

Geographical and seasonal variation of plant composition detected in feces of *Cervus nippon yakushimae* using plant DNA analysis, in Yakushima Island, Japan

東, 悠斗

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**Geographical and seasonal variation of plant composition detected in feces of  
*Cervus nippon yakushimae* using plant DNA analysis, in Yakushima Island, Japan**

**Yuto Higashi**

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1 **Abstract**

2 Recently, plant DNA analysis of feces has been successfully used to evaluate the food  
3 species composition of sika deer *Cervus nippon*. Here, I applied this method to Yaku-  
4 sika *C. nippon yakushimae* which increased its population and had a great negative  
5 impact on understory vegetation in Yakushima Island. I identified food plants of *C.*  
6 *nippon yakushimae* by sequencing chloroplast DNA fragments (*rbcL* region) of plants  
7 remaining in feces collected in the field in April, June, August, and October 2018, using  
8 a next-generation sequencer. I collected feces from populations in two areas where  
9 populations have been managed by capture and three areas where those were not  
10 managed. The top two food plants in terms of the number of sequences determined were  
11 evergreen tall trees in all five areas. In addition to evergreen trees, understory  
12 herbaceous plants, including ferns, were included in the top 15 taxa in all areas. The  
13 diversity of food plants was higher in the managed areas than in the unmanaged areas.  
14 These results indicate that evergreen tall trees are the primary food plants of *C. nippon*  
15 *yakushimae*, but deer also forage on herbaceous plants as a side dish. Under this side  
16 dish effect, understory vegetation is reduced even under the high availability of food  
17 resources from tall trees. The high diversity of food plants in the managed areas  
18 suggested that understory plant diversity was restored when deer density was reduced  
19 by management.

20 **Keywords:** *Cervus nippon yakushimae*, DNA meta-barcoding, diet, regional difference,  
21 seasonal changes

## Introduction

Since the 1970s, the ecological consequences of Cervidae population growth and range expansion have received increasing attention in North America, Europe, and New Zealand (Côté et al., 2004; Sabo et al., 2017). Consequently, there is evidence that deer foraging promotes the extinction of understory plant species (McGraw & Furedi, 2005) and reduces plant species diversity (Begley-Miller et al., 2001). In Japan, the population of the sika deer (*Cervus nippon*) has been increasing since the 1980s and its range has been expanding (McCullough et al., 2009; Takatsuki, 2009; Yoshikawa et al., 2011; Nakahama et al., 2016; Nakahama et al., 2021). Consequently, deer foraging has reduced vegetation coverage and resulted in understory compositional changes (Kato & Okumura, 2004; Fujiki & Takayanagi, 2008; Suzuki et al., 2008; Nagaike, 2012; Sakio et al., 2013), including a decrease in rare species (Inoue, 2003).

Sika deer feed on hundreds of plants, but often selectively forage for their favorable plants (Kato & Okumura, 2004; Takatsuki, 2009; Anonymous, 2012; Hashimoto & Fujiki, 2014; Research cooperation section of Kanagawa Prefectural Government Nature Conservation Center, 2016; Sakata et al., 2021). In habitats where the populations of their favorite plants are reduced, deer change their feeding habits in two ways (Takahashi & Kaji, 2001; Murata et al., 2009). Firstly, deer feed on unfavorable plant species (Sakaguchi et al., 2012; Nakahama et al., 2020). Especially in winter, the amount of resources available to deer is limited, which increases the feeding on unfavorable plant species (Nakahama et al., 2021). In the Ashiu Research Forest of Kyoto University, a deer-unfavorable species *Arachniodes standishii* expanded its distribution in 2004 but disappeared by 2011 due to frequent deer foraging after 2007 (Sakaguchi et al., 2012). Secondly, deer eat fallen leaves, especially in the winter when food resources are scarce (Takahashi & Kaji, 2001; Miyaki & Kaji, 2004; see also Tremblay et al., 2005 for a case in Canada). These changes in feeding habits have been recorded mainly through visual observation in the field. However, it is often difficult to identify the species of plants eaten by deer and to quantify the composition of food species by visual observation alone. To evaluate and predict the damage to vegetation caused by deer feeding and take effective measures against feeding damage, it is necessary to quantitatively evaluate which plants are used as food resources by deer. Recently, Matsuki et al. (2008), Nakahama et al. (2020), and Sakata et al. (2021) have employed the plant DNA analysis

1 of feces to evaluate the food species composition of sika deer. This method is expected to  
2 advance the study of deer food plants.

3 The composition of deer food plants can be evaluated quantitatively using  
4 stomach contents, as done by Kuroiwa (2017) on Yakushima Island, where the population  
5 of *C. nippon yakushimae* is controlled by extermination. This study revealed that the  
6 seven plants used most frequently by deer were all tall trees, including *Daphniphyllum*  
7 *teijsmannii*, used by 81 out of 88 individuals, despite being toxic and listed as an  
8 unfavorable species for *C. nippon yakushimae* (Anonymous, 2012). In addition,  
9 individual deer accumulated renal fats even at relatively high densities. This study also  
10 showed that individual deer varied in the composition of food plants, and this variability  
11 was higher in denser populations. These results suggest that there was no shortage of food  
12 resources for the dense populations of *C. nippon yakushimae*.

13 However, these assessments using stomach contents require exterminated  
14 individuals and cannot be applied to areas where deer are not managed, such as the  
15 western part and highlands of Yakushima Island. Thus, the foraging habits of *C. nippon*  
16 *yakushimae* in western Yakushima were studied by visual observation (Agetsuma et al.  
17 2011, 2019). With this method, however, it is often difficult to identify the species of  
18 plants that deer eat even during the day, and it is almost impossible to observe the foraging  
19 habits at night. The foraging habits of *C. nippon yakushimae* in the highlands of  
20 Yakushima were studied by microscopic observation of fecal samples (Takatsuki, 1990),  
21 but it requires well-trained morphological observation skills; nevertheless, the accuracy  
22 of species identification is often low (Pompanon et al., 2012). In addition to these  
23 difficulties, both visual tracking and microscopic identification are time-consuming and  
24 a limited number of animals or samples can be processed.

25 To overcome these difficulties faced in previous studies in examining the feeding  
26 habits of *C. nippon yakushimae*, I sequenced the DNA of food plants contained in their  
27 feces. In this analysis, DNA was extracted from undigested plant fragments in the feces.  
28 The DNA barcoding region was amplified by polymerase chain reaction (PCR), and the  
29 sequences were determined to identify the plant species eaten by individual deer (Matsuki  
30 et al., 2008; Nakahama et al., 2020; Sakata et al., 2021). Since this method does not  
31 require exterminated deer, it applies to deer populations in the western part and highlands  
32 of Yakushima, where deer are not managed. Applying this method to managed and

1 unmanaged populations of *C. nippon yakushimae*, this study addresses the following  
2 questions: (1) Which plant taxa do *C. nippon yakushimae* mainly eat? (2) Does the  
3 composition of food plants vary by area and season? (3) Does the composition of food  
4 plants vary between individual deer in each area?

## 6 **Materials and Methods**

### 7 **Study site**

8 Yakushima (30°20' N, 130°50' E) is an almost circular island located  
9 approximately 60 km south of the southernmost tip of Kyushu Island, with an area of  
10 approximately 500 km<sup>2</sup>. The central part of the island is a mountainous area with peaks  
11 over 1,800 m above sea level. In the lowlands, subtropical plants, including figs, grow  
12 naturally, but their vegetation is classified as a warm-temperate broad-leaved evergreen  
13 forest that extends 900 m above sea level. At higher elevations, a coniferous forest  
14 dominated by *Cryptomeria japonica* is found up to 1,600 m above sea level, shrubland is  
15 located above 1,600 m, and dwarf bamboo *Pseudosasa owatarii* dominates above 1,800  
16 m (Kyushu Regional Environmental Affairs Office, Ministry of the Environment,  
17 <http://www.env.go.jp/park/yakushima/point/index.html>, accessed: December 2018). It is  
18 home to 388 species of ferns and 1,136 species of angiosperms, including 47 endemic  
19 species and two endemic subspecies (Yahara et al., 1987).

20 *Cervus nippon yakushimae* is a subspecies endemic to Yakushima Island. The  
21 estimated population of *C. nippon yakushimae* on the entire island in 2018 was 13,390 to  
22 20,260 (Survey by fecal granule method, Anonymous, 2019a). *Cervus nippon*  
23 *yakushimae* are currently managed by hunting, except in the western part and the  
24 highlands. The deer density is the highest in the western part, where the deer population  
25 is not hunted (Anonymous, 2016a). A total of 1,769 deer were hunted in the fiscal year  
26 2018 (Anonymous, 2019b). In the western part of Yakushima Island, understory  
27 vegetation is decreasing due to foraging under a dense deer population, and some plants  
28 that are not eaten by deer, such as *Alocasia odora* and *Vernicia cordata*, are increasing  
29 (Anonymous, 2016b). Similarly, the decline of understory vegetation and plant species  
30 have been reported in the mountain-top area, where several plant species are endemic to  
31 this area (Yahara et al., 2021).

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## Sampling

Fecal samples of *C. nippon yakushimae* were collected in four seasons (April, June, August, and October 2018) at the following five locations (Figure 1): the western forest road (hereafter W; elevation 56–156 m), the Shiratani forest road (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL; elevation 74–900 m), the high elevation section of Anbo forest road (hereafter AH; elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–1936 m). In AH and AL where forest roads are installed, deer tend to stay around the forest roads (Anonymous 2017), but in M located in an elevation zone higher than the end of Anbo forest road, deer tend to stay around the ridgelines (Anonymous, 2021). Along Anbo forest road, coniferous forests dominated by *Cryptomeria japonica* are developed in the elevation zone above 900 m, and broad-leaved forests dominated by Fagaceae are developed in the elevation zone below 900 m. I divided M, AH, and AL, expecting that plant resources available to deer differ between these three routes. In each route, I searched for deer feces at intervals of 400–600 m. When I found feces, I georeferenced the location using a GPS logger eTrex 30 J (Garmin, Olathe, Kansas, U.S.A). Two to three grains of fresh feces were collected with disposable chopsticks, placed in a non-woven tea bag with an ID, and dried on silica gel in Ziplock. When the silica gel turned red, it was replaced with a new one and allowed to dry for at least one week. The following samples were obtained from W, S, AL, AH, and M, respectively; 19,9,15,14. and 17 in April, 21,11,13,15, and 16 in June, 21,10,13, 15, and 15 in August, and 21,10,12,15, and 16 samples in October. The survey was limited to spring to autumn because deer have a strong tendency to settle from spring to autumn, but they often move along the elevation gradient in winter (Anonymous, 2017). Except in winter, no large-scale seasonal migration is known to *C. nippon yakushimae*. Surveys using GPS collars showed that the range of movement of *C. nippon yakushimae* individuals was generally within the range of 2–3 km during the season and more restricted during the day (Anonymous, 2016c, 2017, 2019c). From these results, it is considered that the plant composition of a fecal sample represents a deer food menu near a point where feces were collected.

## DNA processing

1 For dried field fecal samples, one grain per site was placed between plastic wraps  
2 and crushed with a pestle, and 20 mg of the fecal grains were used for the following  
3 operations. The resulting pieces of feces were frozen in liquid nitrogen and crushed with  
4 a Bead mill, TissueLyser II (QIAGEN, Hilden, Germany), and 5 mm zirconia beads (AZ  
5 ONE, Tokyo, Japan) for a total of 2 min at 30 Hz. DNA was extracted from the crushed  
6 feces using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) (see Supplemental  
7 Method-1). Multiplex amplicon sequencing by two-step PCR was performed using the  
8 MiSeq next-generation sequencer (Illumina Inc., San Diego, California, U.S.A). The 1st  
9 PCR primer was prepared based on the *rbcLa* primer sequence (JBOLI) (see  
10 Supplemental Method-2). The extracted DNA was used as a template for 1st PCR using  
11 the PCR primers described above and Tks Gflex DNA Polymerase (Takara Bio Inc.,  
12 Kusatsu, Japan), and the PCR product was purified using AMPure XP (Beckman Coulter  
13 Inc., Pasadena, California, U.S.A). The purified 1st PCR product was used as a template  
14 for the 2nd PCR using PrimeSTAR GXL DNA Polymerase (Takara Bio Inc., Kusatsu,  
15 Japan) (see Supplemental Method-2). Electrophoresis of the 2nd PCR product was  
16 performed on a microchip electrophoresis system MultiNA (Shimadzu Corp., Kyoto,  
17 Japan), and samples for which PCR amplification of DNA of the target strand length was  
18 not confirmed were excluded at this stage. The remaining 2nd PCR product was purified  
19 twice with AMPure, and the DNA concentration was measured using the Qubit assay kit  
20 (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A). Then, all the samples were  
21 diluted to the same concentration and mixed. The mixed samples were purified again by  
22 AMPure and quantified by real-time PCR; samples were prepared according to Illumina's  
23 protocol and run on a next-generation sequencer using the MiSeq Reagent Nano kit v2  
24 500 cycles (Illumina Inc., San Diego, California, U.S.A) for paired-end 257 bp each and  
25 index (nine and five bp) sequencing. I changed the 'DarkCycle' option setting to skip the  
26 first 3 bases (anchor regions) in both reads.

27

### 28 **Array Filtering and Clustering**

29 Automated molecular identification software Claident (Tanabe & Toju, 2013)  
30 was used to process the sequencing data (Toju, 2016; see Supplemental Method).

31 The *rbcL* database of woody plant species on Yakushima Island (Forestry and  
32 Forest Products Research Institute undated) and *rbcL* sequence data extracted from the



1 Barcode of Life Database (BOLD) (JBIF 2018, <http://www.gbif.jp/bol/>, obtained on  
2 January 31, 2018) was used to identify a plant operational taxonomic unit (OTU).  
3 Forward and reverse homology searches were performed using BLAST+ 2.7.1 (NCBI  
4 2017, <ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/>, obtained on January  
5 31, 2018) for each OTU with more than 20 reads. Homology searches were performed  
6 with both sequences to identify taxa from the OTUs (see Supplemental Method–3). Using  
7 *rbcL* sequences of OTUs, I identified taxa at the species, genus, or family level. In the  
8 case of Fagaceae, I could not discriminate even genera, and thus Fagaceae was treated as  
9 a taxon. In Rosaceae, I could distinguish *Rubus* and other genera, and thus I classified  
10 samples of Rosaceae into two groups: *Rubus* and Rosaceae (except *Rubus*). In some cases,  
11 I identified species using information on elevational distribution; for example, *Kalopanax*  
12 *septemlobus* is the only woody species of Araliaceae native to the higher elevation of  
13 Yakushima, and thus samples of Araliaceae in high-elevation areas AH and M were  
14 identified as this species.

15

#### 16 **Tree species composition in each area**

17 To describe tree species composition, occurrence records of tree species were  
18 extracted from the plant species survey data of Yakushima (Yahara et al. unpublished). In  
19 this data, occurrences of plant species within a 100 m x 4 m plot are recorded at 191 points  
20 in various parts of Yakushima. Along the dung survey route of W, S, AL, AH, and M,  
21 occurrence records of tree species were available from 5, 4, 6, 5, and 15 plots, respectively,  
22 where 50, 41, 47, 31, and 27 tree species were recorded. Among the total 81 species,  
23 *Rhododendron tashiroi*, *Symplocos myrtacea*, *Cryptomeria japonica*, and *Trochodendron*  
24 *aralioides* were recorded in more than 20 plots. For each species of each plot, the  
25 frequency was determined by dividing the number of occurrences by the number of plots.

26

#### 27 **Statistical analysis**

28 The number of OTUs per sample varied from 1 to 65. However, the number of  
29 OTUs per sample increased with the number of reads per sample; therefore, this number  
30 could not be compared directly between samples. The number of reads per sample  
31 varied from 1 to 6,113. To fairly compare the number of OTUs per sample, I selected a  
32 specific number of reads per sample, called the threshold, to standardize the sample

1 size. In this study, I set thresholds of 100, 200, 300, 400, and 500, and compared the  
2 performance (see below). When the number of OTUs per sample at a threshold is  
3 compared, the expected number needs to be used to even out fluctuations of the increase  
4 in the observed number of OTUs with read counts. To meet this need, I performed  
5 rarefaction on the observed count data of reads using Package “vegan” (Oksanen et al.,  
6 2018) of the statistical analysis software R ver. 3.5.0 (R Core Team, 2020). Rarefaction  
7 is the operation of random resampling of reads from each sample at a set threshold.  
8 Samples of reads below the threshold were removed from the data. Therefore, lowering  
9 the threshold increases the number of samples available, but decreases the expected  
10 number of OTUs for each sample, and vice versa. In this study, rarefaction was  
11 performed with read count thresholds of 100, 200, 300, 400, and 500. I drew rarefaction  
12 curves for 203 samples, excluding samples with reads below the threshold of 500, to see  
13 how the expected number of OTUs in each sample increased with the number of reads  
14 (Figure 2). I then calculated the average, minimum, and maximum number of OTUs per  
15 sample for each of the five thresholds (100, 200, 300, 400, and 500) to determine the  
16 optimal threshold.

17 To examine the seasonal and geographical variation of plant OTU composition  
18 in feces, I calculated the relative read abundance (RRA), of each plant OTU  $RRA_i$   
19 (Deagle et al. 2019) for each of the 20 combinations of 4 seasons  $\times$  5 areas, and each of  
20 the five areas by summing the data of four seasons:

$$21 \quad RRA_i = \frac{1}{S} \sum_{k=1}^S \frac{n_{i,k}}{\sum_{i=1}^T n_{i,k}} \times 100\%,$$

22 where  $i$  is the ID of an OTU,  $S$  is the number of samples,  $k$  is the ID of a sample,  $n_{i,k}$  is  
23 the number of reads of OTU  $i$  in sample  $k$ , and  $T$  is the total number of OTUs.

24 To examine the geographical variation of plant OTU diversity in feces, I  
25 determined rank abundance relationships using the sum of the four seasons data in each  
26 area. Further, to examine the seasonal variation of plant OTU diversity in each area, I  
27 calculated Shannon’s diversity index for the four seasons in each area. Both calculations  
28 were made using the R Package “vegan” (Oksanen et al., 2018).

29 To compare the dominance of major taxa between areas, I defined “dominant  
30 taxa” using the cumulative sum of  $RRA_i$  as follows: all species detected in an area were

1 listed in descending order of  $RRA_i$  and cumulative RRA was determined for each  
2 species. A cumulative RRA of 50% was used as the threshold for the dominant taxa.

3 To compare the proportion of life forms detected in the feces of deer between  
4 areas, I classified angiospermous OTUs into the following life forms: tall trees, shrubs,  
5 vines, and herbs (Table S3). I also defined “small plants” as a collective category,  
6 including angiospermous herbs, ferns, and bryophytes. Each angiospermous taxon was  
7 added up for each life form (Table 1). A taxon (Rosaceae) was excluded from this table  
8 because of the difficulty in classifying them into four life forms.

9 The Shannon’s diversity index and the number of dominant taxa were  
10 compared between areas, and between the areas where deer populations were managed  
11 by capture (S, AL) and the areas where deer populations were not managed (W, AH, M).  
12 Observed differences were statistically tested using 95% bootstrap confidence intervals  
13 computed by bootstrapping 10,000 times. In addition, Shannon’s diversity index and its  
14 95% bootstrap confidence intervals were compared between the areas in each of the  
15 four seasons.

16 I selected the 15 taxa that were predominant throughout the dataset to  
17 determine if there were taxa with similar geographical and seasonal RRA variation  
18 patterns. For each OTU, I determined the maximum RRA value in a dataset of 20 (four  
19 seasons  $\times$  five areas). All taxa were then listed in descending order of maximum RRA,  
20 and the top 15 taxa were selected. For these 15 taxa, principal component analysis  
21 (PCA) was performed using 20 RRA data for each OTU. After performing PCA, I  
22 calculated the contributions of each of the 20 RRA variables to the first and second  
23 principal components (PC1 and PC2, respectively) and drew a biplot. In addition,  
24 hierarchical clustering was performed using the Bray-Curtis dissimilarity and  
25 unweighted pair group method with arithmetic mean (UPGMA).

26 To assess the similarity of plant taxa composition between the five areas, PCA  
27 and hierarchical clustering were performed using RRA of all taxa in each of the five  
28 areas. After performing PCA, I calculated the contribution of each plant OTU to PC1  
29 and PC2 and drew a scatter plot of PC1 and PC2. In addition, hierarchical clustering  
30 was performed using the Bray-Curtis dissimilarity and UPGMA.

31 A previous study (Kuroiwa, 2017) showed that deer individuals tend to utilize  
32 different food plants in high-density populations. To examine whether this tendency was

1 also found in our dataset, Bray-Curtis dissimilarity of OTU composition was calculated  
2 for all samples in each of the five areas and averaged in each area; OTU composition is  
3 a vector of the rarefied number of reads for each OTU observed at a threshold of 300.  
4 Spearman's rank correlation coefficient between Bray-Curtis dissimilarity and deer  
5 density was then calculated using dissimilarities for all combinations of individuals  
6 belonging to the same population (n=1,337). Its significance was tested using a  
7 permutation test by permutating density for all 120 (=5!) combinations. Of the 120  
8 permutations, the number of times the correlation coefficient value (absolute value)  
9 exceeded the observed value was counted, and the probability (value divided by 120)  
10 was calculated. When this probability was less than 0.05, the correlation coefficient  
11 value was significant. The densities of *C. nippon yakushimae* in five areas estimated  
12 using the fecal mass count method were as follows (Anonymous, 2019a): W, 100.25  
13 head/km<sup>2</sup>; S, 2.8 head/km<sup>2</sup>; AL, 6.7 head/km<sup>2</sup>; AH, 24.7 head/km<sup>2</sup>; M, 15.6 head/km<sup>2</sup>.

14 Unless otherwise noted, SD indicates the standard deviation. In a box plot, the  
15 top and bottom of a box are the first and third quartiles, respectively, a horizontal line in  
16 a box is the median, and the upper and lower whiskers are the first quartile  $- 1.5 \times$   
17 (third quartile  $-$  first quartile) or more, and the third quartile  $+ 1.5 \times$  (third quartile  
18  $-$  first quartile) or less, respectively. The white circles refer to data points that are larger  
19 or smaller than the whiskers, i.e., outliers, and the crosses refer to the average value.

20 To compare tree species composition between five dung survey routes, PCA  
21 and UPGMA clustering using Bray-Curtis dissimilarity matrix were performed using  
22 frequency data of each route. The species and family names of plants are based on  
23 "GreenList v1.01" (Ito et al., 2016) for angiosperms, "Fern GreenList v1.01" (Ebihara et  
24 al., 2016) for pteridophytes, and "BG Plants Japanese Name - Scientific Name Index"  
25 (Yonekura & Kajita, 2003) for bryophytes and gymnosperms. Regarding taxon names,  
26 species and varieties are referred to by genus name from the second mention onward,  
27 unless other species of the same genus are described in this paper. In addition, the genus  
28 name is described by omitting "spp."

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## Results

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DNA was extracted from all 295 fecal samples, of which 283 were used for PCR  
and sequencing. The MiSeq (Illumina Inc., San Diego, California, U.S.A) sequence data

1 were deposited in DDBJ nucleotide data bank (Accession no. DRA012948). After  
2 removing noisy and chimeric sequences, sequences with 99% or higher homology were  
3 considered identical taxa, and a total of 448 OTUs were distinguished. After removing  
4 chimeric and low frequency taxa, 177 remaining OTUs had a total of 355,890 reads, with  
5  $2,010.68 \pm 7,173.43$  reads for each OTU (12-70,929 reads,  $n = 183$ ), and  $1,266.51 \pm$   
6  $1121.73$  reads (1-6092 reads,  $n = 281$ ) for each sample. Of the 177 OTUs, 79 with fewer  
7 than 20 reads were not used for taxonomic identification. The remaining 98 OTUs were  
8 identified as 69 angiosperm taxa (family, genus, or species), including 56 woody taxa and  
9 13 herbaceous taxa, three gymnospermous taxa, 14 pteridophyte taxa, and 12 bryophyte  
10 taxa (Tables S3 and S4). For these taxa, read data were rarefied at threshold values of 100,  
11 200, 300, 400, and 500, and a threshold of 300 was adopted at which the increase in the  
12 number of taxa decelerated and 228 (80.6%) of 283 samples were included (Table S1 and  
13 S5). These samples included 59 samples of April (W: 15, S: 9, AL: 11, AH: 11, M: 13),  
14 51 of June (W: 17, S: 8, AL: 8, AH: 12, M: 6), 53 of August (W: 18, S: 8, AL: 11, AH: 9,  
15 M: 7), and 65 of October (W: 21, S: 8, AL: 8, AH M: 15, M: 13), including  $10.29 \pm 4.78$   
16 taxa (mean  $\pm$  SD, 1-26)(Table S3).

### 17 **Dominant plant taxa detected in feces of *C. nippon yakushimae***

18 Regarding the proportion of life forms of plants detected in feces (Table 1), tall  
19 trees were the most common in all areas (75.6%), followed by small plants (12.1%),  
20 shrubs (8.22%), and vines (4.09%). Among the top 15 taxa for RRA (Table 2), the top  
21 two taxa comprised of tall trees, including Fagaceae, *Cryptomeria japonica*,  
22 *Daphniphyllum*, *Ficus subpisocarpa*, *Kalopanax septemlobus*, and *Machilus*. In addition,  
23 ferns, shrubs, herbs, or bryophytes were included in the top 15 taxa in all areas (Table 2).  
24 The number of “dominant taxa” (see the definition in Materials and Methods) in each area  
25 (observed [lower 95% confidence limit, upper 95% confidence limit]) was 2 [2,3] at W,  
26 3 [2,4] at M, 4 [3,5] at AH, 6 [4,7] at AL, and 7 [4,8] at S (Table 2).

27 In PCA using RRA of all taxa in each of the five areas, PC1 scores were larger  
28 in three low-elevation areas and smaller in two high-elevation areas (Figure 3). The five  
29 taxa that mainly contributed to PC1 positively were abundant in low-elevation areas  
30 (Fagaceae, *Rubus*, *Kadsura japonica*, *Dicranopteris linearis*, and *Myrsine seguinii*), and  
31 the five taxa that mainly contributed to PC1 negatively were abundant in high-elevation  
32 areas (*Clethera*, Rosaceae excluding *Rubus*, *Trochodendron aralioides*, *Daphniphyllum*,

1 and *Illicium anisatum*; that contributed negatively) (Figure 3, Table S2). While most taxa  
2 were tall trees, *Rubus* is a shrub, *Kadsura* is a vine, and *Dicranopteris* is a fern. In the  
3 UPGMA clustering using RRA of all taxa, five areas were clustered into three groups: AL  
4 and S, W, and AH and M (Figure S1).

5 The diversity of plant taxa was highest in S, followed by AL, AH, M, and W  
6 (Shannon's index [lower 95% bootstrap confidence limit, upper 95% bootstrap  
7 confidence limit]: 2.41 [2.16, 2.53] at W, 3.35 [2.99, 3.37] at S, 3.30 [2.97, 3.33] at AL,  
8 2.86 [2.60, 2.94] at AH, 2.62 [2.33, 2.74] at M; observed value). Rank-abundance plots  
9 using the Zipf-Mandelblot model also showed that W had a steeper slope than S and AL  
10 (Figure 4).

#### 11 12 **Seasonal changes in taxa detected**

13 Among the predominant 15 taxa, notable seasonal changes in RRA were  
14 observed in the top six taxa (Figure 5, Table S6): *Ficus subpisocarpa* at W was the most  
15 abundant in April, the least abundant in June, and increased from August to October;  
16 *Daphniphyllum* at AH and M was most abundant in October; *Cryptomeria* at M was most  
17 abundant in April and decreased from June to August; *Machilus* at W increased from June  
18 to August; Fagaceae in four areas except M increased from April to June, decreased to  
19 August, and increased again to October; and Araliaceae at AH and M increased from June  
20 to August and decreased to October.

#### 21 22 **Inter-individual variation in taxa composition dissimilarities**

23 Bray-Curtis dissimilarities were  $0.726 \pm 0.247$ ,  $0.852 \pm 0.137$ ,  $0.809 \pm 0.166$ ,  $0.725 \pm$   
24  $0.168$ , and  $0.723 \pm 0.190$  (mean  $\pm$  SD) at W, S, AL, AH, and M, respectively (Figure 6).  
25 Bray-Curtis dissimilarity was not significantly correlated with deer density (Spearman's  
26 rank correlation coefficient:  $r = -0.147$ , permutation test,  $p = 0.267$ ).

#### 27 28 **Tree species composition in each area**

29 Most frequent species in each area were as follows: *Camellia japonica*, *Lithocarpus*  
30 *edulis*, and *Myrsine seguinii* in W (frequency: 4.0% each), *Cleyera japonica*, *Eurya*  
31 *japonica*, *Neolitsea aciculata*, and *Rhododendron tashiroi* in S (4.3% each), *Acer*  
32 *morifolium*, *Ca. japonica*, *Cl. japonica*, *Illicium anisatum*, *Machilus japonica*, *M. seguinii*,

1 *R. tashiroi*, *Stewartia monadelpha*, *Symplocos glauca*, and *Trochodendron aralioides* in  
2 AL (4.2% each), *Ca. japonica*, *Cryptomeria japonica*, *Pieris japonica*, *R. tashiroi*, *T.*  
3 *aralioides*, and *Tsuga sieboldii* in AH (5.5% each), and *Cr. japonica*, *P. japonica* and  
4 *Symplocos myrtacea* in M (8.7% each). In PCA using frequencies of tree species in each  
5 of the five areas, PC1 scores were larger in three low-elevation areas and smaller in two  
6 high-elevation areas (Figure 7). In the UPGMA clustering using frequencies of tree  
7 species, five areas were clustered into three groups: AL and S, W, and AH and M (Figure  
8 S2).

## 11 Discussion

12 Tall trees were predominant among plants detected in feces: tall trees were the  
13 most common in all areas (75.6%), and the top two taxa in five areas were comprised of  
14 tall trees (Tables 1 and 2). This result is consistent with those of a previous study using  
15 stomach contents (Kuroiwa 2017), which showed that the following seven taxa that were  
16 detected in most of the 90 individuals of *C. nippon yakushimae* studied were tall trees:  
17 *Machilus thunbergii*, *Cinnamomum japonicum*, *Daphniphyllum teijsmannii*, *Ficus*  
18 *subisocarpa*, *Elaeocarpus japonicus*, *Ardisia quinquegona*, and Fagaceae. However,  
19 Kuroiwa (2017) compared the occurrences of plant taxa between deer individuals and did  
20 not quantify the RRA of each taxon in each sample. This study showed that tall trees were  
21 quantitatively predominant in all 295 samples.

22 While tall trees are predominant in feces, the edible parts of tall trees are located  
23 at a height of 120 cm or higher, beyond the reach of the mouth of *C. nippon yakushimae*.  
24 Therefore, the main food resources eaten by *C. nippon yakushimae* are considered to be  
25 seedlings, saplings, sprouts, fallen leaves, and/or fallen fruits available on the ground. In  
26 addition, deer sometimes eat the leaves of tree branches that are accessible from bridges  
27 over rivers or streams. Among these food resources, fallen leaves are an alternative food  
28 source in winter or when other food resources are depleted for the *C. nippon* population  
29 of eastern Japan (Takahashi & Kaji, 2001; Kaneko et al., 2020). While fallen leaves have  
30 been considered less important foods in western Japan (Jayasekara & Takatsuki, 2000), a  
31 recent study based on visual observations of foraging behavior of the *C. nippon*  
32 *yakushimae* population in western Yakushima showed that 45.6–59.8% of their diet

1 consisted of fallen leaves of trees (Agetsuma et al. 2011).

## 2 **Geographical variation of plant taxa detected in feces of *C. nippon yakushimae***

3 The composition of plant taxa varied between areas (Table 2 for the top 15 taxa;  
4 Figure 3 for all taxa): three areas in the lower elevation (AL, S, and W) are characterized  
5 by the predominance of Fagaceae (tree) and *Rubus* spp. (shrub) (Table 2) and additional  
6 common detection of *Myrsine seguinii* (tree), *Kadzura japonica* (vine), and  
7 *Dichranopteris linearis* (fern) with lower RRA (Figure 3), while two areas in the higher  
8 elevation (AH and M) are characterized by the predominance of four tree taxa (*Clethra*  
9 *barbnervis*, Rosaceae excluding *Rubus* spp., *Trochodendron aralioides*, and  
10 *Daphniphyllum* spp.) and common detection of an additional tree species *Illicium*  
11 *anisatum*. Judging from the distribution in the higher elevation, Rosaceae excluding  
12 *Rubus* spp. and *Daphniphyllum* spp. are considered to be *Sorbus commixta* and  
13 *Daphniphyllum macropodum*, respectively. It is notable that the primary taxa contributing  
14 to the variation of composition were tall trees (Table 2, Figure 3, Table S2). In both PCA  
15 using RRA and PCA using frequencies of tree species, PC1 scores were divided in three  
16 low-elevation areas and two high-elevation areas (Figure 3, 7), suggesting that the  
17 geographical variation of plant composition in feces reflected the geographical variation  
18 of occurrence frequencies in forests. To further characterize and interpret the patterns of  
19 geographical variation in the RRA of six primary tall tree taxa, I compared RRA values  
20 with their occurrence frequencies (Table 3). From this comparison, *Ficus subpisocarpa*  
21 and *Machilus* spp. are considered to be frequently selected in W, because those are  
22 frequently detected from feces despite their low occurrence frequencies in W. Similarly,  
23 Fagaceae is frequently selected in AL and S, *Cr. japonica* and *K. septemlobus* in M, and  
24 *Daphniphyllum* spp. in AH and M. In contrast, most frequently recorded species such as  
25 *Ca. japonica* in W (occurrence frequency: 4.0 %), *Cl. japonica*, *E. japonica*, and *N.*  
26 *aciculata* in S (4.9 % each), *S. glauca* in AL (4.2 %), *P. japonica* and *T. sieboldii* in AH  
27 (5.5 % each), and *S. myrtacea* in M (8.7 %) were not listed among the top 15 taxa for  
28 RRA. These results indicated that the patterns of geographical variation in RRA reflected  
29 geographical variation in tree species composition, but deer selected some favorable  
30 species for their diets.

31 The predominance of *F. subpisocarpa* in feces of W may be associated with the  
32 frequent use of *F. subpisocarpa* by a monkey *Macaca fuscata yakui* (Maruhashi, 1980;



1 Otani, 2001), dropping leaves, large stipules, and fruits of *F. subpisocarpa* on the ground.  
2 According to Agetsuma et al. (2011), 1.7–10.9% of the seasonal diet of *C. nippon*  
3 *yakushimae* were droppings supplied by *M. fuscata yakui*. However, feeding on *F.*  
4 *subpisocarpa* in W was recorded only once among the 59 observations of Agetsuma et al.  
5 (2019) on the feeding behavior of *C. nippon yakushimae*. This discrepancy may be due  
6 to observations made at different locations in different years. Alternatively, the direct  
7 observation of feeding events by Agetsuma et al. (2019) may have underestimated feeding  
8 on *F. subpisocarpa*. In any case, the results of this survey suggest that the high availability  
9 of *F. subpisocarpa* may be one of the factors that maintains high deer density in W where  
10 the population has not been managed.

11 It is notable that *Daphniphyllum* spp. were frequently selected in AH and M,  
12 despite that both *D. macropodum* and *D. teijsmannii* native to Yakushima include toxic  
13 alkaloids and are listed as species avoided by *C. nippon yakushimae* (Anonymous, 2012).  
14 Kuroiwa (2017) also showed that the leaves of *D. teijsmannii* were detected in the  
15 stomach contents of 81 of the 90 hunted individuals. In addition to *Daphniphyllum* spp.,  
16 *Illicium anisatum* detected in AH and M are known to include toxic sesquiterpenes  
17 (Yamada et al. 1968). These findings suggest that *C. nippon yakushimae* can detoxify  
18 toxic compounds of plants, as North American white-tailed deer that can detoxify  
19 alkaloids of *Taxus canadensis* (Windels & Flaspohler, 2011).

20 In addition to selectively foraging on some tall trees that were not always  
21 abundant in each area, *C. nippon yakushimae* were selectively foraging on some small  
22 plants, because ferns, shrubs, herbs, or bryophytes were included in the top 15 taxa in all  
23 areas. Because the availability of these small plants is higher in managed areas where  
24 more species-rich understory vegetation is found (Anonymous, 2020), the dependence of  
25 *C. nippon yakushimae* on food derived from tall trees may be lower there. As expected,  
26 the proportions of tall tree taxa were lower in S and AL than in W (Table 1), despite the  
27 fact that S, AL, and W are located at lower elevations, similarly covered with evergreen  
28 broad-leaved forests. Corresponding to this difference, the slope in the rank-abundance  
29 plots was steeper in W than in S and AL (Figure 4). However, herbs (*Cyperus*) and shrubs  
30 (*Rubus*) were also included in the top 15 plant taxa in W (Table 2). This indicates that  
31 herbs and shrubs are under the feeding pressure of *C. nippon yakushimae*, even in  
32 unmanaged areas where understory vegetation has declined. It is proposed here that the

1 effects of this foraging pressure on understory plants by deer whose main food resources  
2 (i.e. main dish) are seedlings, saplings, sprouts, fallen leaves, and/or fallen fruits of tall  
3 trees is called the “side dish effect”. More generally, the side dish effect can be defined  
4 as a phenomenon in which infrequent feeding by herbivores poses a great threat to the  
5 survival of plants with a small population size or small biomass. Under this effect, plant  
6 coverage and diversity in the understory of evergreen forests in W may have been reduced.  
7 Small plants detected in feces from W, *Rubus* spp., and *Cyperus* spp. (Table 2), maybe  
8 grazing tolerant species and grazing intolerant species may be already lost. This  
9 possibility was demonstrated by the recovery of understory vegetation within the  
10 vegetation protection fence introduced in W (Terada et al., 2013). Inside the two fences  
11 installed, coverage and species richness of understory vegetation had more than doubled  
12 in four years, while outside the two fences, there was no change in coverage and species  
13 richness of understory vegetation. Similar recoveries of understory vegetation within the  
14 vegetation protection fence have been observed also in the unmanaged area along the  
15 Anbo forest road extending from AL to AH. For all four fences, the understory vegetation  
16 coverage inside the fences increased in 10 years, while the understory vegetation coverage  
17 outside decreased or remained unchanged (Higashi & Yahara, 2021). It is desirable to  
18 install more vegetation protection fences to elucidate how understory vegetations are  
19 reduced under deer foraging pressure and to restore understory vegetation, including  
20 threatened plants.

### 21 **Seasonal variation of plant taxa mainly detected in feces of *C. nippon yakushimae***

22 Agetsuma et al. (2011) reported that 45.6%–59.8% of the *C. nippon yakushimae*  
23 diet consisted of fallen leaves of trees and 8.7%–23.7% were fallen reproductive parts of  
24 trees. More recently, Agetsuma et al. (2019) reported that *C. nippon yakushimae*  
25 predominantly fed on the fallen leaves, fruits, seeds, and flowers of woody plants (up to  
26 82% of food items). These observations suggest that the seasonal change in the number  
27 of leaves and reproductive parts falling to the ground results in seasonal changes in plant  
28 taxon RRA.

29 In fact, seasonal changes in RRA of the following predominant taxa (Figure 5)  
30 were associated with their leafing and fruiting phonologies. (1) In W, *Ficus subpisocarpa*  
31 was most frequently detected in April when leafing occurred, and a large number of green  
32 leaves produced in the previous year fell on the ground (observed by the authors), and the

1 second most frequently detected in October when abundant fruits were produced (Otani  
2 & Kanetani, 2008), consumed by *M. fuscata yakui* (Hamada & Hanya, 2016), and often  
3 fell to the ground. (2) In W, *Machilus* was most frequently detected in June and August,  
4 which occurred during the 3.25 months when the fruits fell to the ground (Hanya & Aiba,  
5 2011). (3) In W, S, and AL, Fagaceae was the most frequently detected in June when the  
6 leaves of *Lithocarpus edulis* and *Quercus salicina* fell on the ground most abundantly  
7 (Tsujino & Yumoto, 2018), and was second most frequently detected in October when  
8 Fagaceae started to drop acorns. (4) In AH and M, *Daphniphyllum* was most frequently  
9 detected in October when its fruits matured. (5) In AH and M, *Kalopanax* was most  
10 frequently detected in August, when it started to drop leaves.

11 In contrast to these five taxa, RRA of *Cryptomeria* in M was as high as 41.2% in  
12 April, and its RRA decreased after June (Figure 5). This seasonal change is probably  
13 because *C. nippon yakushimae* eat young shoots on *Cryptomeria* trees instead of fallen  
14 leaves. In M, short trees, including saplings of *Cryptomeria*, are common, and the young  
15 leaves on these trees are often within the reach of the deer's height.

16 The associations between the seasonal change in RRA of the major plant taxa in  
17 feces and the phenology of leafing and fruiting are, however, only suggested. To test this  
18 hypothesis, it is necessary to observe which parts of the plants are actually eaten by *C.*  
19 *nippon yakushimae*. The plants eaten by *C. nippon yakushimae* have been observed in the  
20 western area by Agetsuma et al. (2011, 2019). Agetsuma et al. (2019) observed that the  
21 fruits of *Lithocarpus edulis* and *Quercus myrsinifolia* were eaten in the summer and fall,  
22 respectively, which is consistent with the results of this study. However, there are some  
23 discrepancies between the observations of Agetsuma et al. (2019) and the results of this  
24 study in W. First, as already mentioned, the use of *Ficus subpisocarpa* was observed only  
25 in one case by Agetsuma et al. (2019). Second, the use of *Machilus thunbergii* was  
26 observed only in spring, but not in summer (Agetsuma et al., 2019). To resolve these  
27 discrepancies, it is necessary to observe more deer individuals because the study by  
28 Agetsuma et al. (2019) was obtained by tracking six individuals, while this study  
29 identified plants from 71 samples from the western area. Further, observations based on  
30 suggestions from our study will be useful in resolving these discrepancies. First, in April,  
31 when *F. subpisocarpa* was most frequently detected, *F. subpisocarpa* dropped not only  
32 the old leaves of the previous years but also large soft stipules to the ground. *Cervus*

1 *nippon yakushimae* may use stipules as food. Second, the RRA of *Machilus* in feces was  
2 higher in August than in June, but the fruits of *M. thunbergii* ripen mainly from May to  
3 June in the western area and are quickly consumed by animals (Noma & Yumoto, 1997).  
4 Hanya et al. (2003) also reported that *M. thunbergii* fruits in low-elevation areas mature  
5 from May to July. Thus, *C. nippon yakushimae* may utilize not only fruits but also fallen  
6 leaves and inflorescences, possibly dropped by *M. fuscata yakui*. Examining these  
7 possibilities based on field observations will provide a more accurate understanding of  
8 the major food resources used by *C. nippon yakushimae*.

9 **Are there differences in the inter-individual variability of the plant taxa composition**  
10 **in *C. nippon yakushimae* feces in different areas?**

11 Unlike the results of Kuroiwa (2017), the dissimilarity in plant taxa composition  
12 and deer density was not correlated (Figure 6). This discrepancy may be partly due to the  
13 difference in deer densities in studied areas: while Kuroiwa (2017) studied an area of  
14 about 0.56 km<sup>2</sup> at Cape Yahazu and the area of 20.3 km<sup>2</sup> including urban areas and farms  
15 at Koseda as the highest density area (deer density: 73.5 head/km<sup>2</sup> and 92.5 head/km<sup>2</sup>,  
16 respectively), I studied W (deer density: 100.25 head/km<sup>2</sup>), an area along the forest road  
17 extending over 13 km, where availability of fallen leaves and fruits may have been higher.  
18 In W, the partitioning of food resources may not have occurred because the availability  
19 of edible plant resources was sufficiently high. This discrepancy may have also resulted  
20 from the difference in data type: while Kuroiwa (2017) used the occurrence data of plant  
21 taxa, I used RRA data to calculate the Bray-Curtis dissimilarity. The method of Kuroiwa  
22 (2017) may be more sensitive to the occurrence of rare food resources, while I neglected  
23 plant taxa detected at low frequencies. Further studies are needed to determine whether  
24 the partitioning of food resources occurs in *C. nippon yakushimae* populations under high  
25 densities.

26 **Conclusion**

27 DNA analysis has made it possible to quantitatively evaluate the question of which  
28 plants are eaten and how much. The results showed that *C. nippon yakushimae* depended  
29 heavily on plant resources derived from a small number of tall tree taxa in each area.  
30 Because the availability of these plant resources is high, it is unlikely that the population  
31 of *C. nippon yakushimae* is starved even at high densities. The same conclusion is derived  
32 for a deer population in a temperate deciduous forest in Kyoto where litterfalls of tall

1 trees play a significant role in maintaining the population density of *C. nippon* (Nakahama  
2 et al., 2021). Despite the high availability of tall tree resources, deer continue to eat  
3 understory plants as a side dish. It is necessary to install more vegetation protection fences  
4 in unmanaged areas to prevent the disappearance of endangered understory species and  
5 conserve understory vegetation.

6 In this study, I obtained a food menu of the *C. nippon yakushimae* population,  
7 which seems to be close to the actual eating habits. However, why *C. nippon yakushimae*  
8 mainly feeds on certain tall tree species such as *Ficus subpisocarpa* and *Machilus*  
9 *thunbergii* needs further studies. Further, the results of this study are based on yearly fecal  
10 samples, and future studies need to clarify how much the deer diet fluctuates from year  
11 to year. It is notable that *C. nippon yakushimae* may be selectively foraging on the leaves  
12 of *Daphniphyllum* and *Illicium* containing toxic compounds. An approach that combines  
13 the detailed dietary information obtained above with nutritional analysis would be  
14 promising to reveal the basis on which *C. nippon yakushimae* choose their diet.

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25

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**Table 1** Proportions of four life forms in five areas

“W”, “S”, “AL”, “AH”, and “M” in the table refer to the following five areas sampled; the western forest road (hereafter W; elevation 56–156 m), the Shiratani forest road (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL; elevation 74–900 m), the high elevation section of Anbo forest road (hereafter AH; elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–1936 m). Small plants include herbaceous plants, pterophytes, and bryophytes. Proportions in total were calculated from the relative read abundance (RRA) of all pooled samples.

	Tall trees	Small plants	Shrubs	Vines
W	89.16	3.52	2.15	5.17
S	62.25	21.09	10.72	5.94
AL	66.12	23.07	5.69	5.13
AH	74.90	9.76	12.91	2.43
M	71.65	12.14	15.07	1.14
Total	75.57	12.12	8.22	4.09

1 **Table 2** The Top 15 taxa in terms of relative read abundance (RRA) in each of the five study areas  
 2 “W”, “S”, “AL”, “AH”, and “M” in the table refer to the following five areas sampled; the western forest road (hereafter W; elevation 56–  
 3 156 m), the Shiratani forest road (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL; elevation 74–900  
 4 m), the high elevation section of Anbo forest road (hereafter AH; elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–  
 5 1936 m). The RRA in each area is the percentage of the average OTU (operational taxonomic unit) reads of each area. Numbers in  
 6 parentheses indicate the cumulative RRA.

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W (n=71)		S (n=32)		AL (n=37)		AH (n=47)		M (n=39)	
taxa	RRA(%)	taxa	RRA(%)	taxa	RRA(%)	taxa	RRA(%)	taxa	RRA(%)
1 <i>Ficus subpisocarpa</i>	29.37 (29.37)	Fagaceae	14.66 (14.66)	Fagaceae	20.34 (20.34)	<i>Daphniphyllum spp.</i>	26.53 (26.53)	<i>Daphniphyllum spp.</i>	26.61 (26.61)
2 <i>Machilus spp.</i>	22.68 (52.05)	<i>Machilus spp.</i>	10.33 (24.99)	<i>Cryptomeria japonica</i>	9.43 (29.77)	<i>Kalopanax septemlobus</i>	8.91 (35.45)	<i>Cryptomeria japonica</i>	20.00 (46.61)
3 Fagaceae	12.06 (64.1)	<i>Elaeocarpus spp.</i>	8.06 (33.05)	<i>Machilus spp.</i>	9.30 (39.07)	Fagaceae	8.12 (43.57)	<i>Kalopanax septemlobus</i>	8.04 (54.64)
4 <i>Elaeocarpus spp.</i>	8.78 (72.89)	<i>Oreocnide pedunculata</i>	5.96 (39.01)	<i>Ficus subpisocarpa</i>	5.96 (45.03)	<i>Cryptomeria japonica</i>	7.58 (51.15)	<i>Eurya yakushimensis</i>	6.56 (61.2)
5 <i>Toxicodendron succedaneum</i>	3.74 (76.63)	<i>Ficus subpisocarpa</i>	4.54 (43.55)	<i>Distylium racemosum</i>	4.17 (49.19)	<i>Rhododendron tashiroi</i>	7.43 (58.58)	<i>Clethra barbinervis</i>	4.57 (65.77)
6 <i>Gynochthodes umbellata</i>	2.46 (79.08)	<i>Daphniphyllum spp.</i>	3.85 (47.41)	<i>Uncaria rhynchophylla</i>	3.02 (52.21)	<i>Machilus spp.</i>	6.73 (65.31)	Rosaceae	2.98 (68.75)
7 <i>Daphniphyllum spp.</i>	1.60 (80.68)	<i>Cryptomeria japonica</i>	3.66 (51.06)	<i>Rubus spp.</i>	2.89 (55.1)	<i>Clethra barbinervis</i>	3.59 (68.9)	<i>Trochodendron aralioides</i>	2.94 (71.69)
8 Araliaceae	1.56 (82.24)	<i>Rubus spp.</i>	3.50 (54.56)	<i>Elaeocarpus spp.</i>	2.77 (57.87)	<i>Cleyera japonica</i>	3.11 (72.01)	<i>Rhamnus crenata</i>	2.90 (74.6)
9 Rosaceae	1.47 (83.71)	<i>Boehmeria sieboldiana</i>	3.35 (57.92)	<i>Rhododendron tashiroi</i>	2.58 (60.45)	Rosaceae	2.12 (74.13)	<i>Stewartia monadelphica</i>	2.79 (77.39)
10 <i>Rubus spp.</i>	1.46 (85.17)	<i>Vitis spp.</i>	3.22 (61.14)	<i>Neanotis hirsuta</i>	2.34 (62.79)	<i>Hydrangea spp.</i>	1.81 (75.94)	<i>Zoysia matrella</i>	2.10 (79.48)



11	<i>Diospyros spp.</i>	1.28	(86.45)	<i>Neanotis hirsuta</i>	3.09	(64.23)	<i>Brachythecium plumosum</i>	2.32	(65.11)	<i>Hypnum tristoviride</i>	1.77	(77.71)	<i>Illicium anisatum</i>	1.99	(81.47)
12	<i>Pinus densiflora</i>	1.24	(87.69)	<i>Carpinus spp.</i>	2.86	(67.09)	<i>Fissidens dubius</i>	2.23	(67.33)	<i>Camellia spp.</i>	1.76	(79.47)	<i>Juncus spp.</i>	1.98	(83.46)
13	<i>Cyperus spp.</i>	1.21	(88.9)	<i>Ilex rotunda</i>	2.47	(69.56)	<i>Ilex rotunda</i>	2.03	(69.36)	<i>Trochodendron aralioides</i>	1.74	(81.21)	<i>Elliottia paniculata</i>	1.95	(85.4)
14	<i>Actinidia rufa</i>	1.19	(90.09)	<i>Distylium racemosum</i>	2.01	(71.57)	<i>Diplopterygium glaucum</i>	1.86	(71.22)	<i>Vitis spp.</i>	1.48	(82.7)	<i>Haloragis micrantha</i>	1.94	(87.34)
15	<i>Ardisia sieboldii</i>	1.00	(91.08)	<i>Lysimachia spp.</i>	1.99	(73.56)	Araliaceae	1.73	(72.95)	Hypnales	1.48	(84.18)	<i>Rhododendron tashiroi</i>	1.69	(89.04)

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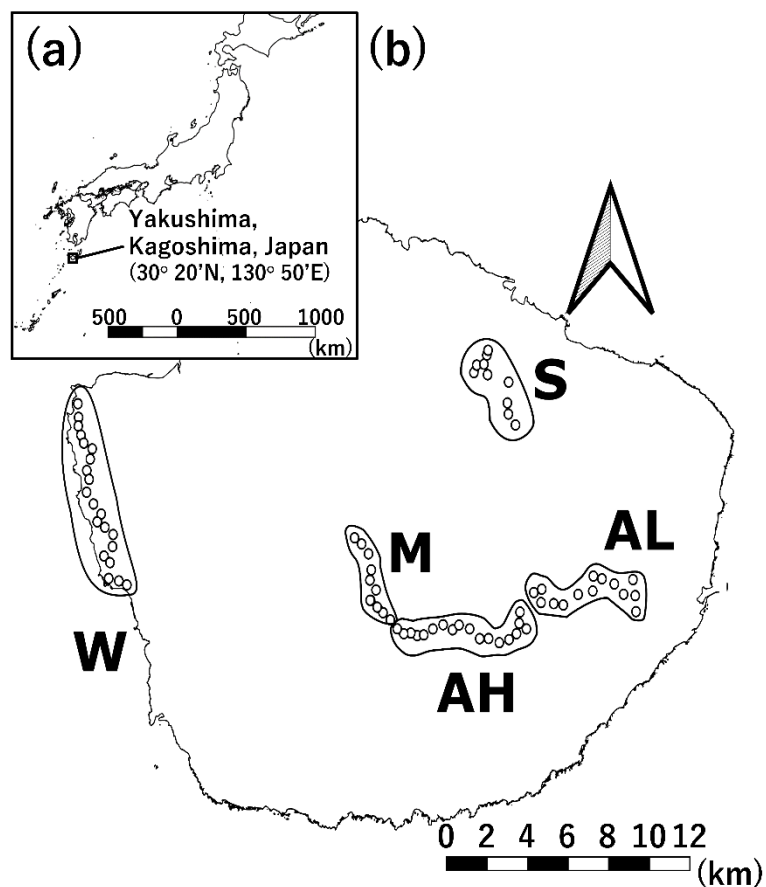
1 **Table3** Comparison between Relative Read Abundance (RRA) in feces and frequency of occurrence per forest plot for six tree taxa in  
 2 five areas.  
 3 “W”, “S”, “AL”, “AH”, and “M” in the table refer to the following five areas sampled; the western forest road (hereafter W; elevation  
 4 56–156 m), the Shiratani forest road (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL; elevation  
 5 74–900 m), the high elevation section of Anbo forest road (hereafter AH; elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter  
 6 M; 1334–1936 m). Selectivity is a summary of the results of a comparison of RRA and frequency of occurrence to determine how  
 7 frequently six tree taxa are selected as food by deer.

Taxa	RRA	Frequency of occurrence	Selectivity
<i>Ficus subpisocarpa</i>	frequently detected in W (29.4%) but less frequently in AL (5.96%) and S (4.54%), and not detected in AH, S, and M.	not recorded in 35 plots of 5 areas.	frequently selected in W
<i>Machilus</i> spp.	frequently detected in W (22.6%) and S (10.3%), less frequently detected in AL (9.3%) and AH (6.7%), and negligibly detected in M (0.07%).	recorded in W (3.0%), S (3.7%), and AL (4.9%), but not recorded in AH and M.	frequently selected in W
Fagaceae	detected in AL (29.34%), S (14.66%), W (12.6%), and AH (8.12%), but not in in top 15 of M (RRA: 2.42%)	recorded in AL(4.2%), S(8.6%), W(10%), AH(1.1%), but not recorded in M.	frequently selected in AL and S
<i>Cryptomeria japonica</i>	detected in M (20.0%,) AL (9.43%), and AH (7.58%) but not in top 15 of S and W (RRA: 3.66% and 0.066% in S and W).	recorded in M (8.7%), AH (5.5%), and AL (2.8%), but not in S and W.	frequently selected in M

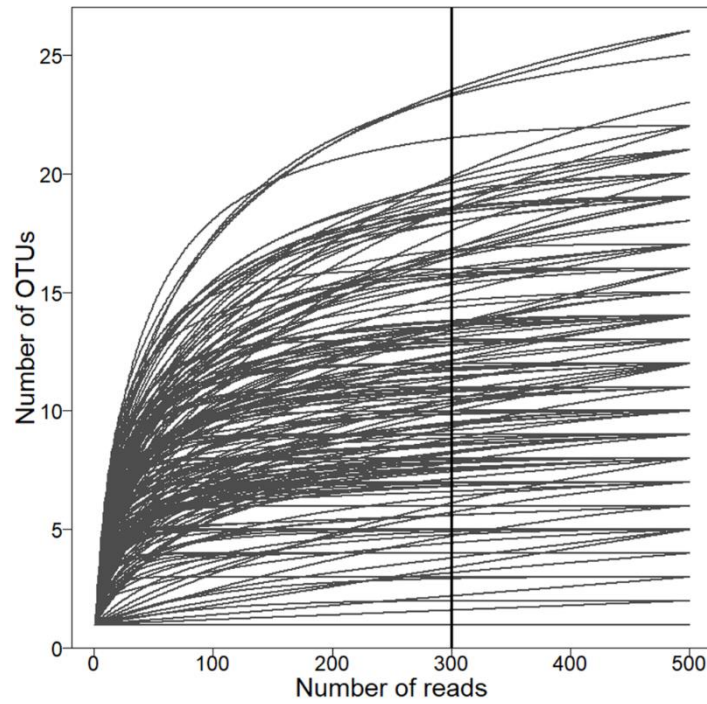
<i>Daphniphyllum</i> spp.	detected frequently in AH (26.5%) and M (26.6%) but not in top 15 of AL, S, and W (RRA: 1.38%, 3.85%, and 1.60% in AL, S and W).	recorded in AH (3.3%) and M (6%) and also in AL (1.4%) , S (2.5%) , and W (2%) .	frequently selected in AH and M
<i>Kalopanax septemlobus</i>	detected in AH (8.91%) and M (8.04%) but not in top 15 of AL, S, and W.	recorded in AH (3.3%), M (3.3%) and S (1.2%) but not in AL and W.	frequently selected in M

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1 **Figure 1** Location of the study area, Yakushima island, Kagoshima, Japan (a), and  
 2 where fecal samples were collected in August 2018 (b).  
 3 “W”, “S”, “AL”, “AH”, and “M” in the figure refer to the following five areas sampled;  
 4 the western forest road (hereafter W; elevation 56–156 m), the Shiratani forest road  
 5 (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL;  
 6 elevation 74–900 m), the high elevation section of Anbo forest road (hereafter AH;  
 7 elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–1936 m). The  
 8 sampling points in other months are similar to this. Outline map source: National Land  
 9 Numerical Information download service  
 10 (National Land Information Division, National Spatial Planning and Regional Policy  
 11 Bureau, and Ministry of Land, Infrastructure, and Transport (2017),  
 12 <http://nlftp.mlit.go.jp/ksj/index.html>).  
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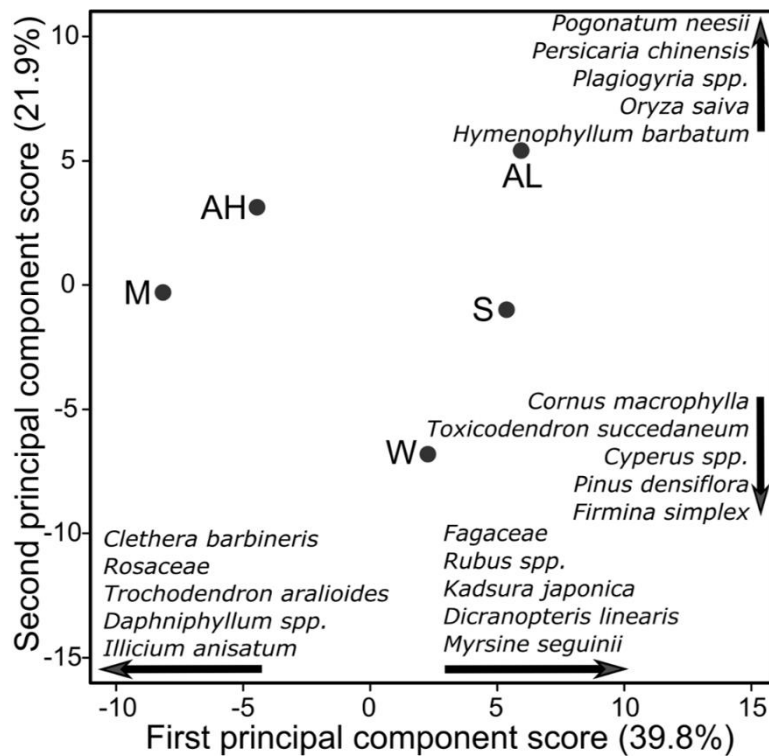


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2 **Figure 2** Rarefaction curves for 203 fecal samples at a threshold of 500

3 This curve shows how the number of OTUs (operational taxonomic units) increased  
4 with the number of reads in each sample. The actual threshold used for rarefaction, 300  
5 reads, is included as a line in the figure.

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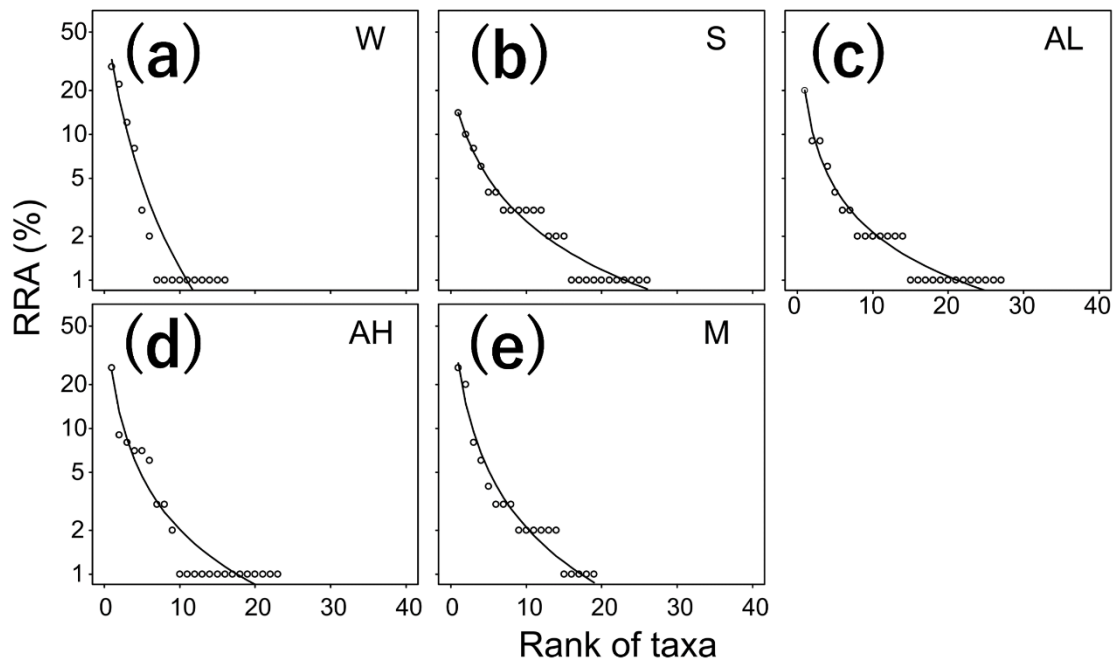


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**Figure 3** Biplot from the principal component analysis for five areas using relative read abundance (RRA).

“W”, “S”, “AL”, “AH”, and “M” in the figure refer to the following five areas sampled; the western forest road (hereafter W; elevation 56–156 m), the Shiratani forest road (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL; elevation 74–900 m), the high elevation section of Anbo forest road (hereafter AH; elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–1936 m). The percentage of variance explained by PC1 or PC2 is shown in parentheses on the axis label. Five taxa with large positive or negative contributions to PC1 and PC2 were identified.

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2

3 **Figure 4** Rank abundance plots of relative read abundance (RRA) of taxa detected in  
4 each area.

5 (a), (b), (c), (d), (e) in the figure refer to the following five areas sampled; (a): the

6 western forest road (hereafter W; elevation 56–156 m), (b): the Shiratani forest road

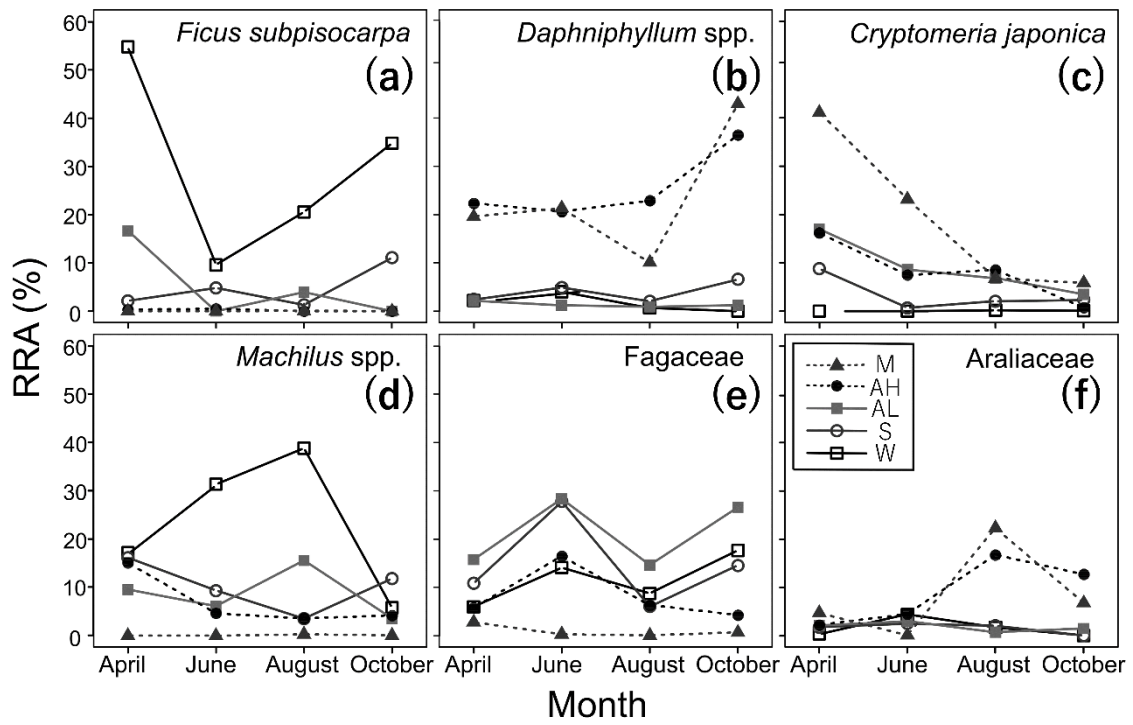
7 (hereafter S; 218–623 m), (c): the low elevation section of Anbo forest road (hereafter

8 AL; elevation 74–900 m), (d): the high elevation section of Anbo forest road (hereafter

9 AH; elevation 900–1334 m), (e): and the Mt. Miyanoura trail (hereafter M; 1334–1936

10 m). The curve is fitted to the data using the Zipf-Mandelblot model.

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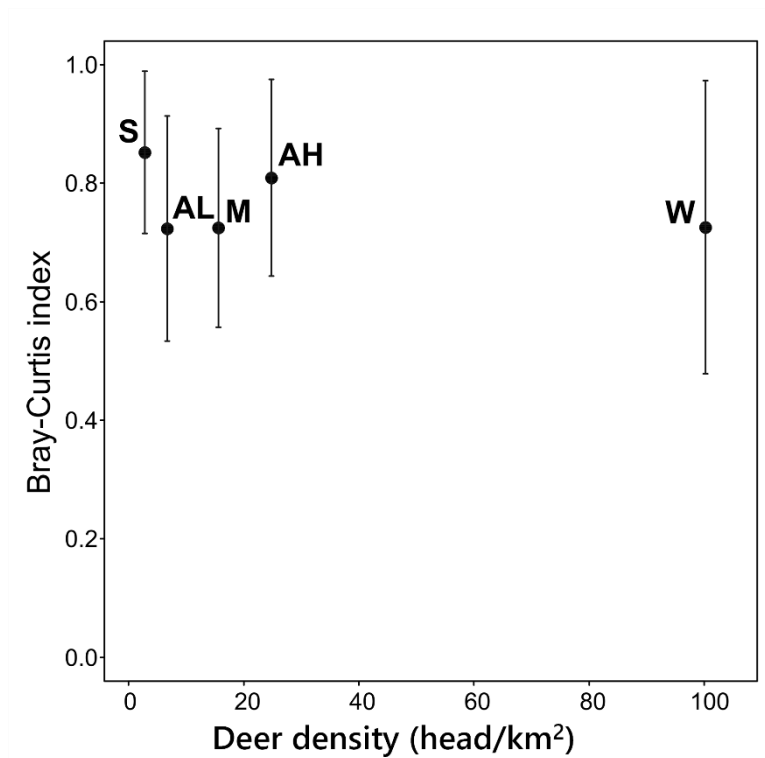
2 **Figure 5** Seasonal changes of relative read abundance (RRA) in 6 predominant plant  
 3 taxa

4 (a): *Ficus subpisocarpa*, (b): *Daphniphyllum* spp., (c): *Cryptomeria japonica*, (d):

5 *Machilus* spp., (e): Fagaceae, (f): Araliaceae

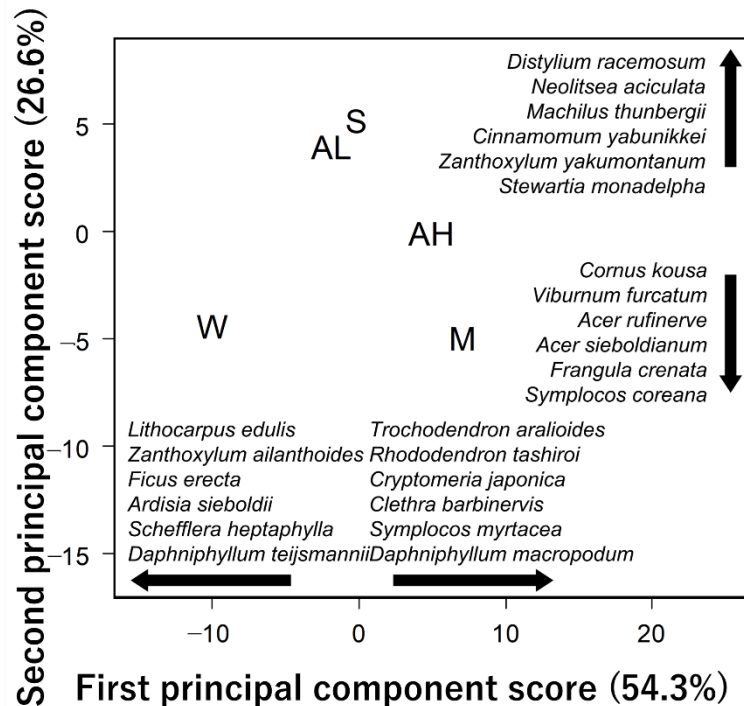
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2 **Figure 6** Relationship between Bray-Curtis dissimilarity and deer density among  
3 samples within areas.  
4 “W”, “S”, “AL”, “AH”, and “M” in the figure refer to the following five areas sampled;  
5 the western forest road (hereafter W; elevation 56–156 m), the Shiratani forest road  
6 (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL;  
7 elevation 74–900 m), the high elevation section of Anbo forest road (hereafter AH;  
8 elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–1936 m). The  
9 points represent the mean of the Bray-Curtis dissimilarity, and the whiskers represent  
10 the standard deviation. The deer density was based on the Kagoshima Prefecture Nature  
11 Conservation Division (2019).

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1 **F Figure 7** Biplot from the principal component analysis for five areas using  
2 frequencies of occurrence of each taxon in 5 areas.  
3 “W”, “S”, “AL”, “AH”, and “M” in the figure refer to the following five areas surveyed;  
4 the western forest road (hereafter W; elevation 56–156 m), the Shiratani forest road  
5 (hereafter S; 218–623 m), the low elevation section of Anbo forest road (hereafter AL;  
6 elevation 74–900 m), the high elevation section of Anbo forest road (hereafter AH;  
7 elevation 900–1334 m), and the Mt. Miyanoura trail (hereafter M; 1334–1936 m). The  
8 percentage of variance explained by PC1 or PC2 is shown in parentheses on the axis  
9 label. Five species with the largest positive or negative contributions to PC1 and PC2  
10 were shown in the figure. The x-axis (PC1) is inverted, that is, it decreases toward the  
11 right and increases toward the left to compare this figure with Figure 3.  
12

- 1 **The Support information (Supplemental Methods, Figure S1-S2, and Table S1-S4)**
- 2 **can be downloaded from the web links below.**
- 3 [https://docs.google.com/document/d/1Sr-3vZ4QOWAAZ5ZEhvTmHhg-](https://docs.google.com/document/d/1Sr-3vZ4QOWAAZ5ZEhvTmHhg-imfW3kZo/edit?usp=sharing&oid=101489418039772797854&rtpof=true&sd=true)
- 4 [imfW3kZo/edit?usp=sharing&oid=101489418039772797854&rtpof=true&sd=true](https://docs.google.com/document/d/1Sr-3vZ4QOWAAZ5ZEhvTmHhg-imfW3kZo/edit?usp=sharing&oid=101489418039772797854&rtpof=true&sd=true)