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**Foraging patterns of bumble bees across
natural forest–human residential landscape**

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Contents

Summary	1
General Introduction.....	4
Materials and Methods	7
Study sites.....	7
Land cover composition	8
Flowering phenology.....	8
Floral traits.....	9
Observation of bumble bees	10
Sampling and identification of pollen grains from the body of bumble bees.....	10

Chapter 1

Flowering structure and floral preferences of bumble bees in the natural forest and human residential environments.....

1.1 Introduction	27
1.2 Analysis	28
Land cover composition.....	28
Functional trait diversity	29
Resource space.....	30
Seasonal change in the floral resources	30
Preference of bumble bees	31
1.3 Results	32
Land cover composition.....	32
Number of available plant species	32
Flower abundance	34
Floral traits.....	35
Functional diversity of floral traits.....	36
Effects of floral shape and floral reward level on bumble bee visits	38
Preferred pollen-source species	38
1.4 Discussion.....	39
Comparisons between habitats.....	39
Comparisons between the urban-forest and natural-forest landscapes	40
Preference of bumble bees and quality of the residential habitat.....	41

1.5 Conclusions	42
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Chapter 2

Foraging habitat preference of bumble bees: Effects of garden flowers on pollinator visits to native flowers	65
2.1 Introduction	65
2.2 Analysis	68
Bumble bee density	68
Choice of targeting flowers	68
Visitation frequency	69
Habitat choice for pollen resources	69
Habitat-use fidelity of bumble bees	70
2.3 Results	71
Bumble bee communities	71
Bumble bee density	72
Choice of targeting flowers	72
Visitation frequency	73
Habitat choice for pollen resources	74
Habitat-use fidelity of individual bumble bees	75
2.4 Discussion	76
Preference to the residential habitat	76
Seasonal change in the habitat usage	78
Effects of garden flowers on the visitation patterns to natural vegetation	80
2.5 Conclusions	81

Chapter 3

Spatiotemporal variability in the diet breadth and overlap among bumble bee species	95
3.1 Introduction	95
3.2 Analysis	97
Diet breadth	97
Diet overlap	98
Floral resource space	99
Effects of floral diversity within the habitat on diet breadth	99
Effects of floral diversity of surrounding habitats on diet breadth at the forest habitat	100
Comparison of the diet overlap	101

3.3 Results	102
Effects of floral diversity within the habitat on diet breadth.....	102
Effects of floral diversity of surrounding habitats on diet breadth at the forest habitat.....	102
Diet overlap between habitats in the urban-forest landscape	103
Seasonal change in the diet overlap	103
Comparing diet overlap between body and load pollen.....	104
Effects of tongue length on the diet overlap	104
3.4 Discussion.....	105
Effects of floral diversity within the habitat on the diet breadth.....	105
Diet segregation between species	106
Effects of surrounding habitats on the diet breadth at the forest habitat.....	108
3.5 Conclusions	108
 General Discussion	 126
Effects of garden flowers at local (habitat) scale	126
Effects of garden flowers at the landscape scale	127
 Acknowledgements	 130
References	131

Summary

Bumble bees are very important pollinators in the cool-temperate ecosystems, and they also contribute to the pollination of agricultural crops as well as wild plants. Recent anthropogenic land-use change has decreased the floral resources in natural vegetation, thus pollinators increasingly rely on the agricultural fields or green spaces in the urban area. During the past decades, effects of the land-use change on the diversity and composition of pollinator communities have been evaluated in many studies. On the other hand, studies on the pollinators in the urban landscapes are limited. In the urban green spaces, floral structures are very different from that of the native vegetation due to the diverse floral composition of garden flowers including exotic plants. With long-flight ability, bumble bees are able to forage across habitats within the urban landscape. However, it is still unclear how the foraging decision of bumble bees is affected by the temporally and spatially heterogeneous floral environment in the urban landscapes. Ecological function of gardens is increasingly recognized as an important component of urban ecosystems, but its ecological significance is still underestimated. In the present study, I evaluated the effects of gardens on the bumble bee community and foraging behaviour at the local and landscape scales based on the monitoring of foraging patterns of bumble bees in two landscapes; 1) the urban-forest landscape including gardens in the residential area and 2) the natural-forest landscape without gardens.

In the first chapter, I compared temporal and spatial distributions of floral resources between the urban-forest and natural-forest landscapes, and between the habitats (forest, grassland, residential) within each landscape. Floral resources were evaluated by species richness, functional traits (growth form, corolla type, nectar and pollen volume, nectar sugar concentration), and functional diversity (Rao's quadratic entropy; based on the functional traits of floral species) of each habitat. The functional traits that influence the floral choice of bumble bees were also evaluated by

analyzing the visitation frequency to individual floral species. The forest habitat in the two landscapes was similar in floral species richness, functional traits, and functional diversity. Functional diversity did not differ between the forest and residential habitats in the urban-forest landscape, but the residential habitat hosted more floral species until early November. Although flowering of abundant species occurred from spring to early summer at the forest habitat, abundant species bloomed throughout the season at the residential habitat. Bumble bees preferred tubular or nectar-rich flowers, and the proportion of those flowers were higher at the residential habitat than at the forest habitat. Because of the diverse and high-quality resources until late season, the residential habitat was thought to be a preferable foraging habitat for bumble bees.

In the second chapter, I examined the habitat preference of bumble bees and the effect of residential habitat on the visitation patterns to natural vegetation. First, bumble bee density, proportion of visited floral species, and visitation frequency to each floral species were compared between the habitats within the urban-forest landscape. Next, proportion of visited floral species was compared between the forests of the urban-forest and natural-forest landscapes. The residential habitat attracted abundant and diverse bumble bees in the urban-forest landscape, and relatively large number of floral species was utilized in the gardens compared to the forest habitat. However, bumble bees at the forest habitat visited floral species at similar proportions in both landscapes, and forest flowers in the urban-forest landscape received similarly frequent visits by bumble bees as garden flowers did. Thus, gardens did not have a negative effect on the native vegetation in terms of pollinator attraction. Bumble bee density became quite low in the later season at the forest habitat while kept high at the residential habitat. Decrease in the abundant floral resources might make bumble bees move out of the forest to the gardens. The residential habitat might support bumble bee community, compensating for the decrease in the floral resource availability at the forest habitat in the urban-forest landscape.

In the third chapter, I examined diet breadth and diet overlap of bumble bee species at the forest and residential habitats by assessing pollen species composition on the body of bumble bees. Diet breadth of individual species at each habitat expanded with the increase in the floral diversity of the habitat. Because of the similarity in the floral diversity between the forest and residential habitats, diet breadth did not differ between the habitats. Diet overlap among species was also similar between the forest and residential habitats, but a seasonal variation in the diet overlap was detected only in the residential habitat. At the residential habitat, diet overlap was lowest in the floral-rich middle season. Larger increase in the floral richness toward middle season at the residential habitat might cause an increase in diet segregation, which was not detected at the forest habitat. Thus, diverse pollinators might be able to coexist at the floral rich residential habitat. Floral diversity or richness of the surrounding habitats (including gardens) did not affect the diet breadth of bumble bees foraging at the forest habitat. Ecological impacts of garden flowers may be limited to the local scale rather than landscape scale.

From these results, gardens in the urban ecosystem served as important foraging habitats for bumble bees, where diverse and high-quality floral resources are provided for a long period. Floral resources at the residential habitat might compensate for the decreasing resources in the later season at the forest habitat, and contribute to the growth and persistence of diverse bumble bee communities in the urban-forest landscape. Furthermore, foraging patterns of bumble bees at the forest habitat was not affected by the existence of floral-rich gardens outside the forest, suggesting limited ecological impacts of garden flowers on pollination in the natural vegetation. Gardens might have facilitative effects on the maintenance of pollinator communities in the urban ecosystems.

General Introduction

Animal pollination is one of the important components of ecosystem function (Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Potts *et al.* 2010). About 87% of the angiosperms benefit from animal pollination (Ollerton *et al.* 2011), and about 35% of the global crop production depends on pollinators (Klein *et al.* 2007). Although cultured honeybees have often been applied to crop pollination, recent studies revealed that diverse wild pollinator communities enhance and stabilize crop yields (Garibaldi *et al.* 2013).

Because of the crucial pollination service, pollinator decline has become a global concern (Kearns *et al.* 1998; Potts *et al.* 2010). Several factors are suggested as potential drivers causing the pollinator decline, such as habitat degradation, climate change, exposure to agrochemicals, and pathogens (Goulson *et al.* 2008a; Potts *et al.* 2010; Roulston & Goodell 2011; Weiner *et al.* 2014; Moffat *et al.* 2015; Baude *et al.* 2016). Among these factors, the loss of natural and semi-natural habitats due to land use change could be the primary cause of the decline (Goulson *et al.* 2008a; Winfree *et al.* 2009; Roulston & Goodell 2011). Degradation or loss of natural habitats is often accompanied by the reduction of floral resources. Recent works reported that the historical decline in the floral resource in the UK conformed to the decline in the diversity of pollinators (Carvell *et al.* 2006; Baude *et al.* 2016).

Agricultural reclamation and urbanization may have different ecological impacts on wild pollinators. Farmland may have a positive effect of mass-flowering crops by providing large floral resources to pollinators (Westphal *et al.* 2003), but crop monoculture, loss of landscape heterogeneity, agrochemicals, and degradation of surrounding natural habitats often result in the decrease in the biodiversity of pollinators (Le Féon *et al.* 2010; Kennedy *et al.* 2013). In contrast, urbanization has more complex effects on pollinator communities. Some studies reported little effects of urbanization on the abundance, richness or diversity of pollinators (T. Sattler *et al.* 2010;

Banaszak-Cibicka & Żmihorski 2012; Baldock *et al.* 2015), but other studies found negative effects on particular taxa (Ahrné *et al.* 2009; Bates *et al.* 2011; Geslin *et al.* 2013). These contradictory results might be partly due to the difference of spatial scale used for analyses (Glaum *et al.* 2017) and/or species-specific response of pollinators to the urban environment (Banaszak-Cibicka & Żmihorski 2012). Urban green spaces, such as parks and gardens provide a wide variety of ornamental and exotic plant species (Thompson *et al.* 2003; Frankie *et al.* 2005) and can support abundant and diverse pollinators that can benefit from those managed flowers (Mcfrederick & Lebuhn 2006; Matteson & Langellotto 2009; Goddard *et al.* 2010; Fortel *et al.* 2014; Baldock *et al.* 2015; Sirohi *et al.* 2015; Foster *et al.* 2016; Normandin *et al.* 2017). Considering the continuous natural habitat loss, urban green spaces are becoming an important refuge for native wildlife including pollinator insects (Goddard *et al.* 2010; Wray & Elle 2015). Although private gardens often prevail and account for the largest area of urban green spaces, their ecological significance is still underestimated or paid little attention (Gaston *et al.* 2005; Hernandez *et al.* 2009; Goddard *et al.* 2010).

Bumble bees, important generalist pollinators in the cool temperate ecosystems (Heinrich 1976a), are also reported to be declining partly because of land use change (Goulson *et al.* 2008a). On the other hand, they may benefit from urban flowers under urbanized environment (Hernandez *et al.* 2009; Matteson & Langellotto 2009). Mcfrederick & Lebuhn (2006) reported that several bumble bee species are abundantly foraging in urban gardens in accordance with the decrease in the natural vegetation. Furthermore, higher colony growth rate and comparable colony density are reported for bumble bee species in urban gardens in comparison with rural habitat (Frankie *et al.* 2005; Mcfrederick & Lebuhn 2006). While pollinators inhabiting agricultural environment are expected to contribute to the pollination of not only crops but also wild flowers in the native vegetation (Herrmann *et al.* 2007; Hagen & Kraemer 2010), the habitat use and foraging patterns of urban

pollinators across urban area and surrounding natural environments are poorly understood. Spatiotemporally species-rich environment in the urban gardens may influence the seasonal activity (i.e., life cycle and phenology) of bumble bees (Stelzer *et al.* 2010), resulting in the modification of foraging patterns. Bumble bees have high cognitive and long flight abilities, and they flexibly change targeting species and locations to maximize the foraging efficiency (Goulson 2010). By monitoring foraging patterns of bumble bees in the human residential–natural forest landscape, I aimed to evaluate the local- and landscape-scale responses of pollinators to the urban garden environment.

In the first chapter, I evaluated the spatial and temporal distributions of floral resources in two landscapes, one is a combination of natural forest and adjacent residential area (urban-forest landscape) and the other is a natural forest apart from a residential area (natural-forest landscape). Then, I discussed the effects of floral traits (corolla type and reward level) on visitation frequency of bumble bees. In the second chapter, I focused on the habitat preference of bumble bees, and compared the proportion of floral species visited by bumble bees and the habitat-use fidelity of bumble bees between habitats (forest, grassland, and residential habitats) and between landscapes (urban-forest and natural-forest landscapes). In the last chapter, I examined the effects of floral diversity within and outside of the foraging habitat on the foraging diversity (diet breadth) and the diet overlap among bumble bee species. Based on these results, I discussed the effects of urban gardens on the foraging patterns of bumble bees in the urban landscape.

Materials and Methods

Study sites

This study was conducted in two landscapes in the central part of Hokkaido, northern Japan during three years (2010–2012) (Fig. 0-1). One was an urban-forest landscape including adjacent residential area (Nopporo Forest Park, Ebetsu) and the other was a natural-forest landscape isolated from residential area (Toyoha natural forest, Jozankei). Observations in the urban-forest landscape were conducted at five locations; two places in a deciduous forest (N 43° 03' 16", E 141° 31' 1" and N 43° 03' 24", E 141° 31' 36", 57–64 m elevation), one place in a grassland nearby the forest (N 43° 03' 28", E 141° 30' 31"), and two places in a residential area nearby the forest (N 43° 03' 39", E 141° 30' 24" and N 43° 03' 44", E 141° 30' 03").

Observations in the natural-forest landscape were conducted at three locations; one place in a mixed forest of conifers and broad-leaved trees (N 42° 59' 31", E 141° 06' 36", 363–376 m elevation) and two places at a grassland nearby the forest (N 42° 58' 56", E 141° 07' 44" and N 42° 59' 25", E 141° 06' 44"). Study site in the natural-forest landscape was apart from the nearest residential area (Jozankei town) about 3 km. I selected two places in each of the forest and residential habitats in the urban-forest landscape, and in the grassland habitat in the natural-forest landscape to follow seasonally heterogeneous flower compositions. In the urban-forest landscape, distance between the places within the forest habitat was 450 m, and that within the residential habitat was 350 m. In the natural-forest landscape, distance between the places within the grassland habitat was about 450 m. The grassland habitat in the urban-forest landscape was dominated by non-native plants; *Trifolium pretense* and *Trifolium repens*, and partially *Solidago canadensis* and *Solidago gigantea* grew in stands. The grassland habitat in the natural-forest landscape was dominated by tall grass and *T. pretense*. Distances between the forest and grassland habitats were

500–900 and 500–1800 m in the urban-forest and natural-forest landscapes, respectively. Distances between the grassland and residential habitats were 500–900 m, and those between the forest and residential habitats were 600–1200 m in the urban-forest landscape. Snow usually melts in early to middle April and early May in the urban-forest and natural-forest landscapes, respectively.

Land cover composition

To quantify the land cover composition in each landscape, vegetation polygon data, which was made by Conservation GIS-consortium Japan (CGIS Japan) based on the vegetation survey data by Biodiversity Center of Japan was downloaded from CGIS Japan (<http://cgisj.jp/>).

Flowering phenology

To quantify the flowering structure at community scale, flowering phenology of plant species visited by bumble bees (available flower species) were monitored weekly from late April or early May to the end of October or early November in the urban-forest landscape (in total, 46, 26 and 22 times in 2010, 2011 and 2012, respectively), and from early May to early October in the natural-forest landscape (in total, 23, 19 and 18 times in 2010, 2011 and 2012, respectively). For plant species at flowering, flowering stage in each species (classified into three classes: <20%, 20–60%, and $\geq$ 60% of the full bloom) and flower abundance at full bloom (classified into three classes: large, medium, small) were recorded. Flowers of large canopy trees, common shrubs or dominant herbs that bloom in large and dense patches were classified as “large”, sparsely-distributed shrubs or relatively common herbs that form many small patches as “medium”, and sparsely-distributed herbs with small flowering patches as “small”. In each observation locations, every flower species within an area of 100 m square were recorded.

Floral traits

To determine the functional traits of individual plant species, depth of floral tube (flower morphology), growth form, potential nectar volume and concentration, and the number of pollen grains were recorded during the flowering season of 2010–2012. Additional sampling was conducted for several plant species in the campus of Hokkaido University and Botanical Garden of Hokkaido University in Sapporo in 2016. Except for the nectar sugar concentration, traits were classified into categories or ordered classes; six categories of morphology, four categories of growth form, four ordered classes of pollen volume, and five ordered classes of nectar volume (Table 0-1). For 29 plant species producing flower heads, such as Asteraceae and *Trifolium* spp., the number of flowers within a head was counted for 2–232 flower heads in each species (Table 0-S1 & 0-S2). Nectar volume was measured using 1 or 2 μ L-Microcaps® (Drummond Scientific Company, U.S.A) for 2–466 individual flowers of 91 flower species. Because some flowers might be foraged by visitors prior to the nectar measurement, the maximum nectar volume obtained in each species was used as a potential nectar availability. Sugar concentration of extracted nectar was immediately measured using a refractometer (Eclipse refractometer for 0–50°Brix (for low volume), Bellingham and Stanley). Because of too small nectar volume for several species, sugar concentration could be measured for 69 out of 91 species. Undehiscent anthers were collected from 1–13 flowers of 73 species. All anthers from a single flower were sampled and stored in a 5-mL Eppendorf tube, brought back to the laboratory, and dried until anthers dehiscence at room temperature. Dehiscent anthers were filled with 70% ethanol and sonicated for 90 min to release pollen from the anthers. These samples were diluted by diluent (ISOTON II, Beckman Coulter, Inc.) to 70 mL to count the number of pollen grains by a particle counter (Z2 Coulter Counter®, Beckman Coulter, Inc.). The range of pollen diameter for counting was determined by the size distribution of pollen particles in

each species, which was calculated by the particle counter. The total volume of pollen grains per flower was calculated using mean particle size and grain numbers, assuming that pollen grains are spheres with a diameter of the mean particle size. For flowers that shape flower heads, nectar and pollen volume per flower was multiplied by the mean number of flowers per head to represent the amount of resource per unit of flower. Samples from different site and year were pooled, so each flower species had one value for each trait.

Observation of bumble bees

During the weekly observation of flowering structure in 2010 to 2012, I recorded the species and behaviour (flying or foraging, and for foragers, visited plant species) of observed bumble bees. In 2010 and 2011, bee observation was made in two methods; a sit-and-wait method in which I waited for bumble bee visits at fixed floral patches, and a searching method in which I walked slowly around the observation sites searching for foraging bumble bees. In the first year, the sit-and-wait method was given the first priority over the searching method, in order to identify the available plant species as floral resources of bumble bees. Some of the plant species observed in the sit-and-wait method were excluded from the searching method. Observation in 2012 was solely made by the searching method. In both methods, I did not distinguish bumble bee individuals, but followed their movement to avoid duplicated recording. In total, 14,583 and 10,154 min were spent for the urban-forest and natural-forest landscapes, respectively (Table 0-2). Because of the unexpected mowing at the grassland in June and August in 2011 and 2012, the observation in the urban grassland was restricted to the period during which flowers were available.

Sampling and identification of pollen grains from the body of bumble bees

Bumble bees were captured during the weekly observation in 2010 and 2011 for the sampling of pollen grains on the body of bumble bees. In total, 945 (*Bombus hypocrita sapporoensis*: 99, *B. terrestris*: 108, *B. hypnorum koropokkrus*: 103, *B. ardens sakagami*: 250, *B. pseudobaicalensis*: 117, *B. diversus tersatus*: 258) and 440 (*B. hypocrita sapporoensis*: 95, *B. terrestris*: 1, *B. hypnorum koropokkrus*: 89, *B. ardens sakagami*: 49, *B. diversus tersatus*: 248, *B. yezoensis*: 52) individuals of bumble bees were captured in the urban-forest and natural-forest landscapes, respectively. Captured bumble bees were immediately anesthetized by CO₂ and rubbed over throughout the body except for the hind legs by a small cube of glycerin jelly (150cc of fuchsine-stained glycerin was solidified with gelatin dissolved in 175cc water; Kearns & Inouye 1993) to collect pollen grains on the body (hereafter body pollen). When the bumble bee had pollen loads of the corbiculae, one of them was sampled (if pollen loads were too small, both of the loads were sampled; hereafter load pollen). Samples were identified with bumble bee ID accompanied by the behaviour (foraging plant species or flying) and sampling location, and kept in a 5-mL Eppendorf tube. In the laboratory, pollen loads were stained by fuchsine, stored in about 3 mL of isotonic sucrose (0.4mol/l) and sonicated for 90 min for homogenization. About 30 µL of the homogenized samples or the cube of glycerin jelly was mounted on glass slides, and examined under a microscope at 400×. Pollen grains within three randomly selected fields were identified to species or group with the aid of reference pollen collection from the studied landscapes, and the number of pollen grains was counted for each species or group. When the total number of pollen grains was less than 100, additional fields were inspected until the total number of grains reached 100. Because the pollens were quite similar between wild and cultivated *Hydrangea* spp. and between *Impatiens textori* and *I. noli-tangere*, these pollens were pooled in groups. Some garden species could not be identified to species level, and the pollens from these flowers were pooled at genus level (*Rhododendron* spp., *Iris* spp., *Rosa* spp., *Cerasus* spp.).

Table 0-1: Classification of floral traits

Traits	Class	For GLMM in Chapter 1	Description	References
Corolla type	LL	Long	≥20 mm with narrow entrance	Based on the predicted tongue length distribution (Fig. 0-S1), long tongued bees were distinguished from medium and short tongued bees at the length of 10 mm. All bumble bee species in the study sites were expected to be able to use flowers whose depth less than 6 mm. Flowers with ≥20 mm-tube length and narrow entrance could not be utilized legitimately by medium and short tongued bees assuming the body length of workers as 13.6 mm (average body length of workers of the seven species calculated from data in Kinoda 2012 * ¹) and the tongue length of 6 mm. The prediction of tongue length was based on the allometry published by Kinoda K. (2012).
	Relaxed	Medium	≥20 mm with wide entrance (bees can easily scroll into the corolla)	
	L	Medium	10–20 mm with narrow entrance	
	M	Short	6–10 mm with narrow entrance	
	S	Short	<6 mm	
	Open	Open	Exposed (easily accessible)	
Nectar	SS	0	Empty	According to Goulson (2002)* ² , the minimum load of nectar or pollen, which was brought back to the nest was around 10 mg for every size of bumble bees. Based on this, 10 µL (nearly 10 mg) was assumed to be large for a single bout. This classification is consistent with Potts et al. (2004)* ³ , in which nectar volume was classified into three classes (<0.1, 0.1–1.0, >1.0 µL).
	S	1	<0.1 µL	
	M	2	0.1–1 µL	
	L	3	1–10 µL	
	LL	4	≥10 µL	
Pollen	S	1	<0.1 µL	
	M	2	0.1–1 µL	
	L	3	1–10 µL	
	LL	4	≥10 µL	

*1 Kinoda K. 2012. 北海道産マルハナバチ全種の計測比較—下唇長、頭幅、体長及び触角長. つねきばち 20: 37-52.

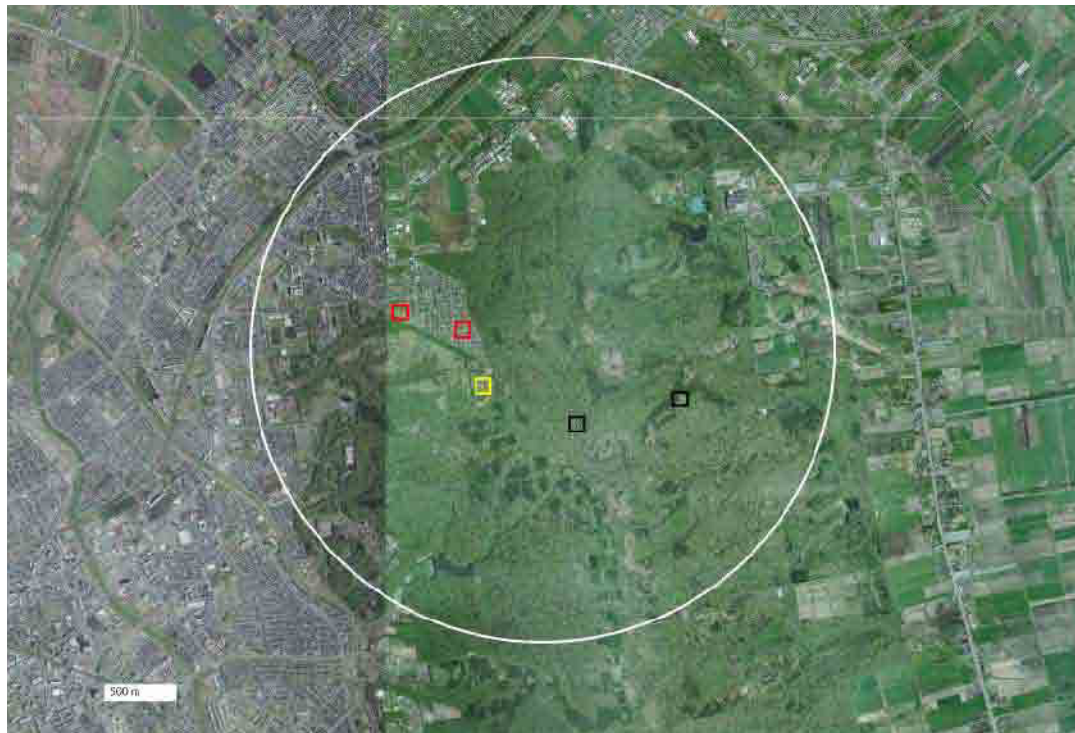
*2 Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC, Hughes WOH. 2002. Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Animal Behaviour* 64: 123-130.

*3 Potts SG, Vulliamy B, Roberts S, O'Toole C, Dafni A, Ne'eman G, Willmer PG. 2004. Nectar resource diversity organises flower - visitor community structure. *Entomologia Experimentalis et Applicata* 113: 103-107.

Table 0-2: Periods (min) for bumble bee visit observation in each habitat (forest, grassland, residential) in each landscape (urban forest, natural, forest). Observations were conducted by two methods; “sit-and-wait” at fixed places and “searching” by walking within fixed areas.

	Forest		Grassland		Residential		Sum
	Sit-and-wait	Searching	Sit-and-wait	Searching	Sit-and-wait	Searching	
Urban	3340	4350	1190	361	2379	2963	14583
Natural	3571	3119	1405	2059			10154

a)



b)

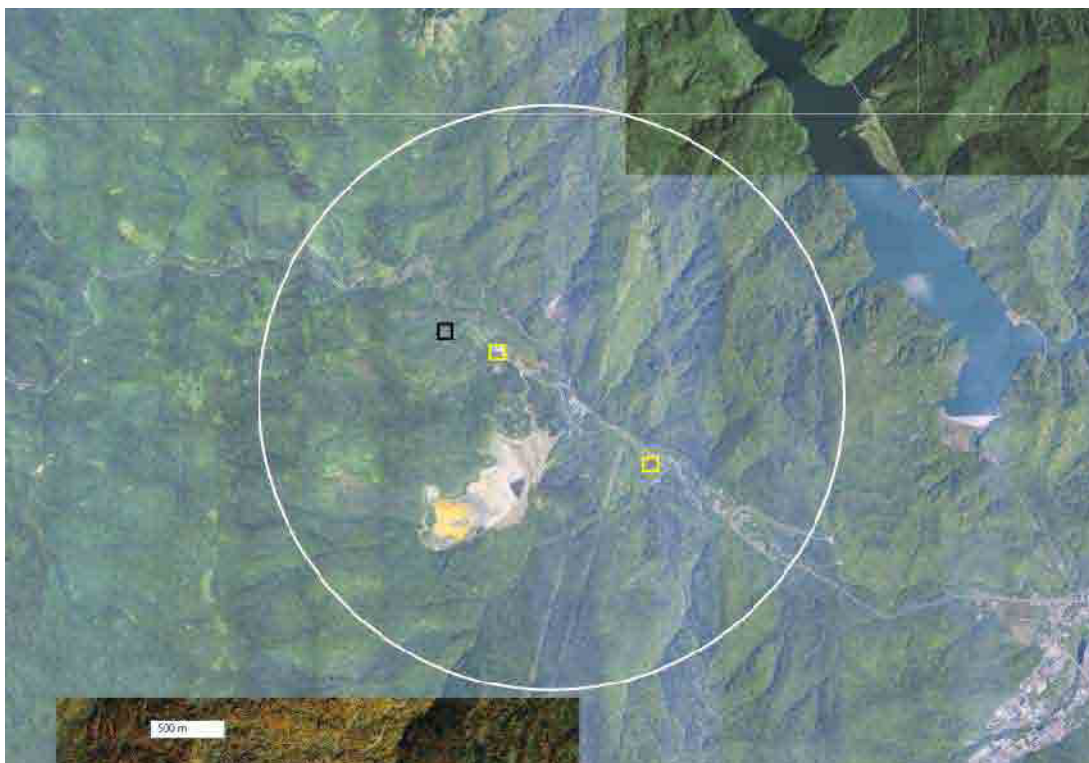


Fig. 0-1: Aerial photographs of the urban-forest (a) and natural-forest (b) landscapes. The locations of observation places are represented in open squares (black: forest habitat, yellow: grassland habitat, red: residential habitat). White circles represent the area in which the landscape composition was calculated. In the natural-forest landscape, Jozankei town can be seen in the lower right.

These seamless pictures were taken from the open-source aerial pictures (Geospatial Information Authority of Japan). The pictures of the urban-forest landscape were taken in May to July 2008 for the left half and in May to June 2007 for the right half. The pictures of the natural-forest landscape were taken in 1984–86 for the lower left end, May to July in 2008 for the upper right, and 2004– for the main part.

Table 0-S1: List of floral species visited by bumble bees. Scientific names conform to Yonekura & Kajita (2003)^{*1} (Ylist; <http://ylist.info>).

Habitat× SpID ^{*2}	PollID ^{*3}	Japanese name	Scientific name	Urban ^{*4}	Natural ^{*5}
co01.1	co01	エゾヤマザクラ	<i>Cerasus sargentii</i> (Rehder) H. Ohba	NF	NF
co01.2	co01	エゾヤマザクラ	<i>Cerasus sargentii</i> (Rehder) H. Ohba	residential	NF
co02.1	co02	オオハンゴンソウ	<i>Rudbeckia laciniata</i> L.	forest	forest
co02.2	co02	オオハンゴンソウ	<i>Rudbeckia laciniata</i> L.	grassland	NF
co03.1	co03	サルナシ	<i>Actinidia arguta</i> (Siebold et Zucc.) Planch. ex Miq.	forest	forest
co03.2	co03	サルナシ	<i>Actinidia arguta</i> (Siebold et Zucc.) Planch. ex Miq.	NF	NF
co04.1	co04	ツリバナ	<i>Euonymus oxyphyllus</i> Miq. var. <i>oxyphyllus</i>	NF	forest
co04.2	co04	ツリバナ	<i>Euonymus oxyphyllus</i> Miq. var. <i>oxyphyllus</i>	residential	NF
co05.1	co17	ツリフネソウ	<i>Impatiens textorii</i> Miq.	forest	NF
co05.2	co17	ツリフネソウ	<i>Impatiens textorii</i> Miq.	residential	NF
co06.1	co06	ナナカマド	<i>Sorbus commixta</i> Hedi.	forest	NF
co06.2	co06	ナナカマド	<i>Sorbus commixta</i> Hedi.	residential	NF
co07.1	co07	ノリウツギ	<i>Hydangea paniculata</i> Siebold	forest	forest
co07.2	co07	ノリウツギ	<i>Hydangea paniculata</i> Siebold	residential	NF
co08.1	co08	ハウチワカエデ	<i>Acer japonicum</i> Thunb.	forest	forest
co08.2	co08	ハウチワカエデ	<i>Acer japonicum</i> Thunb.	residential	NF
co09.1	co09	ヤマブドウ	<i>Vitis coignetiae</i> Pulliat ex Planch.	forest	forest
co09.2	co09	ヤマブドウ	<i>Vitis coignetiae</i> Pulliat ex Planch.	residential	NF
co10.1	co10	エゾミソハギ	<i>Lythrum salicaria</i> L.	grassland	NF
co10.2	co10	エゾミソハギ	<i>Lythrum salicaria</i> L.	residential	NF
co11.1	co11	ギボウシ	<i>Hosta</i> spp.	forest	NF
co11.2	co11	ギボウシ	<i>Hosta</i> spp.	residential	NF
co12.1	co12	マーガレット	<i>Argyranthemum frutescens</i> (L.) Sch.Bip.	grassland	grassland
co12.2	co12	マーガレット	<i>Argyranthemum frutescens</i> (L.) Sch.Bip.	residential	NF
co13.1	co13	エゾゴマナ	<i>Aster glehnii</i> F. Schmidt var. <i>glehnii</i>	NF	forest
co13.2	co13	エゾゴマナ	<i>Aster glehnii</i> F. Schmidt var. <i>glehnii</i>	NF	grassland
co14.1	co14	エゾニユウ	<i>Angelica ursina</i> (Rupr.) Maxim.	NF	forest
co14.2	co14	エゾニユウ	<i>Angelica ursina</i> (Rupr.) Maxim.	NF	grassland
co15.1	co15	オドリゴンウ	<i>Lamium album</i> L. var. <i>barbatum</i> (Siebold et Zucc.) Franch. et Sav.	NF	forest
co15.2	co15	オドリゴンウ	<i>Lamium album</i> L. var. <i>barbatum</i> (Siebold et Zucc.) Franch. et Sav.	NF	grassland

Continued

co16.1	co16	オニシモツケ	<i>Filipendula camtschatica</i> (Pall.) Maxim.	forest	forest
co16.2	co16	オニシモツケ	<i>Filipendula camtschatica</i> (Pall.) Maxim.	residential	NF
co17.1	co17	キツリフネ	<i>Impatiens noli-tangere</i> L.	forest	forest
co17.2	co17	キツリフネ	<i>Impatiens noli-tangere</i> L.	NF	grassland
co18.1	co18	タチツボスミレ	<i>Viola acuminata</i> Ledeb.	forest	forest
co18.2	co18	タチツボスミレ	<i>Viola acuminata</i> Ledeb.	residential	NF
co19.1	co19	ツタウルシ	<i>Toxicodendron orientale</i> Greene	forest	NF
co19.2	co19	ツタウルシ	<i>Toxicodendron orientale</i> Greene	residential	NF
co20.1	co20	オオアワダチソウ	<i>Solidago gigantea</i> Aiton subsp. <i>serotina</i> (Kuntze) McNeill	forest	NF
co20.2	co20	オオアワダチソウ	<i>Solidago gigantea</i> Aiton subsp. <i>serotina</i> (Kuntze) McNeill	grassland	grassland
co20.3	co20	オオアワダチソウ	<i>Solidago gigantea</i> Aiton subsp. <i>serotina</i> (Kuntze) McNeill	residential	NF
co21.1	co21	ゲンノシヨウコ	<i>Geranium thunbergii</i> Siebold ex Lindl. et Paxton	forest	NF
co21.2	co21	ゲンノシヨウコ	<i>Geranium thunbergii</i> Siebold ex Lindl. et Paxton	grassland	NF
co21.3	co21	ゲンノシヨウコ	<i>Geranium thunbergii</i> Siebold ex Lindl. et Paxton	residential	NF
co22.1	co22	セイタカアワダチソウ	<i>Solidago altissima</i> L.	forest	NF
co22.2	co22	セイタカアワダチソウ	<i>Solidago altissima</i> L.	grassland	grassland
co22.3	co22	セイタカアワダチソウ	<i>Solidago altissima</i> L.	residential	NF
co23.1	co23	セイヨウタンポポ	<i>Taraxacum officinale</i> Weber ex F.H.Wigg.	forest	NF
co23.2	co23	セイヨウタンポポ	<i>Taraxacum officinale</i> Weber ex F.H.Wigg.	grassland	grassland
co24.1	co24	ヒメジョオン	<i>Erigeron annuus</i> (L.) Pers.	forest	NF
co24.2	co24	ヒメジョオン	<i>Erigeron annuus</i> (L.) Pers.	grassland	grassland
co25	co25	ヤナギラン	<i>Chamaerion angustifolium</i> (L.) Holub	residential	grassland
co26	co26	ルビナス	<i>Lupinus polyphyllus</i> Lindl.	residential	grassland
cu01	cu01	アスバラガス	<i>Asparagus officinalis</i> L.	residential	NF
cu02	cu02	アマドコロ	<i>Polygonatum odoratum</i> (Mill.) Druce var. <i>pluniflorum</i> (Miq.) Ohwi	residential	NF
cu04	cu04	アヤメspp	<i>Iris</i> spp.	residential	NF
cu05	cu05	イチゴsp	<i>Rubus</i> spp.	residential	NF
cu06	cu06	イボタノキ	<i>Ligustrum obtusifolium</i> Siebold et Zucc.	residential	NF
cu07	cu07	インゲンマメ	<i>Phaseolus vulgaris</i> L.	residential	NF
cu08	cu08	ウメsp	<i>Armeniaca mume</i> (Siebold et Zucc.) de Vriese	residential	NF
cu09	cu09	エゴノキ	<i>Styrax japonica</i> Siebold et Zucc.	residential	NF
cu10	cu10	エゾクガイソウ	<i>Veronicastrum sibiricum</i> (L.) Pennell subsp. <i>yezoense</i> (H.Hara) T.Yamaz.	residential	forest
cu11	cu11	エゾノコリンゴ	<i>Malus baccata</i> (L.) Borkh. var. <i>mandshurica</i> (Maxim.) C.K.Schneid.	residential	NF
cu12	cu12	エリカ	<i>Erica</i> spp.	NF	NF

Continued

cu13	cu13	エリンジウム	<i>Eryngium</i> sp.		residential	NF
cu14	cu14	オレガノ	<i>Origanum vulgare</i> L.		residential	NF
cu16	cu16	カボチャ	<i>Cucurbita maxima</i> Duchesne ex Lam.		residential	NF
cu17	cu17	カラミンサ	<i>Clinopodium nepeta</i> (L.) Kuntze		residential	NF
cu18	cu18	カンパズラ	<i>Campanula glomerata</i> L. subsp. <i>glomerata</i>		residential	NF
cu19	cu19	ギク spp	Asteraceae spp.		residential	NF
cu20	cu20	キャットミント	<i>Nepeta</i> × <i>faassenii</i>		residential	NF
cu21	cu21	キュウリ	<i>Cucumis sativus</i> L.		residential	NF
cu23	cu23	クリ	<i>Castanea crenata</i> Siebold et Zucc.		residential	NF
cu24	cu24	クロバナフウロソウ	<i>Geranium phaeum</i>		residential	NF
cu25	cu25	コスモス	<i>Cosmos bipinnatus</i> Cav.		residential	grassland
cu26	cu26	コバギボウシ	<i>Hosta sieboldii</i> (Paxton) J.W.Ingram var. <i>sieboldii</i> f. <i>spathulata</i> (Miq.) W.G.Schmid		residential	NF
cu27	cu27	コモンタイム	<i>Thymus vulgaris</i>		residential	NF
cu28	cu28	サクラ spp	<i>Cerasus</i> spp.		residential	NF
cu29	cu29	サラサドウダン	<i>Enkianthus campanulatus</i> (Miq.) G.Nicholson		residential	NF
cu30	cu30	ジギタリス	<i>Digitalis purpurea</i> L.		residential	NF
cu31	cu31	シソ科	Lamiaceae spp.		residential	NF
cu32	cu32	シバザクラ	<i>Phlox subulata</i> L.		residential	NF
cu33	cu33	シヤクナゲ	<i>Rhododendron</i> spp.		residential	NF
cu34	cu34	ジャコウアオイ	<i>Malva moschata</i> L.		residential	NF
cu35	cu35	スイートピー	<i>Lathyrus odoratus</i> L.		residential	NF
cu36	cu36	スグリ	<i>Ribes</i> sp.		residential	NF
cu37	cu37	スペアミント	<i>Mentha spicata</i> L.		residential	NF
cu39	cu39	アジサイ spp	<i>Hydrangea</i> spp.		residential	NF
cu40	cu40	セイヨウオダマキ	<i>Aquilegia vulgaris</i> L.		residential	NF
cu41	cu41	セントーレア	<i>Centaurea montana</i>		residential	NF
cu42	cu42	ダリア	<i>Dahlia</i> spp.		residential	NF
cu43	cu43	チコリ	<i>Cichorium intybus</i> L.		residential	NF
cu48	cu48	ツツジ spp	<i>Rhododendron</i> spp.		residential	NF
cu49	cu49	ドウダンツツジ	<i>Enkianthus perulatus</i> (Miq.) C.K.Schneid.		residential	NF
cu50	cu50	トマト	<i>Lycopersicon esculentum</i> Mill.		residential	NF
cu51	cu51	ナツグミ	<i>Elaeagnus multiflora</i> Thunb. f. <i>orbiculata</i> (Makino) Araki		residential	NF
cu52	cu52	ニューギニアインパチエンス	<i>Impatiens hawkeri</i>		residential	NF
cu54	cu54	ハスカップ	<i>Lonicea caerulea</i> L. subsp. <i>edulis</i> (Regel) Hultén var. <i>emphyllacalyx</i> (Maxim.) Nakai		residential	NF

Continued

cu55	ハナマメ	<i>Phaseolus coccineus</i> L.	residential	NF
cu56	ハマナス	<i>Rosa rugosa</i> Thunb.	residential	NF
cu57	バラ spp.	<i>Rosa</i> spp.	residential	NF
cu58	ヒペリカム	<i>Hypericum</i> sp.	residential	NF
cu59	ヒマワリ	<i>Helianthus</i> spp.	residential	NF
cu60	カンパネラ sp.	<i>Campanula</i> sp.	residential	NF
cu61	フジ	<i>Wisteria floribunda</i> (Willd.) DC.	residential	NF
cu62	プリムラ sp.	<i>Primula</i> sp.	residential	NF
cu63	プルモナリア	<i>Pulmonaria</i> sp.	residential	NF
cu64	ベゴニア	<i>Begonia</i> sp.	residential	NF
cu65	ベチユニア	<i>Petunia</i> x <i>hybrida</i> (Hook.f.) Vilm.	residential	NF
cu66	ボンバウンラン	<i>Linaria vulgaris</i> Mill.	residential	NF
cu70	ミヤマオダマキ	<i>Aquilegia flabellata</i> Siebold et Zucc. var. <i>pumila</i> (Huth) Kudô	residential	NF
cu71	ムクゲ	<i>Hibiscus syriacus</i> L.	residential	NF
cu72	ムラサキ科	Boraginaceae sp.	residential	NF
cu73	ムラサキシキブ	<i>Callicarpa japonica</i> Thunb.	residential	NF
cu74	ムラサキツユクサ	<i>Tradescantia ohiensis</i> Raf.	residential	NF
cu76	モントブレチア	<i>Crocosmia</i> x <i>crocosmiiflora</i> (Lemoine) N.E.Br.	NF	grassland
cu77	ヤマツツジ	<i>Rhododendron kaempferi</i> Planch. var. <i>kaempferi</i>	residential	NF
cu78	エキヤナギ	<i>Spiraea thunbergii</i> Siebold ex Blume	residential	NF
cu79	ラスベリー	<i>Rubus idaeus</i> L. subsp. <i>idaeus</i>	residential	NF
cu80	ラベンダー	<i>Lavandula</i> spp.	residential	NF
cu81	ラミウム	<i>Lamium</i> sp.	residential	NF
cu82	ラムズイヤー	<i>Stachys byzantina</i>	residential	NF
cu83	リナリア	<i>Linaria</i> sp.	residential	NF
cu84	リンゴ sp.	<i>Malus</i> sp.	residential	NF
cu85	リンドウサキカンバヌラ	<i>Campanula glomerata</i> L. subsp. <i>glomerata</i>	residential	NF
cu86	ルドベキア	<i>Rudbeckia</i> sp.	residential	NF
cu87	ウスレナグサ	<i>Myosotis</i> sp.	residential	NF
cu88	タデ科	Polygonaceae sp.	residential	NF
fo01	アキノキリンソウ	<i>Solidago virgaurea</i> L. subsp. <i>asiatica</i> (Nakai ex H.Hara) Kitam. ex H.Hara	NF	forest
fo02	アスキナシ	<i>Aria alnifolia</i> (Siebold et Zucc.) Decne.	NF	forest
fo03	イタヤカエデ	<i>Acer pictum</i> Thunb. subsp. <i>dissectum</i> (Wesm.) H.Ohashi	forest	forest
fo04	エゾアジサイ	<i>Hydrangea serrata</i> (Thunb.) Ser. var. <i>yessoensis</i> (Koidz.) H.Ohba	forest	forest

Continued

fo05	fo05	エソエンゴサク	<i>Corydalis fumariifolia</i> Maxim. subsp. <i>azurea</i> Lidén et Zetterlund	forest	forest	forest
fo06	fo06	エソトリカブト	<i>Aconitum sachalinense</i> F.Schmidt subsp. <i>yezoense</i> (Nakai) Kadota	forest	forest	forest
fo07	fo07	エビガライチゴ	<i>Rubus phoenicolasius</i> Maxim.	forest	forest	NF
fo08	fo08	オオアマドコロ	<i>Polygonatum odoratum</i> (Mill.) Druce var. <i>maximowiczii</i> (F.Schmidt) Koidz.	forest	forest	forest
fo09	fo09	オオウバユリ	<i>Cardiocrinum cordatum</i> (Thunb.) Makino var. <i>glehnii</i> (F.Schmidt) H.Hara	forest	forest	forest
fo10	fo10	オオカメノキ	<i>Viburnum furcatum</i> Blume ex Maxim.	forest	forest	forest
fo11	fo11	オオハナウド	<i>Heracleum lanatum</i> Michx. var. <i>lanatum</i>	forest	forest	forest
fo12	fo12	オオバボダイジュ	<i>Tilia maximowicziana</i> Shiras.	NF	NF	forest
fo13	fo13	オオミミナグサ	<i>Cerastium fontanum</i> Baumg. subsp. <i>vulgare</i> (Hartm.) Greuter et Burdet	residential	NF	NF
fo14	fo14	オオレイジンソウ	<i>Aconitum inumae</i> Kadota	NF	NF	forest
fo15	fo15	カタクリ	<i>Erythronium japonicum</i> Decne.	NF	NF	forest
fo16	fo16	キハダ(雄株)	<i>Phellodendron amurense</i> Rupr.	NF	NF	forest
fo17	fo17	キヨスミウツボ	<i>Phacellanthus tubiflorus</i> Siebold et Zucc.	NF	NF	forest
fo18.1	fo18	キンミズヒキ	<i>Agrimonia pilosa</i> Ledeb. var. <i>japonica</i> (Miq.) Nakai	forest	forest	forest
fo18.2	fo18	キンミズヒキ	<i>Agrimonia pilosa</i> Ledeb. var. <i>japonica</i> (Miq.) Nakai	NF	NF	grassland
fo19	fo19	クロイチゴ	<i>Rubus mesogaeus</i> Focke	forest	forest	NF
fo20	fo20	コンロンソウ	<i>Cardamine leucantha</i> (Tausch) O.E.Schulz	forest	forest	forest
fo21	fo21	サイハイラン	<i>Cremata appendiculata</i> (D.Don) Makino	forest	forest	forest
fo22	fo22	サラシナシヨウマ	<i>Cimicifuga simplex</i> (DC.) Wormsk. ex Turcz.	forest	forest	NF
fo23	fo23	シウリザクラ	<i>Padus ssiroi</i> (F.Schmidt) C.K.Schneid.	forest	forest	forest
fo24	fo24	シナノキ	<i>Tilia japonica</i> (Miq.) Simonk.	forest	forest	NF
fo25.1	fo25	タニウツギ	<i>Weigela hortensis</i> (Siebold et Zucc.) K.Koch	NF	NF	forest
fo25.2	fo25	タニウツギ	<i>Weigela hortensis</i> (Siebold et Zucc.) K.Koch	residential	NF	NF
fo26	fo26	チシマアザミ	<i>Cirsium kamschatcicum</i> Ledeb. ex DC.	forest	forest	forest
fo27	cu39	ツルアジサイ	<i>Hydrangea petiolaris</i> Siebold et Zucc.	forest	forest	forest
fo28	fo28	トチハニンジン	<i>Panax japonicus</i> (T.Nees) C.A.Mey.	forest	forest	NF
fo29	fo29	ニリンソウ	<i>Anemone flaccida</i> F.Schmidt	forest	forest	forest
fo30.1	fo30	ノブキ	<i>Adenocaulon himalaicum</i> Edgew.	forest	forest	NF
fo30.2	fo30	ノブキ	<i>Adenocaulon himalaicum</i> Edgew.	residential	NF	NF
fo31	fo31	ハエドクソウ	<i>Phyma leptostachya</i> L. subsp. <i>asiatica</i> (H.Hara) Kitam.	forest	forest	forest
fo32	fo32	フッキソウ	<i>Pachysandra terminalis</i> Siebold et Zucc.	forest	forest	NF
fo33	fo33	ボウチャクソウ	<i>Disporum sessile</i> D.Don ex Schult. et Schult.f.	forest	forest	forest
fo34	fo34	ミゾホオズキ	<i>Mimulus nepalensis</i> Benth.	forest	forest	forest
fo35	fo35	ミヤマイボタ	<i>Ligustrum tschonoskii</i> Decne.	forest	forest	NF

Continued

fo36	fo36	ヤブニンジン	<i>Osmorhiza aristata</i> (Thunb.) Rydb.		forest	forest
fo37	fo37	ヤブハギ	<i>Hyloidesmum podocarpum</i> (DC.) H. Ohashi & R. R. Mill subsp. <i>oxyphyllum</i> (DC.) H. Ohashi & R. R. Mill var. <i>mandshuicum</i> (Maxim.) H. Ohashi & R. R. Mill		forest	forest
fo38	fo38	ユキザサ	<i>Maianthemum japonicum</i> (A. Gray) La Frankie		forest	forest
fo39	fo39	ヨブスマンソウ	<i>Parasenecio hastatus</i> (L.) H. Koyama subsp. <i>orientalis</i> (Kitam.) H. Koyama		forest	forest
fo40	fo40	イヌエンジュ	<i>Maackia amurensis</i> Rupr. et Maxim.		NF	grassland
gr01.1	gr01	アカクローバ	<i>Trifolium pratense</i> L.		forest	NF
gr01.2	gr01	アカクローバ	<i>Trifolium pratense</i> L.		grassland	grassland
gr01.3	gr01	アカクローバ	<i>Trifolium pratense</i> L.		residential	NF
gr02	gr02	アキカラマツ	<i>Thalictrum minus</i> L. var. <i>hypoleucum</i> (Siebold et Zucc.) Miq.		residential	NF
gr03	fo06	エゾトリカブト	<i>Aconitum sachalinense</i> F. Schmidt subsp. <i>yezoense</i> (Nakai) Kadota		NF	grassland
gr04	gr04	キイチゴssp	<i>Rubus</i> spp.		NF	grassland
gr05	gr05	クサフジ	<i>Vicia cracca</i> L.		residential	grassland
gr06	gr06	コンフリ	<i>Symphytum</i> x <i>uplandicum</i> Nyman		residential	grassland
gr07	gr07	シナガワハギ	<i>Melilotus officinalis</i> (L.) Pall. subsp. <i>suaveolens</i> (Ledeb.) H. Ohashi		NF	grassland
gr08.1	gr08	シロクローバ	<i>Trifolium repens</i> L.		grassland	grassland
gr08.2	gr08	シロクローバ	<i>Trifolium repens</i> L.		residential	NF
gr09	gr09	シロバナシナガワハギ	<i>Melilotus officinalis</i> (L.) Pall. subsp. <i>albus</i> (Medik.) H. Ohashi et Tateishi		NF	grassland
gr10	gr10	セイヨウノコギリソウ	<i>Achillea millefolium</i> L.		NF	grassland
gr11	gr11	ツユクサ	<i>Commelina communis</i> L.		residential	NF
gr12	gr12	ネジバナ	<i>Spiranthes sinensis</i> (Pers.) Ames var. <i>amoena</i> (M.Bieb.) H. Hara		grassland	NF
gr13.1	gr13	ノラニンジン	<i>Daucus carota</i> L. subsp. <i>carota</i>		grassland	grassland
gr13.2	gr13	ノラニンジン	<i>Daucus carota</i> L. subsp. <i>carota</i>		residential	NF
gr14	gr14	ハルザキヤマガラシ	<i>Barbarea vulgaris</i> R. Br.		NF	grassland
gr22	gr22	ユウゼンギク	<i>Symphiotrichum novi-belgii</i> (L.) G.L.Nesom		residential	NF

*1 米倉浩司・梶田忠 (2003-) BG Plants 和名ー学名インデックス

*2 Habitat × SpID: IDs of each taxonomic group in each habitat

*3 PollID: IDs of taxonomic groups for the analysis of body and load pollen

*4 Urban: Growing habitat in the urban-forest landscape

*5 Natural: Growing habitat in the natural-forest landscape

NF: Not found in the landscape

Table 0-S2: Floral traits of each taxonomic group. For *Hydrangea* spp. (cu39), *Aconitum sachalinense* subsp. *yezoense* (fo06), and *Impatiens* spp. (co17), the results of nectar and pollen volume measurements were provided separately for each species or each habitat.

PollID*1	Gform*2	Depth*3	DepthN*4	Refs*5	Corolla.6*6	Corolla.4*7	NectLFI*8	NectN*9	Sugar*10	SugarN*11	PG.FI*12	PollSize*13	PV.FI*14	PollN*15	Unit*16	FINJInf*17	FINJInfN*18	Nect.U*19	PV.U*20
co01	tree	5.37	NA	6	NA	S	S	3.60	29	33.07	7	35565.6	32.58	0.64	10	FI	NA	NA	0.64
co02	herb	3.80	NA	3	S	S	S	0.00	20	NA	NA	NA	NA	NA	Inf	294.0	2	0.00	NA
co03	vine	NA	NA	NA	Open	Open	Open	0.00	15	NA	NA	482882.9	15.14	0.88	11	FI	NA	NA	0.88
co04	shrub	NA	NA	NA	Open	Open	Open	0.25	21	34.38	8	4034.3	25.25	0.03	12	FI	23.1	10	0.25
co06	tree	NA	NA	NA	Open	Open	Open	0.27	36	50.00	3	89446.0	21.22	0.45	12	Inf	48.4	9	13.16
co07	tree	NA	NA	NA	Open	Open	Open	1.03	16	26.75	8	119221.5	15.26	0.22	11	Inf	446.3	15	458.75
co08	tree	NA	NA	NA	Open	Open	Open	4.97	32	26.77	28	29206.5	24.83	0.23	11	Inf	7.4	63	36.67
co09	vine	NA	NA	NA	Open	Open	Open	0.00	20	NA	NA	11155.7	18.47	0.04	12	Inf	106.3	11	0.00
co10	herb	4-8	NA	1	M	S	S	0.42	19	32.71	7	21091.6	18.77	0.07	11	FI	142.1	10	0.42
co11	herb	NA	NA	NA	L	M	M	NA	NA	NA	NA	NA	NA	NA	FI	11.0	10	NA	NA
co12	herb	NA	NA	NA	S	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co13	herb	NA	NA	NA	S	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co14	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co15	herb	14.96	10	NA	L	M	M	2.13	26	43.20	15	11686.2	25.25	0.10	11	FI	NA	NA	0.10
co16	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co17(co05.1.2)	herb	35-40	NA	1	LL	L	L	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
co17(co17.1.2)	herb	27.80	20	NA	LL	L	L	0.99	57	42.50	7	235240.7	19.25	0.88	11	FI	NA	NA	0.88
co18	herb	8.66	12	NA	M	S	S	1.10	39	28.89	23	14329.0	25.08	0.12	12	FI	NA	NA	0.12
co19	vine	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co20	herb	3.50	NA	3	S	S	S	0.04	21	NA	NA	4144.0	19.33	0.02	11	Inf	1953.8	11	79.37
co21	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
co22	herb	2.80	NA	3	S	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co23	herb	4.80	NA	3	S	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co24	herb	2.60	NA	3	S	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
co25	herb	NA	NA	NA	Open	Open	Open	2.04	32	29.69	18	16677.8	55.25	1.47	11	FI	90.9	49	2.04
co26	herb	NA	NA	NA	NA	NA	NA	0.00	26	NA	NA	37817.8	26.87	0.38	11	FI	157.6	50	0.00
cu01	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
cu02	herb	NA	NA	NA	L	M	M	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
cu04	tree	NA	NA	NA	L	M	M	NA	NA	NA	NA	28052.0	79.30	7.32	2	FI	NA	NA	7.32
cu05	shrub	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
cu06	shrub	7-9	NA	1	M	S	S	0.58	70	48.46	13	14127.3	26.58	0.14	11	FI	39.0	19	0.58

Continued

cu07	herb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu08	tree	NA	NA	NA	Open	Open	5.39	5	2920	5	NA	NA	NA	FI	NA	NA	NA	5.39	NA
cu09	tree	NA	NA	NA	Open	Open	0.48	30	38.00	8	80752.0	34.12	1.68	12	FI	NA	NA	0.48	1.68
cu10	herb	7-8	NA	1	M	S	0.15	29	25.00	1	60539.9	15.22	0.11	10	Inf	330.8	12	48.58	36.94
cu11	tree	4-5	NA	1	S	S	2.19	28	17.32	22	112844.7	28.87	1.42	12	FI	NA	NA	2.19	1.42
cu12	shrub	NA	NA	NA	NA	NA	0.17	15	50.00	5	NA	NA	NA	FI	NA	NA	NA	0.17	NA
cu13	herb	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu14	herb	NA	NA	NA	S	S	0.09	45	NA	NA	78.54	26.01	0.01	5	Inf	636.0	24	59.23	4.60
cu16	herb	NA	NA	NA	Relaxed	L	30.00	50	47.47	38	18900.0	115.00	15.04	10	FI	NA	NA	30.00	15.04
cu17	herb	NA	NA	NA	S	S	0.12	29	33.00	1	193.7	28.80	0.00	12	FI	NA	NA	0.12	0.00
cu18	herb	NA	NA	NA	Relaxed	L	6.02	40	26.33	20	53032.0	38.05	1.53	8	FI	21.6	50	6.02	1.53
cu19	herb	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu20	herb	NA	NA	NA	L	M	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu21	vine	NA	NA	NA	Relaxed	L	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu23	tree	NA	NA	NA	Open	Open	0.74	26	45.25	12	22817.1	11.20	0.02	12	Inf	424.2	12	314.15	7.12
cu24	herb	NA	NA	NA	Open	Open	0.02	23	NA	NA	5157.6	65.90	0.77	10	FI	NA	NA	0.02	0.77
cu25	herb	NA	NA	NA	S	S	0.15	73	36.30	10	37042.4	23.87	0.26	10	Inf	96.0	33	14.67	25.32
cu26	herb	40-50	NA	1	L	M	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu27	herb	NA	NA	NA	S	S	0.03	21	NA	NA	NA	NA	NA	Inf	38.2	14	1.19	NA	NA
cu28	tree	6.32	8	NA	M	S	5.66	31	18.96	26	23849.0	31.00	0.37	4	FI	NA	NA	5.66	0.37
cu29	shrub	NA	NA	NA	M	S	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu30	herb	NA	NA	NA	Relaxed	L	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu31	herb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu32	herb	NA	NA	NA	NA	NA	0.62	10	21.00	4	NA	NA	NA	FI	NA	NA	NA	0.62	NA
cu33	tree	NA	NA	NA	Relaxed	L	36.66	7	35.79	7	1092.0	32.36	0.02	2	FI	NA	NA	36.66	0.02
cu34	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu35	herb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu36	shrub	NA	NA	NA	Open	Open	3.04	2	33.50	2	NA	NA	NA	FI	NA	NA	NA	3.04	NA
cu37	herb	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu39 (fo04)	shrub	NA	NA	NA	Open	Open	0.00	20	NA	NA	82145.0	12.54	0.08	12	Inf	309.0	21	0.00	26.18
cu39 (fo27)	vine	NA	NA	NA	Open	Open	0.20	54	17.30	10	350403.5	12.65	0.37	11	Inf	509.8	12	102.92	189.00
cu39	shrub	NA	NA	NA	Open	Open	0.00	15	NA	NA	127411.2	12.58	0.13	10	Inf	387.2	20	0.00	51.39
cu40	herb	20.83	10	NA	LL	L	77.67	10	45.44	9	854712.7	18.93	3.04	11	FI	NA	NA	77.67	3.04
cu41	herb	11.17	10	NA	L	M	0.07	31	50.00	3	13288.3	41.33	0.49	12	Inf	48.4	14	3.51	23.75

Continued

cu42	herb	NA	NA	NA	S	S	0.07	13	NA	NA	NA	NA	NA	NA	NA	Inf	202.7	10	13.37	NA
cu43	herb	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
cu48	shrub	NA	NA	NA	Relaxed	L	3.26	28	38.63	20	26498.5	46.83	1.42	8	FI	NA	NA	NA	3.26	1.42
cu49	shrub	7.98	12	NA	M	S	3.48	15	43.18	14	17892.0	26.92	0.18	12	FI	NA	NA	NA	3.48	0.18
cu50	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu51	tree	8.95	13	NA	M	S	0.17	22	31.38	8	NA	NA	NA	NA	FI	NA	NA	NA	0.17	NA
cu52	herb	NA	NA	NA	L	M	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu54	shrub	NA	NA	NA	L	M	0.03	6	50.00	2	NA	NA	NA	NA	FI	NA	NA	NA	0.03	NA
cu55	vine	NA	NA	NA	NA	NA	0.35	5	21.67	3	NA	NA	NA	NA	FI	NA	NA	NA	0.35	NA
cu56	shrub	NA	NA	NA	Open	Open	0.00	4	NA	NA	2610280.0	26.20	24.58	10	FI	NA	NA	NA	0.00	24.58
cu57	shrub	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu58	shrub	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu59	herb	6.50	NA	3	M	S	0.20	24	13.00	3	215033.7	27.97	2.46	10	Inf	924.2	10	181.37	2280.00	
cu60	herb	NA	NA	NA	Relaxed	L	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu61	vine	15-20	NA	1	L	M	1.29	27	29.98	21	26068.0	27.68	0.29	11	FI	NA	NA	NA	1.29	0.29
cu62	herb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu63	herb	3.83	10	NA	S	S	0.60	10	49.64	7	9161.6	32.74	0.17	5	FI	NA	NA	NA	0.60	0.17
cu64	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu65	herb	NA	NA	NA	Relaxed	L	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu66	herb	13.43	10	NA	L	M	4.41	40	39.46	24	51727.5	17.17	0.14	11	FI	35.8	60	4.41	0.14	
cu70	herb	24.90	15	NA	LL	L	5.58	3	49.33	3	NA	NA	NA	NA	FI	NA	NA	NA	5.58	NA
cu71	tree	NA	NA	NA	Open	Open	0.00	20	NA	NA	64140.8	156.73	129.00	10	FI	NA	NA	NA	0.00	129.00
cu72	herb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu73	shrub	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu74	herb	NA	NA	NA	Open	Open	0.47	34	3.00	3	104212.0	27.08	1.08	10	FI	NA	NA	NA	0.47	1.08
cu76	herb	12.12	10	NA	L	M	0.14	22	26.69	13	NA	NA	NA	NA	FI	NA	NA	NA	0.14	NA
cu77	shrub	NA	NA	NA	Relaxed	L	3.13	20	38.62	13	28982.3	39.44	0.93	12	FI	NA	NA	NA	3.13	0.93
cu78	shrub	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu79	shrub	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu80	herb	7.22	10	NA	M	S	0.99	40	24.38	12	2517.7	30.38	0.04	12	Inf	60.9	42	60.04	2.25	
cu81	herb	NA	NA	NA	L	M	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA	NA
cu82	herb	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA	NA
cu83	herb	8.98	10	NA	M	S	0.29	14	26.67	3	26253.8	14.49	0.04	11	FI	54.7	10	0.29	0.04	
cu84	tree	NA	NA	NA	NA	NA	2.12	10	19.10	10	NA	NA	NA	NA	FI	NA	NA	NA	2.12	NA

Continued

cu85	herb	NA	NA	NA	Relaxed	L	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
cu86	herb	3.80	NA	3	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
cu87	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
cu88	herb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
fo01	herb	NA	NA	NA	S	S	0.25	31	28.33	3	NA	NA	NA	FI	NA	NA	0.25	NA
fo02	tree	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo03	tree	NA	NA	NA	Open	Open	0.00	32	NA	NA	10379.4	22.94	0.07	13	Inf	57.6	21	0.00
fo05	herb	NA	NA	NA	L	M	0.99	54	47.50	2	22005.8	27.12	0.23	NA	FI	8.0	140	0.99
fo06 (forest)	herb	24.60	22	NA	LL	L	0.90	83	39.83	18	232573.6	23.98	1.68	10	FI	NA	NA	0.90
fo06 (grass)	herb	24.60	22	NA	LL	L	7.42	174	46.24	87	232573.6	23.98	1.68	10	FI	NA	NA	7.42
fo07	shrub	NA	NA	NA	Open	Open	14.06	9	45.78	9	14255.1	20.82	0.07	9	FI	NA	NA	14.06
fo08	herb	21.74	8	NA	LL	L	5.00	39	24.12	17	97020.0	32.23	1.70	10	FI	NA	NA	5.00
fo09	herb	27.55	10	NA	LL	L	84.58	22	16.53	20	670472.7	43.69	29.27	11	FI	10.7	12	84.58
fo10	tree	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	85.5	15	NA	NA
fo11	herb	NA	NA	NA	Open	Open	0.00	5	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo12	tree	NA	NA	NA	Open	Open	2.23	28	42.10	10	13120.8	32.52	0.24	10	Inf	15.3	14	34.05
fo13	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
fo14	herb	13.03	10	NA	L	M	0.50	36	42.50	2	95242.0	23.07	0.61	10	FI	28.0	43	0.50
fo15	herb	NA	NA	NA	Relaxed	L	10.33	20	36.42	19	52192.0	42.47	2.09	9	FI	NA	NA	10.33
fo16	tree	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo17	herb	25-30	NA	1	LL	L	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
fo18	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo19	shrub	NA	NA	NA	Open	Open	1.15	8	20.75	8	18483.5	21.70	0.10	8	FI	NA	NA	1.15
fo20	herb	NA	NA	NA	S	S	0.03	35	NA	NA	28515.7	17.55	0.08	12	Inf	29.2	12	1.00
fo21	herb	22.03	10	NA	LL	L	2.00	9	18.33	6	NA	NA	NA	FI	12.5	10	2.00	NA
fo22	herb	NA	NA	NA	Open	Open	0.00	7	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo23	tree	NA	NA	NA	Open	Open	1.64	72	26.86	7	18439.3	25.03	0.15	11	Inf	58.6	13	95.98
fo24	tree	NA	NA	NA	Open	Open	4.00	39	42.29	24	9766.9	30.03	0.14	11	Inf	11.8	26	47.25
fo25	shrub	25-30	NA	1	LL	L	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
fo26	herb	6.23	10	NA	M	S	0.32	53	41.50	2	4080.4	39.18	0.13	11	Inf	201.0	22	63.87
fo28	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
fo29	herb	NA	NA	NA	Open	Open	NA	NA	NA	NA	22624.0	25.00	0.18	1	FI	NA	NA	0.18
fo30	herb	NA	NA	NA	S	S	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo31	herb	NA	NA	NA	S	S	0.00	12	NA	NA	NA	NA	NA	FI	NA	NA	0.00	NA

Continued

fo32	shrub	2.98	10	NA	S	S	S	0.07	10	50.00	1	12790.0	32.46	0.23	12	Inf	18.0	50	1.27	4.11
fo33	herb	26.05	10	NA	LL	L	L	6.66	58	2935	20	113041.1	31.11	1.78	11	FI	NA	NA	6.66	1.78
fo34	herb	18.04	10	NA	L	M	M	0.34	12	5.83	6	28995.6	24.53	0.22	9	FI	NA	NA	0.34	0.22
fo35	shrub	6-7	NA	1	M	S	S	0.03	10	NA	NA	14246.4	24.19	0.11	10	FI	12.6	22	0.03	0.11
fo36	herb	NA	NA	NA	S	S	S	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo37	herb	3-4	NA	2	S	S	S	0.05	10	NA	NA	1767.5	24.16	0.01	8	FI	NA	NA	0.05	0.01
fo38	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
fo39	herb	6.08	10	NA	L	M	M	0.40	108	15.92	13	4723.6	26.15	0.04	10	Inf	10.8	65	4.33	0.47
fo40	tree	10-12	NA	1	L	M	M	0.36	34	41.00	2	NA	NA	NA	NA	FI	46.0	4	0.36	NA
gr01	herb	4.81	10	NA	S	S	S	0.95	466	23.44	36	2875.0	31.76	0.05	12	Inf	89.9	232	85.01	4.33
gr02	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
gr04	shrub	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	FI	NA	NA	NA	NA
gr05	herb	10-12	NA	1	L	M	M	0.37	19	49.50	2	4906.2	25.81	0.04	9	Inf	26.5	21	9.75	1.17
gr06	herb	16.09	10	NA	L	M	M	4.00	30	37.57	21	22985.7	23.31	0.15	12	FI	NA	NA	4.00	0.15
gr07	herb	4.00	NA	3	S	S	S	0.05	10	NA	NA	1780.8	20.22	0.01	10	Inf	NA	NA	NA	NA
gr08	herb	3.11	10	NA	S	S	S	0.19	154	24.33	3	7205.3	21.79	0.04	12	Inf	61.4	70	11.50	2.39
gr09	herb	3.20	NA	3	S	S	S	0.51	9	5.44	8	3270.4	20.37	0.01	10	Inf	NA	NA	NA	NA
gr10	herb	NA	NA	NA	Open	Open	Open	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
gr11	herb	NA	NA	NA	Open	Open	Open	0.00	20	NA	NA	6000.4	40.45	0.21	10	FI	NA	NA	0.00	0.21
gr12	herb	5.00	NA	1	S	S	S	0.04	11	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
gr13	herb	NA	NA	NA	Open	Open	Open	0.00	20	NA	NA	9658.8	16.75	0.02	11	Inf	1662.4	13	0.00	39.47
gr14	herb	NA	NA	NA	S	S	S	NA	NA	NA	NA	NA	NA	NA	NA	Inf	NA	NA	NA	NA
gr22	herb	NA	NA	NA	S	S	S	0.31	81	20.56	32	5012.5	24.30	0.04	11	Inf	29.0	83	8.98	1.09

*1 PollID: IDs for pollen analyses which confirm to Table 0-S1

*2 GForm: Growth form

*3 Depth: Mean floral depth

*4 DepthN: Sample sizes for floral depth

*5 Ref: References for floral depth. 1: 佐竹義輔, 大井次三郎, 北村四郎, 亘理俊次, 富成忠夫 編: 日本の野生植物 I - III (1982) 平凡社; 2: 北村四郎, 村田源, 堀勝 著: 原色日本植物図鑑 草本編 II (1957) 保育社; 3: 長田武正 著: 原色日本帰化植物図鑑 (1976) 保育社

*6 Corolla.6: Corolla type in six categories which confirm to Table 0-1

*7 Corolla.4: Corolla type in four categories which confirm to Table 0-1

*8 Nect.FI: Maximum nectar volume (μL) in an individual flower

*9 NectN: Sample sizes for nectar volume measurements

*10 Sugar: Mean sugar concentration (%) in nectar

*11 SugarN: Sample sizes for sugar concentration measurements

*12 PG.FI: Mean number of pollen grains per individual flower

*13 PollSize: Mean pollen size of the species

*14 PV.FI: Mean pollen volume (μL) in an individual flower

*15 PollN: The number of sampled individual flowers for the measurement of the number of pollen grains. Mean number of pollen grains and mean pollen size were calculated for each individual flower as a mean of 5 trails by particle counter, and then averaged among the same species to calculate means of the species.

*16 Unit: Unit that bumble bees would regard as a single flower. Inf: Flower head or inflorescence as a single unit; FI: Individual flower as a single unit

*17 FIN.Inf: Mean number of individual flowers within an inflorescence

*18 FIN.InfN: Sample sizes for the mean number of individual flowers within an inflorescence

*19 Nect.U: Maximum nectar volume per unit of flower (maximum nectar volume per individual flower × the number of flowers within a flower head) (μL)

*20 PV.U: Mean pollen volume (μL) per unit of flower (mean pollen volume per individual flower × the number of flowers within a flower head) (μL)

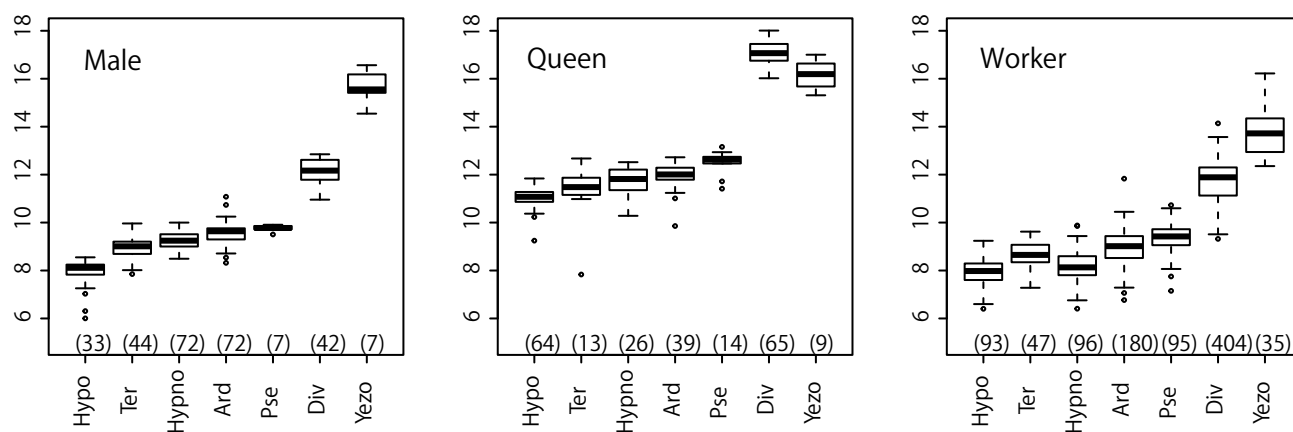


Fig. 1-S1: Tongue length of captured bumble bees.

Tongue length was calculated using the head width-tongue length allometry reported by Kinoda (2012)*.

Figures in the parentheses represent sample size. Hypo: *Bombus hypocrita*, Ter: *B. terrestris*, Hypno: *B. hypnorum*, Ard: *B. ardens*, Pse: *B. pseudobaicalensis*, Div: *B. diversus*, Yezo: *B. yezoensis*. *B. hypocrite* and *B. terrestris* are short tongued, *B. hypnorum*, *B. ardens*, and *B. pseudobaicalensis* are medium tongued, and *B. diversus* and *B. yezoensis* are long tongued species.

* Kinoda K. 2012. 北海道産マルハナバチ全種の計測比較—下唇長、頭幅、体長及び触角長. つねきばち 20: 37-52.

Chapter 1.

Flowering structure and floral preferences of bumble bees in the natural forest and human residential environments

1.1 Introduction

Pollinators having long foraging period require continuous supply of floral resources throughout the season (Goulson *et al.* 2008a). For example, limitation of available floral resources during the growth period may result in a reduced colony growth and small production of bumble bees (Williams *et al.* 2012; Persson & Smith 2013). However, distribution of floral resources highly varies in space and time under natural conditions (Alarcón *et al.* 2008; Delaney *et al.* 2015; Szigeti *et al.* 2016). In cool temperate forests, temporal distribution of floral resources varies between vegetation strata (Kawarasaki & Hori 2001; Kudo *et al.* 2008). Although trees provide abundant floral resources, flowering of trees commonly occurs from spring to summer in the temperate forests (Inoue *et al.* 1990; Abe & Kamo 2003; Inari *et al.* 2012). Under such variable environment, pollinators seasonally change foraging strata, following the flowering phenology of plant communities (Inari *et al.* 2012). Pollinators also change their foraging habitats in response to the seasonal variation in floral resources (Beekman & Ratnieks 2000; Ushimaru 2008). Thus, habitat choice by bumble bees is largely influenced by the spatial and temporal distribution of floral resources.

As mentioned in the General Introduction, urban green spaces are becoming more important foraging habitats for pollinators in accordance with the loss of natural habitats. Because diverse and abundant flowering species (most of them are exotic plants) are kept continuously in urban gardens, temporal distribution of floral resources should be different from that of natural

vegetation (Harrison *et al.* 2015). Phenological shift of pollinators is reported in some species to match the urban floral environment (Gotlieb *et al.* 2011; Wray & Elle 2015). However, temporal variations in floral resources of urban environment have not been analyzed adequately in comparison with the flowering structure of natural plant communities.

Pollinators with high cognitive abilities are able to choose preferable floral species to maximize foraging efficiency. For example, flower abundance, display size, and the quality and quantity of floral rewards affect floral use of bumble bees (Dreisig 1995; Cnaani *et al.* 2006; Makino *et al.* 2007; Jones *et al.* 2015). Floral shape is also an important factor for forage decision made by pollinators (Dafni & Kevan 1997; Balamurali *et al.* 2015). Thus, differences in floral abundance, rewards, and shape within the communities may affect the habitat choice of pollinators between gardens and natural vegetation.

In this chapter, I evaluated the spatial and temporal distributions of floral resources in two landscapes; one includes urban private gardens (hereafter “urban-forest landscape”, consisting of natural forest, semi-natural grassland and residential habitats) and the other without gardens (hereafter “natural-forest landscape”, consisting of natural forest and semi-natural grassland habitats). Then, I clarified the floral preference of bumble bees, which are important pollinators in the cool temperate ecosystems (Heinrich 1976a).

1.2 Analysis

Land cover composition

Land cover composition within the 2 km radius from the center of two most apart observation locations in each landscape was calculated based on the vegetation polygon data. QGIS software was used to calculate the area (m²) of each vegetation polygons. Land cover was re-categorized into five

categories: forest, grassland, open water area, developed land, and crop fields. Forest included native deciduous or mixed forest and exotic plantation; grassland included natural grassland, cut-over area of less than 2 m height of grasses or saplings, and grass-covered road side or vacant lots; open water area included ponds, rivers, and dams; developed land included residential area and non-vegetative land due to human activities; crop fields included rice paddies, dry fields, and meadows. The radius of 2 km was determined considering the flight distance of bumble bees (Nagamitsu *et al.* 2012) and previous studies of pollinators at a landscape level (Ahrné *et al.* 2009; Fortel *et al.* 2014).

Functional trait diversity

Season was divided into seven 4-week terms, and plant species that bloomed in each term was pooled for the analysis. Functional diversity of floral traits in each term was expressed by Rao's quadratic entropy (Rao's Q; Rao 1982), which was based on the functional distances between floral species, weighted by the estimated floral abundances. Rao's Q is calculated as

$$\text{Rao's } Q = \sum_{i=1}^{S-1} \sum_{j=i+1}^S d_{ij} p_i p_j$$

in which d_{ij} is a distance between floral species i and j , p_i is the relative abundance of flower i , and S is the total number of plant species. Distance between species was determined by Gower's dissimilarity (Gower 1971) using measured five functional traits: floral morphology (six categories), growth form (four categories), nectar and pollen volume per flower unit (five and four ordered classes, respectively), and sugar concentration of nectar (%) (for details about the classification, see Materials and Methods). To estimate the relative abundance of each floral species in each term, I assigned the following value for each abundance class; species classified as "large" was assumed to have 10,000 flowers at the peak period (Inari *et al.* 2012), "medium" to have 100, and "small" to have 10. Weekly abundance of each floral species was calculated by multiplying the values of flower

abundance by flowering stage (20%, 60% or 100%), and weekly values were summed up to represent estimated abundance within term. In the calculation of Rao's Q, plant species that lacked both nectar and pollen measurements were excluded. The number of floral species available for this calculation was 62–78% of the available floral species for each site and year (Table 1-1, 1-S1).

Resource space

To visualize floral resource space, PCoA axes values were calculated based on the distances between floral species (Gower's dissimilarity). Since negative eigenvalues are imaginary axes that cannot be represented in a Euclidean space, correction method described by (Cailliez 1983) was adopted for negative values. Floral resource space was visualized by the first and second axes.

Seasonal change in the floral resources

To examine the difference in the number of floral species between landscapes, I performed GLMs assuming a Poisson error distribution for the response variable of the number of species at flowering in each term in each habitat. Landscape, term, quadratic term, and year were included as fixed factors. This analysis was separately carried out for the forest and grassland habitats. For the comparison between habitats, I used similar GLMs but the fixed factor of landscape was replaced with habitat, and the analysis was carried out separately for each landscape. To test the between-abundance differences in the seasonality of the number of available floral species, I separately performed similar GLMs for each habitat in each landscape. To examine the difference in the functional diversity between sites, I performed similar GLMs as the analysis of the number of floral species, but assumed a Gamma error distribution with log-link function. For the analyses of functional diversity, terms with functional diversity = 0 (i.e., blooming of only one species) were excluded.

Preference of bumble bees

The effect of floral shape and floral rewards on the preference (visitation frequency) of bumble bees were tested by GLMMs, assuming a Poisson error distribution. The number of observed foraging bumble bees on each floral species in each term in each habitat during the searching-method observation (2010–12) was used for the analyses. Because of the incomplete data set of floral traits, I conducted three types of GLMMs depending on the focal floral traits (i.e., corolla type, nectar reward, and pollen reward) as determinants of bumble bee visits. To examine the effects of corolla type (four categories, Table 0-1 in Materials and Methods), flower abundance (three ordered classes), year (three categories) and landscape were included as fixed factors. Floral species was included as a random factor, and logarithm of observation duration (hours) in each term in each habitat was included as an offset term. This analysis was restricted to 72 floral species that accompanied by the data of corolla type and that bloomed during the peak season (term 3 and 4) when the floral richness was high and bumble bee workers were most active. To examine the effects of reward levels on the preference of bumble bees, nectar or pollen volume per unit of flower was added as a fixed factor in the GLMM. Due to the lack of measurements for nectar and pollen, 46 floral species was included in the analysis of nectar effect, and 42 floral species was included in the analysis of pollen effect.

To reveal the floral traits of preferred pollen-source species, I defined major pollen-source species as those whose pollen grains were included more than 50% (in numbers) in the pollen load of individual bumble bees. The annual frequency of detection as a major pollen-source was analyzed by a zero-inflated GLMM assuming a Poisson error distribution in which flower abundance (three ordered classes), corolla type (four categories), flowering duration (week), landscape, and year were included as fixed factors, and flower species was included as a random factor. Considering the sampling bias, the number of individual bumble bees captured on a same flower species was

controlled up to five within the week.

To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used for every GLM and GLMM. All statistical analyses are performed using R version 3.1.2 (R Core Team 2014).

1.3 Results

Land cover composition

According to the vegetation survey by Biodiversity Center of Japan, the land cover within 2 km radius of the center of the observation locations in the urban-forest landscape consisted with 66.9% of forest, 4.1% of open grassland, 0.7% of open water area, 13.8% of developed land, and 14.5% of crop fields (Fig. 0-1 in Materials and Methods). The land cover of the natural-forest landscape consisted with 95.2% of forest, 2.8% of open grassland, 1.7% of the open water area, and 0.2% of developed land.

Number of available plant species

Total number of floral species available as floral resources of bumble bees was 42.0 ± 1.7 (mean $\pm$ SD), 9.7 ± 2.1 , and 72.0 ± 22.6 at the forest, grassland, and residential habitats in the urban-forest landscape, respectively, while it was 37.0 ± 2.0 and 16.3 ± 6.1 at the forest and grassland habitats in the natural-forest landscape, respectively (Table 1-1). The number of common species between landscapes was 26.0 ± 2.0 and 6.0 ± 1.7 at the forest and grassland habitats, respectively.

The number of plant species at flowering increased toward the middle of season (significant positive effect of term; within-landscape comparison: $p < 0.01$, $z = 10.50$ in the urban-forest landscape, $p < 0.01$, $z = 9.24$ in the natural-forest landscape; between-landscape comparison: $p < 0.01$,

$z = 5.80$ at the forest habitat, $p < 0.01$, $z = 4.46$ at the grassland habitat), then decreased in the late season (significant negative effect of quadratic term, within-landscape comparison: $p < 0.01$, $z = -12.93$ in the urban-forest landscape, $p < 0.01$, $z = -10.30$ in the natural-forest landscape; between-landscape comparison: $p < 0.01$, $z = -6.63$ at the forest habitat, $p < 0.01$, $z = -4.30$ at the grassland habitat; Fig. 1-1, Table 1-2). In the between-landscape comparisons, floral species in the natural-forest landscape showed steeper seasonal increase and decrease compared to the urban-forest landscape at both forest and grassland habitats (significant positive interaction between landscape and term: $p = 0.09$, $z = 1.70$ at the forest habitat, $p = 0.04$, $z = 2.09$ at the grassland habitat; significant negative interaction between landscape and quadratic term: $p = 0.04$, $z = -2.05$ at the forest habitat, $p = 0.01$, $z = -2.52$ at the grassland habitat).

In the within-landscape comparisons, grassland habitats had less floral species compared to the forest habitat ($p < 0.01$, $z = -7.02$ in the urban-forest landscape; $p < 0.01$, $z = -6.00$ in the natural-forest landscape). In contrast to the early end of the seasonal increase at the forest habitats, however, seasonal increase at the grassland habitats continued until late season (significant positive interaction between habitat and term: $p < 0.01$, $z = 5.26$ in the urban-forest landscape; $p < 0.01$, $z = 5.13$ in the natural-forest landscape; Table 1-2, Fig. 1-1). The residential habitat had different seasonality of the number of floral species from that at the forest habitat in which the increase in species number was much larger at the residential habitat (significant positive interaction between habitat and term: $p < 0.01$, $z = 3.61$). Because the number of floral species at the residential habitat was almost twice of that at the forest habitat at the peak season, and the rate of seasonal decrease was similar between the habitats (interaction between habitat and quadratic term was excluded by AIC), the number of floral species at the residential habitat was larger than that at the forest habitat in the middle to late season (Table 1-2, Fig. 1-1).

Flower abundance

When the flower abundance distribution was compared between landscapes, the forest habitat in the urban-forest and natural-forest landscapes was similar (Fig. 1-2), but at the grassland habitat seemed different between landscapes. The number of species of class “S” at the grassland habitat was larger in the natural-forest landscape compared to that in the urban-forest landscape. When the flower abundance distribution was compared between habitats in the urban-forest landscape, the residential habitat had lower proportion of class “L” species compared to the forest habitat (12% at the residential habitat, 20% at the forest habitat), although the number of class “L” species was similar (8.7 ± 3.5 at the residential habitat, 8.3 ± 1.5 at the forest habitat). When the forest and grassland habitats were compared within a landscape, the grassland habitat had relatively higher proportion of class “L” species than at the forest habitat in both landscapes (45% species was class “L” at the grassland habitat, while 20% at the forest habitat in the urban-forest landscape; 26% species was class “L” at the grassland habitat, while 16% at the forest habitat in the natural-forest landscape).

Between-abundance comparisons in the seasonality of the number of floral species revealed significant differences between abundance classes at the forest and residential habitats (Fig. 1-3). At the forest habitat, the number of large-abundance species (class “L”) was larger than the small-abundance species in the beginning of season ($p = 0.04$, $z = 2.04$ in the urban-forest landscape; $p < 0.01$, $z = 3.59$ in the natural-forest landscape; Table 1-3). However, the decrease in floral species started earlier in the large-abundance species compared to the small-abundance species (significant negative interaction between abundance class and quadratic term in the urban-forest landscape: $p < 0.01$, $z = -3.59$ in abundance “M”, $p < 0.01$, $z = -4.81$ in abundance “L”; significant negative interaction between abundance class and term in the natural-forest landscape $p = 0.04$, $z = -2.01$ in abundance “M”, $p < 0.01$, $z = -5.34$ in abundance “L”). The species of class “L” at the forest habitat were trees, which extensively bloomed from the mid-spring to early summer, and spring ephemerals

(Fig. 1-4). At the residential habitat, the number of floral species of class “L” and “M” were relatively small compared to the species with class “S” ($p = 0.07$, $z = -1.83$ in class “M”, $p = 0.06$, $z = -1.86$ in class “L”). Seasonally, the large-abundance species at the residential habitat showed smaller increase compared to the small-abundance species ($p = 0.04$, $z = -2.06$), and it was relatively stable (around three species) throughout the season (Fig. 1-3). At the grassland habitat, no difference was detected in the seasonality of the number of available flower species between abundance classes (interactions between abundance class and term effects were excluded by AIC). At the grassland and residential habitats, abundant floral species (class “L”) were distributed from early to late season, and some of them showed long flowering period up to five months (Fig. 1-4).

Floral traits

The composition of growth forms at the forest and grassland habitats was similar between landscapes (Fig. 1-5). Herbaceous species was most abundant at all habitats, with the highest relative frequency at the grassland habitat (96%). In the urban-forest landscape, relative frequency of herbaceous species was similar between the residential and forest habitats (66%).

The composition of corolla types at the forest and grassland habitats was similar between the landscapes (Fig. 1-6). Within the urban-forest landscape, the residential habitat had diverse corolla type composition compared to the grassland and forest habitats, with the existence of trumpet like flowers (class “Relaxed”). Most floral species at the grassland habitat had small flowers (class “S”), i.e., 78% in the urban-forest landscape and 57% in the natural-forest landscape. In the urban-forest landscape, the frequency of open flowers was smaller at the residential habitat (32%) than at the forest habitat (47%), indicating that tubular flowers were common at the residential habitat.

Plant species with little nectar contents ($\leq 0.1 \mu\text{L}$, class “S”) were not common throughout

the habitats. The distribution of nectar volume per flower was similar between the landscapes when the forest and grassland habitats were compared separately (Fig. 1-7a, b). In the urban-forest landscape, nectar-rich ($\geq 1.0 \mu\text{L}$, class “M” to “LL”) flowers were more abundant at the residential habitat (84%) compared to those at the forest habitat (73%). When the frequency of nectar volume class was separately calculated for each corolla type, open flowers had highest frequency of nectar-less flower species (33%), and flowers with deep corolla ($\geq 6 \text{ mm}$) had no nectar-less species; thus 91% of the tubular floral species were nectar-rich ($\geq 1.0 \mu\text{L}$, class “M” to “LL”), whereas 63% of the open floral species were nectar-rich (Fig. 1-7c). Except for *Rubus phoenicolasius*, all open and small flower species that had the largest nectar volume ($\geq 10 \mu\text{L}$, class “LL”) per unit of flower constructed flower heads.

The distribution of sugar concentration was similar between the landscapes when the forest and grassland habitats were compared separately (Fig. 1-8). In the urban-forest landscape, the number of species with high sugar concentration ($>30\%$) was larger at the residential habitat (55%) than at the forest habitat (42%). The distribution of pollen volume per flower in each habitat type was similar between the landscapes (Fig. 1-9).

Functional diversity of floral traits

The PCoA result revealed that flowers at the residential habitat occupied most of the space covered by the forest and grassland flowers in the urban-forest landscape (Fig. 1-10). Distribution of the forest flowers within the resource space was similar between the landscapes. In the GLM conducted for individual habitats to examine the differences between the landscapes, functional diversity of floral traits at the forest habitat did not differ between the landscapes even after considering seasonal variation (term effect: $p < 0.01$, $z = 7.74$; quadratic term effect: $p < 0.01$, $z = -8.94$, landscape effect was excluded by AIC; Table 1-4, Fig. 1-11). Functional diversity at the grassland habitat was more

diverse in the natural-forest landscape than in the urban-forest landscape ($p < 0.01$, $z = 3.57$); it showed unimodal transition as season progressed (term effect: $p < 0.01$, $z = 7.37$; quadratic term effect: $p < 0.01$, $z = -7.47$) and the seasonal transition was more moderate in the natural-forest landscape in comparison with the urban-forest landscape (significant interaction between landscape and term, $p < 0.01$, $z = -3.11$).

The GLM results conducted in each landscape were also summarized in Table 1-4. In the natural-forest landscape, seasonal trend of functional diversity showed unimodal transition as season progressed (term effect: $p < 0.01$, $z = 6.88$; quadratic term effect: $p < 0.01$, $z = -8.02$) and varied between years (2011: $p = 0.78$, $z = 0.28$; 2012: $p = 0.02$, $z = 2.46$). The grassland habitat in the natural-forest landscape was less diverse than the forest habitat ($p < 0.01$, $z = -5.06$) but the functional diversity increased rapidly from early to the middle of season more than that at the forest habitat (significant interaction between habitat and term, $p < 0.01$, $z = 4.57$), and the decline of functional diversity occurred later in the season than at the forest habitat (Fig. 1-11b).

Functional diversity in the urban-forest landscape also showed unimodal transition as season progressed (term effect: $p = 0.01$, $z = 2.69$; quadratic term effect: $p < 0.01$, $z = -3.30$) and differed between years (2011: $p = 0.02$, $z = 2.41$; 2012: $p < 0.01$, $z = 4.19$). The grassland habitat was less diverse ($p < 0.01$, $z = -6.48$) compared to the forest habitat but the seasonal changes in functional diversity were more apparent at the grassland habitat in comparison with the forest habitat (Fig. 1-11a; significant interaction between habitat and term: $p < 0.01$, $z = 4.50$; significant interaction between habitat and quadratic term: $p < 0.01$, $z = -3.14$). Functional diversity at the residential habitat did not differ from that of the forest habitat even after considering seasonal variations (habitat effect: $p = 0.87$, $z = -0.16$; interaction between habitat and term: $p = 0.66$, $z = -0.44$; interaction between habitat and quadratic term: $p = 0.31$, $z = 1.02$).

Functional diversity of floral traits increased with the increase in the species richness at

flowering, but it was saturated when the species richness reached around 15, which was the maximum richness at the forest habitat (Fig. 1-S1).

Effects of floral shape and floral reward level on bumble bee visits

In the GLMM including floral shape as a focal explanatory factor, visitation frequency of bumble bees was affected by flower abundance ($p < 0.01$, $z = 9.70$) and corolla type (Fig. 1-12, Table 1-5). Bumble bees preferred short- and medium-corolla flowers to open flowers ($p = 0.04$, $z = 2.00$ in short corolla; $p = 0.03$, $z = 2.15$ in medium corolla), while there was no significant difference in visitation frequency between long-corolla flowers and open flowers ($p = 0.15$, $z = 1.43$). Visitation frequency was larger in the natural-forest landscape than in the urban-forest landscape ($p = 0.01$, $z = 2.66$), and varied among years ($p < 0.01$, $z = 4.14$ in 2010; $p < 0.01$, $z = 8.98$ in 2012).

In the GLMM including nectar reward level as a focal explanatory factor, the positive effect of nectar volume was detected ($p = 0.01$, $z = 2.64$; Fig. 1-13) in addition to the significant effects of flower abundance ($p < 0.01$, $z = 9.12$), landscape ($p < 0.01$, $z = 3.03$), and year ($p < 0.01$, $z = 3.51$ in 2010; $p < 0.01$, $z = 9.41$ in 2012). Corolla type was excluded as an explanatory variable by AIC.

In the GLMM including pollen reward level as a focal explanatory factor, the significant effects of flower abundance ($p < 0.01$, $z = 9.12$), landscape ($p < 0.01$, $z = 3.03$), and year ($p < 0.01$, $z = 3.51$ in 2010; $p < 0.01$, $z = 9.41$ in 2012) was detected, but corolla type and pollen reward were excluded as explanatory variables by AIC.

Preferred pollen-source species

In the urban-forest landscape, 45 plant species out of 85–116 species (when years were pooled) were chosen as major pollen source based on the observation of 386 pollen-collecting bumble bees. In the

natural-forest landscape, 24 plant species out of 46–52 species (when years were pooled) were chosen as major pollen source based on the observation of 258 pollen-collecting bumble bees. Frequently utilized pollen-source flowers commonly had long flowering period (more than one month); they were *Hydrangea* spp., *Tradescantia* sp., *Trifolium pretense*, and *Trifolium repens* in the urban-forest landscape, and *Aconitum sachalinense* subsp. *yezoense*, *T. pretense*, *Lupinus polyphyllus*, and *Hydrangea* spp. in the natural-forest landscape (Fig. 1-14).

According to the GLMM, positive effects of flower abundance ($p < 0.01$, $z = 5.40$) and flowering duration ($p < 0.01$, $z = 8.78$) were detected on the choice of pollen source flowers by individual bumble bees. Landscape ($p < 0.01$, $z = 6.35$) and year ($p < 0.01$, $z = 5.30$) also affected the probability of being chosen as pollen source, while corolla type was excluded by AIC.

1.4 Discussion

Comparisons between habitats

Forest, grassland, and residential habitats had specific seasonality of available floral resources. The forest habitat was characterized by the flowering peak in the earlier season than other habitats, especially in the species of large flower abundance (Fig. 1-3). Major plant species tended to bloom from spring to early summer in the forest, and this trend was consistent with previous studies (Inoue *et al.* 1990; Abe & Kamo 2003). Such pattern resulted in the quick decline of floral resources in the later season at the forest habitat.

At the grassland habitat, the number of available floral species and functional diversity were small throughout the season compared to the forest and residential habitats. However, the grassland species tended to have large flower abundance for a long period (Fig. 1-3). In contrast to the forest habitat, furthermore, the number of available floral species at the grassland habitat reached at

maximum late in the season, when floral resources at the forest habitat became scarce. Considering the small area of the grassland habitat in both landscapes, however, the compensation effects of the grassland habitat on floral resources at landscape level might be limited.

At the residential habitat, the number of available floral species was especially large throughout the season (Fig. 1-1), where some flowers were retained until the onset of frost in late autumn (Fig. 1-4). Although the floral functional diversity at the residential habitat was similar to that at the forest habitat (Fig. 1-10 & 1-11), the residential habitat provided species-rich foraging environment until later season. In addition, species with large amount of flowers at the residential habitat were scattered throughout the season (Figs. 1-3 & 1-4). With these community-scale flowering phenologies, the residential habitat could be able to support bumble bees throughout the season, resulting in the expansion of their foraging periods (Harrison *et al.* 2015).

Comparisons between the urban-forest and natural-forest landscapes

Although the seasonal changes in the number of available floral species at the forest habitat slightly differed between the landscapes (Table 1-2; Fig. 1-1), seasonal changes in the functional diversity of floral traits at the forest habitats were similar between the landscapes (Fig. 1-11). In addition, the distributions of forest-flower species within a resource space were similar between the landscapes (Fig. 1-10). These similarities are mainly because of the high overlap of species composition between the landscapes (Table 1-1). On the other hand, seasonal changes in the number of floral species and functional diversity of floral traits at the grassland habitat differed between the landscapes reflecting the species-rich floral composition at the natural-forest landscape compared to that in the urban-forest landscape.

The major pollen-source species differed between the landscapes. Bumble bees highly relied on *A. sachalinense* subsp. *yezoense* (fo06) in the natural forest landscape (Fig. 1-14). This species also

grew at the forest habitat in the urban-forest landscape, but it was more abundant at the grassland habitat in the natural-forest landscape. In the urban-forest landscape, *Tradescantia* sp. (cu74), which was a medium-abundance garden species, and *Hydrangea* spp. (cu39), that were abundant both in the residential and forest habitats but available for longer period in the gardens, were frequently used as pollen source. These major pollen-source species are of large or medium flower abundance having long flowering periods. The GLMM revealed that species with large flower abundance and/or long flowering period were preferred for pollen source. Although the preference of bumble bees might be species specific for pollen source (Goulson *et al.* 2008b), each species might choose flower species that are abundantly available for a long period.

At the grassland and residential habitats, species with longer flowering period were more abundant compared to the forest habitat, and some of the species with large flower abundance bloomed throughout the season (Fig. 1-4). For these reasons, bumble bees might rely more on the open habitats (grassland and gardens) for pollen foraging, and it might result in the between-landscape difference in the preferred major pollen-source species.

Preference of bumble bees and quality of the residential habitat

Floral visits of bumble bees were consistently related to the flower abundance in all GLMMs, while the significance of corolla type was detected only when the effects of nectar and pollen rewards were not included as explanatory factors. Although it was difficult to figure out whether bumble bees preferentially visited abundant flower species or the opportunistic visitation resulted in this pattern in the present study, many studies report that flower abundance and/or display size contribute to increase pollinator visits (Klinkhamer & De Jong 1990; Dreisig 1995; Goulson *et al.* 1998). Nectar reward level and floral shape also influence the foraging pattern of pollinators (Klinkhamer & De Jong 1990; Dafni & Kevan 1997; Biernaskie *et al.* 2002; Balamurali *et al.* 2015). Also in the present

study, the species with open flowers had higher proportion of nectar-less flowers, and they received lower frequency of bumble bee visits in the corolla type model. Bumble bees seemed to avoid open flowers because of lower nectar rewards. Direct effect of nectar reward was detected in the nectar reward model but effect of corolla type was not significant in this model, indicating that nectar reward level is more important than simple floral shape for floral choice of bumble bees. Corolla type was not selected also in the pollen reward model in which the factor of pollen reward was also excluded. Because floral species that were measured for nectar or pollen volume might be biased toward attractive species (which received large visitations), the differences in the quality and/or quantity of resources might had more importance on the visitation of bumble bees than the differences in corolla types. Bumble bees are species specific for pollen source flowers (Goulson *et al.* 2008b), and able to choose pollen species of preferred nutrient contents (Ruedenauer *et al.* 2016; Vaudo *et al.* 2016). As for the pollen source, quality of pollen might be more important than the gain per unit of flower.

At the residential habitat, the proportion of nectar-rich flowers was higher than at the forest habitat (Fig. 1-7a) because species with tubular flowers containing larger amount of nectar were common here (Figs. 1-6 & 1-7c). In addition, flowers at the residential habitat tended to have higher sugar concentrations (Fig. 1-8). Floral quality, as well as quantity is an important factor for the foraging decision of pollinators (Pappers *et al.* 1999; Cnaani *et al.* 2006; Szegedi *et al.* 2016). Thus, residential habitat could be a preferred foraging habitat for bumble bees.

1.5 Conclusions

Existence of multiple habitat types within a landscape could compensate for the seasonal fluctuations of floral resources of individual habitats. However, its compensative function depends

on the scale of qualitative and quantitative floral availability of individual habitats. Although the grassland communities could phenologically compensate for the decline of floral resources of the forest communities late in the season, the amount of floral resources at the grassland habitat seemed to be insufficient for bumble bees in both landscapes of this study. In contrast, floral resources of the residential habitat fully compensated for the seasonal dynamics of floral resources of the forest community, both for qualitatively and quantitatively. Nectar-rich, diverse (species-rich) flowers at the residential habitat might be able to support bumble bees for longer period in the urban-forest landscape.

Table 1-1: The number of available plant species as floral resources of bumble bees in each landscape.

Landscape	Year	Habitat			Total (unique)
		Forest	Grassland	Residential	
Urban	2010	40	12	47	85
	2011	43	9	78	116
	2012	43	8	91	126
	Mean $\pm$ SD	42.0 $\pm$ 1.7	9.7 $\pm$ 2.1	72.0 $\pm$ 22.6	109.0 $\pm$ 21.4
Natural	2010	39	15	-	52
	2011	35	11	-	46
	2012	37	23	-	56
	Mean $\pm$ SD	37.0 $\pm$ 2.0	16.3 $\pm$ 6.1	-	51.3 $\pm$ 5.0
Common species	2010	28	5	-	35
	2011	26	5	-	35
	2012	24	8	-	37
	Mean $\pm$ SD	26.0 $\pm$ 2.0	6.0 $\pm$ 1.7	-	35.7 $\pm$ 1.2

Table 1-2: Results of GLMs for the seasonal change in the number of available floral species. Landscape (urban, natural) or habitat (forest, grassland, residential), term (seven 4-week period), quadratic term, year (2010, 2011, 2012) and the interactions between landscape or habitat and term effects were considered as explanatory variables. To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used.

	N	AIC	Estimate	Std. Error	z value	Pr(> z)
Within-landscape comparison						
Urban	63	330.2				
Intercept (Habitat = forest, Year = 2010)			0.79	0.23	3.47	<0.001
Habitat (grassland)			-2.60	0.37	-7.02	<0.001
Habitat (residential)			0.07	0.21	0.33	0.74
Term			1.11	0.11	10.50	<0.001
Quadratic term			-0.17	0.01	-12.93	<0.001
Year (2011)			0.34	0.09	3.61	<0.001
Year (2012)			0.55	0.09	6.11	<0.001
Habitat (grassland)×Term			0.45	0.08	5.26	<0.001
Habitat (residential)×Term			0.20	0.06	3.61	<0.001
Natural	42	187.8				
Intercept (Habitat = forest, Year = 2010)			0.13	0.34	0.37	0.71
Habitat (grassland)			-2.31	0.39	-6.00	<0.001
Term			1.78	0.19	9.24	<0.001
Quadratic term			-0.28	0.03	-10.30	<0.001
Year (2011)			-0.11	0.15	-0.73	0.46
Year (2012)			0.26	0.13	1.93	0.05
Habitat (grassland)×Term			0.51	0.10	5.13	<0.001
Between-landscape comparison						
Forest	42	191.4				
Intercept (Landscape = urban)			1.11	0.32	3.41	<0.001
Landscape (natural)			-0.71	0.50	-1.41	0.16
Term			1.12	0.19	5.80	<0.001
Quadratic term			-0.17	0.03	-6.63	<0.001
Landscape (natural)×Term			0.52	0.31	1.70	0.09
Landscape (natural)×Quadratic term			-0.09	0.04	-2.05	0.04
Grassland	42	169.3				
Intercept (Landscape = urban, Year = 2010)			-1.41	0.70	-2.02	0.04
Landscape (natural)			-1.22	1.01	-1.21	0.23
Term			1.50	0.34	4.46	<0.001
Quadratic term			-0.17	0.04	-4.30	<0.001
Year (2011)			-0.25	0.18	-1.35	0.18
Year (2012)			0.27	0.16	1.66	0.10
Landscape (natural)×Term			1.05	0.50	2.09	0.04
Landscape (natural)×Quadratic term			-0.15	0.06	-2.52	0.01

Table 1-3: Results of GLMs for the seasonal change in the number of available floral species in each abundance class. Abundance class (S, M, L), term (seven 4-week period), quadratic term, year (2010, 2011, 2012) and the interactions between abundance class and term effects were considered as explanatory variables. To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used.

	N	AIC	Estimate	Std. Error	z value	Pr(> z)
Urban-forest landscape						
Forest	63	201.1				
Intercept (Abundance = S)			-0.74	0.44	-1.69	0.09
Abundance (M)			0.41	0.27	1.55	0.12
Abundance (L)			0.69	0.34	2.04	0.04
Term			1.51	0.22	6.81	<0.01
Quadratic term			-0.19	0.03	-6.89	<0.01
Abundance (M) × Quadratic term			-0.06	0.02	-3.59	<0.01
Abundance (L) × Quadratic term			-0.17	0.03	-4.81	<0.01
Grassland	63	169.9				
Intercept (Abundance = S)			-2.48	0.69	-3.58	<0.01
Term			1.50	0.34	4.46	<0.01
Quadratic term			-0.17	0.04	-4.30	<0.01
Residential	63	285.2				
Intercept (Abundance = S, Year = 2010)			-0.11	0.30	-0.37	0.71
Abundance (M)			-0.49	0.27	-1.83	0.07
Abundance (L)			-0.71	0.38	-1.86	0.06
Term			1.33	0.14	9.58	<0.01
Quadratic term			-0.18	0.02	-10.32	<0.01
Year (2011)			0.56	0.13	4.27	<0.01
Year (2012)			0.90	0.12	7.29	<0.01
Abundance (M) × Term			0.07	0.06	1.07	0.28
Abundance (L) × Term			-0.21	0.10	-2.06	0.04
Natural-forest landscape						
Forest	63	190.1				
Intercept (Abundance = S)			-1.67	0.56	-2.99	<0.01
Abundance (M)			0.39	0.49	0.79	0.43
Abundance (L)			2.13	0.59	3.59	<0.01
Term			2.29	0.30	7.62	<0.01
Quadratic term			-0.32	0.04	-8.07	<0.01
Abundance (M) × Term			-0.28	0.14	-2.01	0.04
Abundance (L) × Term			-1.20	0.23	-5.34	<0.01
Grassland	63	166.7				
Intercept (Abundance = S, Year = 2010)			-3.28	0.76	-4.34	<0.01
Abundance (M)			-1.36	0.26	-5.14	<0.01
Abundance (L)			-0.78	0.21	-3.67	<0.01
Term			2.55	0.37	6.83	<0.01
Quadratic term			-0.32	0.05	-6.96	<0.01
Year (2011)			-0.38	0.27	-1.42	0.15
Year (2012)			0.56	0.21	2.62	<0.01

Table 1-4: Results of GLMs for the seasonal change in the floral functional diversity (Rao's quadratic entropy). Landscape (urban, natural) or habitat (forest, grassland, residential), term (seven 4-week period), quadratic term, year (2010, 2011, 2012) and the interactions between landscape or habitat and term effects were considered as explanatory variables. To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used.

	N	AIC	Estimate	Std. Error	z value	Pr(> z)
Within-landscape comparison						
Urban	54	-155.4				
Intercept (Habitat = forest, Year = 2010)			-3.86	0.73	-5.29	<0.01
Habitat (grassland)			-9.92	1.53	-6.48	<0.01
Habitat (residential)			-0.16	0.99	-0.16	0.87
Term			1.28	0.47	2.69	0.01
Quadratic term			-0.23	0.07	-3.30	<0.01
Year (2011)			0.56	0.23	2.41	0.02
Year (2012)			0.95	0.23	4.19	<0.01
Habitat (grassland) × Term			3.58	0.79	4.50	<0.01
Habitat (residential) × Term			-0.27	0.61	-0.44	0.66
Habitat (grassland) × Quadratic term			-0.30	0.10	-3.14	<0.01
Habitat (residential) × Quadratic term			0.08	0.08	1.02	0.31
Natural	31	-127.9				
Intercept (Habitat = forest, Year = 2010)			-4.14	0.35	-11.82	<0.01
Habitat (grassland)			-2.07	0.41	-5.06	<0.01
Term			1.48	0.22	6.88	<0.01
Quadratic term			-0.25	0.03	-8.02	<0.01
Year (2011)			0.04	0.16	0.28	0.78
Year (2012)			0.40	0.16	2.46	0.02
Habitat (grassland) × Term			0.45	0.10	4.57	<0.01
Between-landscape comparison						
Forest	34	-113				
Intercept			-3.66	0.27	-13.41	<0.01
Term			1.36	0.18	7.47	<0.01
Quadratic term			-0.23	0.03	-8.94	<0.01
Grassland	31	-119				
Intercept (Landscape = urban, Year = 2010)			-14.03	1.41	-9.97	<0.01
Landscape (natural)			3.36	0.94	3.57	<0.01
Term			4.73	0.64	7.37	<0.01
Quadratic term			-0.51	0.07	-7.47	<0.01
Year (2011)			0.88	0.36	2.45	0.02
Year (2012)			1.63	0.35	4.65	<0.01
Landscape (natural) × Term			-0.65	0.21	-3.11	<0.01

Table 1-5: Results of GLMMs for the visitation frequency to floral species in term3 and 4 (late July to middle August). To examine the effects of corolla type, flower abundance (S, M, L), corolla type (open, S, M, L), landscape (urban, natural), and year (2010, 2011, 2012) were considered as explanatory variables, floral species as a random factor, and observation duration (hours) as an offset term. To examine the effects of nectar or pollen volume, nectar (five ordered classes) or pollen (four ordered classes) volume was added as one of the explanatory variables. Classification of the explanatory variables conforms to Table 0-1 in Materials and Methods. To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used.

	N	AIC	Estimate	Std. Error	z value	Pr(> z)
Corolla-type model	166	1249.1				
Intercept (Corolla type = open, Landscape = urban, Year = 2010)			-2.56	0.23	-10.97	<0.01
Abundance			0.56	0.06	9.70	<0.01
Corolla (S)			0.49	0.24	2.00	0.05
Corolla (M)			0.67	0.31	2.2	0.03
Corolla (L)			0.42	0.30	1.43	0.15
Landscape (natural)			0.27	0.10	2.66	0.01
Year (2011)			0.45	0.11	4.14	<0.01
Year (2012)			0.89	0.10	8.98	<0.01
Nectar-volume model	131	1093.3				
Intercept (Landscape = urban, Year = 2010)			-2.80	0.35	-7.95	<0.01
Abundance			0.54	0.06	9.12	<0.01
Nectar volume			0.22	0.08	2.64	0.01
Landscape (natural)			0.32	0.11	3.03	<0.01
Year (2011)			0.40	0.11	3.51	<0.01
Year (2012)			0.95	0.10	9.41	<0.01
Pollen-volume model	124	1058.2				
Intercept (Landscape = urban, Year = 2010)			-2.02	0.20	-10.34	<0.01
Abundance			0.52	0.06	8.70	<0.01
Landscape (natural)			0.29	0.11	2.66	0.01
Year (2011)			0.38	0.11	3.35	<0.01
Year (2012)			0.92	0.10	8.78	<0.01

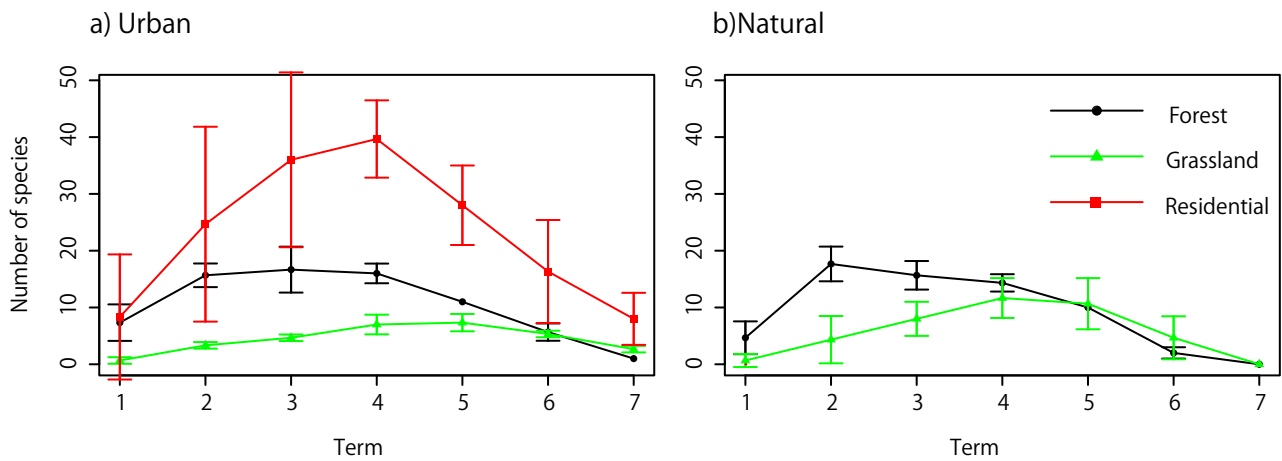


Fig. 1-1: Seasonal change in the number of available flower species in each habitat. a) urban-forest landscape, b) natural-forest landscape. Points and error bars represent means and SDs of three years (2010-2012).

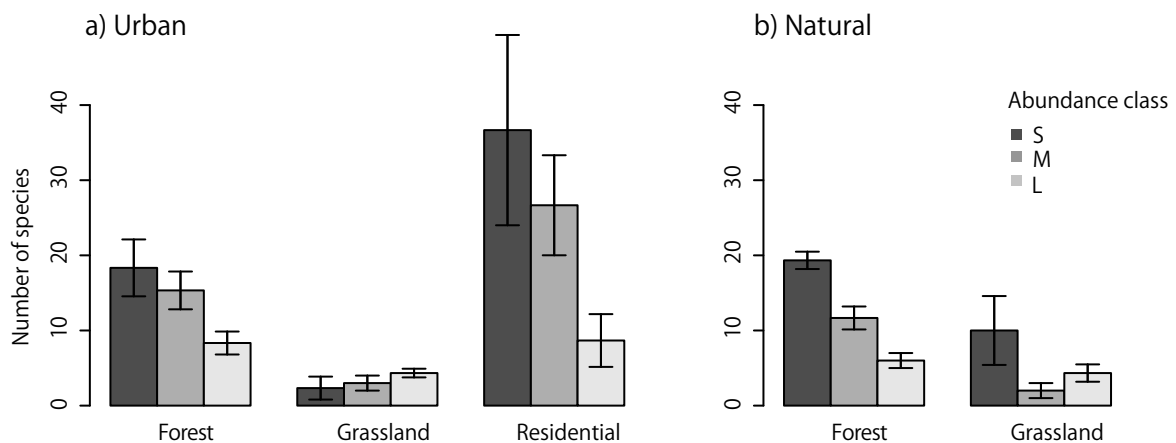


Fig. 1-2: Distribution of flower abundance in each habitat. Mean frequency of three years (2010-2012) in the urban-forest (a) and natural-forest (b) landscapes is represented by barplots with SDs (error bars). L: Flowers of large trees, common shrubs, and herbs that bloom in large carpet-like patches, M: shrubs or herbs that form many small patches, S: rare herbs with small flowering patches.

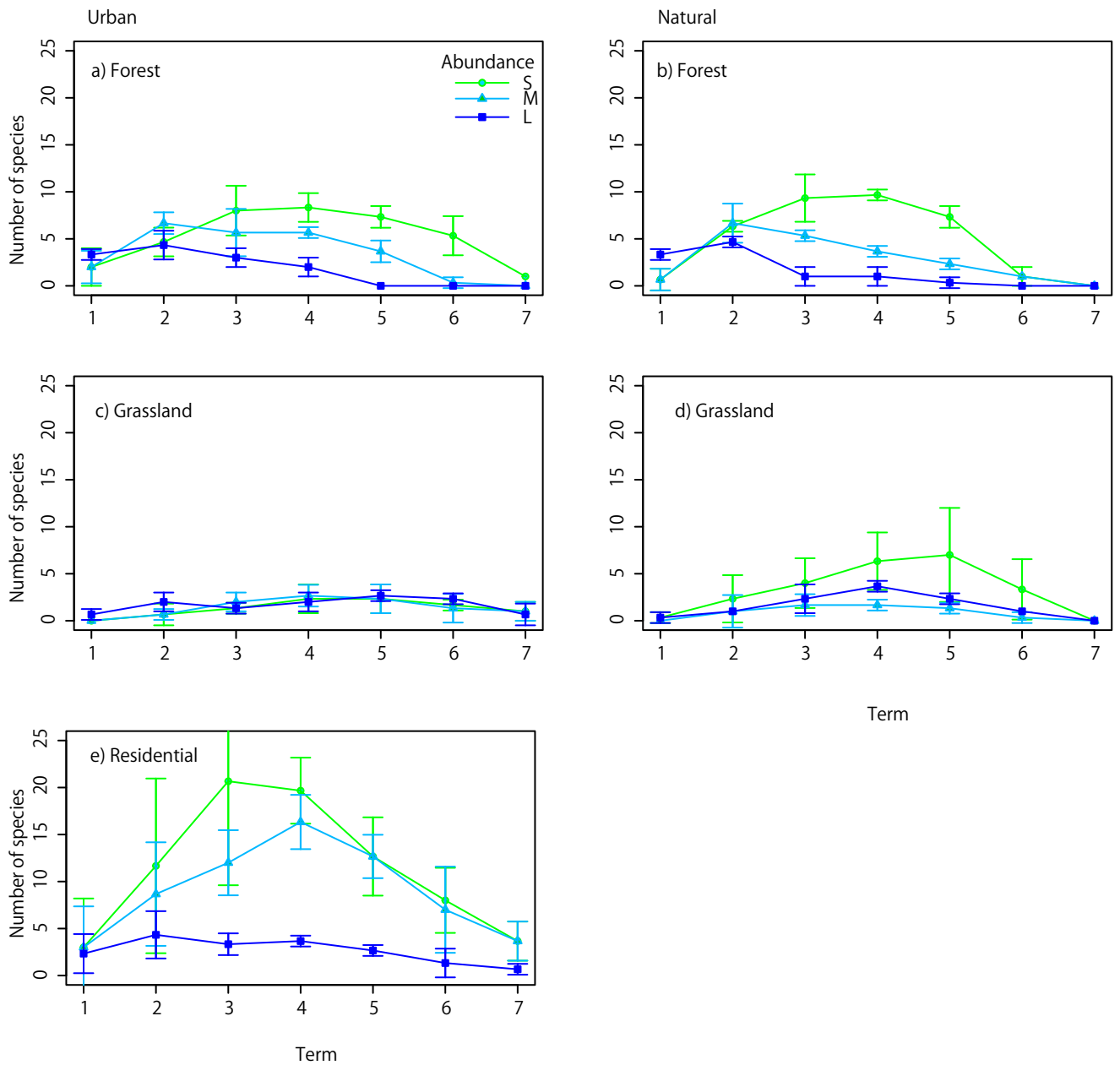


Fig. 1-3: Seasonal changes in the number of species at flowering in each flower abundance class. Forest habitat in the urban-forest (a) and natural-forest (b) landscapes, grassland habitat in the urban-forest (c) and natural-forest (d) landscapes, and residential habitat (e). Points and error bars represent means and SDs of three years (2010-2012).

a) Urban-forest landscape in 2010

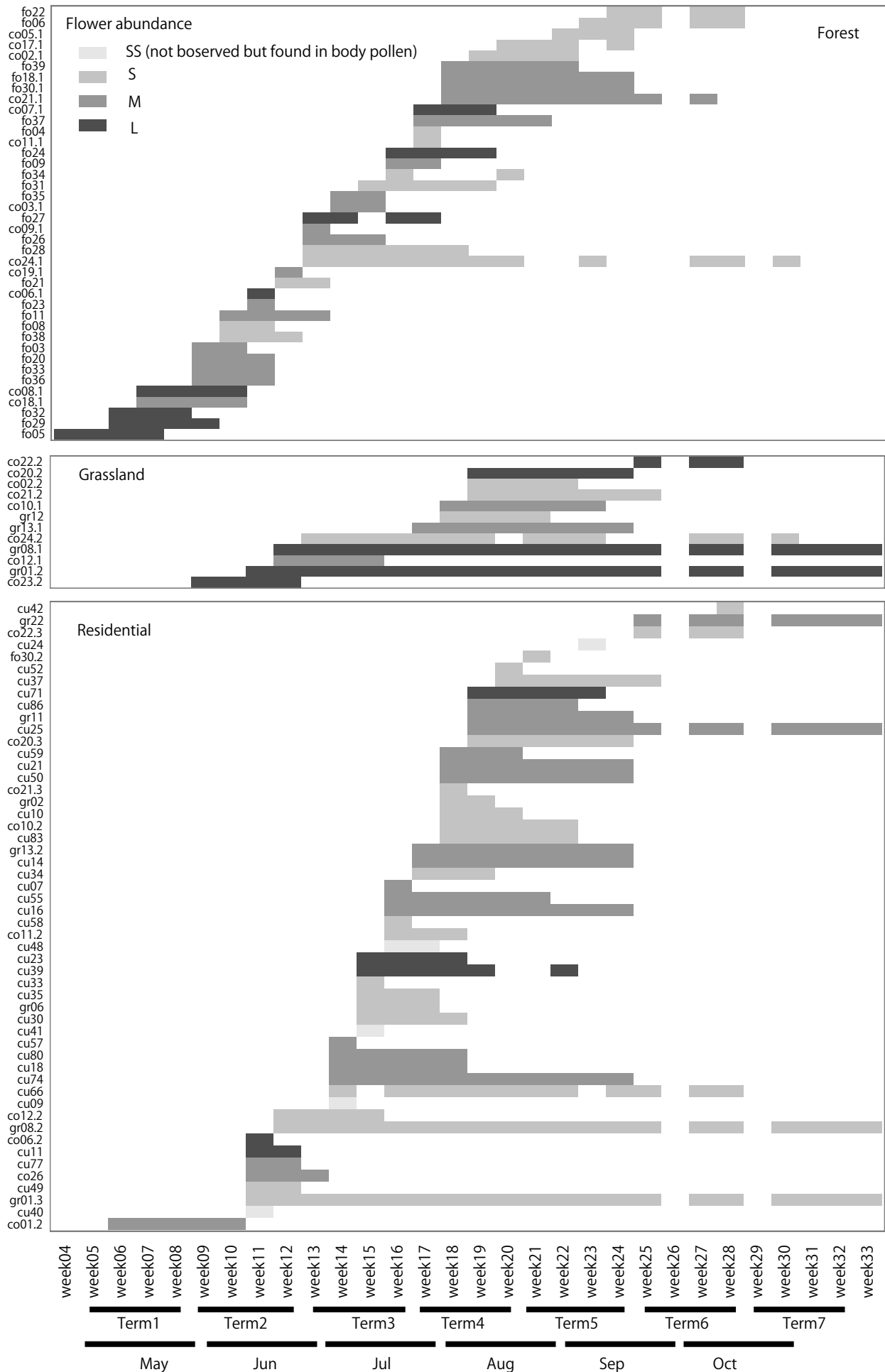
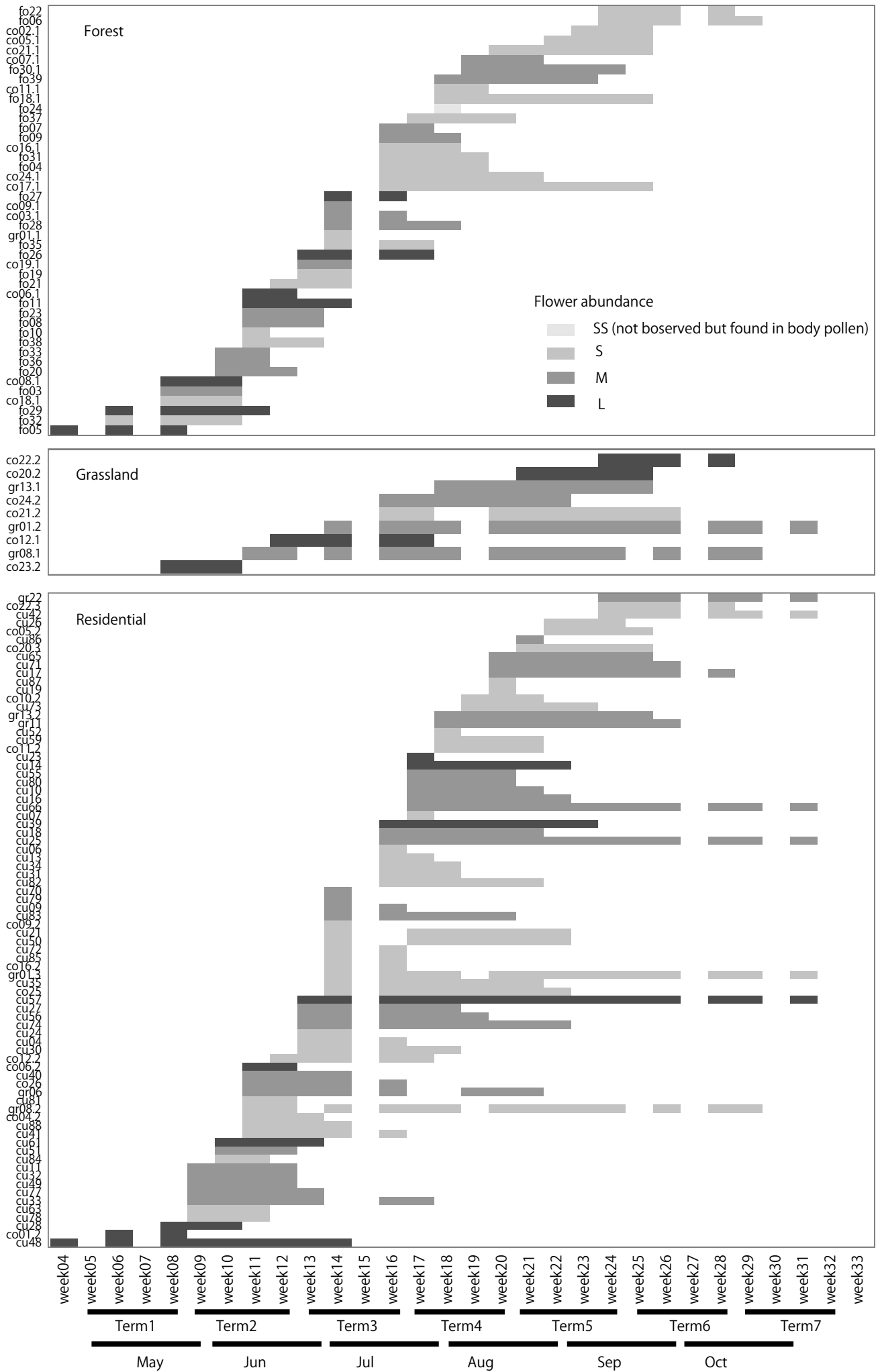
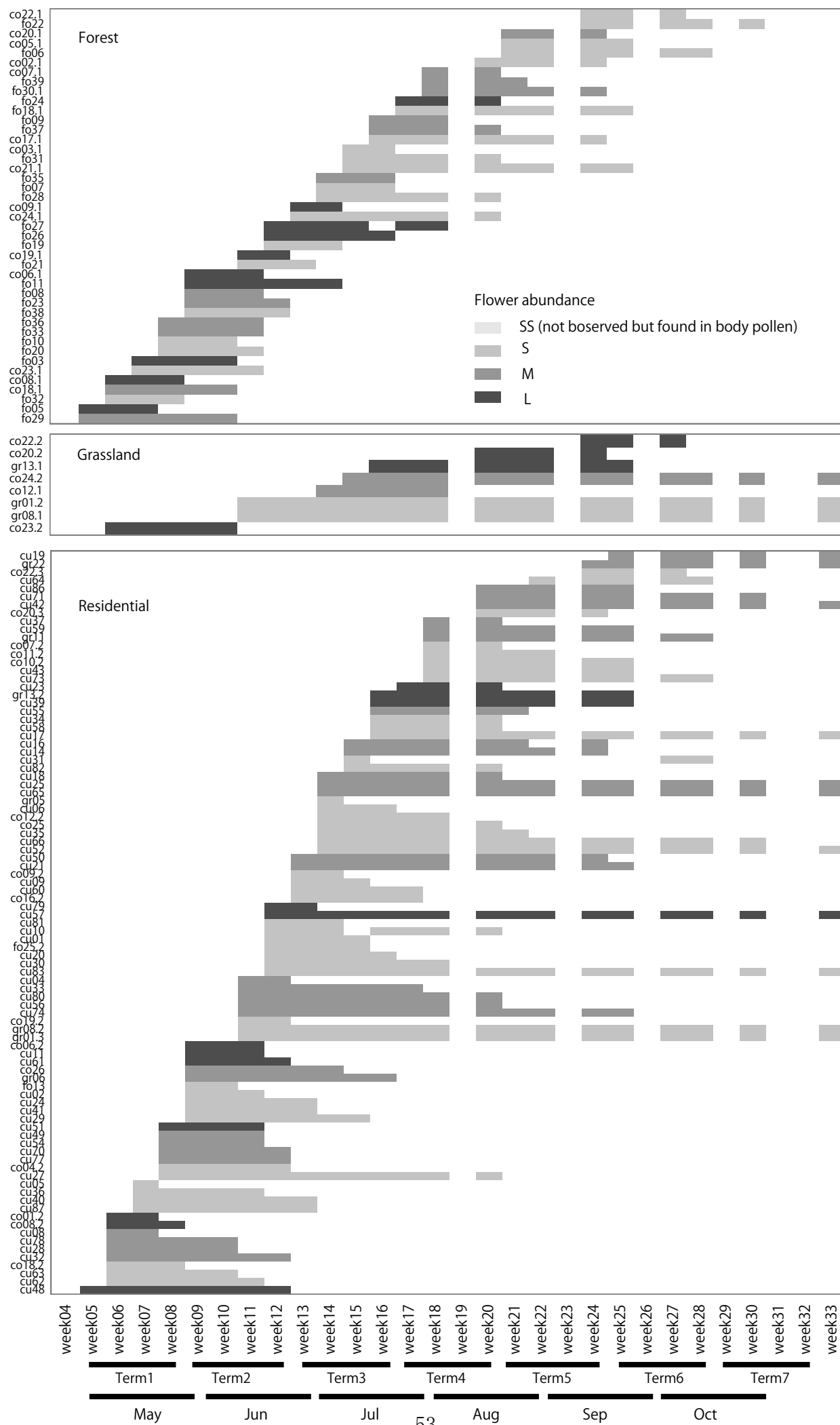


Fig. 1-4: Floral phenology in each site and year. The urban-forest landscape in a) 2010, b) 2011, c) 2012, and natural-forest landscape in d) 2010, e) 2011, f) 2012. Week numbers start from the beginning of April in each year. Flower IDs in the vertical axes correspond to that in the Table 0-S1.

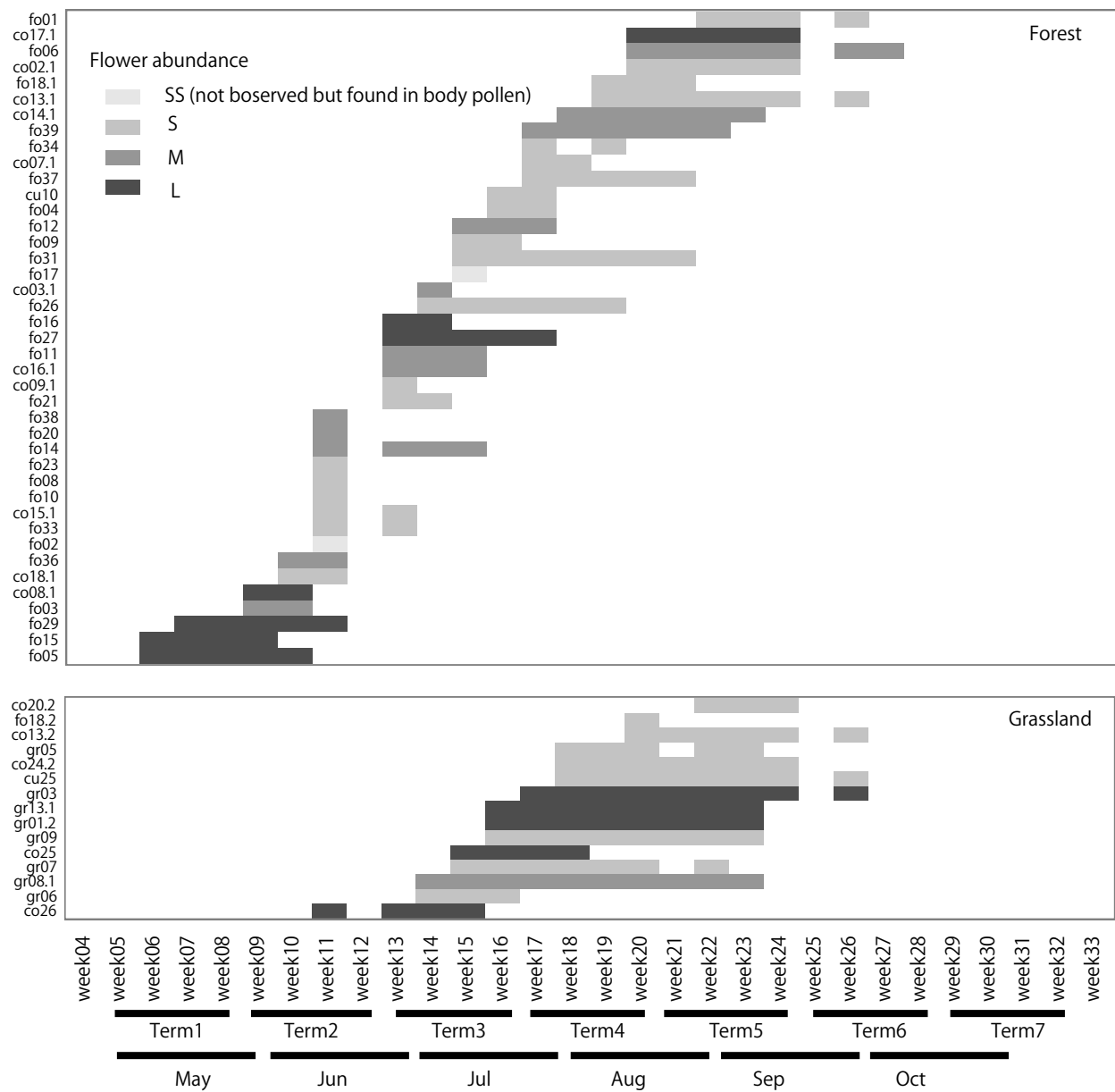
b) Urban-forest landscape in 2011



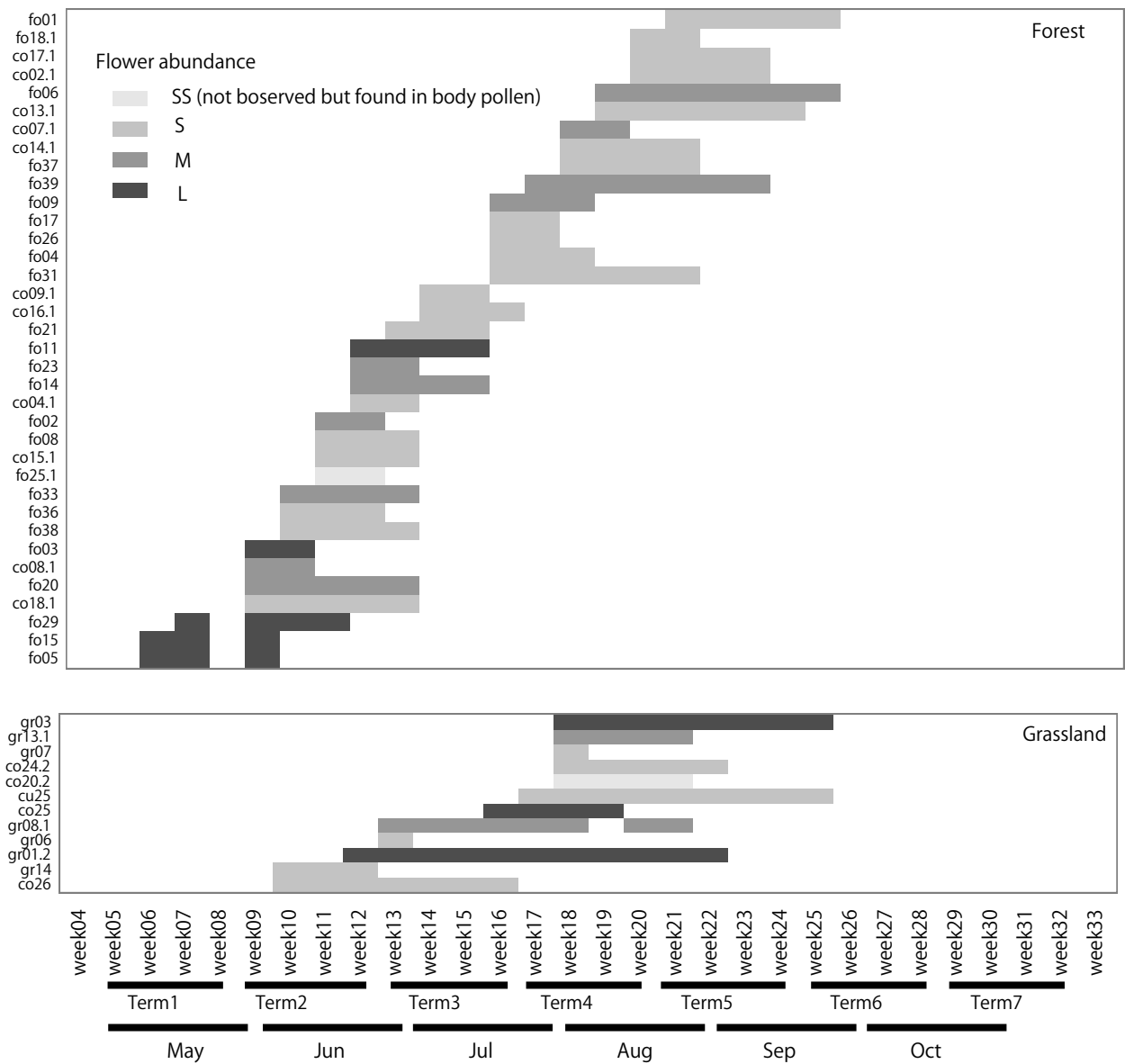
c) Urban-forest landscape in 2012



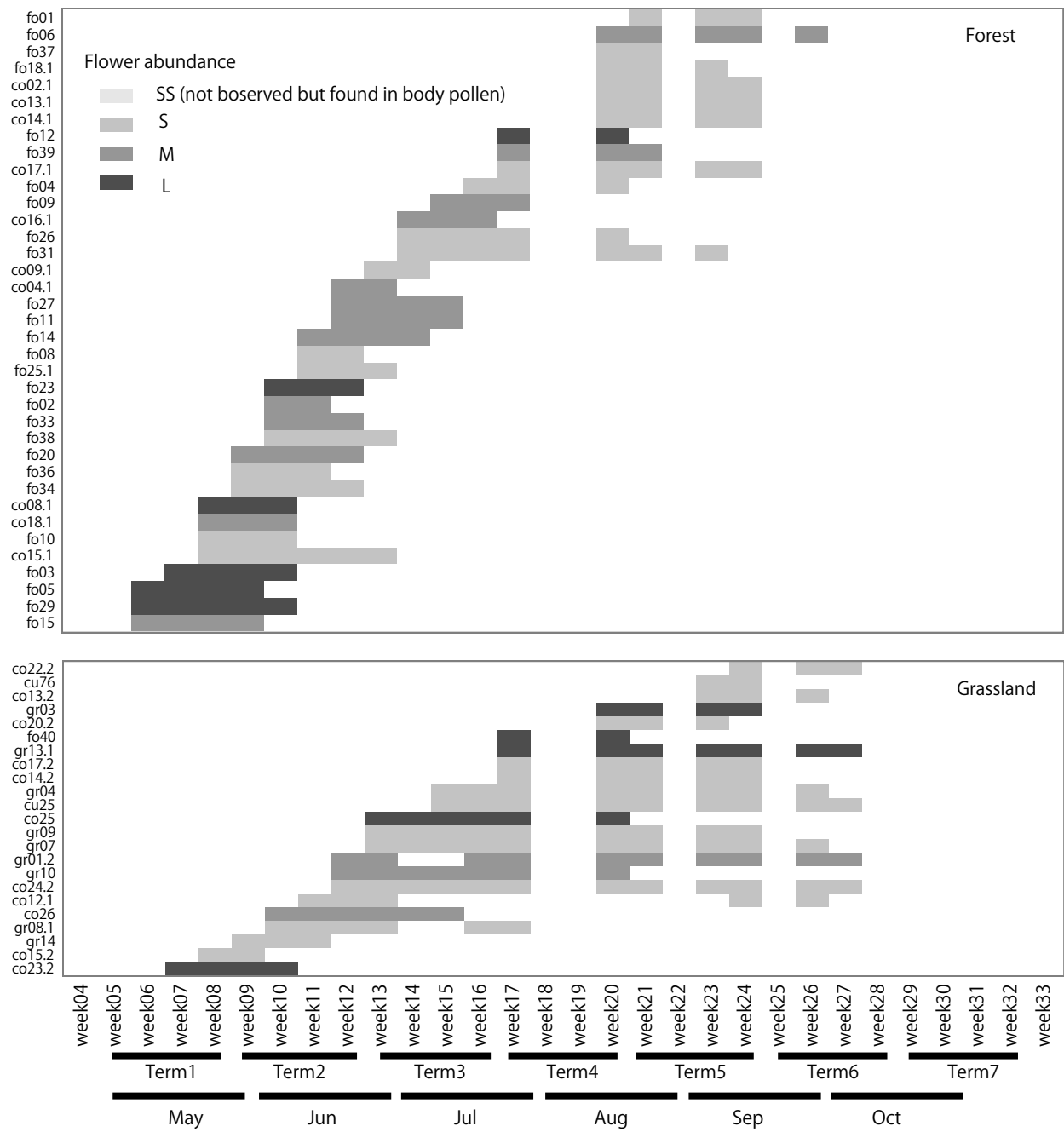
d) Natural-forest landscape in 2010



e) Natural-forest landscape in 2011



f) Natural-forest landscape in 2012



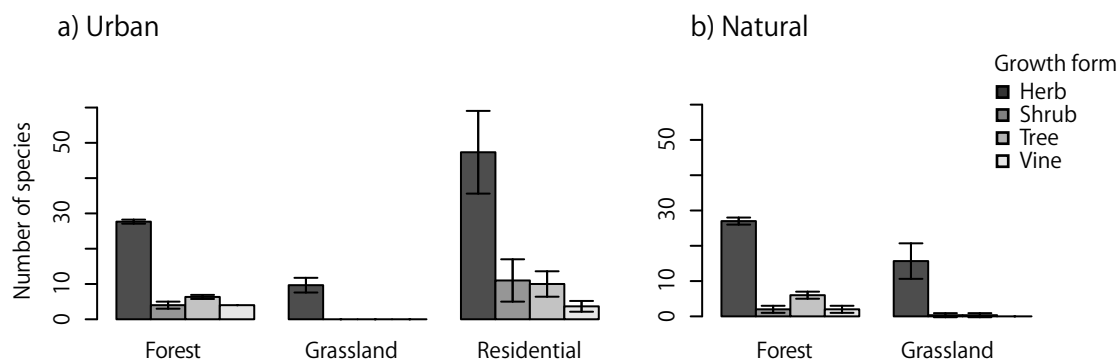


Fig. 1-5: Distribution of growth forms in each habitat. a) urban-forest landscape, b) natural-forest landscape. Bars and error bars represent means and SDs of three years (2010-2012).

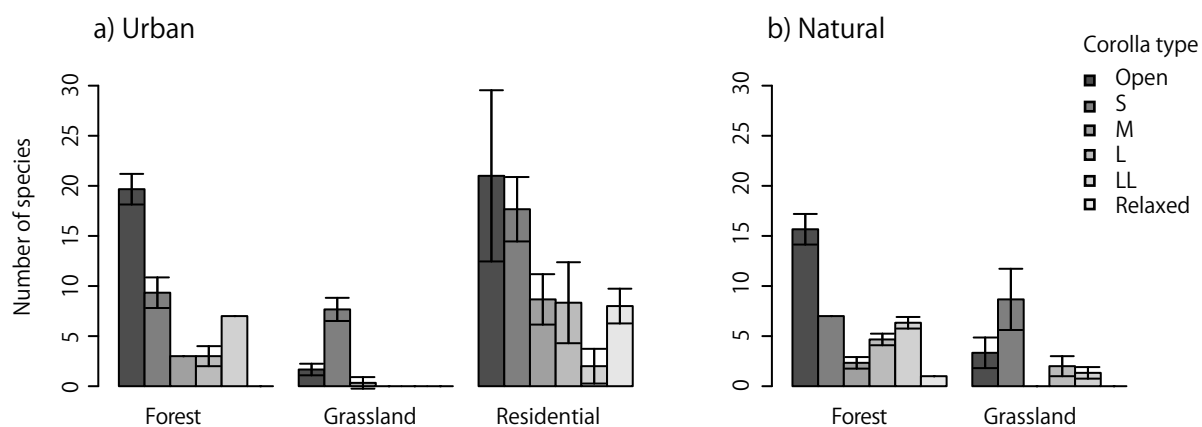


Fig. 1-6: Distribution of corolla types in each habitat. a) urban-forest landscape, b) natural-forest landscape. Classification of corolla types conforms to Table 0-1 in Materials and Methods. Bars and error bars represent means and SDs of three years (2010-2012).

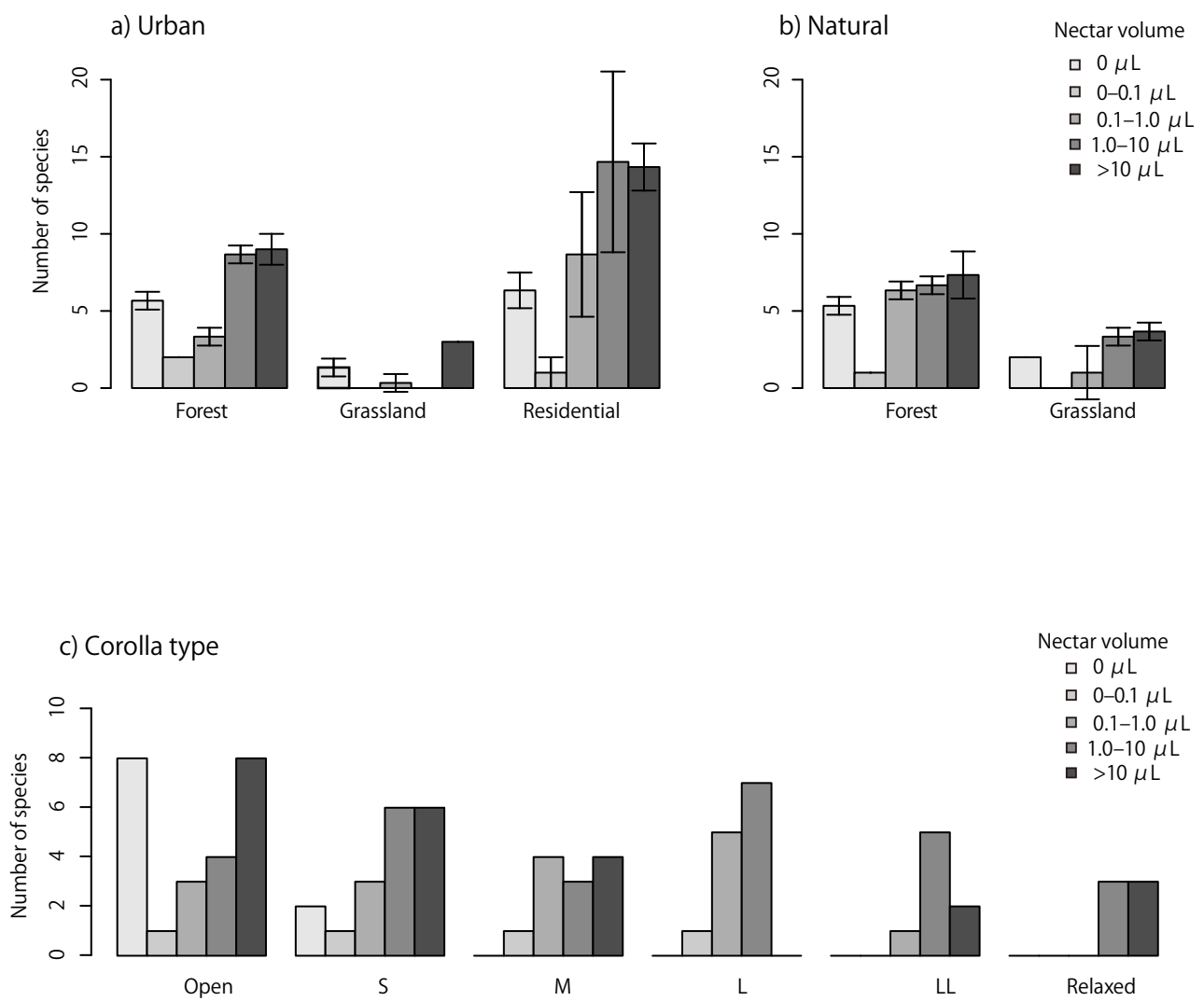


Fig. 1-7: Distribution of nectar volume in each habitat (in the urban-forest (a) and natural-forest (b) landscapes) and each corolla type (c). Classification of corolla types conforms to Table 0-1 in Materials and Methods. Bars and error bars represent means and SDs of three years (2010-2012).

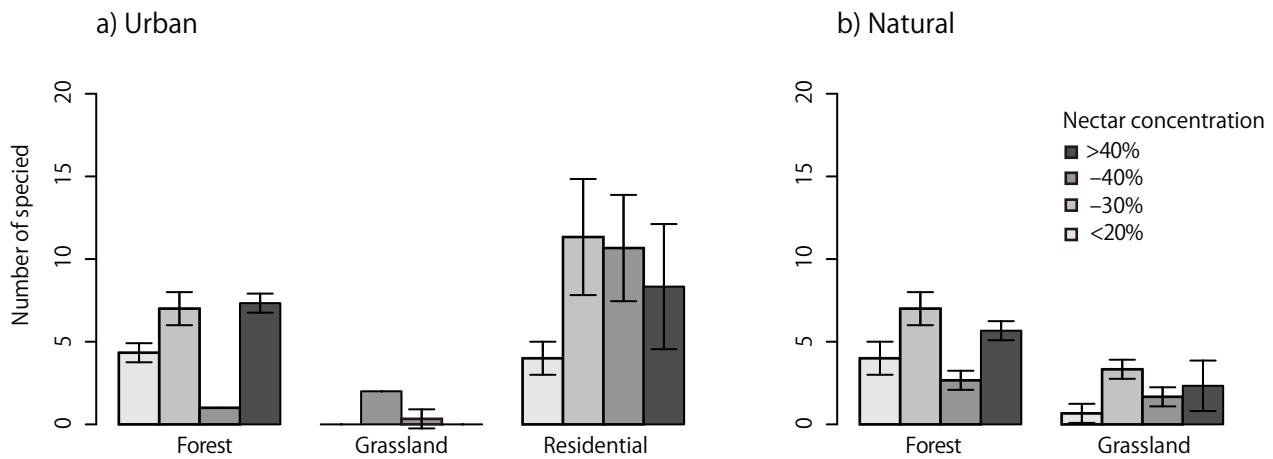


Fig. 1-8: Distribution of sugar concentration in the urban-forest (a) and natural-forest (b) landscapes. Bars and error bars represent means and SDs of three years (2010-2012).

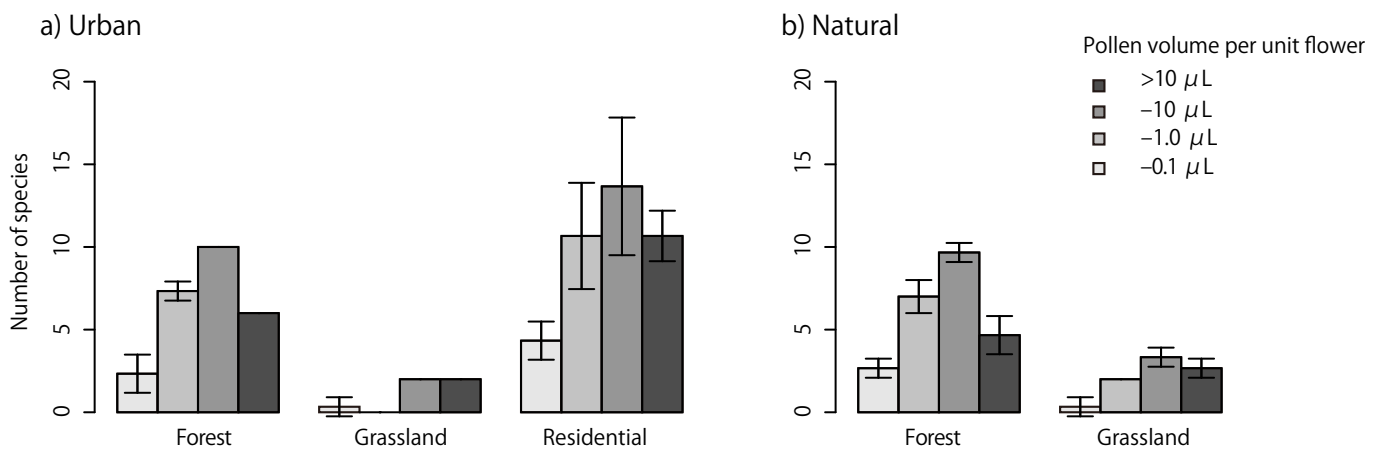


Fig. 1-9: Distribution of pollen volume in the urban-forest (a) and natural-forest (b) landscapes. Bars and error bars represent means and SDs of three years (2010-2012).

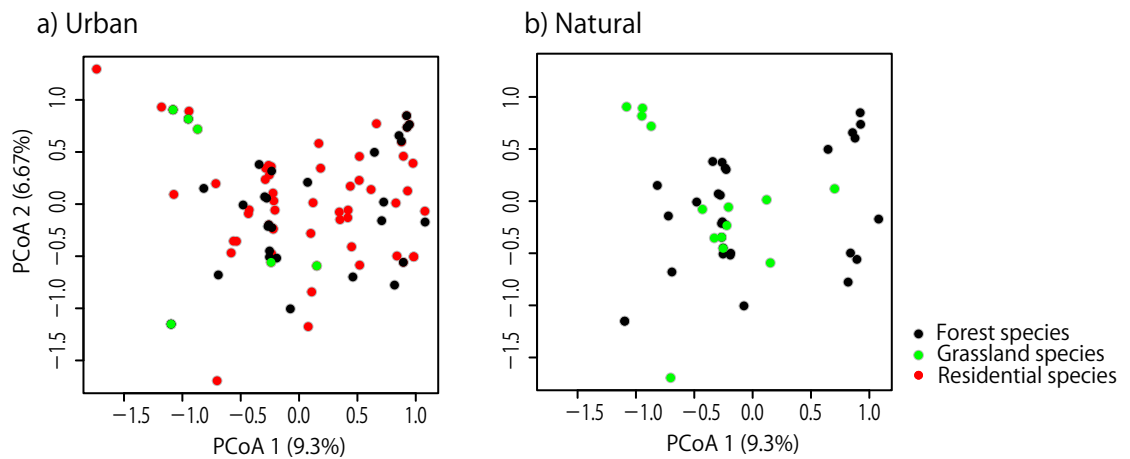


Fig. 1-10: Floral resource space in the urban-forest (a) and natural-forest (b) landscapes.

PCoA axes values are calculated using five floral traits (growth form, corolla type, nectar and pollen volume per unit flower, and sugar concentration of nectar). Flowers that lacked data of either nectar or pollen volume were excluded.

Each dot represents single species that bloomed in either of the three years (2010-12).

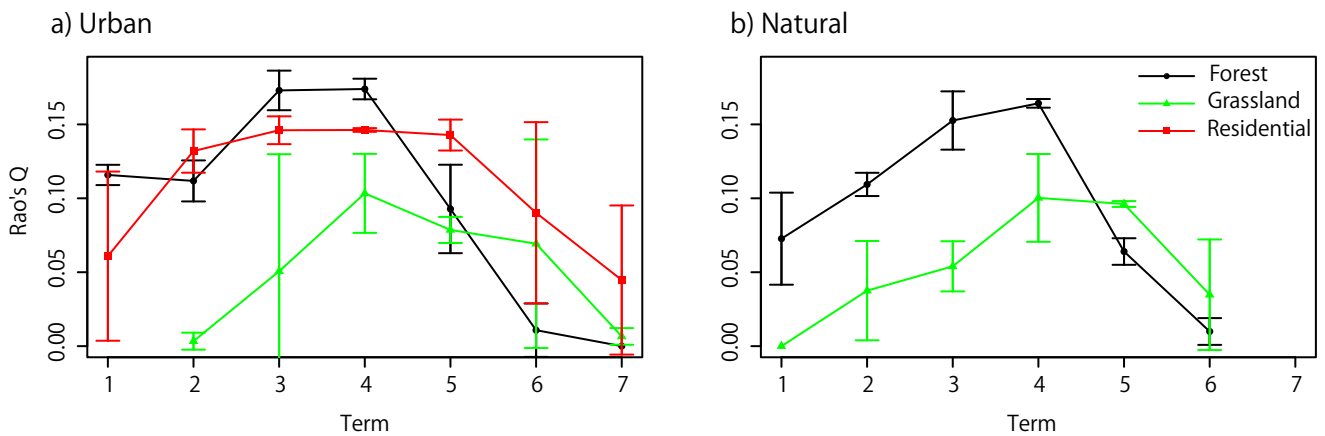


Fig. 1-11: Seasonal changes in the functional diversity of floral traits (Rao's quadratic entropy) in each habitat in the urban-forest (a) and natural-forest (b) landscapes. Points and error bars represent means and SDs of three years (2010-2012).

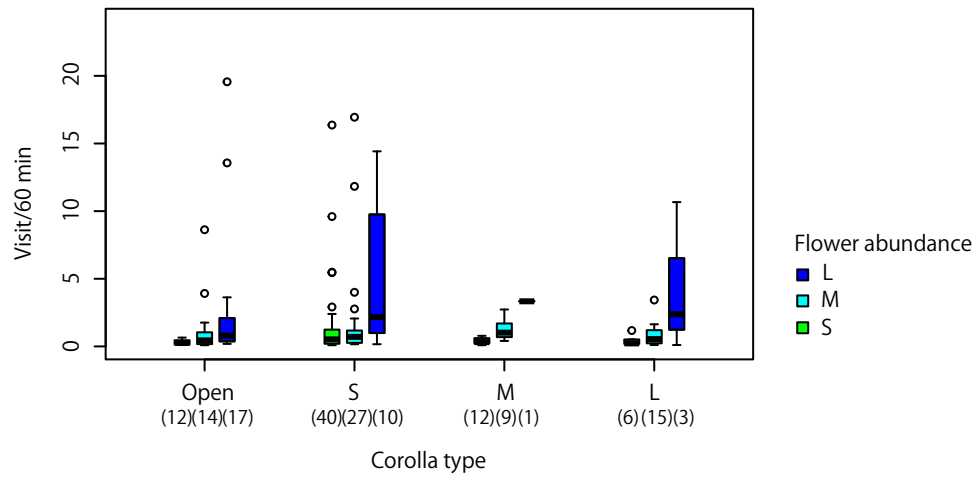


Fig. 1-12: Differences in the visitation frequencies to each flower species in each term among corolla types. Searching method was used for the observation in 2010-12. Figures in the parentheses represent sample sizes.

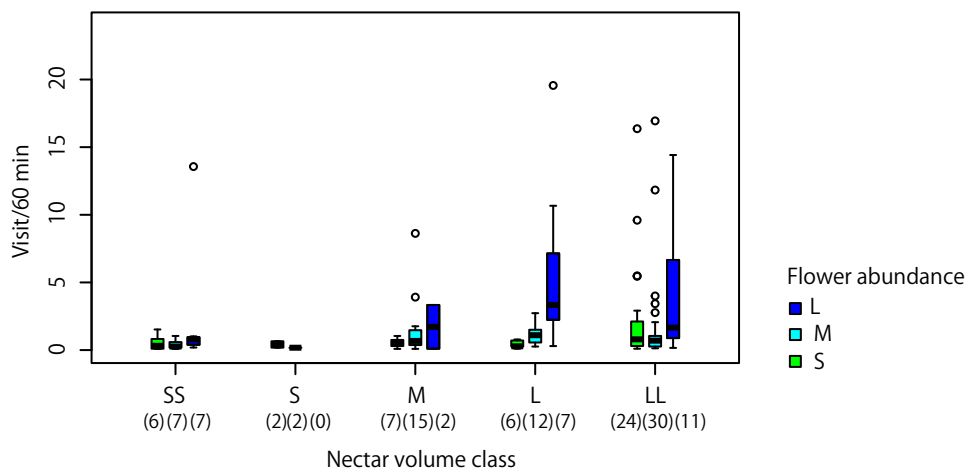


Fig. 1-13: Differences in the visitation frequencies to each flower species in each term among nectar volume classes. Searching method was used for the observation in 2010-12. Figures in the parentheses represent sample sizes.

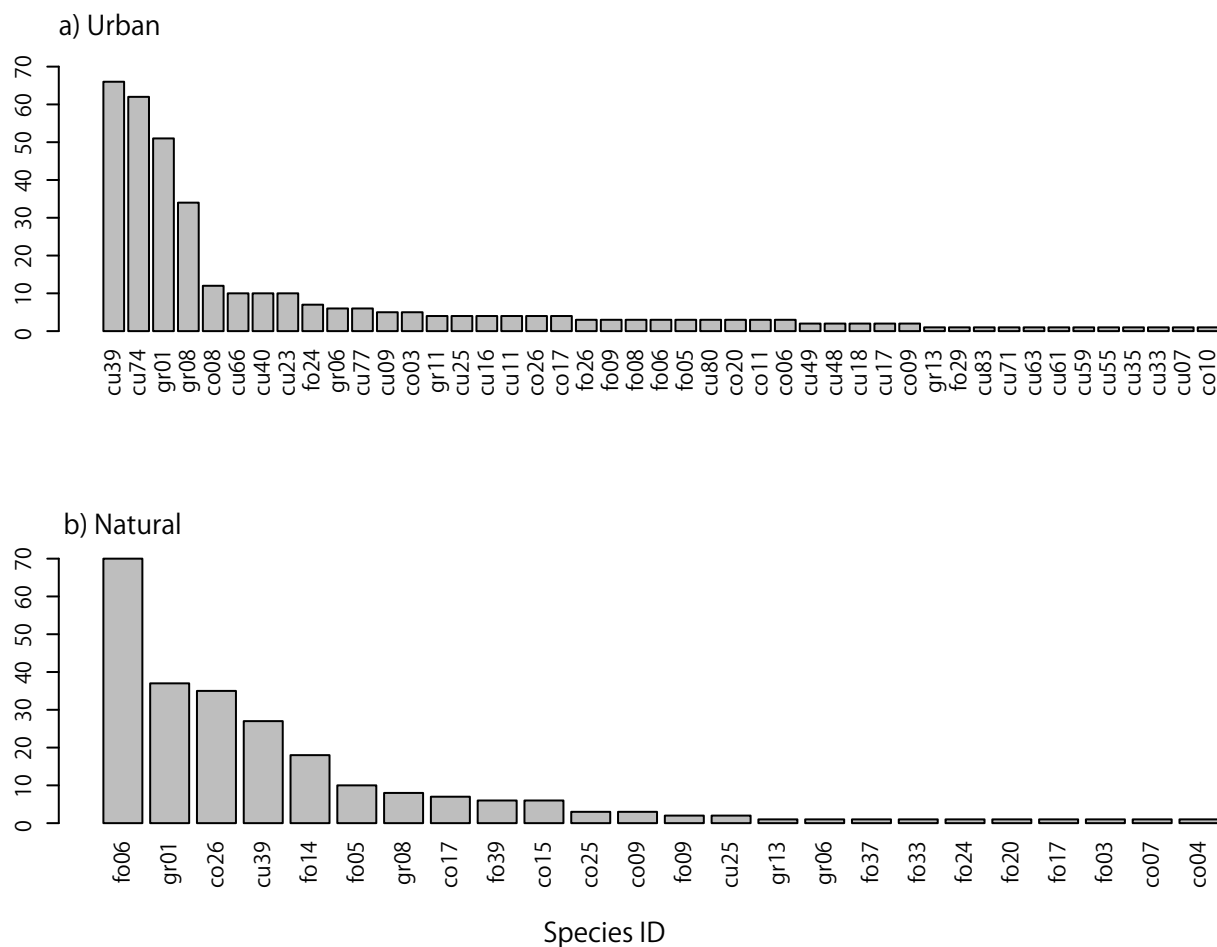


Fig. 1-14: Frequency of major pollen source flower species utilized by captured bumble bees (2010-11) in the urban-forest (a) and natural-forest (b) landscapes. Flower IDs conform to Table 0-S1 in Materials and Methods.

Pollen species that were included more than 50% in the pollen load of individual bumble bees were defined as major pollen source. The number of individual bumble bees captured on a same flower species was restricted up to five within the week.

Table 1-S1: The number of floral species of which the data of pollen and nectar is obtained.

Landscape	Year	Habitat												All habitats						
		Forest						Grassland										Residential		
		Nectar	Pollen	Both	Nectar	Pollen	Both	Nectar	Pollen	Both	Nectar	Pollen	Both	Nectar	Pollen	Both	Nectar	Pollen	Both	Nectar or Pollen
Urban	2010	28	25	24	6	5	5	30	28							28	54	49	48	55
	2011	29	26	25	4	4	4	50	43							42	72	63	61	74
	2012	29	26	25	4	4	4	55	47							46	76	65	63	78
	Mean ± SD	28.7 ± 0.6	25.7 ± 0.6	24.7 ± 0.6	4.7 ± 1.2	4.3 ± 0.6	4.3 ± 0.6	45.0 ± 13.2	39.3 ± 10.0							38.7 ± 9.5	67.3 ± 11.7	59.0 ± 8.7	57.3 ± 8.1	69.0 ± 12.3
Natural	2010	29	26	25	10	9	9	-	-							-	37	34	33	38
	2011	25	22	21	8	7	7	-	-							-	35	31	30	36
	2012	26	24	23	12	9	9	-	-							-	39	35	34	40
	Mean ± SD	26.7 ± 2.1	24.0 ± 2.0	23.0 ± 2.0	10.0 ± 2.0	8.3 ± 1.2	8.3 ± 1.2	-	-							-	37.0 ± 2.0	33.3 ± 2.1	32.3 ± 2.1	38.0 ± 2.0

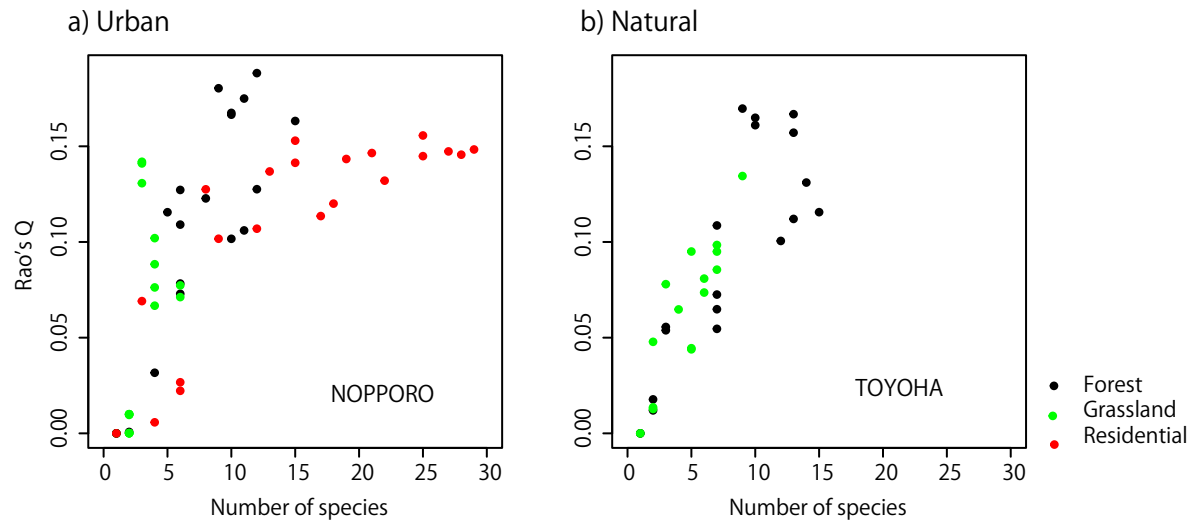


Fig. 1-S1: Relationship between floral functional trait diversity (Rao' s quadratic entropy) and the number of species included in the calculation. a) urban-forest landscape, b) natural-forest landscape. Each points represent the values in each term in each year (2010-2012).

Chapter 2.

Foraging habitat preference of bumble bees: Effects of garden flowers on pollinator visits to native flowers

2.1 Introduction

In recent years, fragmented natural or semi-natural vegetation, nature reserves, and gardens within urban area have been considered to be important components for the maintenance of sustainable ecological services in ecosystems with anthropogenic impacts (Lovell & Johnston 2009; Mcclintock 2010; Miller *et al.* 2015). In the urban landscapes, pollinators can utilize both of the artificial and natural vegetation habitats. Mass-flowering crops are especially important resources for pollinators including bumble bees in the agricultural landscapes, where fragmented natural habitats are scattered within crop fields (Westphal *et al.* 2003; Herrmann *et al.* 2007). Hagen & Kraemer (2010) suggested that crop fields could act as a “pollinator rescue” that supports diverse pollinator communities inhabiting natural forests. On the other hand, mass-flowering crops may lure pollinators from the natural habitats, and negatively affect reproductive success of wild plants inhabiting the natural or semi-natural habitats (Lander *et al.* 2011; Montero-Castaño *et al.* 2016). Magrach *et al.* (2017) assessed 177 plant-pollinator networks in the semi-natural grassland landscapes including crop fields and found that more than half of pollinator species were attracted to the mass-flowering crops.

Foraging decision of pollinators is influenced by not only the species-specific traits (e.g. matching between the length of corolla tube and proboscis of pollinators; Ranta & Lundberg 1980) but also floral structure of the surrounding environment (Kunin 1997; Chittka *et al.* 2001; Hanoteaux *et al.* 2013). In the urban landscape, where fragmented natural vegetation is scattered within a residential area, artificial gardens could positively affect wild plants by spillover of pollinators from gardens to natural vegetation. However, they may also negatively affect wild plants by taking

pollinators away from the natural vegetation. Studies on pollinator movements between habitats have focused on the risk of pollinator limitation for isolated plant populations in the fragmented habitat due to the reduced pollinator visits (Fahrig 2003; Cranmer *et al.* 2012). If pollinator movements to the natural habitats are restricted due to the existence of gardens, the risk of extinction of wild plants in the natural fragments may increase. However, little is known about the habitat preference of pollinators at landscape scale (but see Magrach *et al.* 2017), and it is still unclear how the gardens in the urban landscape affect the habitat choice of pollinators.

In consistent with the previous studies (Dreisig 1995; Goulson *et al.* 1998), bumble bees frequently utilized nectar-rich flowers, and plant species providing abundant and/or long-lasting flowers were preferred by pollinators as pollen source (Chapter 1). Gardens in the urban landscape provide species-rich floral resources with a high proportion of nectar-rich tubular flowers (Chapter 1). In addition, flowering periods of many floral species at the residential habitat are longer than native plants of the forest habitat, and abundant floral species are available throughout the season at the residential habitat (Chapter 1). For these superiorities as a foraging habitat, the residential habitat may be preferred over the forest habitat by bumble bees, and bumble bees' reliance for the pollen source may be stronger for the residential habitat.

Considering the effects of gardens on the foraging decision of pollinators in the urban landscape, many pollinators may stay at the gardens and do not visit native flowers as frequently as pollinators in the natural landscape (where gardens are not available) do. As for the pollen collection activity, if pollinators highly rely on gardens for pollen resource in the urban landscape, pollen collection at the native plant communities might be more active in the natural landscape than that in the urban landscape.

Pollen grains remain on the body of pollinators relatively for long period (Courtney *et al.* 1982). Thus, the composition of pollen on pollinator's body constructs a record of the floral species

previously visited by the pollinator (Bosch *et al.* 2009). Bumble bees often show site fidelity and constantly return to the same foraging locations (Ogilvie & Thomson 2016). If the forest habitat is less attractive compared to the residential habitat, however, habitat fidelity of bumble bees foraging at the forest habitat may not be as strong as bumble bees foraging at the residential habitat. In contrast to the pollen stored in the corbiculae (hereafter load pollen), which reflects exclusively pollen source flowers, pollen composition deposited on the pollinator's body (hereafter body pollen) reflects overall visitation records (both of the nectar and pollen source flowers). By analyzing body pollen, therefore, it would be possible to reveal the habitat fidelity of individuals.

In this chapter, I compared the habitat choice and the foraging pattern of bumble bees between the urban-forest landscape (a landscape including residential area) and the natural-forest landscape (a landscape not including residential area) and between habitats within the landscapes in order to examine the following hypotheses:

- 1) Habitat choice in the urban-forest landscape: bumble bee density will be higher at the residential habitat compared to the forest habitat because of the qualitatively and quantitatively rich floral resources.
- 2) Foraging patterns between the forest and residential habitats in the urban-forest landscape: (1) the proportion of visiting floral species (i.e., choice of targeting flowers) and the visitation frequency to the floral species are higher at the residential habitat compared to the forest habitat, (2) more foraging bumble bees carry pollen loads at the residential habitat compared to the forest habitat, and (3) habitat fidelity is stronger for the foraging bumble bees at the residential habitat compared to the forest habitat.
- 3) Foraging patterns at the forest habitat between the urban-forest and natural-forest landscapes: (1) the proportion of visiting floral species at the forest habitat is lower in the urban-forest landscape compared to the natural-forest landscape, (2) pollen foraging at the forest habitat is more active in

the natural-forest landscape than that in the urban-forest landscape, and (3) habitat fidelity of individuals foraging at the forest habitat is weaker in the urban-forest landscape compared to the natural-forest landscape.

2.2 Analysis

The number of bumble bee species observed in each floral species was similar between the observation methods (i.e., sit-and-wait or searching by walking; see Fig. 2-S1). Therefore, the data of both methods were pooled in case of observation method was not particularly mentioned in the following analyses.

Bumble bee density

From the data of 2012, when the observation was solely made by the searching method, the density of bumble bees for each 4-week period (term) was compared between the habitats. The number of observed bumble bees in each term was analyzed by a GLM assuming a Poisson error distribution in which habitat, term, and quadratic term were included as fixed factors, and observation period (min) in each term was included as an offset term. *Bombus pseudobicalensis* was excluded from the analysis because they foraged flowers only outside the forest.

Choice of targeting flowers

To compare the proportion of floral species visited by bumble bees (i.e., choice of targeting flowers) between habitats, I analyzed the binary data of bumble bee visitation to individual floral species in each year by GLMMs assuming a binomial error distribution in which habitat and year were included as fixed factors, and plant species as a random factor. This analysis was separately conducted for the urban-forest landscape and the natural-forest landscape. For the comparisons of

floral choice between the landscapes, similar analyses were performed but the fixed factor of habitat was replaced with the factor of landscape, and analysis was conducted in each of the forest habitat and the grassland habitat, separately.

Visitation frequency

To compare the seasonal trends in the floral use of bumble bees between habitats, I analyzed the visitation frequencies in individual floral species throughout the season by GLMMs assuming a Poisson error distribution. In the GLMMs, habitat, term, quadratic term, year, and interactions between habitat and term effects were included as fixed factors, plant species as a random factor, and logarithmic observation period (hours) in each term as an offset term. Floral species visited more than once was included, and the GLMM was conducted in each of the urban-forest landscape and the natural-forest landscape, separately. For the comparison of floral use patterns between the landscapes, similar GLMMs were performed but the fixed factor of habitat was replaced with the factor of landscape, and analysis was conducted in each of the forest habitat and the grassland habitat, separately.

Habitat choice for pollen resources

To examine the habitat choice of bumble bees for pollen resources, the ratio of bumble bees carrying pollen loads to all captured females (queens and workers) was compared between habitats. The number of bumble bees carrying pollen loads in each term was analyzed by GLMMs assuming a Poisson error distribution in which habitat and year were included as fixed factors, bumble bee species as a random factor, and logarithmic number of captured individuals of each bumble bee species in each term as an offset. This analysis was conducted in each of the urban-forest landscape and the natural-forest landscape, separately. Data of term 3 and 4 were used for the analysis because workers were active and available floral species was abundant in both landscapes. To examine the

difference between the landscapes, similar GLMMs were performed but the fixed factor of habitat was replaced with the factor of landscape, and the analysis was conducted in each of the forest habitat and the grassland habitat, separately. Because the ratio of pollen collecting females might be biased in case of small sample size, similar analyses were carried out for the selected data sets in which sample sizes more than or equal to four.

Habitat-use fidelity of bumble bees

Differences in the compositions of body-pollen and load-pollen were checked prior to the test of habitat-use fidelity of bumble bee individuals. The number of pollen species included in the body or load pollen of individuals was analyzed by a GLMM assuming a Poisson error distribution in which pollen type (body or load pollen), year, and landscape were included as fixed factors, and bumble bee species as a random factor. For individuals that carried two or more pollen species, species diversity of pollen grains (Shannon's diversity) was compared between habitats by a GLMM assuming a Gamma error distribution with the similar fixed and random factors as above. Pollen grains failed in the identification were excluded from the analysis, and these analyses were restricted for term 3 and 4, when worker bees were active and available floral species were abundant in both landscapes.

For the comparisons of foraging patterns of bumble bees between the habitats, the ratio of individuals having body pollen derived from outside of the captured habitat (hereafter outside pollen) was tested by Fisher's exact test. In this analysis, I compared the ratio of bumble bees having outside pollen between the habitats within landscapes and between the landscapes in each of the forest and grassland habitats.

To examine the habitat-use fidelity of bumble bees more exactly, the number of outside pollen species on the bumble bee body was analyzed by GLMMs assuming a negative-binomial

error distribution in which habitat, term, and year were included as fixed factors, bumble bee species as a random factor, and total pollen species number as an offset term. The analysis of the between-habitat comparison was conducted only for the urban-forest landscape including three habitats, i.e., forest, grassland and residential habitats. To examine the difference between the landscapes, furthermore, different GLMM was performed in which landscape, habitat, term, year, and an interaction between landscape and habitat were included as fixed factors, bumble bee species as a random factor, and total pollen species number as an offset term. In the between-landscape comparison, data of the residential habitat in the urban-forest landscape was excluded.

To evaluate the effectiveness of the explanatory variables in every GLMM, I used Akaike's information criterion (AIC). Statistical analyses are performed using R version 3.1.2 (R Core Team 2014).

2.3 Results

Bumble bee communities

In total, 3127 and 2119 bumble bees were observed throughout the weekly observations in 2010–2012 (Table 2-1). Observed species were *Bombus hypocrita sapporoensis*, *B. ardens sakagami*, *B. hypnorum koropokkrus*, *B. diversus tersatus*, *B. pseudobaicalensis*, *B. yezoensis*, and invasive *B. terrestris*. Of these species, *B. yezoensis* was not found in the urban-forest landscape. *Bombus terrestris* and *B. pseudobaicalensis* were very rare in the natural-forest landscape; they were detected only once throughout the observation period. Bumble bees were observed from the end of April to early November in the urban landscape, and from early May to the end of September in the natural-forest landscape (Fig. 2-1). Earliest appearance of bumble bees was recorded at the forest habitat in both landscapes. *Bombus ardens sakagami* and *B. hypnorum koropokkrus* had shorter active season compared to other species, and their life cycles were finished by early August. In the

natural-forest landscape, all species (except for rare *B. pseudobaicalensis* and *B. terrestris*) foraged at both of the forest and grassland habitats. In the urban-forest landscape, *B. pseudobaicalensis* did not forage at the forest habitat throughout the active season. *Bombus hypocrita sapporoensis* and *B. terrestris* were rarely observed at the forest habitat after early August, while they actively foraged at the residential habitat. Only *B. diversus tersatus* foraged at the forest habitat after early August. In the urban-forest landscape, possible maximum species number at the forest habitat of the urban-forest landscape was five excluding *B. pseudobaicalensis*, but coexistence of all five species was not observed throughout the season (Fig. 2-1).

Bumble bee density

The number of bumble bees increased as seasonal progress and decreased late in the season (Fig. 2-2): significant positive effect of term (urban: $z = 8.26$, $p < 0.01$, natural: $z = 6.69$, $p < 0.01$) and negative effect of quadratic term (urban: $z = -8.44$, $p < 0.01$, natural: excluded by AIC) were detected by the GLMMs. The density of bumble bees was higher at the grassland habitat than at the forest habitat in both landscapes (urban grassland: $z = 5.40$, $p < 0.01$, natural grassland: $z = 8.91$, $p < 0.01$). Bumble bee density was continuously higher at the residential habitat in the urban-forest landscape ($z = 13.91$, $p < 0.01$). The number of bumble bees in the natural-forest landscape reached at maximum relatively late in the season (term 5; late August to early September) at both of the forest and grassland habitats, when *A. sachalinense* subsp. *yezoense* reached at full bloom there. Foraging by workers was most active in term 3 and 4 in the urban-forest landscape and in term 2 to 5 in the natural-forest landscape (Fig. 2-2b).

Choice of targeting flowers

In total, 16.3 ± 2.5 (mean $\pm$ SD), 4.3 ± 3.2 , and 54.7 ± 13.1 plant species were visited by bumble bees at the forest, grassland, and residential habitats, respectively, in the urban-forest landscape; and

17.3 $\pm$ 0.6 and 9.7 $\pm$ 4.0 species were visited at the forest and grassland habitats, respectively, in the natural-forest landscape. These represented for 39.1 $\pm$ 7.6% (mean $\pm$ SD), 41.7 $\pm$ 22.0%, and 77.4 $\pm$ 7.0% of the available species at the forest, grassland, and residential habitats, respectively, in the urban-forest landscape; and 46.9 $\pm$ 1.5% and 58.5 $\pm$ 3.4% of the available species at the forest and grassland habitats, respectively, in the natural-forest landscape.

In the between-habitat comparisons of the proportion of plant species visited within the habitat, significant effects of habitat and year on floral choice were detected in the urban-forest landscape. Plant species at the residential habitat were more frequently visited by bumble bees compared to the plant species at the forest habitat ($z = 5.52$, $p < 0.01$), while plant species at the grassland habitat were visited similarly as plants at the forest habitat ($z = 1.42$, $p = 0.16$). The proportion of plant species visited differed between years (2011: $z = -2.37$, $p = 0.02$; 2012: $z = -3.11$, $p < 0.01$). In the natural-forest landscape, on the other hand, the proportion of plant species visited did not differ between the habitats or between years (all of the explanatory factors were excluded by AIC). Therefore, the proportion of floral species used by bumble bees was especially high at the residential habitat.

In the between-landscape comparison in each habitat, the proportion of plant species visited did not differ between the landscapes or between years at both of the forest and grassland habitats (all of the explanatory factors were excluded by AIC). These results indicate that the floral choice of bumble bees was similar at the forest and grassland habitats irrespective of the existence of residential habitat.

Visitation frequency

Visitation frequency of bumble bees to individual floral species was significantly larger at the grassland habitat than at the forest habitat ($p < 0.01$) and varied between years ($p < 0.01$) in both

landscapes (Tables 2-2 & 2-3). However, there was no significant difference in the visitation frequency between the forest habitat and the residential habitat in the urban-forest landscape ($p = 0.36$; Table 2-3). In the natural-forest landscape, visitation frequency slightly decreased toward middle season (term effect: $p < 0.01$), and slightly increased later on (quadratic term effect: $p < 0.01$; Fig. 2-3, Table 2-3). The increase in the late season at the grassland habitat was slow compared to that at the forest habitat (significant interaction between habitat and quadratic term: $p < 0.01$) in the natural-forest landscape. In the urban-forest landscape, seasonal change in the visitation frequency was not detected (term effect: $p = 0.17$; quadratic term effect: $p = 0.28$) but the visitation frequency at the grassland habitat showed different trend compared to the forest habitat (significant interaction between grassland habitat and term: $p < 0.01$).

Habitat choice for pollen resources

The total number of captured female bumble bees during the active season (term 3 and 4) were 331 (29 queens and 362 workers) in the urban-forest landscape and 237 (24 queens and 213 workers) in the natural-forest landscape. The GLMM conducted for the urban-forest landscape revealed that relatively large number of individuals carried pollen at the grassland ($z = 1.72$, $p = 0.09$) and residential ($z = 1.69$, $p = 0.09$) habitats compared to the forest habitat (Fig. 2-4a), and there was no difference between years (year effect was excluded by AIC). On the other hand, the proportion of pollen collecting females to all captured females did not differ between the habitats and years in the natural-forest landscape (both of the effects were excluded by AIC). Similar results were confirmed for the sample-size restricted data (Fig. 2-4b) in which the number of pollen-collecting bumble bees was larger at the grassland ($z = 2.06$, $p = 0.04$) and residential ($z = 1.75$, $p = 0.08$) habitats than at the forest habitat with no difference between years in the urban-forest landscape, while there were no differences between the habitats and between years in the natural-forest landscape.

In the comparisons between landscapes, the proportion of pollen collecting females to all captured females at the forest habitat varied between years ($p = 0.025$, $z = 2.24$), and did not differ between the landscapes (landscape effect was excluded by AIC; Fig. 2-4a). At the grassland habitat, the proportion of pollen collecting females to all captured females did not differ between landscapes and between years (landscape and year effects were excluded by AIC). Similar trend was detected in the sample-size restricted data (Fig. 2-4b). In the forest habitat, the number of pollen-collecting bumble bees did not differ between the landscapes ($p = 0.10$, $z = 1.63$), and slightly differed between years ($p = 0.09$, $z = 1.69$). In the grassland habitat, the proportion of pollen collecting females did not differ between landscapes and between years (landscape and year effects were excluded by AIC).

Habitat-use fidelity of individual bumble bees

Before examining habitat-use fidelity of individual bumble bees, floral species diversity of pollen was compared between body and load pollen. The number of pollen species included in the body pollen in term 3 and 4 was 1.64 ± 0.78 (mean $\pm$ SD; $n = 770$), and it was larger than that included in the load pollen (1.47 ± 0.69 , $n = 449$; $z = -2.85$, $p < 0.01$). The diversity of body pollen was also larger even when individuals containing single pollen species were excluded ($z = -3.19$, $p < 0.01$). The effects of year and landscape did not affect the number of pollen species or diversity of pollen species included in the body pollen (both factors were excluded by AIC).

The numbers of individuals that carried outside pollen in the urban-forest landscape were 15 out of 153 at the forest habitat, 33 out of 113 at the grassland habitat, and 58 out of 524 individuals at the residential habitat. The numbers of individuals that carried outside pollen in the natural-forest landscape were 11 out of 187 at the forest habitat and 73 out of 228 individuals at the grassland habitat (Fig. 2-5). When the ratio of individual bumble bees that carried outside pollen was compared between the habitats, it was higher at the grassland habitat in both landscapes (Fisher's

exact test, urban: $p < 0.01$; natural: $p < 0.01$). When the ratio was compared between the landscapes, there were no differences between the urban-forest and natural-forest landscapes at both of the forest and grassland habitats (Fisher's exact test, urban: $p = 0.22$; natural: $p = 0.62$).

The number of pollen species included in the body pollen was around 1.4–1.7 at all habitats throughout the bee-active terms (Table 2-4, Fig. 2-6). In the between-habitat comparison of the urban-forest landscape, the number of outside pollen species on the bumble bee body was significantly larger at the grassland habitat ($p < 0.01$) than at the forest habitat, but there was no difference between the forest and residential habitats ($p = 0.29$; Table 2-5). This indicates that the habitat-use fidelity of bumble bees foraging at the grassland was lower than that foraging at other habitats. In the between-landscape comparison of body pollen, the number of outside pollen was larger at the grassland and varied among terms ($p < 0.01$) but the effect of landscape was excluded from the explanatory variables (Table 2-5). Therefore, the habitat-use fidelity of bumble bees did not differ between the urban-forest and the natural-forest landscape.

2.4 Discussion

Preference to the residential habitat

Density of bumble bees and proportion of visited plant species in the urban-forest landscape were higher at the residential habitat compared to the forest habitat. These results are consistent with my expectation in which the residential habitat is preferred over the forest habitat, and with previous studies reporting abundant bumble bees at urban gardens (McFrederick & Lebuhn 2006; Matteson & Langellotto 2009). The preference to the residential habitat may be due to the floral composition in the gardens where nectar-rich tubular flowers with high sugar concentration were abundant (Chapter 1). According to Lázaro & Totland (2010), dense and floral species rich communities were preferred by pollinators. Diverse floral species in urban gardens throughout the season (Chapter 1; Garbuzov

et al. 2014; Harrison *et al.* 2015) might also resulted in the high preference to the residential habitat.

Although a lot of bumble bees foraged at the residential habitat, visitation frequency to individual floral species did not differ between the residential habitat and the forest habitat in the urban-forest landscape (Table 2-2). This might be partly because floral species with no visits were excluded in this analysis. In contrast to the similar visitation frequency to the targeting floral species, bumble bees utilized much more floral species at the residential habitat than at the forest habitat (54.7 ± 13.1 species were used at the residential habitat, while only 16.3 ± 2.5 species at the forest habitat). On the other hand, visitation frequency to individual floral species at the grassland habitat was high compared to that at the forest habitat in both landscapes. It might be because of the high attractiveness of grassland species. Most of the visited floral species in the grassland habitat were highly attractive, including highly preferred pollen source species; *Trifolium pretense* and *Trifolium repens* in the urban-forest landscape, and *Aconitum sachalinense* subsp. *yezoense*, *T. pretense*, and *Lupinus polyphyllus* in the natural-forest landscape (Fig. 1-14 in Chapter 1).

Body pollen was more diverse than load pollen, which was consistent with the report by Goulson *et al.* (2008b) in which bumble bees visited more diverse floral species for nectar foraging than for pollen collection. Thus, body pollen was suitable to understand the habitat-use of bumble bees. Bumble bees foraging at the residential and forest habitats showed high level of habitat-use fidelity (Fig. 2-5; about 90% of bumble bees carried pollens derived exclusively from the captured habitat). Although a large number of bumble bees were attracted to the residential habitat at the community level, bumble bees captured at the forest habitat highly relied on the floral resources of forest habitat. On the other hand, the habitat-use fidelity of bumble bees at the grassland habitat was lower in both landscapes (29%–32% of them carried outside pollens). It implies that resources gained at the grassland habitat might be small relative to their requirements because of the small grassland area within the landscapes (Fig. 0-1 in Materials and Methods; grassland accounted for

3%–4% of the land cover).

Relatively large number of female individuals carried pollen loads at the residential and grassland habitats compared to the forest habitat (Fig. 2-4). Bumble bees relied more on the residential and grassland flowers for pollen source in the urban-forest landscape. Bumble bees preferred abundant and long-lasting floral species for pollen source, and floral species with such properties were more abundant at the residential and grassland habitats than at the forest habitat (Chapter 1). However, other factors such as nutrient contents could influence the pollen collection by bumble bees (Ruedenauer *et al.* 2016; Vaudo *et al.* 2016). At the forest habitat in the urban-forest landscape, bumble bees mainly focused on abundant *Cirsium kamtschaticum* in late June to early July (term 3) but they did not use this species as a major pollen source (of the 36 females having body pollen of *C. kamtschaticum*, 23 carried pollen loads containing this species, but the contents was limited to $21.7 \pm 29.2\%$).

In the natural-forest landscape, female individuals similarly collected pollens from the forest and grassland habitats. The existence of highly attractive pollen-source species (such as *Aconitum umbrosum* in late June to early July and *A. sachalinense* subsp. *yezoense* in mid-August; Chapter 1) might promote the pollen collection at the forest habitat of the natural-forest landscape.

Seasonal change in the habitat usage

In the urban-forest landscape, bumble bee density after the middle season (term 4) was quite low at the forest habitat, but it remained at high level at the residential habitat (Fig. 2-2). At the forest habitat of the urban-forest landscape, the number of floral species with large- or medium-abundance decreased with the progress of season. In contrast, the number of species with large-abundance was rather stable throughout the season, and the number of species with medium-abundance increased until the middle of season at the residential habitat (Fig. 1-3 in Chapter 1). The foraging patterns of

pollinators often varied depending on the floral species composition within the floral community (Ghazoul 2006; Lázaro & Totland 2010), and the distribution of floral resources at landscape level affect the abundance of pollinators (Westphal *et al.* 2006). Seasonal decrease in the rewarding floral species at the forest habitat and the corresponding increase in relative importance of the residential habitat as a foraging place might accelerate the movement of bumble bees from the forest habitat to the residential habitat late in the season.

In spite of the seasonal decrease in the number of floral species at the forest habitat of the natural-forest landscape, on the other hand, the density of bumble bees did not change as clearly as in the urban-forest landscape. Because *A. sachalinense* subsp. *yezoense*, important nectar and pollen resources, bloomed late in the season at the forest habitat of the natural-forest landscape, it might attract and keep bumble bees within the forest habitat.

In the natural-forest landscape, visitation frequency was high at early and late season (Fig. 2-3). However, such trend was not detected at the forest and residential habitats of the urban-forest landscape. In the natural-forest landscape, visits of bumble bees were concentrated on a few attractive species in term 1 (mainly *Corydalis fumariifolia* subsp. *azurea* at the forest habitat) and term 6 (exclusively *A. sachalinense* subsp. *yezoense* at the forest habitat, and mainly *A. sachalinense* subsp. *yezoense* and *T. pretense* at the grassland habitat). On the other hand, in the urban-forest landscape, bumble bees had many choices of attractive species within the landscape in both term 1 (mainly *Corydalis fumariifolia* subsp. *azurea* and *Acer japonicum* but *Pachysandra terminalis* also showed medium attractiveness at the forest habitat, and 12 garden species were similarly attractive at the residential habitat) and in term 6 (mainly *A. sachalinense* subsp. *yezoense* at the forest habitat, mainly *T. pretense* at the grassland habitat, and mainly *Cosmos bipinnatus* but six other garden species showed medium attractiveness at the residential habitat). Such difference in the number of attractive species might result in the difference in the seasonal change in the visitation frequency to

each floral species between urban-forest and natural-forest landscape. Seasonal variation in the visitation frequency was found at the grassland habitat in the urban-forest landscape, but it might be caused by the mowing in June (term 2 in 2011 and term 3 in 2012) and August (term 4 in 2011 and term 5 in 2012), thus the seasonality of bumble bee activity at the grassland habitat was unclear in the urban-forest landscape.

Inconsistent with the contrasting seasonal trends in bumble bee density between the forest and residential habitats in the urban-forest landscape (Fig. 2-2), seasonal pattern of visitation frequency to individual floral species did not differ between the habitats (Fig. 2-3 & Table 2-3). Visitation frequency at the forest habitat was kept as high as that at the residential habitat throughout the season. It implies that although bumble bee density was quite low late in the season at the forest habitat of the urban-forest landscape, a few highly rewarding species at the forest habitat, like *A. sachalinense* subsp. *yezoense* in small abundance, were visited as frequently as species at the residential habitat. This indicates the importance of key floral species determining the foraging pattern of pollinators.

Effects of garden flowers on the visitation patterns to natural vegetation

Considering the constantly provided high-quality resources in the residential area (Chapter 1) and previous studies reporting the pollinator movements from natural vegetation to crop field (Lander *et al.* 2011; Montero-Castaño *et al.* 2016), I predicted that the residential habitat would have a negative effect on the adjacent native plant communities. Contrary to my expectation, however, the proportion of visiting floral species at the forest habitat, visitation frequency to forest flowers and dependence on pollen-source of the forest habitat did not differ between the urban-forest and natural-forest landscapes (Fig. 2-3). These results imply that although the relative number of using floral species was small at the forest habitat compared to the residential habitat, some rewarding species at the

forest habitat were intensively utilized irrespective of the existence of attractive gardens outside of the forest. The low density of bumble bees at the forest habitat in the natural-forest landscape might be due to the lack of attractive abundant flowers. Although the functional diversity of floral traits was similar between the urban-forest and natural-forest landscapes (Fig. 1-11 in Chapter 1), attractive *A. sachalinense* subsp. *yezoense*, which bloomed late in the season at the forest habitat, was quite rare in the urban-forest landscape. For this reason, preferably used resources at the forest habitat might be low in the urban-forest landscape compared to the natural-forest landscape. Thus, resources at the residential habitat might strongly support bumble bees in the urban-forest landscape especially late in the season.

At the individual level, habitat-use fidelity to the forest habitat did not differ between the urban-forest and natural-forest landscapes (Table 2-5). Thus, individuals at the forest habitat highly relied on the forest flowers independent of the presence of gardens outside of the forest habitat.

2.5 Conclusions

The residential habitat attracted many bumble bees in the urban-forest landscape, and relatively large number of floral species was utilized in the gardens compared to the forest habitat. However, a few plant species at the forest habitat were continuously used by individuals with high habitat fidelity irrespective of the availability of gardens within the landscape. Therefore, gardens did not have a negative effect on the plants of the native vegetation in terms of pollinator attraction. The residential habitat might support bumble bee community by providing preferable resources throughout the season, and compensate for the decrease in the resource availability of preferred species at the forest habitat in the urban-forest landscape. These results indicate the facilitative effects of gardens on the maintenance of pollinator communities in the urban ecosystems.

Table 2-3: Results of GLMMs for the visitation frequency in each landscape. Habitat (forest, grassland, residential), term (seven 4-week periods in ordered variable), quadratic term, year (2010, 2011, 2012) and interactions between habitat and term effects were included as fixed factors, plant species was included as a random factor, and logarithm of observation duration in each term (hours) was included as an offset. Flower species that received more than one visit was included. To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used.

	N	AIC	Estimate	Std. Error	z value	Pr(> z)
Urban-forest landscape	258	1529.5				
Intercept (Habitat = forest, Year = 2010)			-0.79	0.52	-1.53	0.13
Habitat (grassland)			14.55	2.52	5.78	<0.01
Habitat (residential)			-0.54	0.58	-0.92	0.36
Term			-0.56	0.40	-1.4	0.17
Quadratic term			0.08	0.07	1.09	0.28
Year (2011)			0.64	0.11	6.00	<0.01
Year (2012)			0.79	0.10	7.63	<0.01
Habitat (grassland) × Term			-5.73	1.27	-4.49	<0.01
Habitat (residential) × Term			0.48	0.43	1.12	0.26
Habitat (grassland) × Quadratic term			0.59	0.15	3.88	<0.01
Habitat (residential) × Quadratic term			-0.05	0.07	-0.65	0.52
Natural-forest landscape	94	873.4				
Intercept (Habitat = forest, Year = 2010)			-0.43	0.69	-0.62	0.54
Habitat (grassland)			2.19	0.47	4.66	<0.01
Term			-1.38	0.34	-4.01	<0.01
Quadratic term			0.23	0.04	5.39	<0.01
Year (2011)			1.31	0.13	10.00	<0.01
Year (2012)			2.03	0.13	15.85	<0.01
Habitat (grassland) × Quadratic term			-0.08	0.02	-4.82	<0.01

Table 2-4: The number (means $\pm$ SDs) of outside-pollen species in the body pollen of bumble bee individuals (upper) and the total number of pollen species in the body pollen (lower) in 2010–2011. Figures in parentheses represent (sample sizes of individuals that carried only outside pollen/sample sizes of individuals that carried both outside and within-habitat pollen/total sample sizes)

	Forest	n	Grassland	n	Residential	n
Urban	0.12 $\pm$ 0.38 1.42 $\pm$ 0.65	(5/15/153)	0.37 $\pm$ 0.63 1.64 $\pm$ 0.72	(3/33/113)	0.11 $\pm$ 0.32 1.69 $\pm$ 0.83	(3/58/524)
Natural	0.06 $\pm$ 0.24 1.40 $\pm$ 0.60	(2/11/187)	0.37 $\pm$ 0.59 1.51 $\pm$ 0.73	(14/73/228)		

Table 2-5: Results of the GLMMs for the seasonal change in the habitat-use fidelity of individual bumble bees. For the between-habitat comparison, the number of outside-pollen species was analyzed by a GLMM in which habitat (forest, grassland, residential), term (seven 4-week period in a ordered class), and year (2010, 2011) were considered as fixed factors, bumble bee species was included as a random factor, and the number of total pollen species as an offset term. For the between-landscape comparison, landscape (urban, natural), habitat, term, year, and an interaction between landscape and habitat were considered as fixed factors, bumble bee species was included as a random factor, and the number of total pollen species as an offset term. To evaluate the effectiveness of individual explanatory variables, Akaike's information criterion (AIC) was used.

	N	AIC	Estimate	Std. Error	z value	Pr(> z)
Between-habitat comparison (urban-forest landscape)	790	581.1				
Intercept (habitat = forest, Year = 2010)			-1.52	0.34	-4.54	<0.01
Habitat (grassland)			1.52	0.34	4.48	<0.01
Habitat (residential)			0.34	0.32	1.07	0.29
Term			-0.37	0.10	-3.63	<0.01
Year (2011)			-0.48	0.20	-2.41	0.02
Between-landscape comparison	681	623.1				
Intercept (habitat = forest, Year = 2010)			-2.41	0.33	-7.37	<0.01
Habitat (grassland)			1.56	0.22	7.22	<0.01
Term			-0.21	0.09	-2.36	0.02
Year (2011)			0.31	0.16	1.87	0.06

a) Urban-forest landscape in 2010

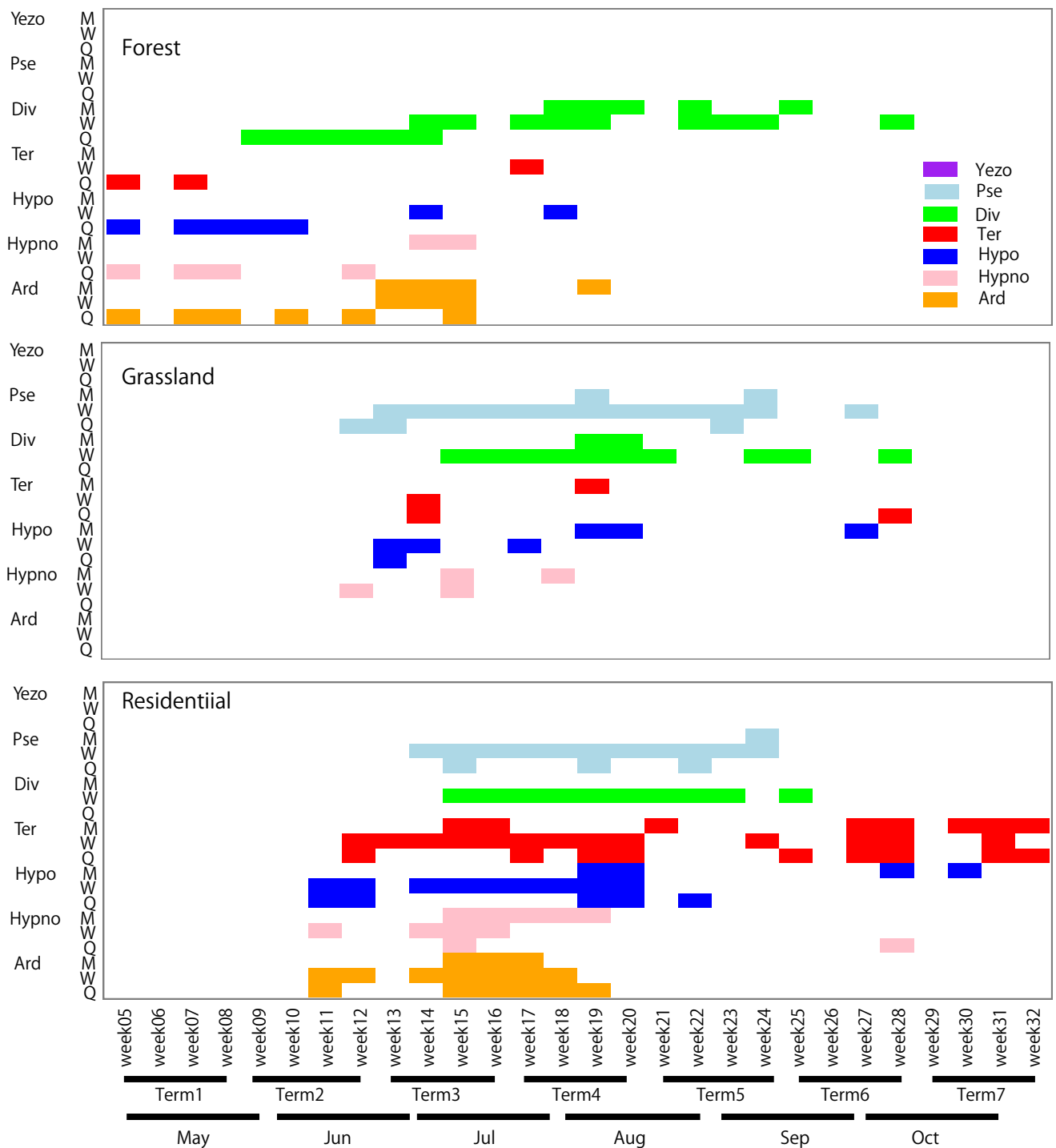


Fig. 2-1: Phenology of bumble bees in each landscape in each year. Urban-forest landscape in a) 2010, b) 2011, c) 2012, and natural-forest landscape in d) 2010, e) 2011, f) 2012.

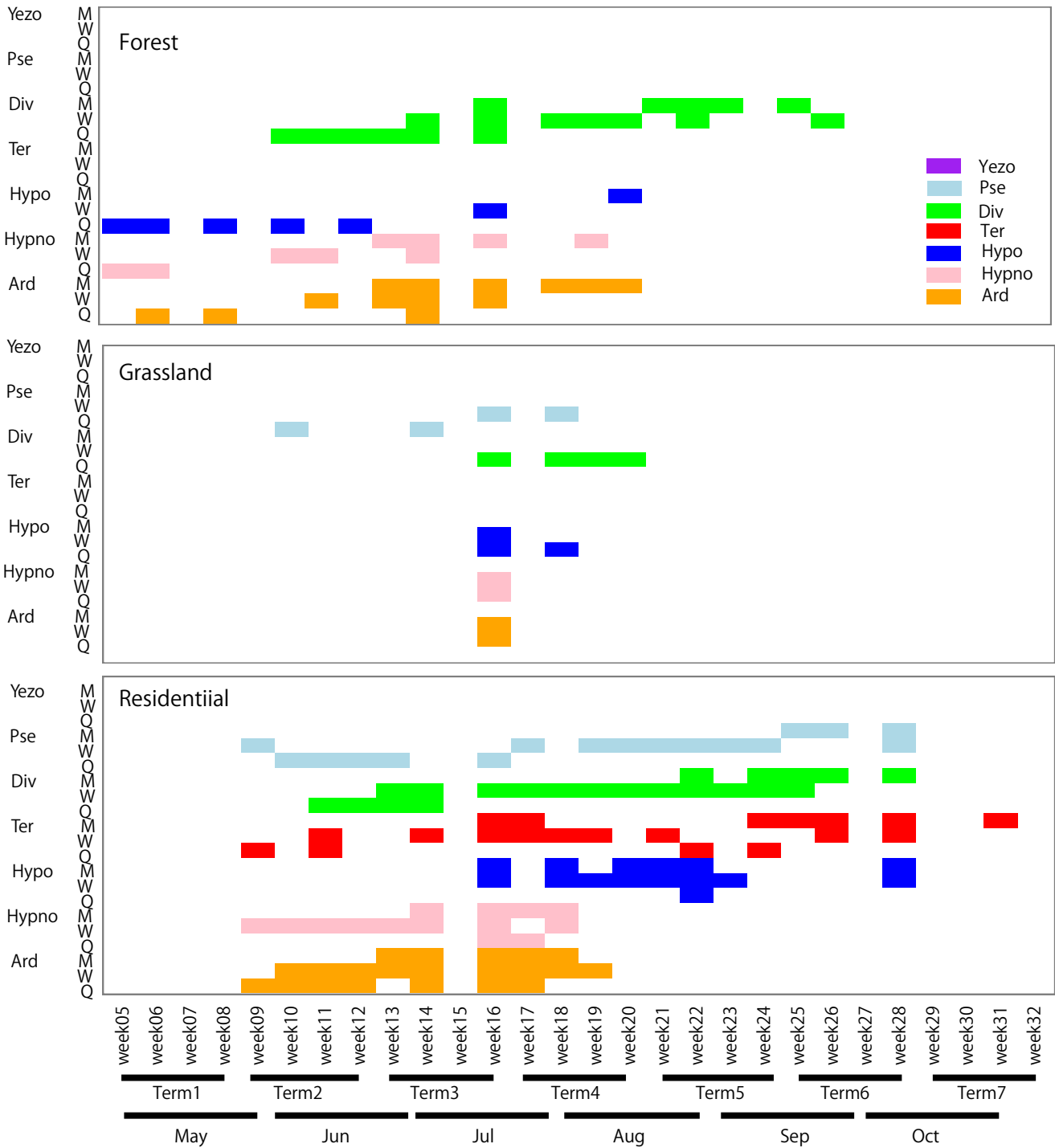
Week numbers start from the beginning of April in each year.

Q: Queen, W: Worker, M: Male

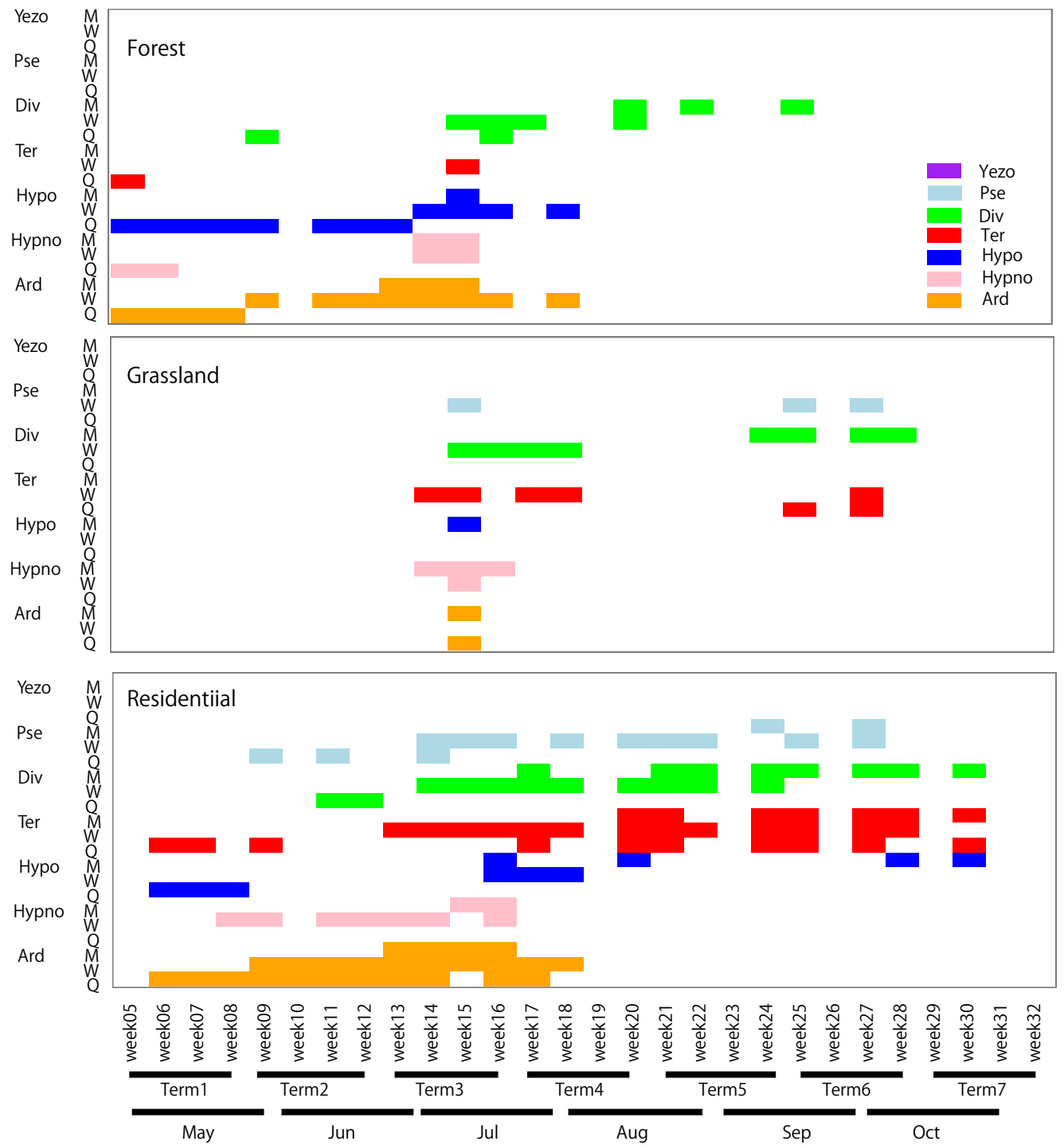
Ard: *Bombus ardens sakagami*, Hypno: *B. hypnorum koropokkrus*, Hypo: *B. hypocrita sapporoensis*,

Ter: *B. terrestris*, Div: *B. diversus tersatus*, Pse: *B. pseudobaicalensis*, Yezo: *B. yezoensis*.

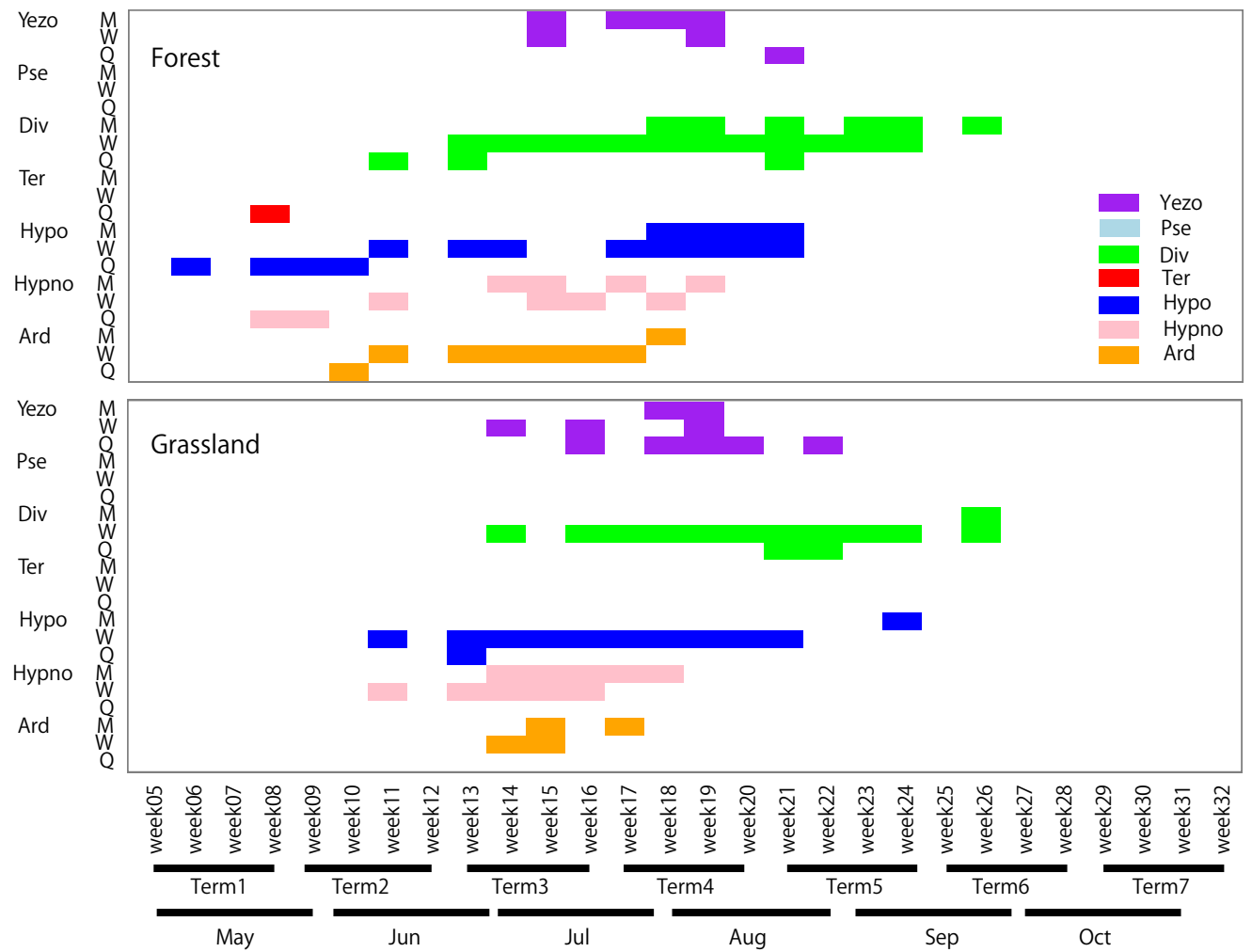
b) Urban-forest landscape in 2011



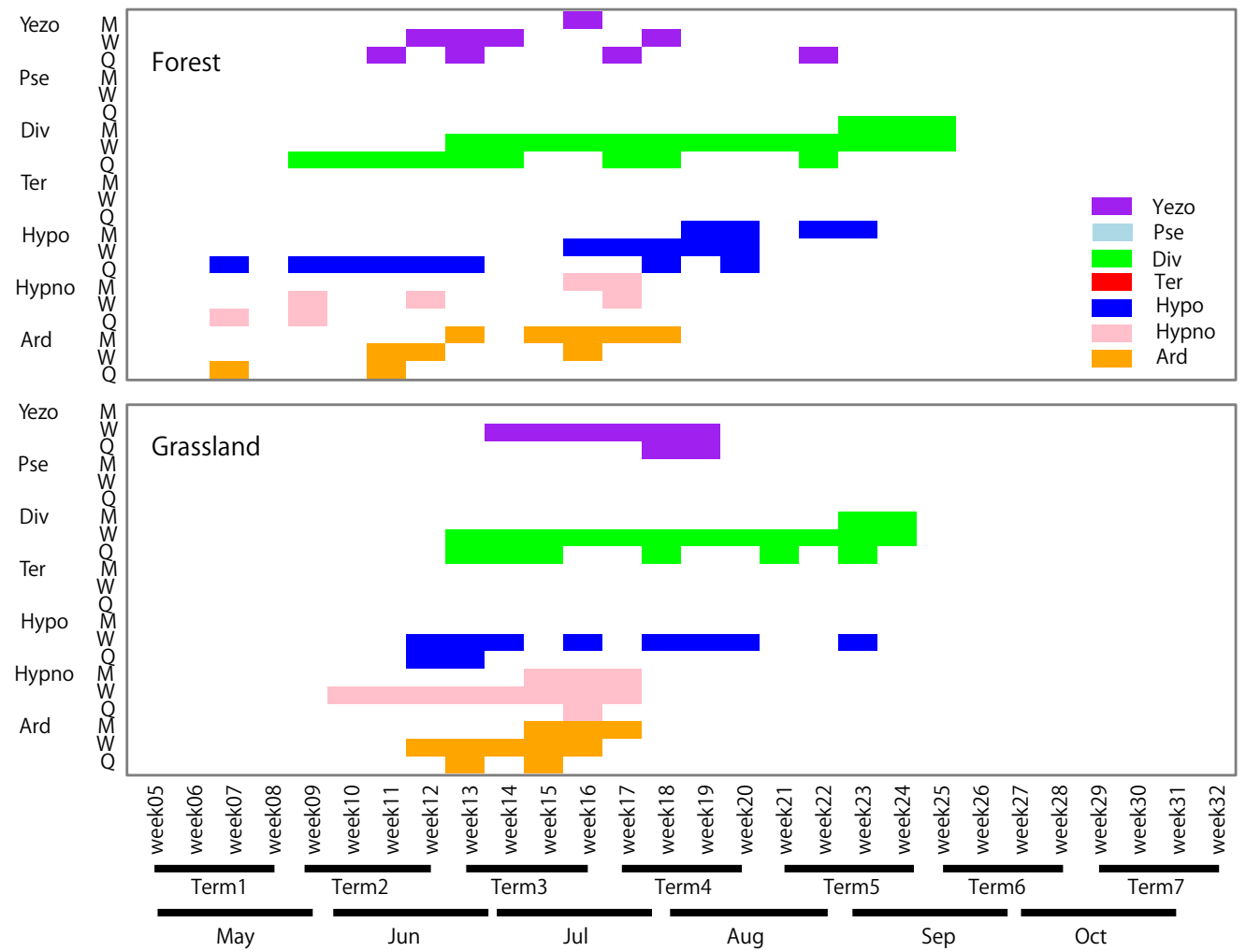
c) Urban-forest landscape in 2012



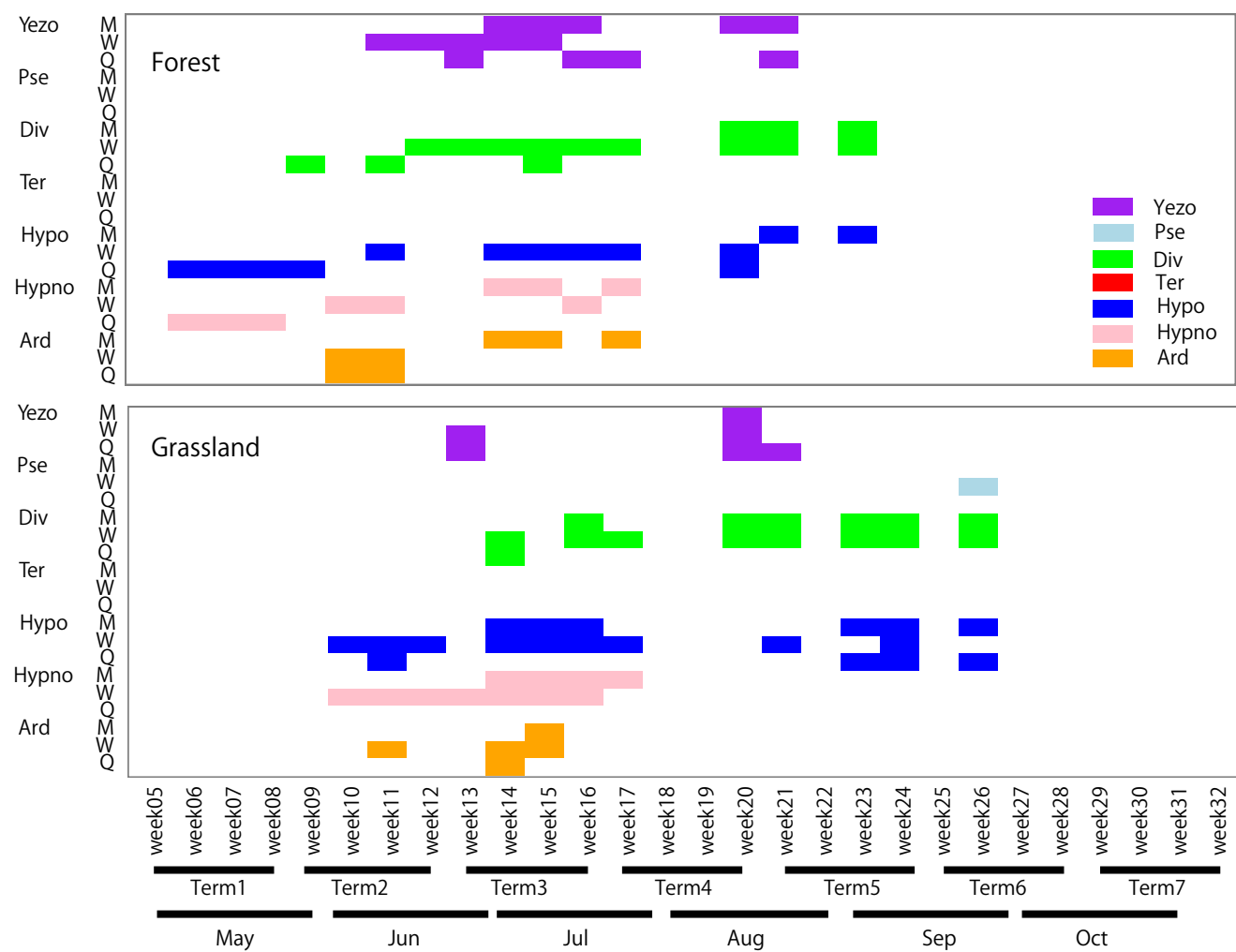
d) Natural-forest landscape in 2010



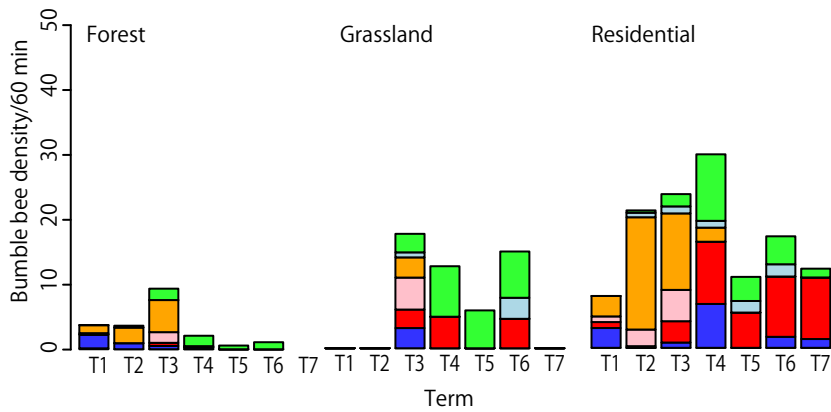
e) Natural-forest landscape in 2011



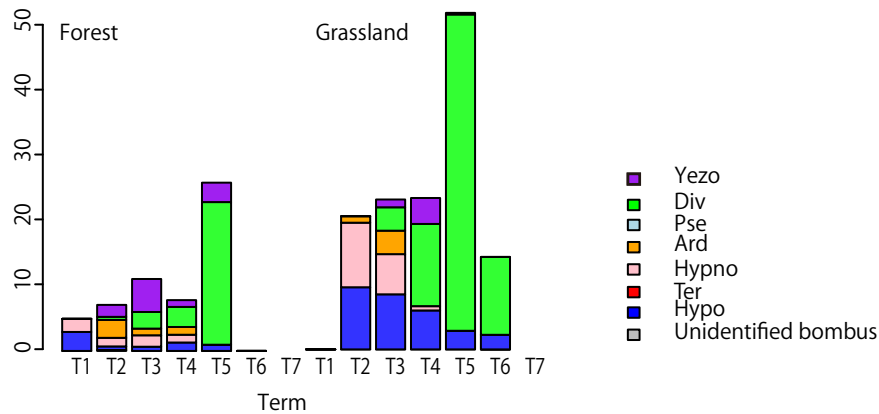
f) Natural-forest landscape in 2012



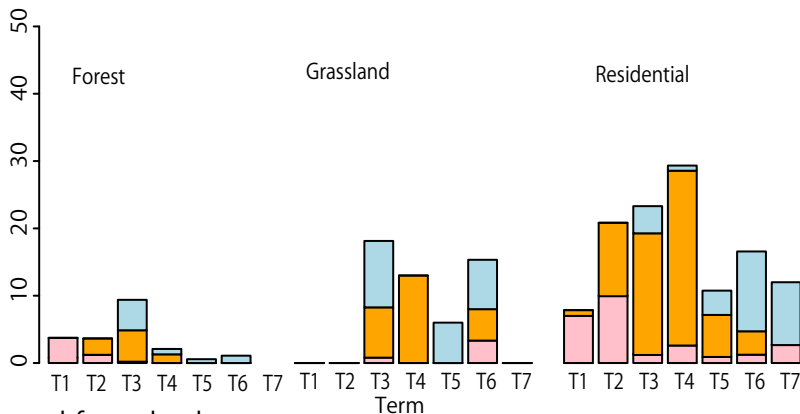
a) Urban-forest landscape



b) Natural-forest landscape



c) Urban-forest landscape



d) Natural-forest landscape

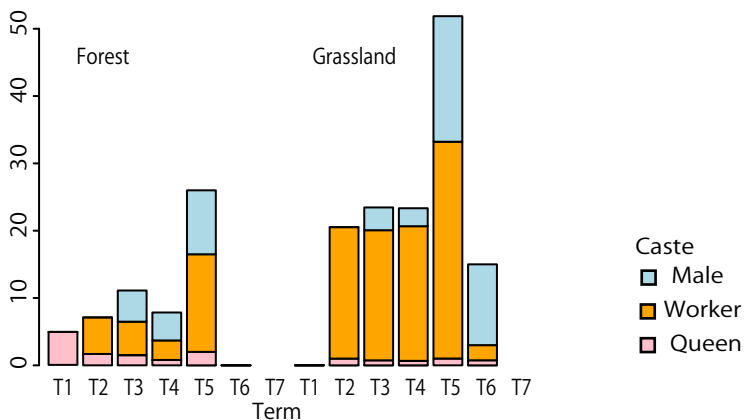


Fig. 2-2: Seasonal change in the bumble bee density in 2012 by bumble bee species (urban-forest landscape(a), natural-forest landscape (b)) and by caste (urban-forest landscape(c), natural-forest landscape (d)).

Bumble bees were counted while walking around the habitats (searching method).

Hypo: *Bombus hypocrita sapporoensis*, Ter: *B. terrestris*, Hypno: *B. hypnorum koropokkrus*, Ard: *B. ardens sakagami*, Div: *B. diversus tersatus*, Pse: *B. pseudobaicalensis*, Yezo: *B. yezoensis*.

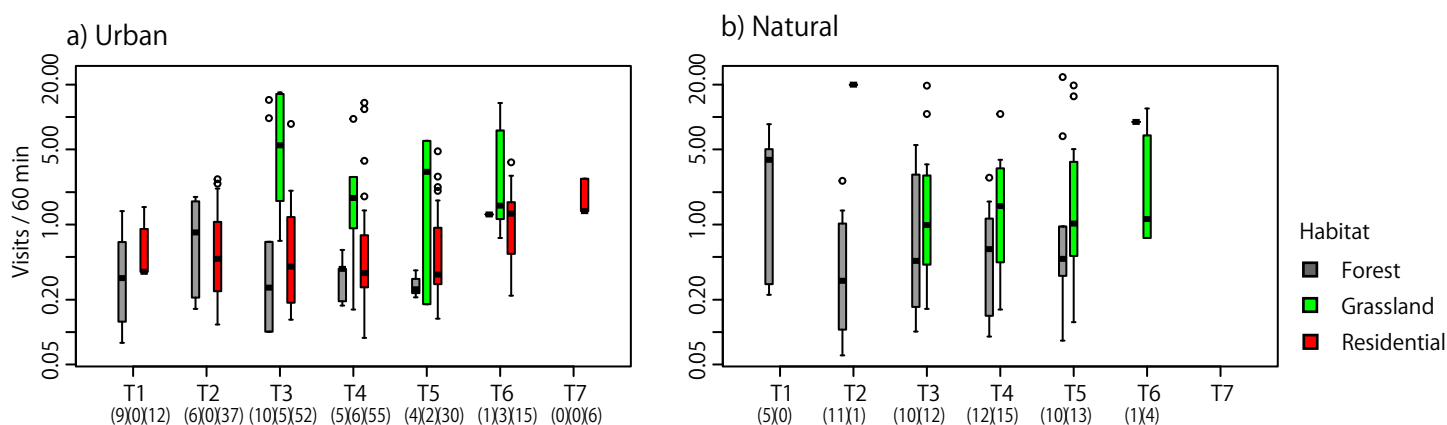


Fig. 2-3: Seasonal change in the visitation frequency of bumble bees to floral species in the urban-forest (a) and natural-forest landscape (b). Vertical axes represent bumble bee visits per 60 min to individual floral species during the searching observation in 2010–2012.

The lower ends of the boxes represent the first quartiles and the uppers the third quartiles, the segments inside the boxes indicate the medians, whiskers above and below the boxes indicate the minimums and maximums, and outliers are represented by open circles. Figure in parentheses represent sample sizes.

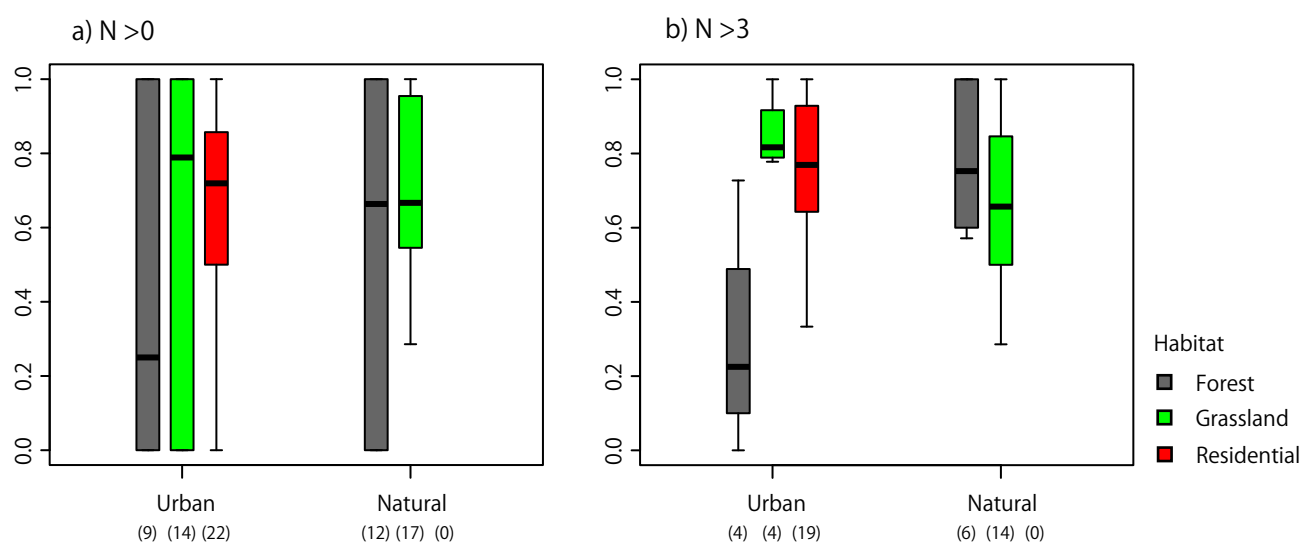


Fig. 2-4: Ratio of captured pollen-collecting females of each bumble bee species to all of the captured females of each bumble bee species in term3 and 4 (late July to middle August). a) Sample sizes in each species in each term were equal to or more than 1 (do not restrict sample sizes), b) Sample sizes in each species in each term were restricted to more than 4.

The lower ends of the boxes represent the first quartiles and the uppers the third quartiles, the segments inside the boxes indicate the medians, whiskers above and below the boxes indicate the minimums and maximums, and outliers are represented by open circles. Figure in parentheses represent sample sizes.



Fig. 2-5: Ratio of individual bumble bees by the number of outside-pollen species in body pollen. Figure in parentheses represent sample sizes.

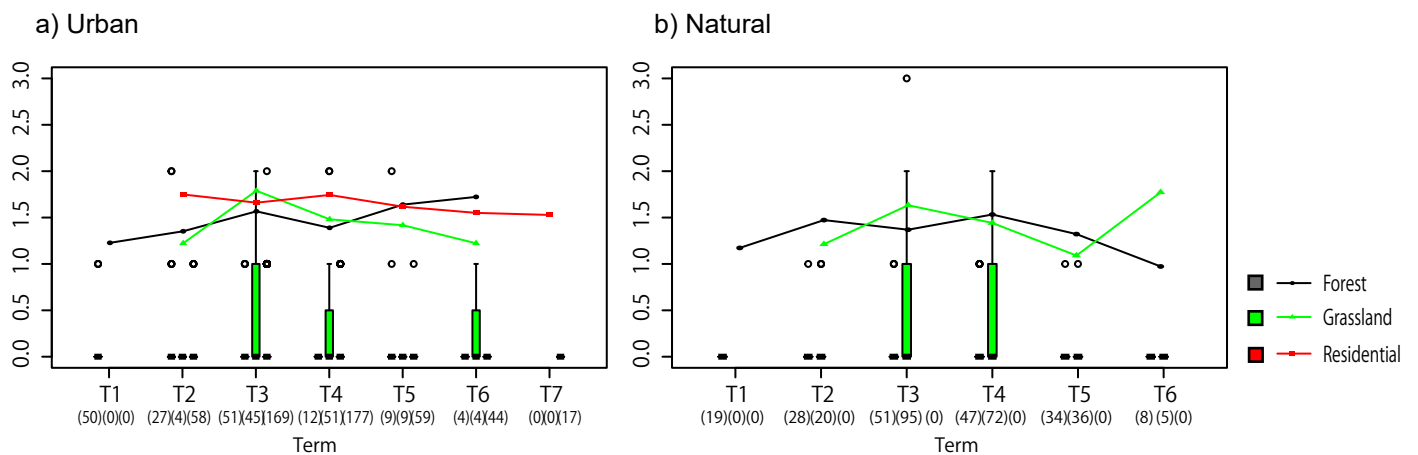


Fig. 2-6: Seasonal change in the number of outside-pollen species (boxes) and total pollen species (lines; points represent means) in the body pollen of individual bumble bees in the urban-forest (a) and natural-forest (b) landscape in 2010–2011. Boxes are of forest, grassland, and residential habitats, from left to right for each term. The lower ends of the boxes represent the first quartiles and the upper the third quartiles, the segments inside the boxes indicate the medians, whiskers above the boxes indicate the minimums, and outliers are represented by open circles. Figure in parentheses represent sample sizes.

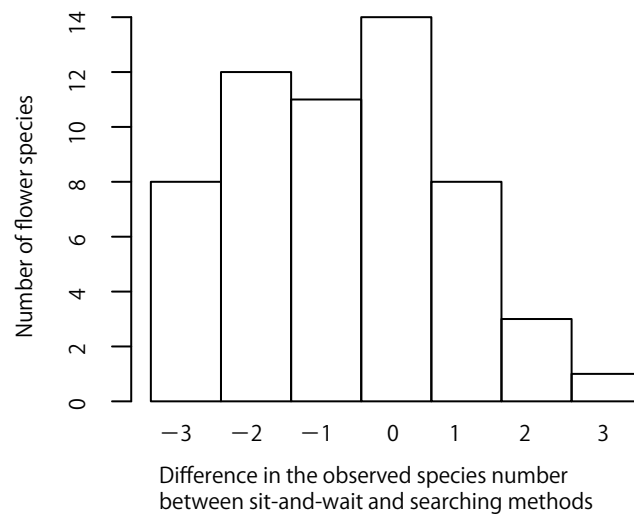


Fig. 2-S1: Comparison between sit-and-wait and searching methods. Differences between the number of bumble bee species observed foraging on a flower species in a sit-and-wait method and that in the searching method are represented in the horizontal line. Thirty-seven flower species that were observed in the both methods were included.

According to the GLMM assuming Poisson error distribution, in which observation method, year and landscape was included as fixed factors and plant species was included as a random factor, all explanatory variables including observation method were excluded by AIC.

Chapter 3

Spatiotemporal variability in the diet breadth and overlap among bumble bee species

3.1 Introduction

Diverse, species-rich or abundant floral resources often have positive effects on the abundance or diversity of pollinator communities (Potts *et al.* 2003; Hines & Hendrix 2005; Roulston & Goodell 2011; Bennett & Gratton 2013). However, the information about how each pollinator species responds to the change in the diversity of available resources is still limited (Kaluza *et al.* 2017). Kaluza *et al.* (2017) reported that the diversity of floral resources collected by generalist pollinators increased (diet expansion) as diversifying available floral resources. According to the network analysis by Spiesman & Gratton (2016), the increase in the niche availability resulted in the increase of modularity and complementary specialization, which would be translated to the acceleration of resource partitioning.

In the urban-forest landscape in which pollinators can access both native forest and managed gardens, urban gardens (residential habitat) hosted a large variety of floral species throughout the season compared to the natural vegetation (forest habitat), and abundant bumble bees foraged at the residential habitat (Chapter 1 & 2). At the residential habitat, bumble bees used larger proportion of floral species than at the forest habitat, while visitation frequency to targeting floral species was similar between the habitats (Chapter 2). Bumble bee species might respond to the diverse floral environment at the residential habitat by expanding diet breadth. At the same time, based on the report by Spiesman & Gratton (2016), more apparent resource partitioning among bumble bee species might be possible in the diverse resource environment at the residential habitat. In addition, the effects of diverse floral resources might become clearer in the middle of season when the floral

diversity becomes highest.

Floral structure at landscape level should affect the foraging decision of pollinators (Kunin 1997; Chittka *et al.* 2001; Hanoteaux *et al.* 2013). Although the proportion of floral species visited at the forest habitat was not affected by the presence of garden flowers, functional diversity of visited flowers within the forest habitat (diet breadth at the forest habitat) might be affected by the floral diversity of surrounding environment. Thus, in the urban-forest landscape where residential gardens are available next to the natural vegetation of forest, diet breadth of bumble bee species at the forest habitat might be affected by the floral diversity at the residential habitat.

The tongue length of bumble bees varies among species, and each species has suitable flower depth for foraging. The floral preferences of nectar foraging bumble bees highly depend on the tongue length of individual species; the larger the dissimilarity of the tongue length, the lower the diet overlap between the bumble bee species (Goulson *et al.* 2008b). Higher diet partitioning (lower diet overlap) between nectar foragers might be expected for species pairs of large tongue length dissimilarity. In contrast to the nectar-foraging, the floral preference of pollen foragers might be independent of the tongue length (Goulson *et al.* 2008b). As explained in Introduction of Chapter 2, pollens on the pollinators' body (body pollen) reflect the nectar and pollen source flowers, while pollens collected in the corbiculae (load pollen) reflect exclusively pollen source flowers. Species-specific preference for pollen source flowers might result in lower diet overlap between bumble bee species based on the load pollen compared to the body pollen.

In this chapter, I described the foraging patterns of bumble bee species based on the record of pollen species and the number of pollen grains found in the body and load pollen. By comparing the diet breadth or overlap of bumble bee species between habitats in the urban-forest landscape, I examined the following hypotheses: 1) diet breadth would be expanded under highly diverse floral environment (residential habitat), 2) diet overlap would be small under highly diverse floral

environment (residential habitat and middle of season). By examining the diet breadth of bumble bee species at the forest habitat, I tested the third hypothesis: 3) diet breadth of bumble bees at the forest habitat would be affected by the floral diversity of surrounding habitats. Finally, hypotheses concerning preferences of bumble bees were examined: 4) diet overlap would be smaller for species pairs with higher tongue length dissimilarity and 5) diet overlap would be smaller for pollen collection than for nectar foraging.

3.2 Analysis

Diet breadth of bumble bee species and diet overlap between bumble bee species within the habitat were calculated for each observation term, based on the body or load pollen and floral traits. The observation period was divided into seven 4-week terms, and floral species blooming and bumble bees captured in each term were pooled. The number of bumble bees captured on a same floral species was controlled up to five per week per species to prevent sampling bias. Individuals that carried no pollen, only unidentified pollen, only outside pollen of the captured habitat or only pollen of species lacking data of nectar or pollen volume were excluded. Floral species lacking both nectar and pollen volume measurements was also excluded from the analyses.

Diet breadth

Diet breadth of bumble bee species was calculated using relative preferences of bumble bee species weighted by functional distances between flowers (De Cáceres *et al.* 2011). Pollen grains unidentified or supposed to be derived from outside of the captured habitat were excluded. Relative preference of bumble bee species to floral species j (f_j) was determined by weighting relative use by the log-transformed flower abundance:

$$f_j = \frac{p_j/q_j}{\sum_j^S p_j/q_j},$$

where p_j is the relative use of flower j by the bumble bee species, which is calculated by summing up the proportion of pollen species j for all bumble bee individuals within the species, and q_j is the relative abundance of log-transformed abundance of flower j (for details about the method for estimating flower abundances, see Analysis in Chapter 1).

Diet breadth, which was derived from Rao's quadratic entropy (Rao's Q; Rao 1982) was calculated as

$$\text{Diet breadth} = \frac{1}{2} \sum_{i=1}^S \sum_{j=1}^S f_i f_j d_{ij}^2 \quad (\text{De Cáceres } et al. 2011),$$

where d_{ij} is a functional distance between the floral species i and j , and S is the total number of floral species. Functional distance between species was determined by Gower's dissimilarity (Gower 1971) using measured five functional traits, i.e., potential nectar and average pollen volume per flower unit, growth form, floral morphology, and sugar concentration of nectar (for the classification details, see Table 0-1 in Materials and Methods).

Diet overlap

Diet overlap between bumble bee species within observation term was calculated taking account of the resemblance between flowers (De Cáceres *et al.* 2011).

$$\text{Diet overlap} = \frac{\sum_{i=1}^S \sum_{j=1}^S f_{1i} f_{2j} (1 - d_{ij}^2)}{\sqrt{\sum_{i=1}^S \sum_{j=1}^S f_{1i} f_{1j} (1 - d_{ij}^2) \sum_{i=1}^S \sum_{j=1}^S f_{2i} f_{2j} (1 - d_{ij}^2)}}$$

This is a generalization of a measure of niche overlap using cosine of the angle between the two vectors of relative resource preferences, and the value ranges from 0 to 1 (complete overlap; De Cáceres *et al.* 2011). The diet overlap will not be 0 even if the species 1 and 2 utilized completely different flowers, because similarity of the floral species is taken into account.

Floral resource space

To visualize the floral resource space and plot bumble bee individuals within the space, PCoA axes values were calculated based on the functional distances between floral species bloomed in 2010–11, when the sampling of body and load pollen was made. Gower dissimilarity (Gower 1971) was used for the functional distances between flowers, based on five functional traits of floral species. Since negative eigenvalues are imaginary axes that cannot be represented in a Euclidean space, correction method described by (Cailliez 1983) was adopted for negative values. Floral resource space was visualized by the first and second axes, and the positions of bumble bee individuals were computed by averaging the coordinates of the flowers preferred by the bumble bee species:

$$\bar{x}_l = \sum_{j=1}^S f_j x_{jl},$$

where x_{jl} is the position of the j -th floral species on the l -th axis, and S is the floral richness (De Cáceres *et al.* 2011). Although pollens of *Hydrangea* species were pooled into one group in the analysis of body and load pollen (see Materials and Methods), floral coordinates of the taxon (cu39) in the resource space were calculated based on the species within the habitat (i.e., floral traits of cultivated *Hydrangea* spp. was used for the coordinate in the residential habitat, and the mean traits of *Hydrangea serrata* var. *yezoensis* and *Hydrangea petiolaris* was used for the coordinate in the forest habitat). Similarly, because the nectar volume of *Aconitum sachalinense* subsp. *yezoense* (fo06) differed between habitats, floral coordinates of the species was separately calculated for forest and grassland habitats. The pollens of *Impatiens* spp. (co17; *Impatiens textori* and *I. noli-tangere*) were also pooled in the pollen analysis, but the coordinate for *Impatiens* spp. was based on *I. noli-tangere* because *I. textori* lacked nectar and pollen data.

Effects of floral diversity within the habitat on diet breadth

To examine whether the diet breadth of bumble bee species is affected by floral diversity within

the foraging habitat, I compared diet breadth between habitats in the urban-forest landscape by GLMMs assuming a Gamma error distribution with log-link function in which habitat, floral functional diversity (Rao's Q; for details, see Chapter 1) or floral richness, and year were included as fixed factors, observation term and bumble bee species as random factors, and sample size (the number of bumble bee individuals in each species, habitat and observation term) as an offset term. Because diet breadth asymptotically increased with sample size and reached at stable value around 10 individuals (Fig. 3-S1), it would underestimate the diet breadth of species with large samples if I simply offset diet breadth by the sample size. Therefore, expected diet breadth at 10 individuals was used for samples more than 10 individuals. Expected diet breadth was calculated using rarefaction in which I randomly sub-sampled 10 individuals without replacement, replicated the procedure 100 times, and averaged the resulting diet breadth of 100 trails. Bumble bee species with sample size 1 was excluded from the GLMM because these data occasionally became outliers. GLMMs were performed separately for body and load pollen.

Effects of floral diversity of surrounding habitats on diet breadth at the forest habitat

To examine the effects of floral diversity of surrounding habitats on the diet breadth of bumble bee species within the forest habitat, I performed a GLMM assuming a gamma error distribution with log-link function in which floral diversity (Rao's Q) or floral species richness of within and outside the forest habitat, landscape and year were included as fixed factors, observation term and bumble bee species as random factors, and sample size as an offset term. Similar to the analyses for the effects of floral diversity within the foraging habitat, expected diet overlap at the sample size 10 was used for samples which included more than 10 individuals. The diet breadth based on body pollen was used because of the limited number of collected load pollen at the forest habitat in the urban-forest landscape.

Comparison of the diet overlap

To examine whether the degree of diet overlap differs between the habitats in the urban-forest landscape, I carried out permutation-based ANOVAs for each habitat pairs within the landscape, with the fixed factor of habitat and the random factor of tongue-length dissimilarity class. Bumble bees were classified into three tongue-length classes based on the allometry between head width and tongue length; *Bombus hypocrita sapporoensis* and *B. terrestris* were classified into short-tongued, *B. hypnorum koropokkrus*, *B. ardens sakagami* and *B. pseudobaicalensis* into medium-tongued, and *B. diversus tersatus* and *B. yezoensis* into long-tongued species (Kinoda 2012). Based on the combination of the tongue-length classes, four tongue-length dissimilarity classes were defined: between the same tongue length, between short and medium, between medium and long, and between short and long. To examine whether the degree of diet overlap differs between the tongue-length dissimilarity classes, seasons, or usage types (body or load pollen), permutation-based ANOVAs were carried out for each habitat in each landscape, in which tongue-length dissimilarity class, observation term, or usage type, respectively, was included as a fixed factor. Observation term was included as a random factor for the comparison between tongue-length dissimilarity classes, and tongue-length dissimilarity class was included as a random factor for the comparisons between seasons and between usage types. As in the analyses of diet breadth, species of sample size 1 was excluded from the analyses.

To evaluate the effects of the explanatory variables and select the best-fit GLMMs, I used Akaike's information criterion (AIC). Statistical analyses are performed using R version 3.1.2 (R Core Team 2014). GLMMs and permutation-based ANOVAs were performed using the *glmer* (package *lme4*) and *aovp* (package *lmPerm*) functions (Wheeler *et al.* 2016; Bates *et al.* 2017). PCoA axes for floral resource space were calculated through *dbFD* function in the package *FD*.

3.3 Results

In total, body and/or load pollen from 804 and 433 bumble bees were included in the analysis in the urban-forest landscape and the natural-forest landscape, respectively. Available body-pollen samples were 1168 (urban: 799, natural: 369), and available load-pollen samples were 616 (urban: 369, natural: 247; Table 3-1). Because of the low density of pollen grains in the samples, total number of pollen grains was less than 100 in 637 and 362 samples of the body and load pollen, respectively (Fig. 3-S2).

Bumble bees visited 33%–73% of the available floral species in each habitat as nectar and/or pollen source (based on body pollen), and visited 19%–72% of the available floral species as pollen source (based on load pollen; Table 3-2).

Effects of floral diversity within the habitat on diet breadth

Individual bumble bees at the residential habitat widely spread within the resource space than individuals at the forest habitat in accordance with the spread of the floral species (Fig. 3-1). In addition, distribution of individuals within the residential habitat showed seasonal change as the number of floral species fluctuated seasonally (Fig. 3-1). Even individuals of the same species widely spread within the resource space, utilizing diverse floral species. Thus, diet breadth of bumble bee species in each habitat of the urban-forest landscape was positively correlated with the floral diversity of the foraging habitat (body pollen: $p < 0.01$, $t = 4.75$; load pollen: $p = 0.01$, $t = 2.58$; Fig. 3-2), while effects of habitat, floral richness and year were excluded by AIC in the best-fit GLMM.

Effects of floral diversity of surrounding habitats on diet breadth at the forest habitat

The diet breadth at the forest habitat positively correlated with the floral richness of the forest habitat ($p = 0.05$, $t = 2.00$). The effects of landscape and year were additionally selected by AIC, but they were not significant (landscape: $p = 0.62$, $t = -0.50$; year: $p = 0.19$, $t = -1.32$), while the floral diversity (Rao's Q) of the forest habitat and the floral diversity or richness of surrounding habitats were excluded by AIC in the best-fit GLMM. This result indicates that the diet breadth at the forest habitat was independent of the floral diversity of surrounding habitats.

Diet overlap between habitats in the urban-forest landscape

Floral-use patterns of bumble bees in the resource space were highly overlapping among species at all habitats across season (Fig. 3-1). Especially at the grassland habitat, most of the individuals highly concentrated in one or two floral species; mainly *Trifolium pretense* (gr01) and *Trifolium repens* (gr08) in the urban-forest landscape. Between-habitat comparison of diet overlap revealed that bumble bee species at the forest and residential habitats had lower diet overlaps based on body-pollen compared to those at the grassland habitat (forest: $p < 0.01$, $df = 1$; residential: $p < 0.01$, $df = 1$; Table 3-3), while there was no difference in diet overlaps between the forest and residential habitats ($p = 0.76$, $df = 1$). On the other hand, the diet overlaps based on load-pollen did not differ in any combination of habitats (forest–residential: $p = 0.07$, $df = 1$; forest–grassland: could not be computed due to small sample size; residential–grassland: $p = 0.055$, $df = 1$).

Seasonal change in the diet overlap

Although the floral-use patterns were highly overlapping among species in the resource space, individual species tended to show specific distributions when the floral species were widely distributed in the resource space (Fig. 3-1). Seasonal changes in the diet overlap were detected at the residential habitat, where the floral species-richness was highest (body pollen: $p = 0.03$, $df = 4$; load pollen: $p < 0.01$, $df = 3$; Fig. 3-3). Although a significant seasonal variation in the diet overlaps

(body-pollen based) was detected also at the grassland habitat of the urban-forest landscape ($p < 0.01$, $df = 2$), the diet overlaps were retained at high level throughout the season (Fig. 3-1 & 3-3). There was no significant seasonality in the diet overlaps (body-pollen based) at the forest habitat ($p = 1.00$, $df = 2$). Seasonal trends in the diet overlaps based on the load-pollen could not be computed at the grassland and forest habitats of the urban-forest landscape because of the small sample sizes. In the natural-forest landscape, seasonal changes in the diet overlaps were not significant at both of the forest habitat (body pollen: $p = 0.22$, $df = 4$; load pollen: $p = 1.00$, $df = 1$) and the grassland habitat (body pollen: $p = 0.52$, $df = 2$; load pollen: $p = 0.94$, $df = 2$).

Comparing diet overlap between body and load pollen

When the floral-use patterns of individual bumble bee species were compared in the resource space, the load-pollen based distribution tended to be aggregated more than the body-pollen based distribution at the residential habitat (Fig. 3-1). The permutation-based ANOVA revealed that the diet overlaps based on the load-pollen were significantly smaller than that based on the body-pollen at the residential habitat ($p < 0.01$, $df = 1$; Fig. 3-3). However, there were no differences between the body-pollen and load-pollen based diet overlaps at the forest habitat ($p = 0.49$, $df = 1$) and the grassland habitat ($p = 0.49$, $df = 1$) of the urban-forest landscape, and at the forest habitat ($p = 0.84$, $df = 1$) and the grassland habitat ($p = 0.56$, $df = 1$) of the natural-forest landscape.

Effects of tongue length on the diet overlap

The tongue-length dissimilarity between bumble bee species was related to the degree of diet overlaps at both of the residential habitat (body pollen: $p < 0.01$, $df = 3$; load pollen: $p < 0.01$, $df = 3$; Table 3-4) and the grassland habitat (body pollen: $p = 0.06$, $d = 3$; load pollen: $p = 0.02$, $df = 3$; Table 3-5) of the natural-forest landscape. Pairs of long- and short-tongued species showed low diet overlaps compared to other pairs. This trend was apparent in the resource space of these habitats in

which the short-tongued *Bombus hypocrita sapporoensis* (blue dots) were distributed far from the long-tongued *B. diversus tersatus* (green dots in Fig. 3-1). Floral use of *B. hypocrita sapporoensis* was strongly limited in the pollen collection from *Hydrangea* spp. (cu39) at the residential habitat compared to other species (Fig. 3-1). Pairs of short- and medium-tongued species had the lowest load-pollen based diet overlaps at the residential habitat (Table 3-4). Diet overlaps were not associated with the difference in the tongue length at the forest habitat (body pollen: $p = 1.00$, $df = 2$; no test for the load pollen because of small sample size) and the grassland habitat (body pollen: $p = 0.26$, $df = 3$; no test for the load pollen because of small sample size) of the urban-forest landscape, and at the forest habitat (body pollen: $p = 0.45$, $df = 3$; load pollen: $p = 1.00$, $df = 1$) of the natural-forest landscape.

3.4 Discussion

Effects of floral diversity within the habitat on the diet breadth

Bumble bees responded to the diverse foraging environment by expanding their diet breadth at the population level. Diet breadth at each habitat was positively correlated with the floral diversity (Rao's Q) of the habitat in the urban-forest landscape. Although high functional diversity of floral resources could be disadvantageous for generalist pollinators because of the cost of identification and handling of different type of flowers (Fornoff *et al.* 2016), individual bumble bees targeted only for a few species (1.64 ± 0.78 species, $n = 770$ in body-pollen; Chapter 2). Thus, the expansion of diet breadth was achieved by diversifying target floral species among bumble bee individuals (Fig. 3-1). This trend was consistent with the report by Heinrich (1976b) in which bumble bees had a large variation in the floral preference among individuals within a same species in addition to the difference among species. Fontaine *et al.* (2008) reported that when resource gained by specialization is not enough for colony growth, bumble bees shifted to more generalized foraging at

the colony level. Similar mechanism might work in the present study when floral environment became diverse spatially or temporally. Therefore, bumble bees can flexibly adjust the floral-use depending on the floral conditions of the foraging habitat.

Floral species richness was higher at the residential habitat than at the forest habitat, while floral diversity did not differ significantly between the habitats (Chapter 1). Because floral diversity (Rao's Q) explained diet breadth better than floral species richness, individual bumble bee species utilized diverse flowers at similar level between the forest and residential habitats even under environments with different floral richness. In the analyses of diet breadth, I included sample size (the number of individuals in each term in each habitat) as an offset term in order to compare diet breadth independent of sampling bias. However, because population sizes at the forest and residential habitat might differ (different bumble bee density; Chapter 2), the effects of floral diversity on the real foraging diversity of bumble bee species might be more complex. Experimental approaches at the colony level are necessary to clarify this possibility.

Diet segregation between species

Floral choice of bumble bee species was very similar to each other, with high diet overlap in all habitats (Table 3-3). The values of diet overlap were higher than those reported by Inoue *et al.* (2007) and Goulson & Darvill (2004). This may be due to the index employed, in which similarity in the floral functional traits are taken into account (De Cáceres *et al.* 2011). Although there was a high similarity in the floral choice between species, diet overlap between species decreased responding to the increasing floral diversity. When the body-pollen based diet overlaps were compared among the habitats, the diet overlap was lower at the forest and residential habitats, where diverse floral species existed, than that at the grassland habitat (Table 3-3). Although the diet overlap did not differ between the residential and forest habitats, seasonal change in the diet overlap was detected only at

the residential habitat (although significant seasonal variation was detected also at the grassland habitat of the urban-forest landscape, it would be ecologically meaningless because the diet overlap kept almost one; Fig. 3-4). The body-pollen based diet overlap at the residential habitat was lowest in the middle of season when floral richness was highest. Diverse floral resources may increase niche complementarity among pollinator groups (Spiesman & Gratton 2016; Venjakob *et al.* 2016), i.e., promotion of niche segregation. Similarly, niche segregation among bumble bee species might be the results of high species diversity of herbaceous plants (Kato *et al.* 1993). Even morphologically quite similar bumble bee species could exhibit different floral preferences (Scriven *et al.* 2016). Such species-specific preference might become clearer under diverse floral environment even with the varying preference among individual bumble bees.

Contrast to the clear seasonal change in the diet overlap among bumble bees at the residential habitat, the diet overlap of hummingbirds did not show seasonality because they kept foraging on flowers with the most suitable depth (Weinstein & Graham 2017). Since bumble bees are rather generalist pollinators (Heinrich 1976a), they could flexibly respond to the resource environment. However, apparent seasonality in the diet overlaps was not detected at the forest and grassland habitats in both landscapes. Seasonal variation in the floral diversity of those habitats might not be large enough to affect the diet overlap between species.

Species with similar tongue length tend to have similar choice of nectar foraging flowers (Goulson & Darvill 2004) but not in the pollen foraging (Goulson *et al.* 2008b). Also in the present study, pairs of short- and long-tongued species showed most dissimilar floral choice based on body-pollen (which reflect both nectar and pollen source flowers) at the residential habitat of the urban-forest landscape and at the grassland habitat of the natural-forest landscape (Tables 3-4 & 3-5). In these habitats, pollen-source flowers that were preferred by short-tongued *B. hypocrita sapporoensis* (*Hydrangea* spp. at the residential habitat of the urban-forest landscape and *Lupinus*

polyphyllus at the grassland habitat in the natural forest landscape) were abundant (Chapter 1), and the functional traits of these plants were quite different from the floral species preferred by other bumble bee species (Fig. 3-1). The preference of *B. hypocrita sapporoensis* to *Hydrangea* spp. was much stronger in the pollen collection at the residential habitat, and almost all *B. hypocrita sapporoensis* concentrated on the visits to *Hydrangea* spp. Thus, the diet of the short-tongued species at the residential habitat was quite different from species of any other tongue-length classes (Table 3-4). Heinrich (1976c) reported that short-tongued species were more reliant on the pollen-source flowers than long-tongued species.

Other bumble bee species than *B. hypocrita sapporoensis* also seemed to show more species-specific foraging for pollen collection compared to nectar foraging at the floral-rich residential habitat (Fig. 3-1); thus diet overlap based on load-pollen became lower than that based on body-pollen at the residential habitat.

Effects of surrounding habitats on the diet breadth at the forest habitat

Although floral diversity within the forest habitat was positively related to the diet breadth within the forest habitat, floral diversity of surrounding habitats did not influence the diet breadth at the forest habitat. Thus, garden flowers did not have any effects on the foraging diversity of bumble bee species at the forest habitat. Similarly, floral choice at the forest habitat (the proportion of floral species visited by bumble bees) was not affected by the presence of gardens (Chapter 2). On the other hand, bumble bees preferred the residential habitat to the forest habitat in which larger proportion of floral species was used in comparison with the forest habitat (Chapter 2). Therefore, garden flowers might affect habitat choice of bumble bees, but not affect the foraging behaviour of bumble bees outside of the gardens.

3.5 Conclusions

Bumble bee species expanded diet breadth in response to the increase in the floral diversity (Rao's Q). Although the diet breadth and diet overlap at the residential habitat did not differ from those at the forest habitat, higher floral richness at the residential habitat might result in the seasonal diet segregation, which was not detected at the forest habitat. In addition, because gardens could host an abundant floral species that was preferred by short-tongued species, pollen-source diet segregation might be further promoted at the residential habitat. Thus, diverse pollinators might be able to coexist at the floral rich residential habitat. Because foraging behaviour of bumble bees at the forest habitat was not affected by the surrounding species-rich residential habitat, ecological impacts of garden flowers may be limited to local scale rather than the landscape scale.

Table 3-1: Number of samples included in the analyses of body and load pollen. Individuals that carried only unidentified pollen, only outside pollen of the captured habitat or only pollen of species lacking data of nectar or pollen volume were excluded.

		Ard			Div			Hypno			Hypo			Pse			Ter			Yezo			Total				
		W	Q	M	W	Q	M	W	Q	M	W	Q	M	W	Q	M	W	Q	M	W	Q	M	W	Q	M	All	
Urban	BP	15	23	20	12	25	8	3	4	10	1	23	2	0	0	0	0	2	0	0	0	0	0	31	77	40	148
	Grassland	2	0	2	30	0	2	3	0	10	2	1	8	41	4	2	1	2	0	0	0	0	0	79	7	24	110
	Residential	78	12	21	142	11	10	28	3	24	34	1	13	42	7	5	38	10	42	0	0	0	362	44	115	521	
	Total	95	35	43	184	36	20	34	7	44	37	25	23	83	11	7	39	14	42	0	0	0	472	128	179	779	
LP	Forest	9	6		7	1		3	8		0	2		0	0		0	0		0	0		19	17	0	36	
	Grassland	0	0		26	0		2	0		1	0		32	2		0	0		0	0		61	2	0	63	
	Residential	54	5		107	7		20	1		30	0		25	1		19	1		0	0		255	15	0	270	
	Total	63	11		140	8		25	9		31	2		57	3		19	1		0	0		335	34	0	369	
Natural	BP	12	3	9	65	19	10	6	7	5	12	12	7	0	0	0	0	1	0	0	9	4	4	104	46	35	185
	Grassland	12	1	0	95	5	5	33	3	1	26	4	0	0	0	0	0	0	0	22	5	2	188	18	8	214	
	Total	24	4	9	160	24	15	39	10	6	38	16	7	0	0	0	0	1	0	31	9	6	292	64	43	399	
	LP	5	0		51	5		6	3		11	9		0	0		0	0		9	1		82	18	0	100	
	Grassland	4	0		75	2		23	0		21	2		0	0		0	0		17	3		140	7	0	147	
	Total	9	0		126	7		29	3		32	11		0	0		0	0		26	4		222	25	0	247	

* BP: Body pollen

* LP: Load pollen

Ard: *Bombus ardens sakagami*, Div: *B. diversus tersatus*, Hypno: *B. hypnorum koropokkrus*, Hypo: *B. hypocrita sapporoensis*, Pse: *B. pseudobaicalensis*, Ter: *B. terrestris*, Yezo: *B. yezoensis*.

Table 3-2: The number of visited floral species by bumble bees within the habitat based on the body and load pollen records. Figures in the parentheses represent the available number of species in which nectar or pollen volume measurements were conducted (= included in the calculation of diet breadth and overlap). The proportion of visited species to the available floral species in each habitat is indicated.

		Nectar or pollen source (body pollen)			Pollen source (load pollen)				
		Forest	%	Grassland	%	Residential	%	Forest	%
Urban	2010	17 (16)	42.5	6 (5)	50.0	29 (27)	67.4	8 (7)	20.0
	2011	17 (17)	39.5	3 (2)	33.3	39 (36)	50.0	8 (8)	18.6
Natural	2010	16 (14)	41.0	11 (10)	73.3			11 (11)	28.2
	2011	20 (16)	57.1	8 (7)	72.7			16 (15)	45.7
								8 (7)	72.7

Table 3-3: Diet overlap between bumble species in each habitat in the urban-forest landscape (mean $\pm$ SD). Diet overlap in each term (term 1–7) in each year (2010, 2011) was pooled. Sample sizes (the number of species pairs) are shown in parentheses, and different letters (a, b) within the same row represent statistical difference at $p < 0.05$ by permutation-based ANOVAs in which habitat was included as fixed effect and tongue-length dissimilarity class as a random term. Species of sample size 1 was excluded from the analyses.

	Forest	N	Grassland	N	Residential	N
Body pollen	0.94 $\pm$ 0.06a	(13)	1.00 $\pm$ 0.01 b	(16)	0.90 $\pm$ 0.12 a	(74)
Load pollen	0.99	a (1)	0.99 $\pm$ 0.01 a	(2)	0.80 $\pm$ 0.19 a	(51)

Table 3-4: Diet overlaps between tongue-length classes in the urban-forest landscape in 2010–11(mean $\pm$ SD). Upper: body-pollen based diet overlap, lower: load-pollen based diet overlap. Values in the parentheses represent the sample sizes (the number of species pairs). The asterisk represent the habitat in which tongue-length dissimilarity was significantly related to the body- and load- pollen based diet overlap at $p < 0.05$. Statistical analysis was performed by permutation-based ANOVAs in which tongue-length dissimilarity class was included as a fixed factor, and tterm was included as a random term.

		Short		Medium		Long	
Forest	Short	0.96	(1)				
		NA	(0)				
	Medium	0.96 $\pm$ 0.03	(6)	0.99 $\pm$ 0.01	(2)		
		0.99	(1)	NA	(0)		
	Long	NA	(0)	0.89 $\pm$ 0.08	(4)	NA	(0)
		NA	(0)	NA	(0)	NA	(0)
Grassland	Short	NA	(0)				
		NA	(0)				
	Medium	1.00 $\pm$ 0.00	(6)	1.00 $\pm$ 0.00	(4)		
		NA	(0)	NA	(0)		
	Long	1.00 $\pm$ 0.01	(2)	1.00 $\pm$ 0.01	(4)	NA	(0)
		NA	(0)	0.99 $\pm$ 0.01	(2)	NA	(0)
Residential *	Short	0.94 $\pm$ 0.09	(5)				
		0.97 $\pm$ 0.03	(3)				
	Medium	0.84 $\pm$ 0.14	(27)	0.96 $\pm$ 0.04	(13)		
		0.69 $\pm$ 0.20	(19)	0.92 $\pm$ 0.06	(7)		
	Long	0.85 $\pm$ 0.15	(12)	0.97 $\pm$ 0.02	(17)	NA	(0)
		0.72 $\pm$ 0.20	(9)	0.93 $\pm$ 0.07	(13)	NA	(0)

Short tongued : *B. hypocrita sapporoensis*, *B. terrestris*

Medium tongued : *B. hypnorum koropokkrus*, *B. ardens sakagami*, *B. pseudobaicalensis*

Long tongued : *B. diversus tersatus*

Table 3-5: Diet overlaps between tongue-length classes in the natural-forest landscape in 2010–11(mean ± SD). Upper: body-pollen based diet overlap, lower: load-pollen based diet overlap. Values in the parentheses represent the sample sizes (the number of species pairs). The asterisk represent the habitat in which tongue-length dissimilarity was significantly related to the body- and load- pollen based diet overlap at p <0.05. Statistical analysis was performed by permutation-based ANOVAs in which tongue-length dissimilarity class was included as a fixed factor, and tterm was included as a random term.

		Short		Medium		Long	
Forest	Short	NA	(0)				
		NA	(0)				
	Medium	0.98 ± 0.04	(4)	0.94 ± 0.05	(4)		
		1.00	(1)	NA	(0)		
	Long	0.89 ± 0.06	(5)	0.93 ± 0.06	(12)	0.97 ± 0.01	(4)
		0.74	(1)	0.91 ± 0.14	(3)	0.97 ± 0.03	(2)
Grassland *	Short	NA	(0)				
		NA	(0)				
	Medium	0.98 ± 0.03	(6)	0.99 ± 0.01	(3)		
		0.99 ± 0.01	(4)	0.99	(1)		
	Long	0.92 ± 0.07	(6)	0.97 ± 0.02	(8)	0.98 ± 0.01	(4)
		0.92 ± 0.05	(6)	0.97 ± 0.02	(6)	0.99 ± 0.01	(4)

Short tongued : *B. hypocrita sapporoensis*, *B. terrestris*
Medium tongued : *B. hypnorum koropokkrus*, *B. ardens sakagami*
Long tongued : *B. diversus tersatus*, *B. yezoensis*

a) Urban-forest landscape in 2010, based on body pollen

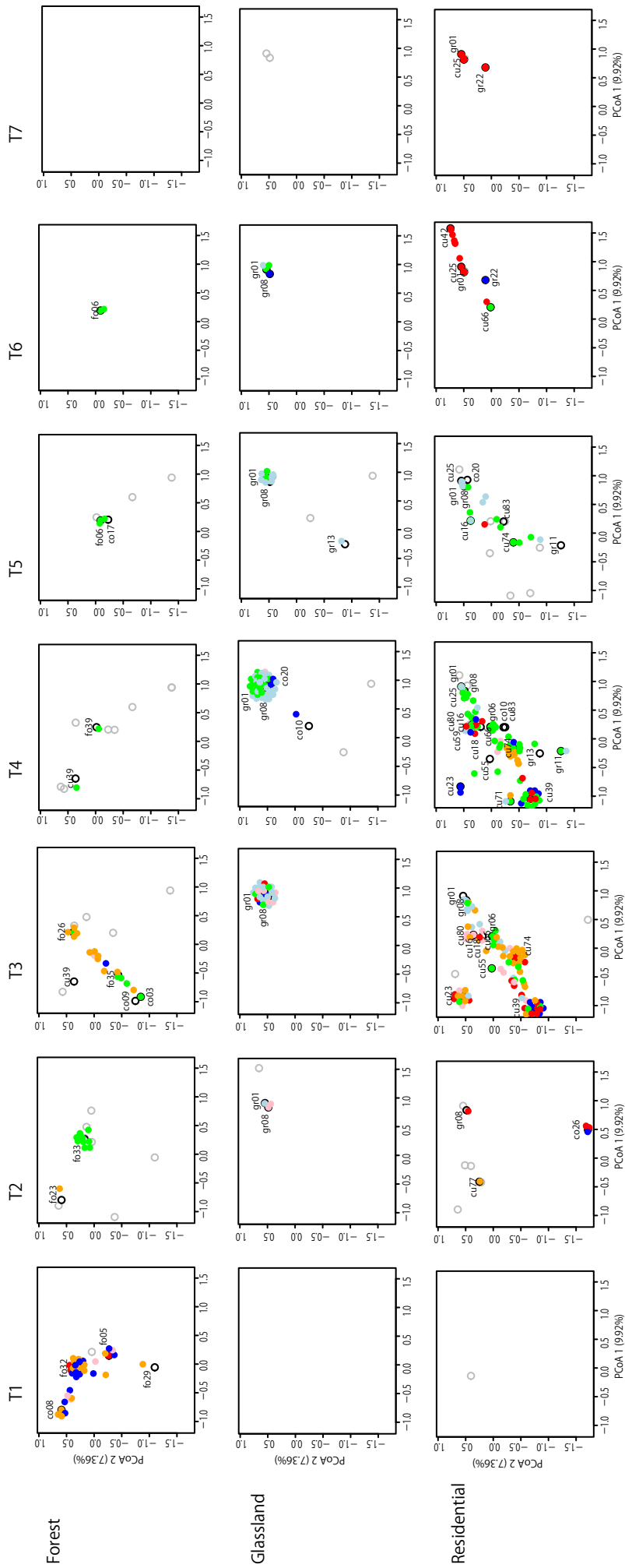
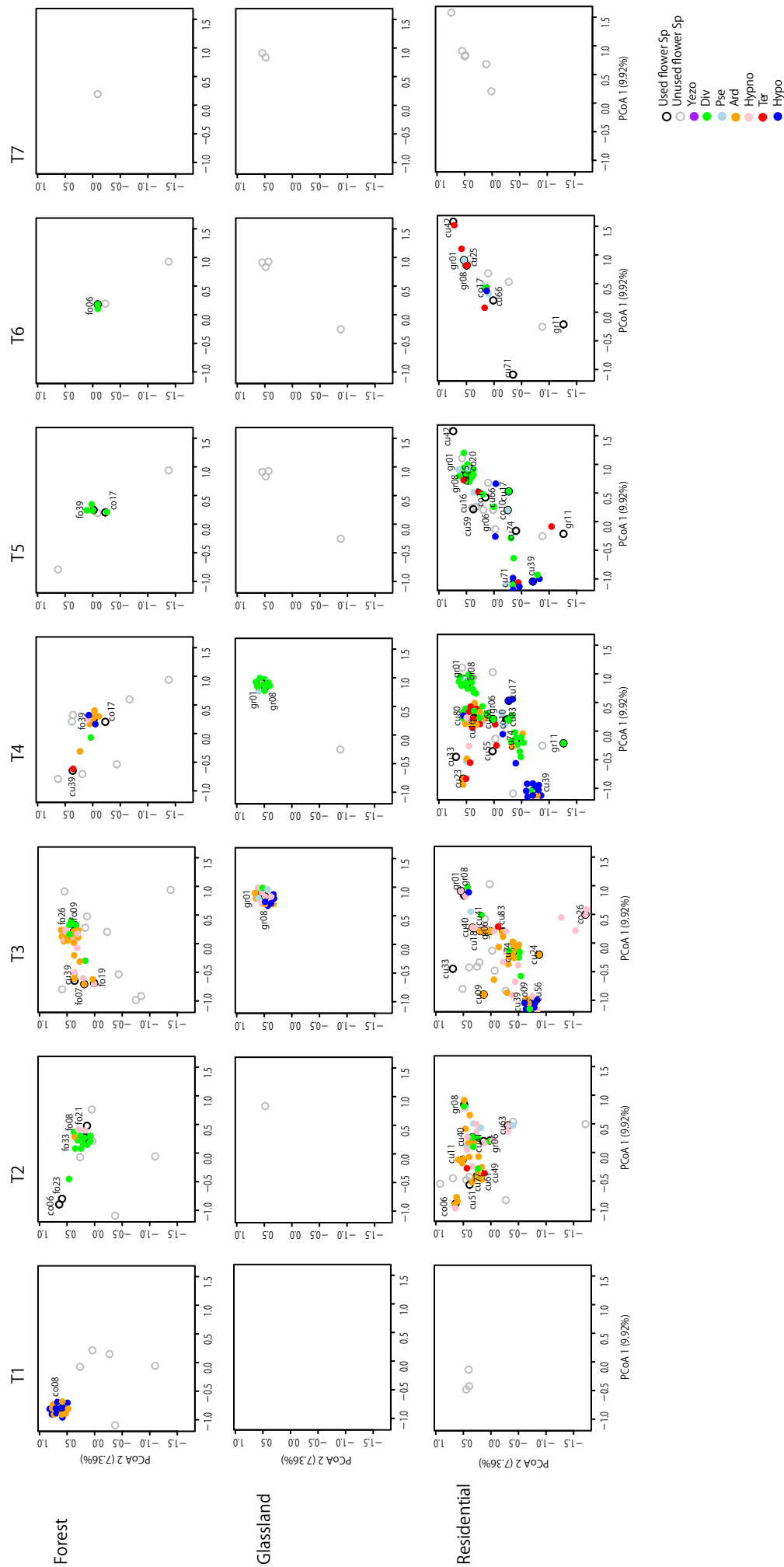


Fig. 3-1: Floral use variations of individual bumble bees in the floral resource space of the urban-forest (a-d) and natural-forest (e-h) landscapes in 2010 (a, c, e, g) and 2011 (b, d, f, h) across observation period (term T1 to term T7).

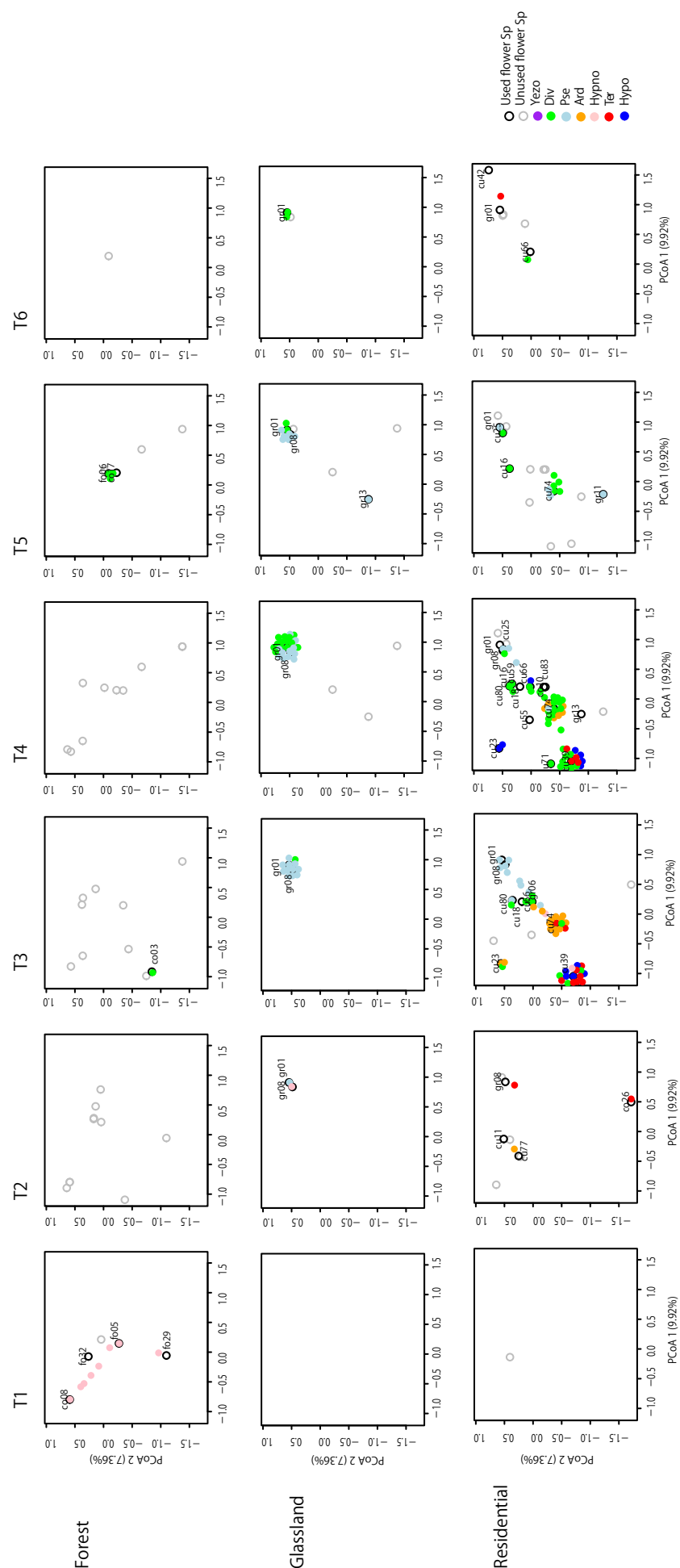
A resource space was constructed based on the dissimilarity among flowers, and bumble bee individuals were plotted based on the body pollen (a, b, e, f) or load pollen (c, d, g, h). If several individuals were located on the same floral species, dots of individuals were scattered around the focal point to show the number of individuals and species composition. Floral coordinates of cu39 were calculated based on the species within the habitat (i.e., floral traits of cultivated *Hydrangea* spp. was used for the coordinate in the residential habitat, and the mean traits of *Hydrangea serrata* var. *yesoensis* and *Hydrangea petiolaris* was used for the coordinate in the forest habitat). Similarly, because the nectar volume of *Aconitum sachalinense* subsp. *yezoense* (fo06) differed between habitats, floral coordinates of the species was separately calculated for forest and grassland habitats.

Plant IDs written in the plates conform to the Table 0-S1 in Chapter 1. Only IDs of used floral species are shown.

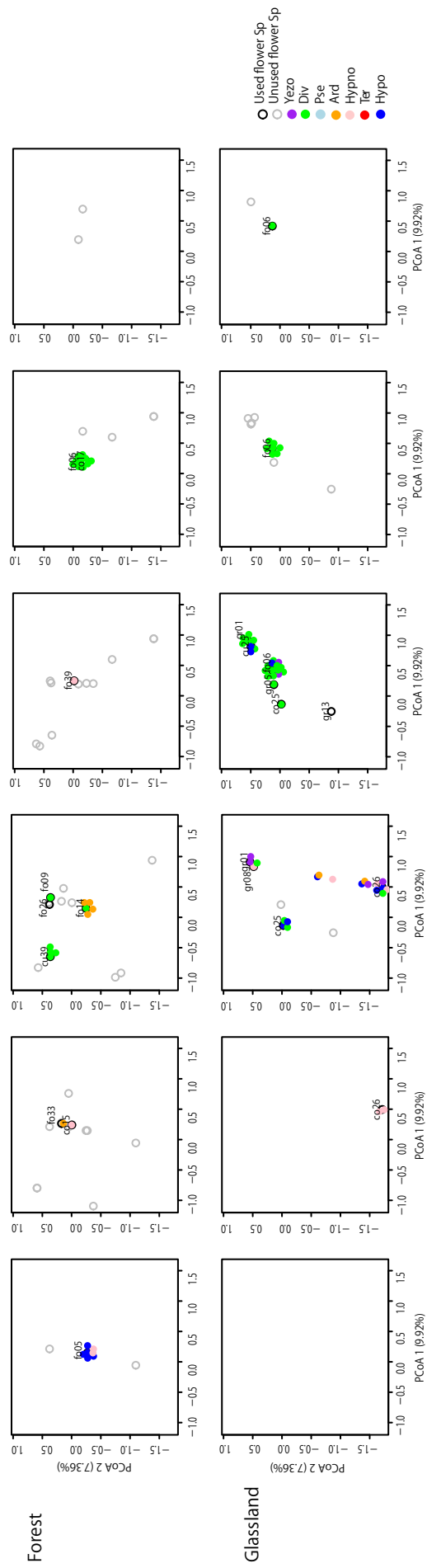
b) Urban-forest landscape in 2011, based on body pollen



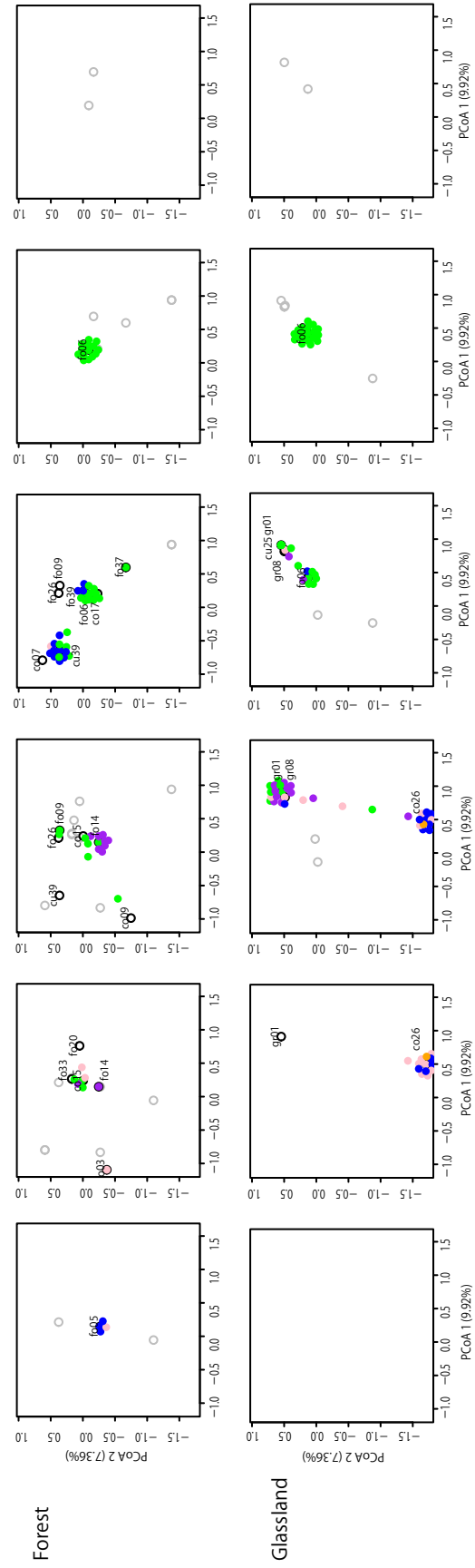
e) Urban-forest landscape in 2010, based on load pollen



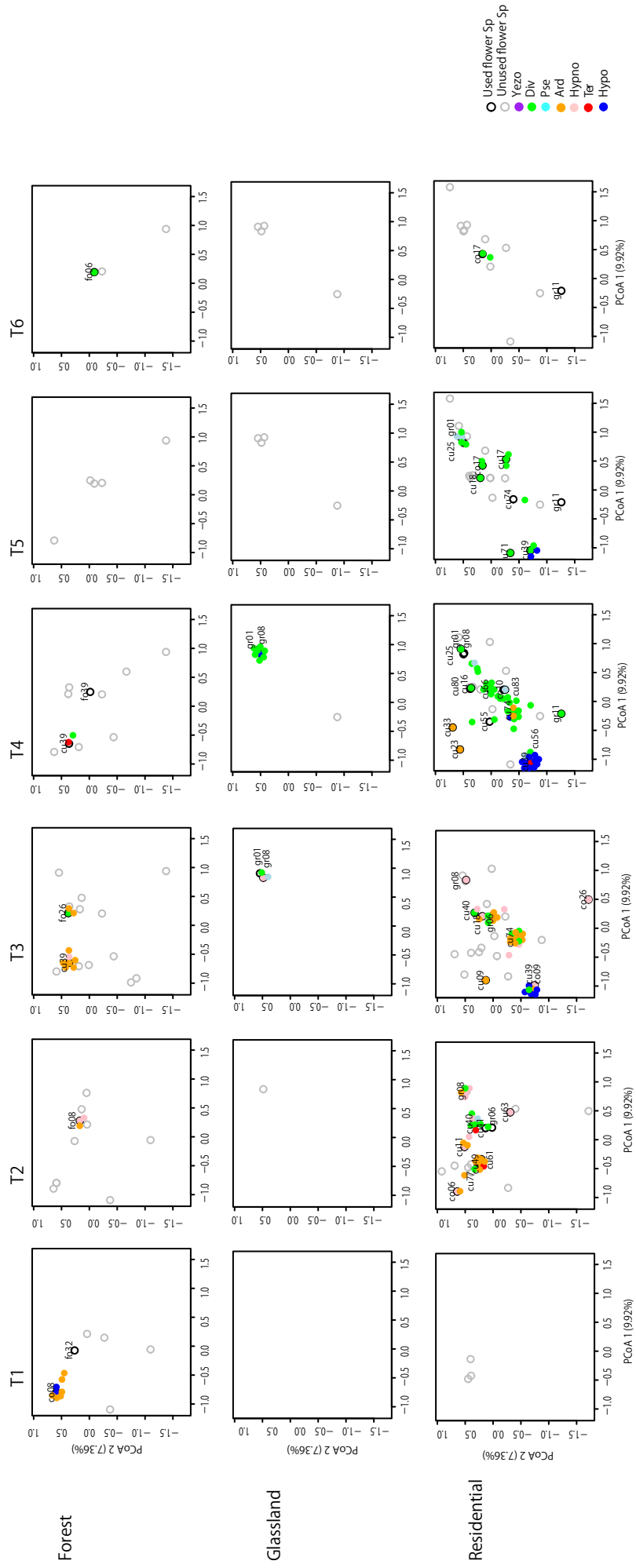
c) Natural-forest landscape in 2010, based on load pollen



d) Natural-forest landscape in 2011, based on load pollen



e) Urban-forest landscape in 2011, based on load pollen



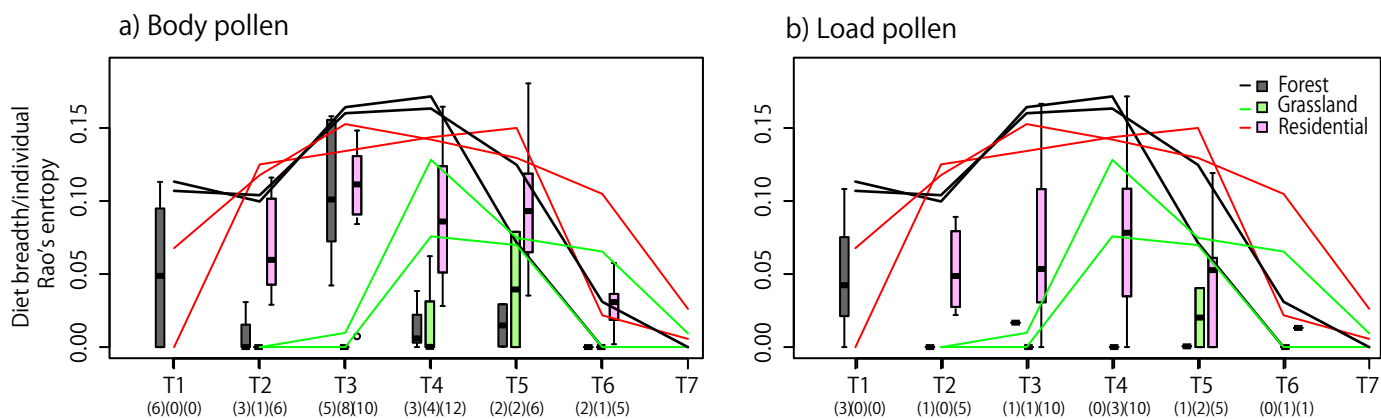


Fig. 3-2: Seasonal changes in the diet breadth of bumble bee species (boxes) and floral diversity (Rao' s quadratic entropy; lines) in the urban-forest landscape based on the body pollen (a) and load pollen (b) in 2010 and 2011. Lines in the same color in each plate represent floral diversity in 2010 and 2011. Sample size of the diet breadth (box) is shown in the parentheses. The lower ends of the boxes represent the first quartiles and the upper the third quartiles, the segments inside the boxes indicate medians, whiskers above and below the boxes indicate the minimums and maximums within the range of 1.5 times of interquartile range from the upper or the lower quartiles.

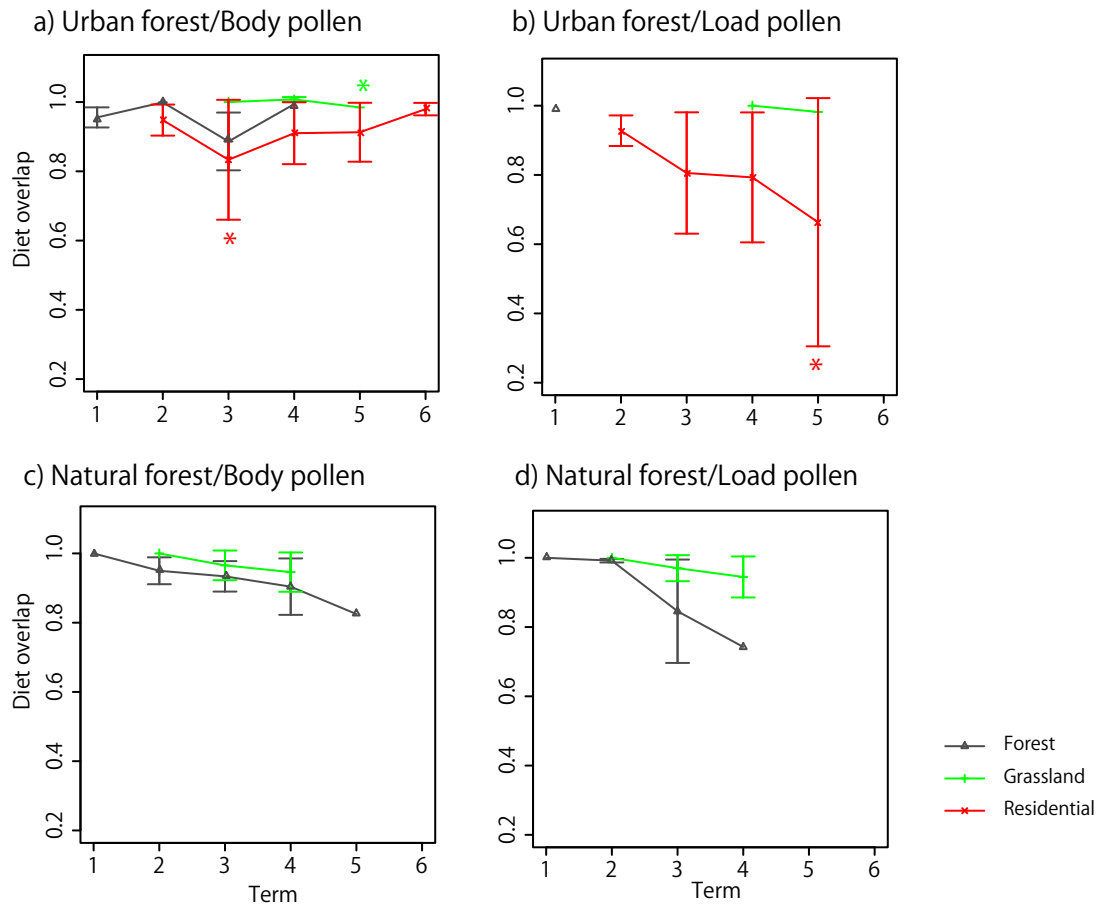
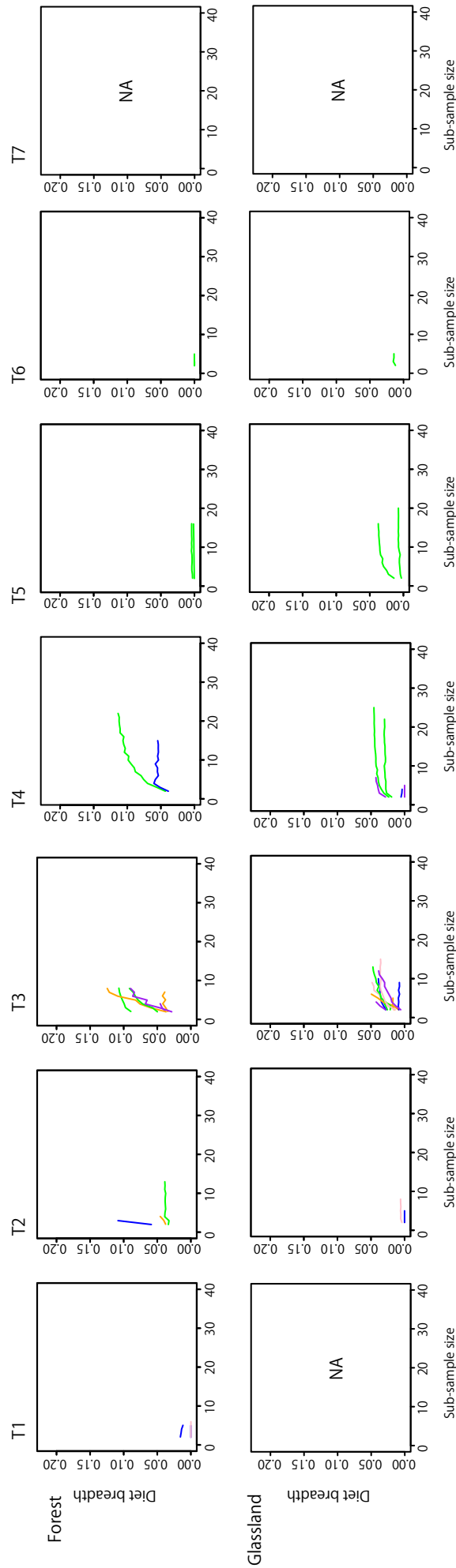


Fig. 3-3: Seasonal changes in the diet overlap in the urban-forest landscape based on the body (a) and load (b) pollen, and in the natural-forest landscape based on the body (c) and load (d) pollen in 2010–11. Each points and error bars represent means and SDs (year pooled). Asterisks represent significant seasonal difference within the habitat at $p < 0.05$, based on the permutation-based ANOVAs in which term was included as a fixed factor, and tongue-length dissimilarity class was included as an random term.

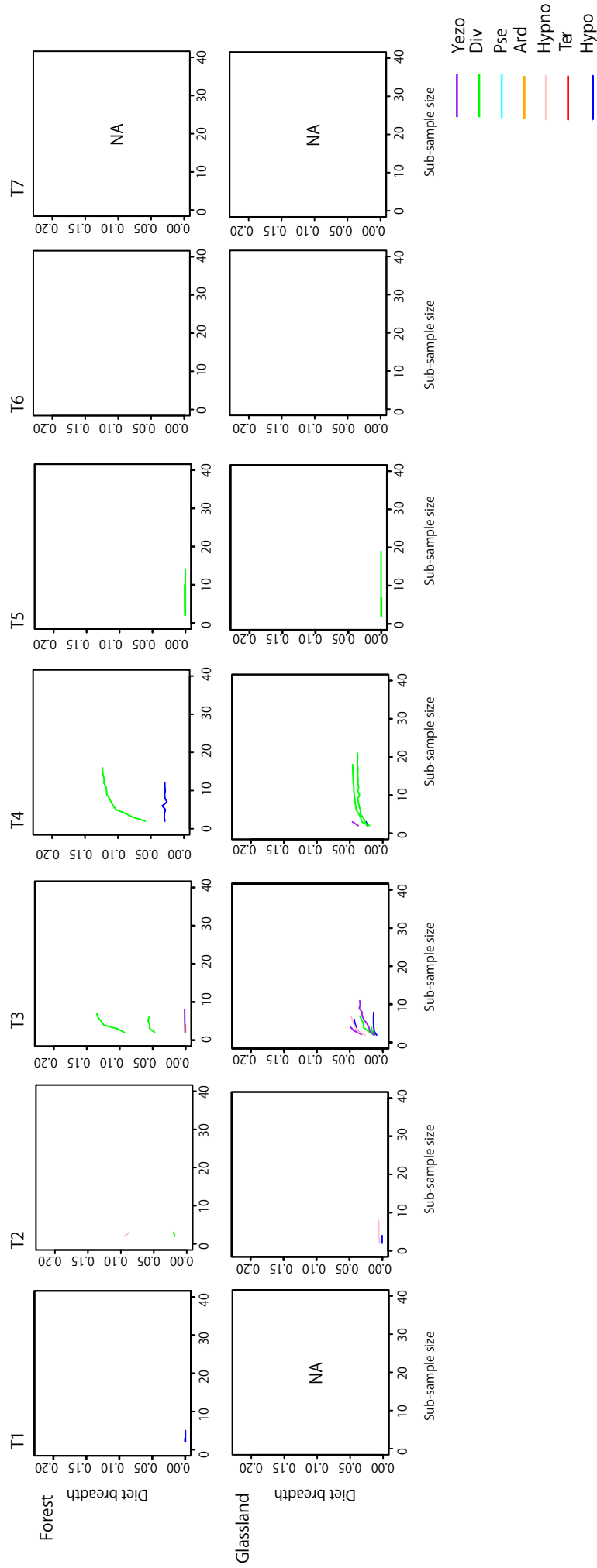
If the sample size less than 3, curves are not available, which might result in a blank plate.

tersatus, Yezo: *B. yezoensis*.

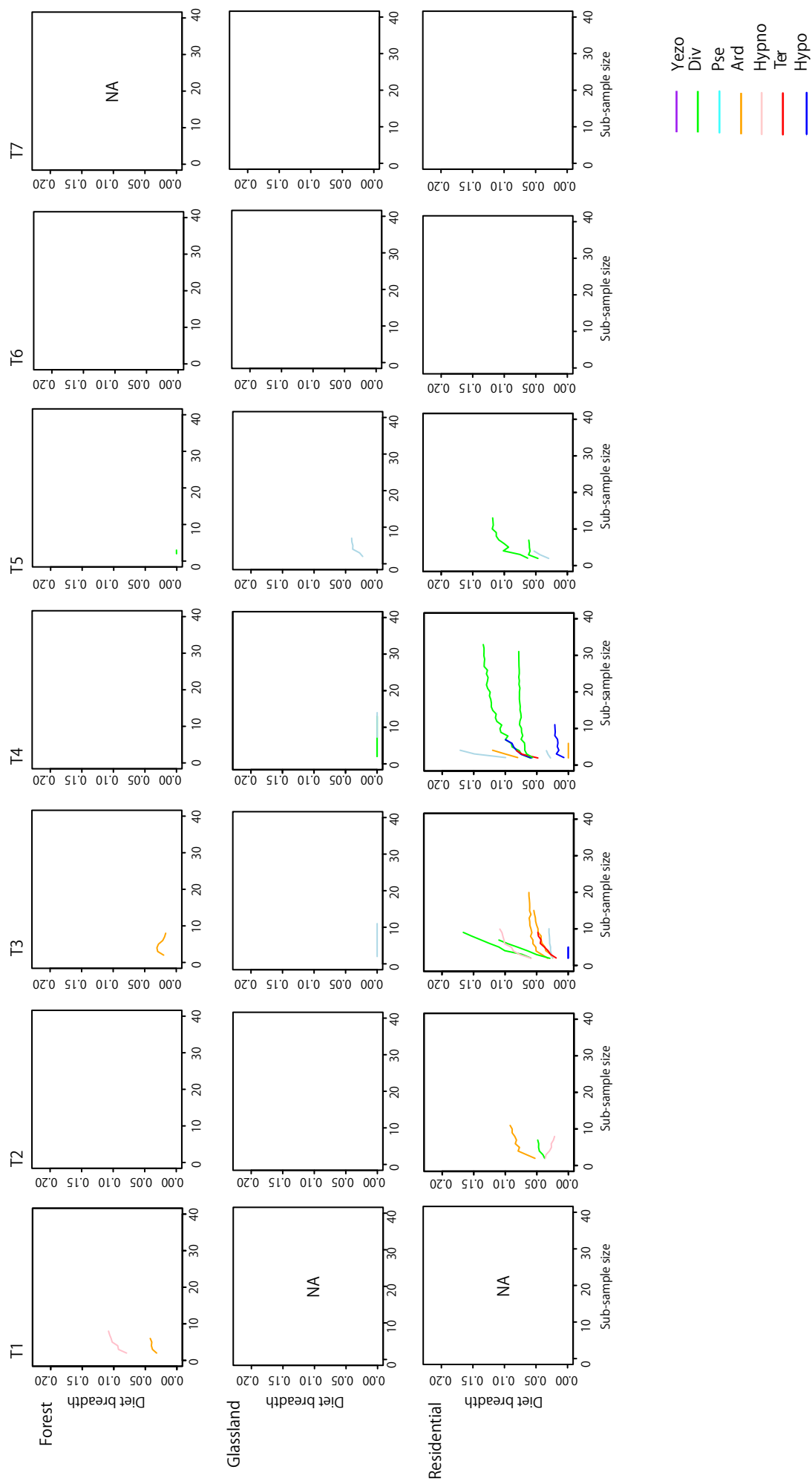
c) Natural forest/Body pollen



d) Natural forest/Load pollen



b) Urban forest/Load pollen



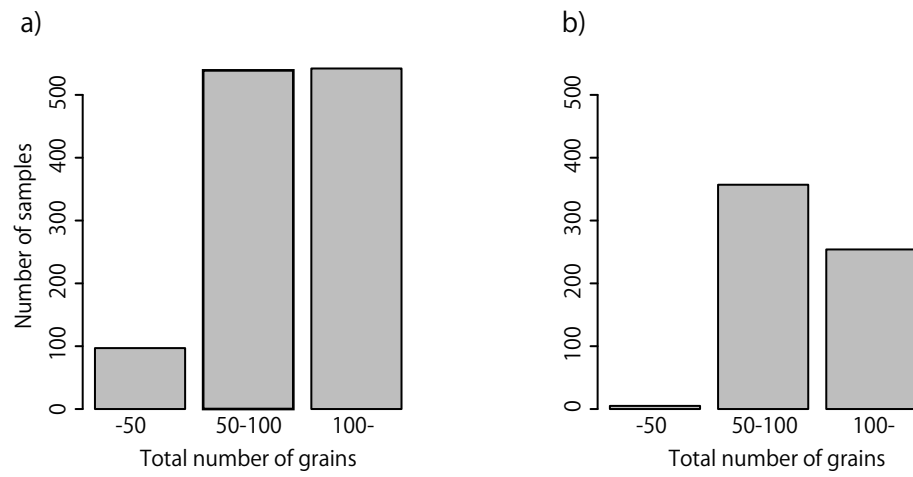


Fig. 3-S2: Distribution of the total number of pollen grains in each sample.
a) Body pollen, b) load pollen.

General Discussion

Effects of garden flowers at local (habitat) scale

Garden plants might be unsuitable for native bumble bees because of the artificial selection which often result in rewardless flowers or due to morphological mismatch between exotic flowers and native pollinators (Corbet *et al.* 2001). However, more abundant and diverse bumble bees were foraging in the gardens, and larger proportion of floral species were used at the garden in comparison with the forest habitat (Chapter 2). Not only diverse species composition, but also nectar-rich and/or high nectar-quality flowers were common at the residential habitat compared to the native vegetation (Chapter 1). Because of these superiorities of gardens as a foraging habitat, bumble bees highly relied on garden flowers in the urban-forest landscape (Chapter 2). These results are consistent with the previous study by Frankie *et al.* (2005), in which they reported diverse and abundant bee communities in gardens, where diverse bee-preferred flowers bloomed simultaneously. Importance of residential habitat relative to natural vegetation should vary depending on the landscape structure. At least in this study, the quality and quantity of floral resources at the residential habitat are better than that of natural vegetation at the forest habitat throughout the active period of bumble bees, resulting in the preference for gardens over wild plant communities as a foraging site.

Responses of foraging diversity (diet breadth) of bumble bee species to the functional diversity of floral community of the habitat were similar between the forest and residential habitats in the urban-forest landscape (Chapter 3). It implies that bumble bees can utilize artificial gardens effectively enough as they utilize native vegetations. Because bumble bees are generalist for floral resource use (Goulson *et al.* 2008a), they flexibly adapt to the garden environment with many exotic species. Insect and bird communities in urban environment were also reported to be generalized (Eremeeva & Sushchev 2005; Devictor *et al.* 2007). Because of the foraging flexibilities, bumble

bees can be effective pollinators of crops in the urban environment (Werrell *et al.* 2009; Lowenstein *et al.* 2015).

Bumble bees require large amount of floral resources during the period of colony development (Williams *et al.* 2012; Persson & Smith 2013). Because flowering periods of major species have finished before summer at the forest habitat, floral resources of the forest habitat might not be enough to support the entire colony growth of bumble bees. Relatively small reliance to the forest habitat for pollen resource during the active season (the proportion of bumble bees carrying pollen load was lower at the forest habitat compared to the residential habitat; Chapter 2) also suggested the low quality and/or quantity of the forest habitat to raise larvae. On the other hand, the residential habitat continuously provided abundant floral resources until the end of fall, and the floral richness was quite high in summer (Chapter 2). Diet segregation among bumble bee species was most apparent in summer at the residential habitat in both nectar and pollen foraging, although each species utilized diverse flowers (large diet breadth under floral diverse environment; Chapter 3). Floral-rich environment of the residential habitat might enable bumble bees to partition resources while ensuring diverse foraging. Thus, floral resources at the residential habitat might compensate for the decreasing resources at the forest habitat, and contribute to the growth and persistence of diverse bumble bee communities in the urban-forest landscape. Mass flowering crops in farmland may also compensate for the seasonally fluctuating floral resources at the natural vegetation (Westphal *et al.* 2003). Because of the limited flowering period of agricultural crops comparing to the garden flowers, however, the compensative effect of agricultural crops might be limited. Holzschuh *et al.* (2016) reported the low pollinator density in the agricultural landscapes with temporal mass flowering of crops.

Effects of garden flowers at the landscape scale

Mass flowering crops may lure pollinators from the natural vegetation (Magrach *et al.* 2017; Saunders & Luck 2014) and influence the pollination situation of wild plants through pollinator competition (Montero-Castaño *et al.* 2016). In the present study, I demonstrated that urban gardens attracted many bumble bees throughout the season, while bumble bee density was quite low at the forest habitat (Chapter 2). However, the proportion of visited floral species at the forest habitat was independent on the presence of garden flower (similar floral-use proportion at the forest habitat between the urban-forest and natural-forest landscapes; Chapter 2), and floral species at the forest habitat received similar level of visitation as garden flowers had. Thus, a few highly attractive floral species at the forest habitat could receive pollination service irrespective of the floral environment outside of the forest habitat. This pattern was also confirmed by the foraging diversity of bumble bee species in which the diet breadth of bumble bee species was not influenced by the floral diversity of surrounding habitats (Chapter 3).

From these results, urban gardens were revealed to be able to support abundant and diverse bumble bee species in the urban-forest landscape, without negative effects on the pollination of wild plants of the native vegetation. However, If some garden flowers were disproportionately beneficial to particular species, it could alter the native plant-pollinator interaction (Diekötter *et al.* 2010). In the present study, short-tongued *Bombus hypocrita sapproensis* showed high preference for particular floral group, which was abundantly available for long period in the gardens. This indicates the importance of species-specific relationships between pollinators and plants in the urban environment (Banaszak-Cibicka & Żmihorski 2012).

Suburbanization or low level of anthropogenic land-use change may contribute to the increase in resource availability and habitat heterogeneity, which are beneficial for pollinators (Winfree *et al.* 2011; Hinnert *et al.* 2012). For example, Winfree *et al.* (2006) found more abundant and diverse pollinator communities in the forested habitat surrounded by agricultural or urbanized

land than that surrounded only by native vegetation. However, high level of urbanization of surrounding landscape negatively influence species richness of bumble bees in floral-rich gardens (Ahrné *et al.* 2009). Thus, gardens could support abundant and diverse pollinators by providing floral-rich, high quality resources, but this positive effects of gardens might not exist without the existence of native habitats within the landscape.

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