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1 **Visualization of the seasonal shift of a variety of airborne pollens in western Tokyo**

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17

19   **Abstract**

20   Airborne pollens cause pollinosis and have the potential to affect microphysics in clouds;

21   however, the number of monitored species has been very limited due to technical difficulties

22   for the morphotype identification. In this study, we applied an eDNA approach to the

23   airborne pollen communities in the suburbs of the Tokyo metropolitan area in Japan, within a

24   mixed urban, rural, and mountain landscape, revealing pollen seasonality of various taxa (a

25   total of 78 families across the period) in the spring season (February to May). Those taxa

26   distinctly shifted in the season, especially in the beginning of February and the middle of

27   April. Air temperature shift was an obvious key factor to affect the airborne pollen

28   community, while the influence of other meteorological factors, such as wind speed,

29   humidity, and precipitation, was not clear. Taxonomic classification of major Amplicon

30   Sequence Variants (ASVs) indicates multiple pollen sources, including natural forest, planted

31   forest, roadside, park lands, and horticultural activities. Most major ASV belongs to Japanese

32   cedar (*Cryptomeria japonica*), which is the most notable allergen that causes pollinosis in

33   Japan, peaking in mid-February to March. Backward trajectory analysis of air masses

34   suggests that the Japanese cedar and other Cupressaceae plantation forests in the western

35   mountains were a significant source of airborne pollen communities detected at our sampling

36   site. Other major plant pollen sources, including Japanese zelkova (*Zelkova serrata*) and

37   ginkgo (*Ginkgo biloba*), emanated from the nearby parks or roadside regions. This study's

38   approach enables us to visualize the phenology of multiple pollen, including timing and

39   duration. Long-term monitoring of this type would provide additional insight into

40   understanding the role of climate change on pollen transmission and links to flowering

41   events.

42

2

Introduction

Aerosolization of pollen is a natural part of the pollination process for the majority of

gymnosperms and a smaller part of angiosperms. Anemophilous (wind pollinationing) plants

generally have more flowers with more pollen grains than animal pollination plants

(Friedman & Barrett 2009), and pollen release and dispersion are controlled primarily by

winds, though also through humidity and temperature (Whitehead 1983; Picornell *et al.*

2019). The dispersal range of the pollen increase as the terminal settling velocity decreases

(Niklas 1985). Terminal settling velocity is determined by the physical parameters of pollens

such as size, shape, and bulk density (Hirose & Osada 2016) and those can change the range

of transportation if once into turbulence. For example, Japanese cedar, which is the most

common pollen causing pollinosis in Japan, can travel and induce symptoms over more than

a 100 km range (Okamoto *et al.* 2009). Furthermore, smaller size pollen such as birch pollen

can stay in the air for a few days and be transported much further: 1,000 km (Sofiev *et al.*

2006). The long-range transportation of these pollens affects human health issues such as

pollinosis.

 Seasonal allergic rhinitis in Japan is caused by pollen of multiple species, including Japanese

cedar, Japanese cypress, birch, alder, beech, oak, elm, grass such as mugwort (Kishikawa &

Yokoyama 2016). The most notable airborne pollen is Japanese cedar because the pollinosis

caused by Japanese cedar pollen dominates in Japan, with 26.5 % of the Japanese population

in 2008 having an allergic reaction to Japanese cedar pollen, and prevalence increasing

recently (Okubo *et al.* 2017). One reason for the high prevalence of Japanese cedar pollinosis

is the large surface area of the Japanese cedar forest (18% of Japan's entire land) (Yamada *et*

al. 2014). More than half of the Japanese cedar forest was planted from the 1950s to 1970s

due to the high demand for timber after World War II. The prevalence of Japanese cedar

68 pollinosis is markedly increasing after the 1980s because male flowers become mature to

69 produce more pollen grains after 30 years old. Japanese cedar pollinosis also has become a

70 health issue in Japan and other countries where Japanese cedar was transplanted (Lee *et al.*

71 2015).

72

73 The flowering phenology and pollen initiation (hereafter "pollen seasonality") are changing

74 due to climate change, especially the consequences of air temperature warming. In North

75 America, the length of ragweed (*Ambrosia* spp.) pollen season has been increasing, and this

76 is associated with a delay in the first frost of the fall and lengthening of the frost free period

77 (Ziska *et al.* 2011). In Japan, the length of the *Cryptomeria japonica* pollen season has been

78 increasing too*,* and the first day of observation has become earlier (Teranishi *et al.* 2000).

79 This pollen season change has been associated with mean air temperatures in the previous

80 July because production of pollen after male flowers bloom during this season is much more

81 in the higher July air temperature.

82 Therefore, understanding pollen seasonality changes due to warming temperatures is

83 important for determining impacts on human health. However, most of the existing pollen

84 seasonal data is for limited species (e.g., major species that cause pollinosis in Japan, such as

85 Japanese cedar and Japanese cypress. Data is publicly accessible from the Association of

86 Pollen Information: http://pollen-net.com/welcome.html). Furthermore, the pollen seasonality

87 of other species was not measured routinely because the traditional microscopic counting

88 method is time-consuming in order to cover multiple species and requires investigators'

89 expertise to identify their morphotype (Núñez *et al.* 2017).

90 Recently, comprehensive detection of pollen using an environmental DNA (eDNA) approach

91 via high throughput sequencing (HTS) has enabled acquisition of fine taxonomical resolution

92 of airborne pollens (Kraaijeveld *et al.* 2015; Núñez *et al.* 2017; Banchi *et al.* 2019; Brennan

93   *et al.* 2019). For example, Brennan et al. (2019) showed spatio-temporal shifts of airborne

94   grass pollen at a high taxonomic resolution (genera or species-level) in the UK. Heretofore,

95   this has been difficult to identify based on morphology alone, and so was generally

96   categorized only at the family level (Poaceae).

97   In this study, we focus on the airborne pollen in the suburbs of Tokyo because there is closer

98   to possible source region of Japanese cedar and including or surrounding various vegetation

99   types (e.g., forest, roadside, park lands, and horticulture) that cause seasonal allergic rhinitis.

100   In order to identify the pollen seasonality (i.e., the start date and duration of pollen dispersal)

101   of multiple species, we applied the eDNA approach (next generation sequencing and

102   bioinformatics) to airborne pollen sampled on a fine time scale (every 24 hours during from

103   February to mid-May) and showed a seasonal shift of multiple taxa at the genera or species-

104   level, including identifying the start date and duration of each pollen type.

105

106   **Method**

107   ***Bioaerosol sampling***

108   Airborne particles were sequentially collected (every 24h interval from 0:00 Japan Standard

109   Time) on the rooftop of the building of the National Institute of Polar Research (26.7 m AGL.

110   N 35°42'44.6", E 139°24'32") in the western suburbs area of Tokyo (Fig. 1) from February to

111   May 2016 using a sequential aerosol sampler (GS-10N, Tokyo Dylec Corp.). Samples were

112   filtered through 47 mm diameter quartz filters (Tissuquartz™ Filters, 2500 QAT-UP, Pall)

113   mounted in sterilized open NILU (Norwegian Institute for Air Research) filter holders at a

114   flow rate of average 30.2 L /min. In order to remove potential contamination, quartz filters

115   were combusted (500 °C for two hours) before using, and filter holders were hand washed

116   with detergent followed by wiping by DNAaway tissue (Thermo Fisher Scientific Inc.) on a

117   clean bench.

118

DNA sequencing

In order to avoid contamination, all processes before Polymerase Chain Reaction (PCR)

amplification were done in a laminar flow clean bench (PCV-1305BNG3-AG, Hitachi). The

clean bench was sanitized with a UV lamp overnight, and pipettes were sterilized in a DNA

cross-linker (CL-1000, UVP) box inside the clean bench. Genomic DNA in bioaerosols

captured on quartz filters was extracted using the FastDNA™ SPIN Kit for Soil (MP

Biomedicals, Santa Ana, CA). The quartz filter was initially pulverized during the bead

beating step, but in order to maximize the yield of DNA, all fragments of the filter were

carried over until the final elution step. The ribulose-bisphosphate carboxylase gene (*rbc*L)

was amplified using the forward primer: 5'-CTTACCAGYCTTGATCGTTACAAAGG-3'

and the reverse primer: 5'-GTAAAATCAAGTCCACCRCG-3' (Erickson *et al.* 2017) with

Illumina overhang adaptor sequences attached to each 5′ end, by Ex-Taq HS (Takara, Shiga,

Japan). PCR reaction conditions comprised 35 cycles of denaturation at 94 °C for 20 s,

annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min and an additional final

elongation at 72 °C for 5 min using a GeneAmp PCR System 9700 (Applied Biosystems,

CA, USA). Subsequent clean-up and indexing of PCR amplicons were performed by

following Illumina standard protocol for 16S metagenomic sequencing library preparation.

All samples were sequenced at NIPR using a MiSeq (Illumina, San Diego, CA). Raw

sequence data are available from the Sequence Read Archive of the National Center for

Biotechnology Information (NCBI): PRJNA676177.

Sequence analysis

All sequence libraries from samples were clustered into amplicon sequence variants

(ASVs) using the R package "DADA2" (Callahan *et al.* 2016). Taxonomy was assigned by

6

143   the Naive Bayes Classifier method in The Ribosomal Database Project (RDP) Classifier

144   (Wang *et al.* 2007) implemented in DADA2 using the *rbc*L database (Bell *et al.* 2017) with

145   sequences of closest relatives of our major ASVs using Basic Local Alignment Search Tool

146   (BLAST) implemented in Geneious R10 (https://www.geneious.com)

147   (https://figshare.com/account/home#/projects/90272). The alpha diversity (the number of

148   ASVs), taxonomy visualization, and Non-metric multidimensional scaling (NMDS) were

149   performed by R package phyloseq (McMurdie & Holmes 2013). Kruskal-Wallis test and

150   Tukey's test were performed by kruskal.test and TukeyHSD functions in R package stat,

151   respectively. The distance-based redundancy discriminate analysis (dbRDA) with

152   environmental data (see "Environmental data" in this section) was analyzed using R package

153   Vegan (Oksanen 2015). The closest relatives of the major 20 ASVs were searched by BLAST

154   and assigned the finest taxonomy for each sequence. The first day of dispersal season of each

155   pollen was defined as the first day of 3 consecutive days, in which relative abundance was

156   more than 1% of maximum relative abundance through the season. And the last day was

157   defined as the day before 3 consecutive days, in which relative abundance was less than 1%

158   of maximum relative abundance through the season. Each pollen period was determined from

159   the days between the first and the last day of the season.

160

161   *Environmental data*

162   Environmental data was taken by the Automated Meteorological Data Acquisition System

163   (AMeDAS), the Japan Meteorological Agency

164   (http://www.data.jma.go.jp/obd/stats/etrn/index.php). Air temperature, moisture, wind speed,

165   and PM10 were measured at the "Tachikawa" station, the adjacent building of the study site

166   (Tachikawa City Hall). Precipitation was measured at the "Ome" station, 15 km northwest of

167   the study site (Fig. 1b).

168

Number of pollen grains

170   The number of pollen grains (grains/m3 of air) was monitored by an automated pollen

171   monitoring system of the Ministry of Environment (see the detail in "Pollen seasonality" in

172   Introduction) at Kodaira City Health Center, 6.5 km northeast from the study site. Hourly

173   total concentration during main pollen season (February - May) since 2006 is publically

174   available at http://kafun.taiki.go.jp/DownLoad1.aspx (in Japanese) and daily sum

175   concentration is shown in Figure 2c. Raw data (Number of pollen.txt) were deposited in

176   https://figshare.com/account/home#/projects/90272.

177

Vegetation map

179   Vegetation data (National surveys on the Natural Environments) managed by the Biodiversity

180   Center for Japan, the Ministry of the Environment, are available as KML and Shape data

181   format are from http://www.biodic.go.jp/trialSystem/EN/kmlddl.html. The 6th vegetation

182   survey data in the 2nd mesh point (red square in SI Fig. 1) was used for analysis. Shape data

183   was edited and redrawn using "MANDARA 10"(http://ktgis.net/mandara/index.php) in

184   Fig.1a.

185

Backtrajectory

187   The three-dimensional trajectories of air masses arriving at the study site (calculation height

188   = 500 m AGL) were calculated by the Hybrid Single Particle Lagrangian Integrated

189   Trajectory Model (HYSPLIT4) (Draxler & Hess 1998; Stein *et al.* 2015) implemented in R

190   package SplitR v0.4 using 1 degree gridded meteorological data from the Global Data

191   Assimilation System (GDAS). Calculation length of backward air trajectories was 12h.

192

194 **Result**

195 *The number of total and unique sequences*

196 We attempted to undertake PCR analysis on samples from all days between February 1 to

197 May 13 2016, and most of the samples were successfully amplified and sequenced. However,

198 since some samples (February 3-5, 10 and 13-15, March 7 and 26, and April 26) were not

199 amplified, we excluded these samples from sequencing and downstream analysis. On

200 average, 60574 and standard deviation (SD) 10758 sequences were retrieved from each

201 sample. These sequences were assigned to 1121 ASVs and an average of 32.0 ASVs (SD:

202 24.6) per sample ranging from 3 ASV on March 10 to 110 ASVs on April 25. These ASVs

203 were classified into 78 families, with an average of 10.2 families (SD: 8.7) per sample

204 ranging from 2 families and 37 families. The major families were seasonally shifted (Fig. 2,

205 described in more detail in "Seasonal change of ASVs and taxonomy")**,** especially three

206 major families Betulaceae, Cupressaceae, and Fagaceae, which dominated in the early

207 February, later February to March, and April through May, respectively.

208

209 *Detailed taxonomy assignment of major ASVs*

210 Twenty ASVs, which were significant in samples (mean relative abundance throughout the

211 whole period> 0.02%) and could be identified at the species, genus, or family level of

212 taxonomy using BLAST against NCBI nr/nt database, were manually selected (SI Table 1,

213 Fig. 3). Taxonomy identification of these ASVs was made as follows. For ASV1, ASV9, and

214 ASV17, a single BLAST search candidate species was categorized with 100% BLAST

215 identity (*Cryptomeria japonica*, *Ginkgo biloba*, and *Morella rubra*, respectively). For ASV2,

216 ASV7, ASV8, ASV15, and ASV23, candidate species were multiple with 100% BLAST

217 identity except for ASV15 (97.1%), but only one species was selectable based on their

218   habitats (*Chamaecyparis obtusa, Chamaecyparis pisifera*, *Zelkova serrata*, *Corylus*

219   *heterophylla*, *Torreya nuciferaa*). For ASV3, ASV4, ASV5, ASV6, ASV10, ASV11,

220   ASV12, ASV14, ASV18, ASV19, ASV33, and ASV34, candidate species or genus were

221   multiple with 100% BLAST identity, and only genus or family was selectable. The total

222   relative abundance of these selected (only) 20 ASVs was 0.89 out of 1 on the average (SD: +-

223   0.10) (SI Fig. 2), and fraction of these representing other pollen and plant tissue was much

224   smaller.

225

226   *Seasonal change of ASVs and taxonomy*

227   The numbers of ASVs showed significant variations during our sampling period (Kruskal-

228   Wallis: p < 0.001), and those in April and May were significantly higher than in February and

229   March based on Tukey's test (SI Table 2). An NMDS ordination plot of beta diversity by

230   Bray–Curtis dissimilarity (SI Fig. 3) shows that the airborne plant community was seasonally

231   shifted by month. The numbers of Families also showed significant variations during our

232   sampling period (Kruskal-Wallis: p < 0.001), with number in April and May were

233   significantly higher than in February and March by Tukey's test (SI Table 3). Seasonality of

234   20 selected are shown in Figure 3. For example, the family *Cupressaceae*, *Cryptomeria*

235   *japonica* (ASV1), the dominant ASV among all ASVs, primarily dominated in Febuary and

236   March. Then, *Cupressaceae's* relative abundance is gradually shifted to *Chamaecyparis*

237   *pisifera* (ASV7) and *Chamaecyparis obtusa* (ASV2) in March. Duration of dispersal period

238   of each pollen was listed in SI Table 4.

239

240   *Number of airborne pollen grains*

241   The number of pollen grains measured by the automated pollen monitoring system is shown

242   in Fig. 2 c. March is the highest month by average number (813 +- 529 grains/m3) followed

243   by April (757 +- 788 grains/m^3), May (392 +- 229 grains/m^3), and February (326 +- 324

244   grains/m^3). Monthly change was significant (Kruskal-Wallis: $p < 0.001$).

245

246   *Environmental factors*

247   Environmental data, measured in nearby weather monitoring stations, show the seasonal

248   change of meteorological factors, including the gradual increase of air temperature (SI Fig.4).

249   Among 5 factors (air temperature, moisture, precipitation, wind speed, and PM10), distance-

250   based redundancy analysis (dbRDA) shows that the only air temperature was significantly

251   related to community change ($F = 23.3$, $p = 0.001$, SI Table. 5).

252

253   **Discussion**

254   *Plant type of airborne pollen*

255   Various families of plants were found and the majority of these were likely originated from

256   airborne palynomorph transport, because the sampling site of this study is located on the

257   rooftop of a 26.7 m high building. We found that the majority of the sequences belong to

258   trees plant (Fig. 2a), while the presence of grass families (e.g., Poaceae, Plantaginaceae,

259   Araceae) and mosses (e.g., Hylocomiaceae, Funariaceae) are also identified.

260   Pollen of tree anemophilous species is commonly found in spring in Japan; however, the

261   main flowering season of grass anemophilous species, including *Urticaceae*, *Poaceae*,

262   and *Ambrosia* is autumn (Kawashima *et al.* 2007). Therefore, grass pollen might be less

263   abundant during our sampling period. Another reason is that our sampling site is relatively

264   close to the mountain region in western Tokyo, where is extensively covered by uniform

265   planted forest (Fig. 1, Cupressaceae: *Cryptomeria japonica*, *Chamaecyparis obtuse, and*

266   *Chamaecyparis pisifera* plantation). As backward trajectory analysis indicates, air (and

267   contained particles) was predominantly passing from over this mountain region to the site,

268   especially in February (Fig. 4); therefore, ASVs of tree pollens: *Cryptomeria japonica,*

269   *Chamaecyparis obtusa and Chamaecyparis pisifera* (ASV1, ASV2, ASV7) might be very

270   common in air samples, especially February and March. It is also noteworthy that

271   *Chamaecyparis pisifera* is very commonly used as a hedge in homes, and some amount of

272   pollen of *Chamaecyparis pisifera* may be originated from cultivars around the sampling site.

273

274   ***The seasonal shift of airborne pollen***

275   The taxonomy of airborne pollen changed seasonally (Fig.2 a & 3), and their diversity

276   increased along with the air temperature rise over the season. The seasonal change of plant

277   community structure shown by Bray-Curtis dissimilarity also shows a shift along with

278   months (SI Fig. 3). Although pollen release and transport are thought to be controlled

279   primarily by wind and humidity (Whitehead 1983), our results indicate that among 5

280   environmental factors (air temperature, precipitation, moisture, wind speed, and PM10), only

281   air temperature is related to the community change. Therefore, the air temperature as a proxy

282   of the seasonal shift from winter to spring is the most influential factor in alpha (number of

283   ASVs) and beta (community structure) diversities.

284   At the beginning of the sampling season (February 2016), the number of observed ASVs is

285   significantly lower than in other months, and 7 samples (February 3-5, 10, and 13-15) during

286   this period had been failed for PCR, likely due to the low concentration of DNA (i.e., pollen,

287   Fig. 2c). After this period, the pollen of *Cryptomeria japonica* (ASV1) became detectable by

288   PCR, and the number of pollen grains significantly increased around one order higher. This

289   timing is similar to the first days of observation of *Cryptomeria japonica* over 15 years of

290   record (Teranishi *et al.* 2000). Other species of the *Cupressaceae (Chamaecyparis obtusa),*

291   which was also from the same planted forest of *Cryptomeria japonica* (Fig.1b), had lagged

292   with *Cryptomeria japonica* by a month, similar to a previous study (Yamada *et al.* 2014).

293   Then, *Chamaecyparis obtusa* gradually took over *Cryptomeria japonica*, and their number of

294   ASVs reached the highest level in early April. Following this season, the

295   family *Fagaceae*, *Ulmaceae*, *Ginkgoaceae* increased their relative abundance. ASVs (ASV3

296   & 6) belonging to Fagaceae were only taxonomy, which could not be classified at the genus

297   level among the major 20 ASVs, and potentially include *Castanea*, *Castanopsis*, *Quercus*,

298   and *Lithocarpus* (only in ASV6). However, these species are generally distributed in the

299   same type of forests, categorized in "Substitutional vegetation of secondary forest" in the

300   vegetation map. This type of vegetation, which is referred to as "Satoyama", is remarkably

301   abundant around reservoirs (Yamaguchi Reservoir and Murayama Reservoir) and in the

302   foothills forest in the City of Ome and Akiruno (5 km north and 10 km west of the study site,

303   respectively) (Fig. 1b). Substitutional vegetation of secondary forest is also spotted in

304   western mountains, especially above 350 m a.s.l. (20 km west of the study site). The

305   backward trajectory of air masses show that the direction of likely particle transport is quite

306   variable in April and May, with no preferred direction. Therefore, these vegetation types

307   would be a significant source of Fagaceae. Otherwise, *Zelkova*

308   *serrata* in *Ulmaceae* and *Ginkgo biloba* in *Ginkgoaceae* are significant species on the

309   roadside in Tachikawa City and in parks, especially in the Showa Commemorative National

310   Government Park (Fig. 1b, SI Fig. 5), which is a neighboring park of sampling site with 180

311   ha total area. Therefore *Zelkova serrata* and *Ginkgo biloba* are likely to have originated from

312   the urban area nearby.

313   Getting information on each pollen dispersal seasonality and their potential source from this

314   study is useful for personal allergic rhinitis treatment. In many cases, the cause of rhinitis is

315   hard to detect except for remarkable seasons such as Japanese cedar. Because pollen types

316   and season are very variable in the country has wide longitudinal range, and very limited

317   information on pollen seasonality is available in public. Otherwise, minor pollen also can

318 cause allergic rhinitis (Kawashima *et al.* 2007), and that information from the eDNA

319 approach can provide what kind of pollen should be cared for each location and person.

320

321 *Detection of the phenology of various species*

322 In this study, we found a distinct phenological pattern for each ASV, for example, early start

323 (February 19) and prolonged period (58 days) for *Cryptomeria japonica* (ASV1), and in

324 contrast, a late start (April 15) and the short period (16 days) for *Ginkgo biloba* (ASV9) (Fig.

325 3). These phenological patterns, such as timing (Clot 2003) and the duration of pollen

326 dispersal (Ziska *et al.* 2011), will be changed by future air temperature increases due to

327 global warming. For example, for *Cryptomeria japonica*, the first day of observation became

328 earlier, and the duration got longer in 15 years of record from 1983 to 1998 due to 394-

329 temperature warming (Teranishi *et al.* 2000). Furthermore, this phenological change follows

330 an increase in the number of pollen grains because the temperature in previous July relates to

331 male flower formation during the next season (Ito *et al.* 2008; Yamada *et al.* 2014), and the

332 average temperature in Tokyo has risen 1.15 °C in the past 100 years, which is higher than

333 0.6 °C as the global average (Yamada *et al.* 2014).

334 This study's approach, using the DNA marker as pollen indicator, enables us to show the

335 timing and duration of various taxonomy from various sources, information that difficult to

336 obtain by classical morphological measurement. Therefore, long-term pollen monitoring

337 using DNA markers can supply detail of phenological changes of forest and urban plant

338 communities. However, for minor types which has lower relative abundance, pollen periods

339 were much shorter due to discontinuous distribution. Therefore, the definition of the period in

340 this study should be considered, especially for minor types.

341 This study supports the potential utility of developing a pollen monitoring system using

342 eDNA measurements. In order to construct a new pollen monitoring system, one needs to

343   consider the standard protocol. For example, in this study, we only used *rbc*L for the DNA

344   marker, and it is difficult to identify some of the taxonomy (e.g., Fagaceae in this study).

345   Furthermore, detection accuracy varies by markers (Brennan *et al.* 2019); therefore, we

346   recommend using multiple markers such as *rbc*L, *trn*L, and ITS for better comparison. Also,

347   high volume air samplers, with sampling volumes about one order higher than used in this

348   study, have been introduced for use in recent bioaerosol studies (e.g., Mbareche *et al.* 2018;

349   Archer *et al.* 2019), and these can reduce contamination in the extraction and enrichment

350   processes, thereby improving the detection accuracy of rare taxa.

351

352   **Conclusion**

353   In the air of suburbs of the Tokyo metropolitan area, 78 families were detected during the

354   spring season (February to May) using the eDNA approach. Japanese cedar (*Cryptomeria*

355   *japonica*) was the most major allergen from mid-February to March, which corresponded to

356   the typical pollinosis season in this region. Backward trajectory analysis of air masses

357   suggests that the Japanese cedar and other Cupressaceae plantation forests in the western

358   mountains were a significant source of airborne pollen communities. And other major plant

359   pollen sources, such as Japanese zelkova (*Zelkova serrata*) and gingo (*Ginkgo biloba*) were

360   distributing nearby parks or roadside regions and emanated from there. The phenology of

361   multiple pollen were detected, for example longer period for *Cryptomeria japonica* (58 days),

362   and the short period for *Ginkgo biloba* (16 days). This approach is very useful to understand

363   the regional difference of multiple pollen dispersal between the major cities and prevention

364   and treatment for allergic rhinitis.

365

15

377

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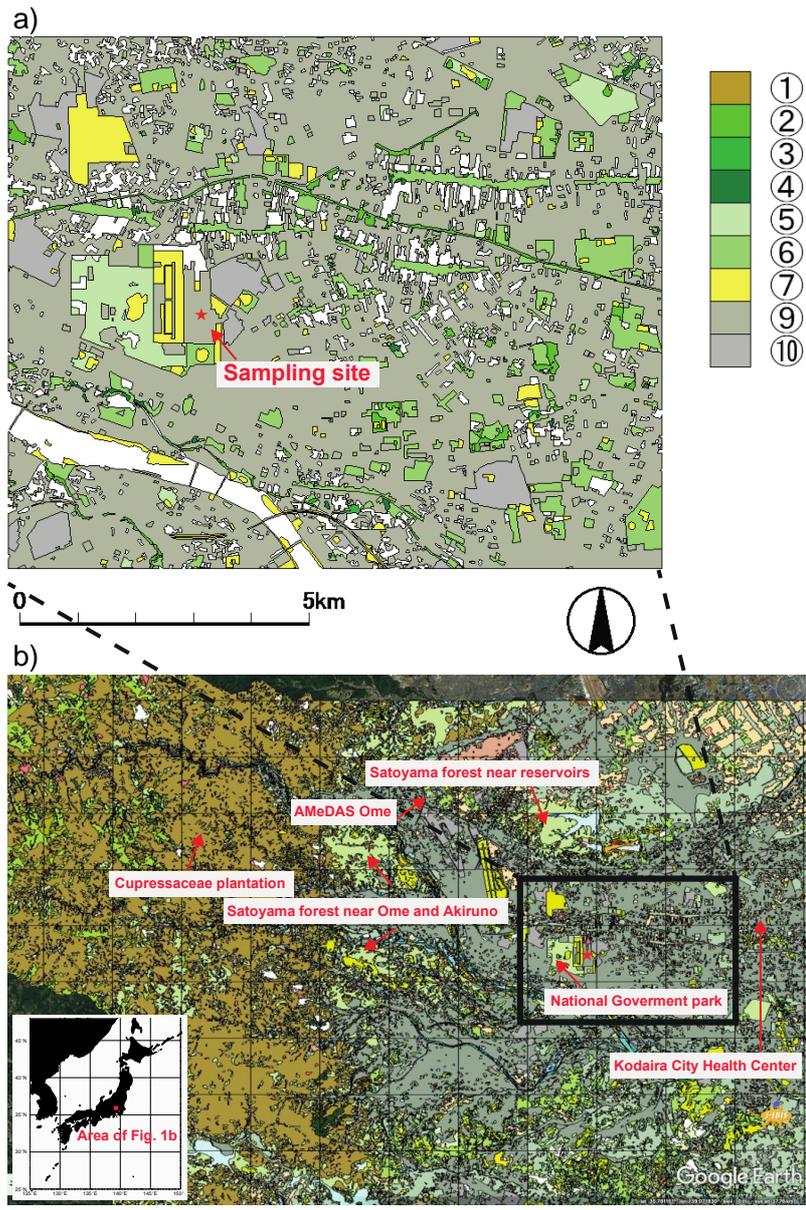
Figure 1: Vegetation map a) near the sampling site, b) in the regional scale. 1: Cupressaceae

plantation (*Cryptomeria japonica, Chamaecyparis obtusa*, *Chamaecyparis pisifera*), 2:

Quercetum acutissimo-serratae, 3: *Quercetum myrsinaefoliae*, 4: *Quercus myrsinaefolia*

premises forest, 5: Park, Graveyard etc. with residual, planted trees, 6: Urban and

residential district with many trees, 7: Golf links and Turf, 8: Plant communities in

clear-cut area, 9: Urban district with a few trees, 10: Factory and industrial area. More

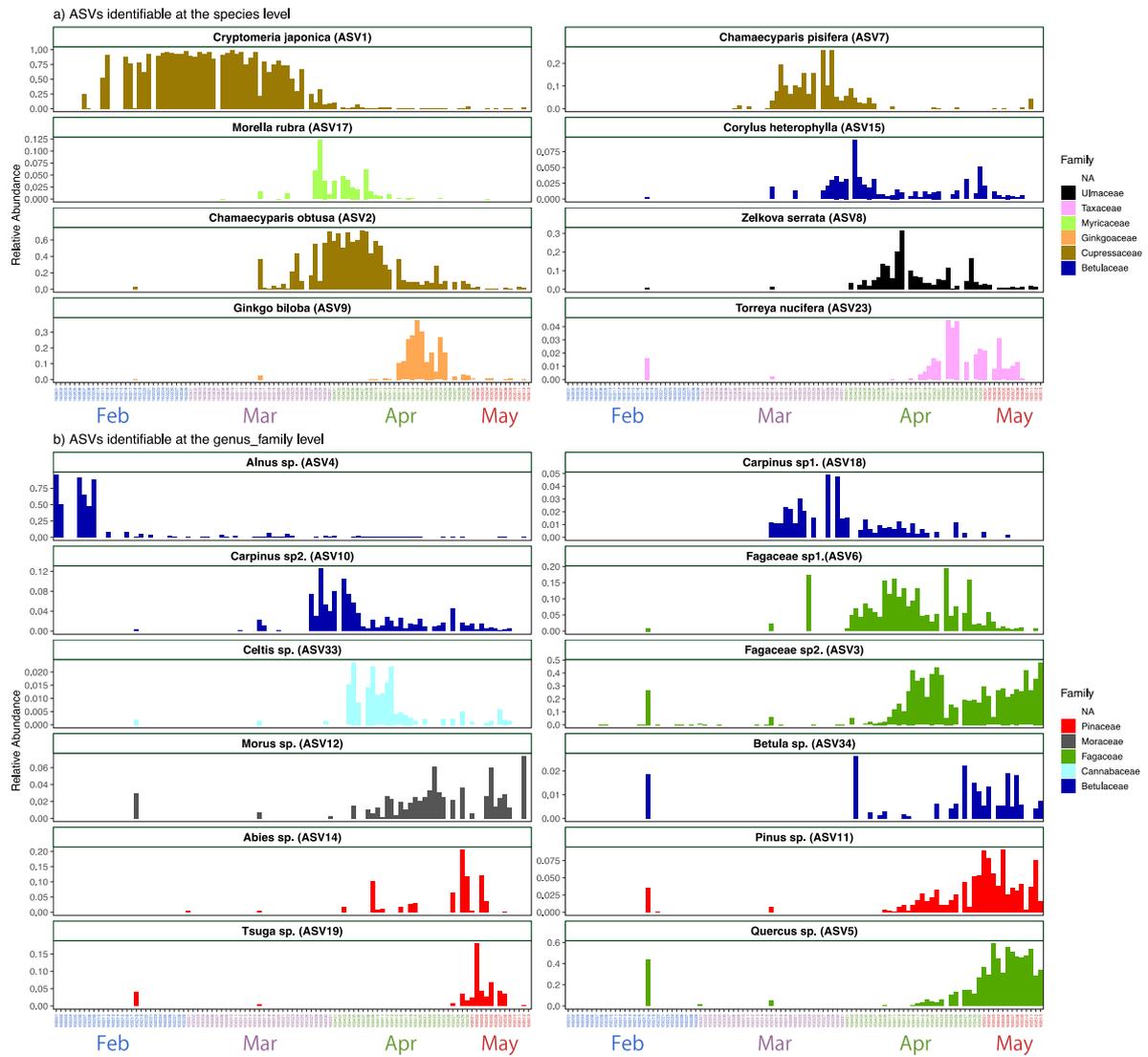detail is available from the Ministry of the Environment (detail in Method section).
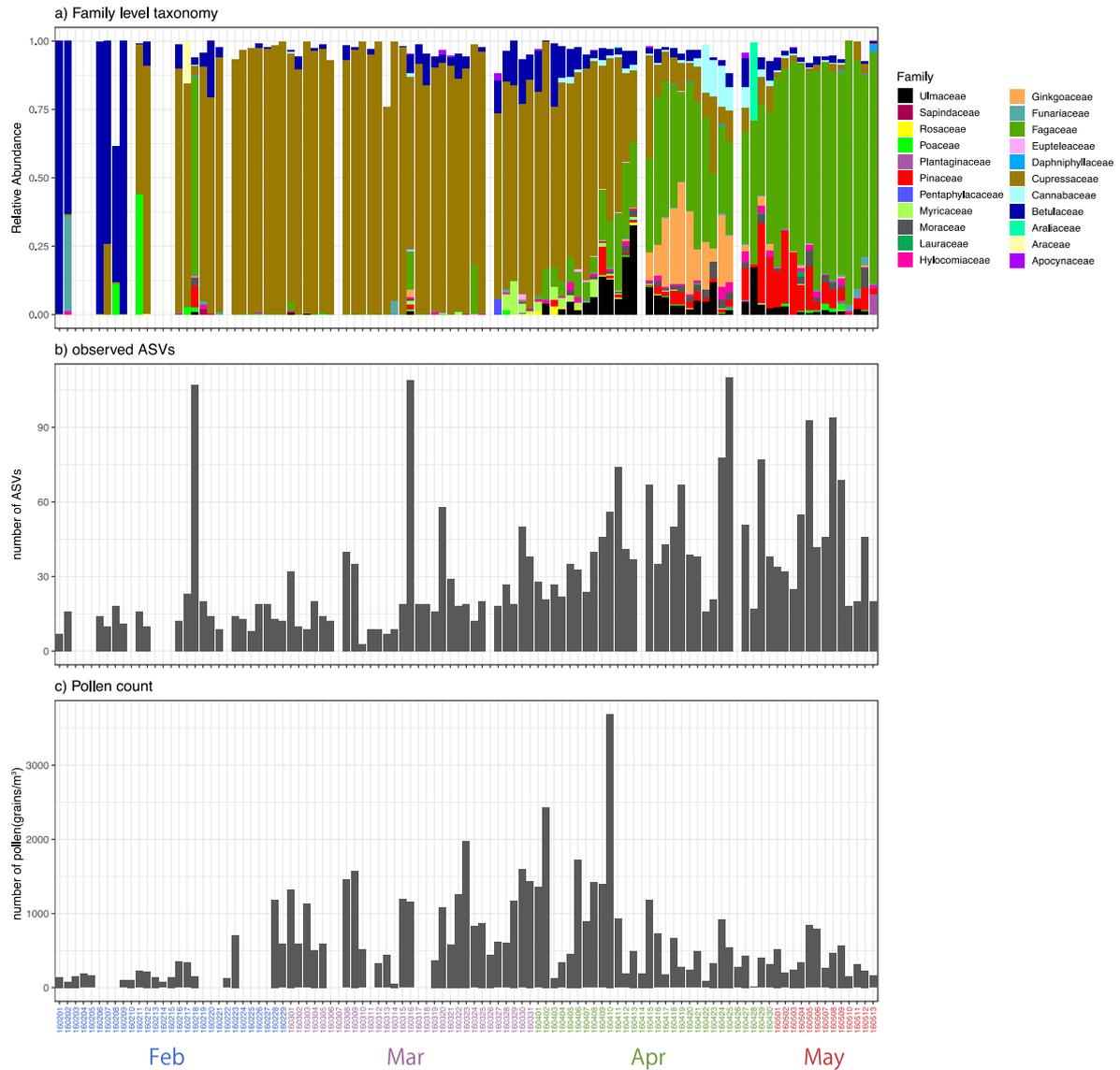
Figure 2: Seasonal change of a) taxonomy at the family level, b) the number of observed ASVs, and c) pollen concentration by an automated pollen monitoring system of the Ministry of Environment.

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475   Figure 3: Seasonal change of 20 major ASVs, identifiable at the species level (a), and at

476   genus-family level (b).
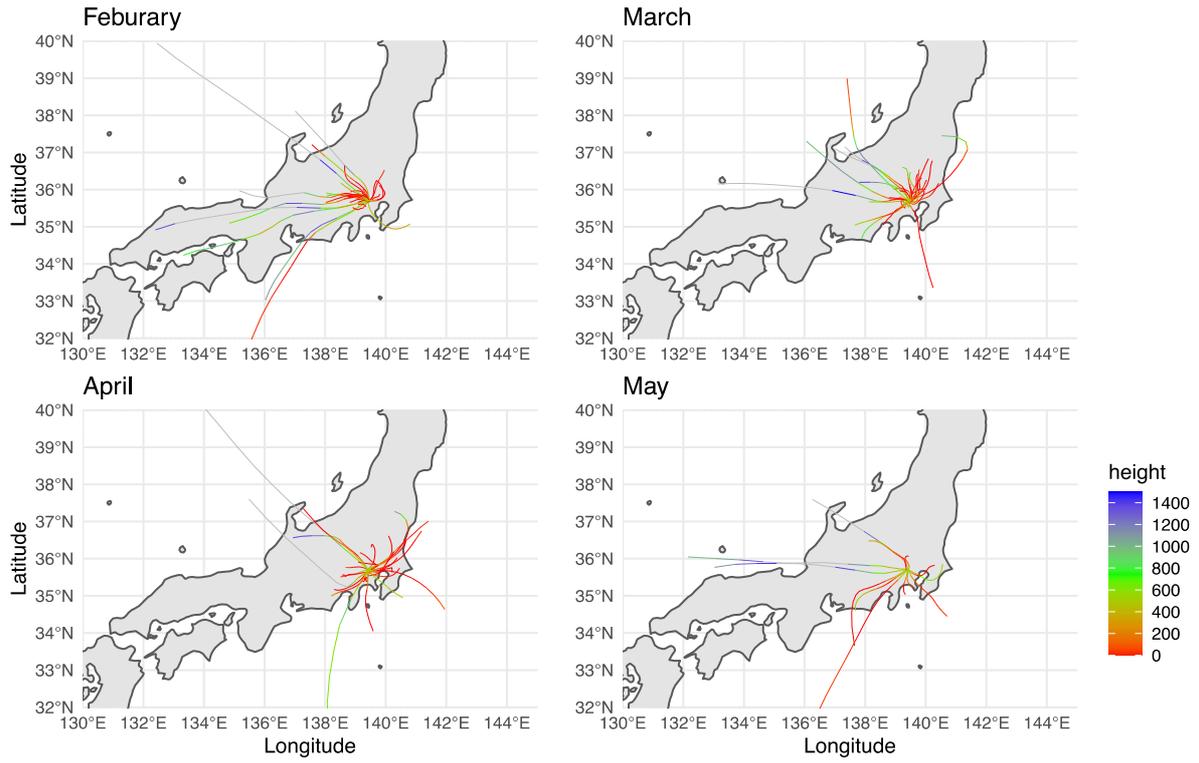
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485   Figure 4: 24 hour back trajectories calculated using HYSPLIT4 with GDAS 1 degree data,

486   initiated above the sampling site (500 m).

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