrata	F																									+	
Lemmaphyllum microphyllum	F											+	+														
Lygodium japonicum	F					+	+																				
Microlepia marginata	F														+												
Onychium japonicum	F					1	+																		+		
Polypodiophyta (Unk. spp.)	F					+	+						+							1			1	+		+	1
Pteris cretica	F					+	+								+					+			+				
Stegnogramma pozoi	F			+					+												+						

Supplementary material A6 (cont.)

Carex lenta	G	+		+		4	20	16	15						+		+	+		+			+		1	+	20
Carex multifolia	G											+											+			2	
Miscanthus sinensis	G					7	15								2	70										+	
Oplismenus undulatifolius	G			+		+	+	+							1	+	+			+			+	1	4		+
Poaceae (grass)	G														+									+			
Alpinia japonica	H							+	2								1	+									3
Arisaema sp.	H			+																		+					+
Asarum nipponicum	H										+	+	1	+	+	+	1	+				+			+		+
Calanthe discolor	H																					+					
Carpesium rosulatum	H																						+	+			
Conyza sumatrensis	H					+																					
Erechtites hieraciifolius	H											+			+					+					+		
Gentiana zollingeri	H								+		+																
Gnaphalium affine	H														+	+											
Houttuynia cordata	H						+																	+			
Hydrocotyle sibthorpioides	H															+									+		
Hypericum erectum	H														+	+									+		
Liparis nervosa	H																					+					
Lysimachia japonica	H															+											
Ophiopogon japonicus	H	+																		+				+			
Oxalis corniculata	H						+																				
Perilla sp.	H										+																
Phytolacca decandra	H					+																					
Platanthera sigeyosii	H																						+				
Salvia japonica	H					+																					

Supplementary material A6 (cont.)

Scutellaria indica	H														+												
Stellaria sp.	H																							+			
Viola grypoceras	H		+			+	+	+				+	+		+	+				+			+	+	+	+	+
Ardisia crenata	S	+	+	+		1	+		1			+											+				
Asteraceae sp.	S		+									+				+				+					+		+
Broussonetia kazinoki	S							2																	+	1	3
Callicarpa japonica	S	+		+		1	3	6	+						2	2	9	2					+		+	2	5
Callicarpa mollis	S																+										2
Damnacanthus indicus	S	3	6	7	1	1	2	1	+				2		5		2										
Daphne kiusiana	S								+																		
Debregeasia orientalis	S						+																				
Deutzia crenata	S						8								1	1									+	+	+
Deutzia scabra	S					2	3								+	+											
Edgeworthia chrysantha	S					1		1																			
Euchresta japonica	S											+															
Lespedeza pilosa	S					+	+																		+		
Ligustrum obtusifolium	S	+																					+				
Maesa japonica	S	1	+	+	+	8	2	1	2		+	+	1		8	10	+	+		+			+	30	25	+	1
Pertya scandens	S								+																		
Pittosporum tobira	S			+				1	2																		
Pourthiaea villosa	S																										+
Rosa luciae	S					2	4	+																			
Rubus microphyllus	S							+							+	+								+			
Rubus miniscululs	S						+								+												
Rubus palmatus	S																								+		1

Supplementary material A6 (cont.)

Viburnum erosum	S														+												
Weigela coraeensis	S																2										2
Zanthoxylum piperitum	S			+	+	+	1								+	2											
Abies firma	T	+				+					+	+	+	+	2	4	2	1					+	2	2	+	+
Acacia mearnsii	T							6																			
Acer mono	T																										2
Albizia julibrissin	T	+						+																	+	1	+
Aralia elata	T					+		2							+	+								+			
Camellia japonica	T												10				+	8		8		20	8			2	4
Castanopsis sieboldii	T		45	45	95						1	60	25					16		45		2	35				
Cerasus jamasakura	T																4									1	
Cinnamomum camphora	T							+																		+	
Cinnamomum tenuifolium	T	40	10	+	5				+		20		5	8				8		1		+	2		8	6	1
Clerodendrum trichotomum	T								+								+										+
Cleyera japonica	T										4	6		20				8					+			11	
Cornus macrophylla	T																							+		+	
Cryptomeria japonica	T							+	+						+	+							30	+	+	+	
Daphniphyllum teijsmannii	T		4	3	5			1							+												
Dendropanax trifidus	T								4				+				+					20	2			3	2
Diospyros japonica	T																					6					1
Eurya japonica	T	7	10	10	15	1	+	6	8		10	40	5	5	2	1	40	20		20	20	14	7	+	+	18	8
Euscaphis japonica	T																									6	2
Ficus erecta	T		+					+															+				

Supplementary material A6 (cont.)

Ilex integra	T		15									5								5							1
Illicium anisatum	T														4			5									
Kalopanax septemlobus	T				+			1	1								1	2									
Lindera umbellata	T			+				+				+					+	4					+	+	+	+	2
Lithocarpus edulis	T							1																			
Litsea coreana	T							+																			
Machilus thunbergii	T										7											18					
Magnolia hypoleuca	T																+	7								3	
Mallotus japonicus	T	+		+	1	+		4							+		3				+			+		+	+
Melia azedarach	T	+	+	+																							
Myrica rubra	T								+																		
Myrsine seguinii	T		4					2																			
Neolitsea sericea	T	+	+	+		5	12	5				+		+		+	+					+					
Osmanthus heterophyllus	T	1	+	+	+	30												1				2			+		
Padus buergeriana	T			+													1										
Pinus densiflora	T																							30	12	+	
Pinus taeda	T			30																							
Podocarpus macrophyllus	T	+	1	+				1																			
Prunus sp.	T							5												8							
Quercus acuta	T	30	1	+	+						50	2	40	75	+		9	8			70	60	8			3	3
Quercus glauca	T	+						20	46				25							+	3						
Quercus myrsinifolia	T																							+			
Quercus salicina	T						3	5												15	20						
Quercus serrata	T							2																			

Supplementary material A6 (cont.)

Rhus javanica	T							5	4						+									+	+	1	18
Stachyurus praecox	T							8	2						+		4	14								+	4
Styrax japonica	T		8	+	+	+	6	2							+	+		1									+
Swida controversa	T																8										
Symplocos prunifolia	T														+												
Toxicodendron trichocarpum	T																								+		
Zanthoxylum ailanthoides	T						8	6	4			+					2	5						+	+	4	6
Cucurbitaceae	V						+																				
Actinidia arguta	V		1		+												+						+				1
Actinidia polygama	V																	+									
Akebia trifoliata	V			+				+	+					+									+			+	+
Ampelopsis glandulosa	V	+		+			+	+							+	+	+	+					+			+	
Anodendron affine	V	+	+	+	+	+	3		+																		
Berchemia racemosa	V					+		+																		+	
Celastrus orbiculatus	V																										+
Dioscorea tokoro	V					+			+																		
Dioscorea tokoro	V			+				+														+					
Ficus nipponica	V		+		+	+		+																			
Hedera rhombea	V							+				+	+		+								+				
Jasminanthes mucronata	V	+	+			+																					
Kadsura japonica	V	+			+																						
Parthenocissus tricuspidata	V																+										
Piper kadsura	V			+				+																			

Supplementary material A6 (cont.)

Pueraria lobata	V																										+
Rubus buergeri	V																1										4
Rubus hakonensis	V																						+				
Smilax china	V					+			+							+				+				+		+	+
Stauntonia hexaphylla	V												+	+	+		1									+	+
Toxicodendron orientale	V			+		+	+								+			+									
Trachelospermum asiaticum	V		+	+	+	+	+	+	+		+	+	1	30	+		2	+		2	1	+	+		+	+	2
Vitis flexuosa	V							+																			
Wisteria floribunda	V			+										+	+	+	5	7									+