Science of the Total Environment 799 (2021) 149254

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Relative abundance of saccharides, free amino acids, and other compounds in specific pollen species for source profiling of atmospheric aerosol

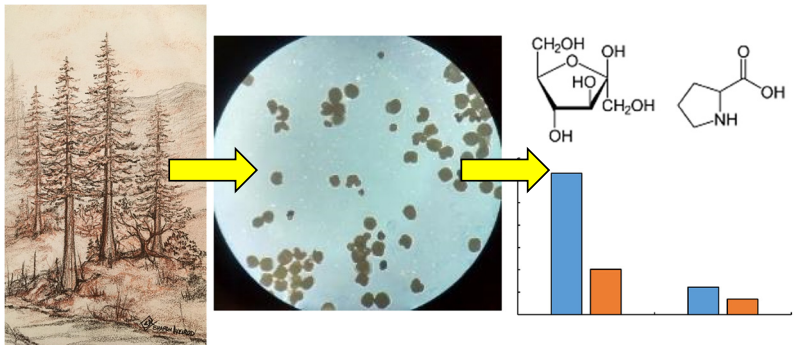
Kevin Axelrod, Vera Samburova, Andrey Y. Khlystov *

Desert Research Institute, 2215 Raggio Parkway, Reno, NV 89512, USA

HIGHLIGHTS

- Several pollen species were analyzed for saccharide, free amino acid, anhydrosugar, and resin acid content.
- Saccharides and free amino acids show a significant, species-specific contribution to the total pollen weight.
- Saccharides alone are not sufficient for species identification, but together with amino acids, species are better identified.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 April 2021
Received in revised form 23 June 2021
Accepted 21 July 2021
Available online 27 July 2021

Editor: Philip K. Hopke

Keywords:
Atmospheric aerosol
Bioaerosol
Pollen
Saccharide
Anhydrosugar
Resin acid
Amino acid

ABSTRACT

Though studies in bioaerosols are being conducted with increasing frequency over the past decade, the total breadth of knowledge on bioaerosols and their role in atmospheric processes is still minimal. In order to better characterize the chemical composition of fresh biological aerosol for purposes of source apportionment and tracing in the atmosphere, several plant pollen species were selected for detailed chemical analyses. For this purpose, different pollen species were purchased and collected around Reno, Nevada, USA, for further extraction and detailed chemical analysis. These species included aspen, corn, pecan, ragweed, eastern cottonwood, paper mulberry, rabbitbrush, bitterbrush, lodgepole pine, and Jeffrey pine. Saccharides, free amino acids, and various other polar compounds (e.g., anhydrosugars and resin acids) were quantitatively analyzed using gas chromatography and ultra-high performance liquid chromatography coupled with mass spectrometry techniques (GC–MS and UPLC-MS), with the purpose to identify differences and nuances in chemical composition of specific pollen species. The saccharides β-D-fructose, α-D-glucose, and β-D-glucose were ubiquitously found across all pollen samples (10), and sucrose was found in five samples. D-galactose was also found in pine species. Total saccharides were 4.0 to 29% of total dry weight across all samples. Total free amino acids were 0.29% to 15% of total dry weight across all samples, with the most common amino acid being proline. Chemical profiles (including both saccharides and amino acids) of surface-deposited aerosol in the Lake Tahoe area correlated most closely with pine pollen than other analyzed pollen species, indicating that chemical profiles of pollen can be used to infer its contribution to local aerosols.

* Corresponding author.
E-mail address: andrey@dri.edu (A.Y. Khlystov).

https://doi.org/10.1016/j.scitotenv.2021.149254
0048-9697/

1. Introduction

Biological aerosols, or bioaerosols, are defined as any aerosol in the atmosphere that is biological in origin. Common examples of bioaerosols include bacteria, fungal spores, pollen, pieces and fragments of plants or animals, and aerosols that contain biological materials (Estillore et al., 2016; Fröhlich-Nowoisky et al., 2016; Graham et al., 2018; Wang et al., 2015). Conventionally, the size of bioaerosols may range from 10^{-9} m (e.g., biological molecules) to 10^{-4} m (e.g., pollen) in diameter (Fröhlich-Nowoisky et al., 2016). The term can also be applied to aerosols in which biological materials contribute to part but not all of their composition (e.g., sea spray aerosols (Graham et al., 2018)). Bioaerosols have been found abundant in both rural and urban air, with number concentrations ranging from tens to hundreds (Andreae and Rosenfeld, 2008) and even thousands (Steiner and Solmon, 2018) per cm^3 across a variety of environments, with the concentrations likely underestimated due to difficulties with identifying aerosols as bioaerosols (Andreae and Rosenfeld, 2008). Bioaerosols can be emitted in a variety of fashions. One common means of emission is the creation of sea spray aerosol containing biological organisms or materials (Fröhlich-Nowoisky et al., 2016; Graham et al., 2018; Wilson et al., 2015). There are several mechanisms of land-based aerosolization, including active release from biological organisms, suspension via wind (Fröhlich-Nowoisky et al., 2016), and release via rain droplet impaction on biological-containing materials (Joung et al., 2017). In addition, bioaerosols may be released into the air via human activity, such as composting facilities (Wéry, 2014), animal/livestock production facilities (Anderson et al., 2016; Douglas et al., 2018; Millner, 2009), and agricultural activities (Hansen et al., 2012; Lee et al., 2006).

Bioaerosols play a critical role in many aspects of the environment and human life; they serve an important role in reproduction and spread of plants and other organisms (Fröhlich-Nowoisky et al., 2016), as well as impacting human health in ways such as spreading bacterial and viral diseases like influenza, tuberculosis, measles, and streptococcus among many others (Anderson et al., 2016; Bonifait et al., 2014; Fröhlich-Nowoisky et al., 2016; Triadó-Margarit et al., 2017) and causing seasonal allergies (D'Amato et al., 2007) and exacerbating other respiratory conditions like asthma (Taylor et al., 2007). Bioaerosols can make a significant contribution to the total aerosols mass in the atmosphere (Fröhlich-Nowoisky et al., 2016; Monks et al., 2009), as well as serve as cloud condensation nuclei (CCN) (Whitehead et al., 2016; Zhang et al., 2021), impacting cloud formation, weather, and climate, with studies indicating that primary bioaerosols have an effect on the number of ice nuclei (IN) in forest ecosystems (Andreae and Rosenfeld, 2008; Tobo et al., 2013; Whitehead et al., 2016). Several types of bioaerosols have been determined to be effective CCN, owing to their relatively large size in comparison to other types and high amounts of water-soluble constituents (Andreae and Rosenfeld, 2008). Pollen, the male gamete and haploid entity produced by most land plants, has been demonstrated effective as CCN in laboratory experiments (Pope, 2010). In addition, pollen grains can rupture under high-humidity conditions, producing subpollen particles (SPPs) that may also act as CCN and IN, and are considered more climatologically relevant due do their smaller size and greater numbers than whole pollen grains (Steiner et al., 2015; Steiner and Solmon, 2018; Tang et al., 2019). Pollen is critical in the reproductive cycle of plants and can be affected by many factors, with air pollution (Sénéchal et al., 2015) and heat stress (Jiang et al., 2015) being among important atmospheric phenomena among them. In addition, pollen are among the most common allergens among humans (affecting ~40% of population in industrialized nations (Fröhlich-Nowoisky et al., 2016)) and thus are considered to be a major factor in overall air quality (D'Amato et al., 2007; Fröhlich-Nowoisky et al., 2016). Studies have assessed the allergenic components of pollen and asserted them to be an important point of study within the public health field (Dedic et al., 2009; Gong et al., 2015; Stanic et al., 2009).

There is a diverse range of pollinating plant species, and as such, many studies do not focus on specific pollen species when analyzing chemical composition. In fact, a large number of studies focused on the chemical composition of bee pollen (pollen mixed with enzymes such as amylase and catalase in bee saliva) (Denisow and Denisow-Pietrzyk, 2016), since it has been used and marketed for nutritional value and health benefits throughout history (Chen et al., 2018; Marghitas et al., 2009; Nogueira et al., 2012; Stabler et al., 2018; Yang et al., 2007). Pollen is known to contain many biological molecules, including carbohydrates/saccharides (Pacini et al., 2006), fatty acids (DeGrandi-Hoffman et al., 2018; Ischebeck, 2016; Lawson et al., 2021), proteins (Gong et al., 2015), of which include allergens (Dedic et al., 2009; Stanic et al., 2009), amino acids (DeGrandi-Hoffman et al., 2018; Lawson et al., 2021; Schwacke et al., 2020), and minerals (Stabler et al., 2018; Yang et al., 2013), though carbohydrates/saccharides are known to be the primary constituent (Carrizo García et al., 2010; Komosinska-vassev et al., 2015; Pacini et al., 2006). Carbohydrate/saccharide composition of pollen can come in many varieties, including starches (generally in fresher, unreleased pollen) (Pacini et al., 2006), pectin, and disaccharide and monosaccharide sugars. They are also known as important energy sources for pollen germination and tube growth. It has been found in vitro that large di- and oligosaccharides play a crucial role in pollen germination, while smaller monosaccharides such as glucose and fructose do not, and in fact may inhibit germination (Hirsche et al., 2017), suggesting the importance of polysaccharides in early pollen development. As such, pollen is also considered a major overall contributor of the saccharide sucrose, which has been used as a tracer in the atmosphere (Fu et al., 2012; Zhu et al., 2015). Still, monosaccharides are known to be common in several pollen species, among them being glucose, galactose, and arabinose (Nakamura and Suzuki, 1981).

Overall, the role that the chemical constituents of pollen have in atmospheric chemistry is not well known. In many atmospheric processes, aerosols and their chemical constituents play an important role in atmospheric heterogeneous chemistry (multiphase chemistry), with implications on cloud/ice nucleation and air quality (Ravishankara, 1997). Atmospheric chemistry has been shown to alter bioaerosol properties; for example, IN abilities of tree pollen can be affected by atmospheric oxidation (Gute et al., 2020). But, the total breadth of knowledge on bioaerosols' roles in these atmospheric chemical processes remains limited (Estillore et al., 2016; Pan et al., 2021). As such, it is important to understand the chemical composition of certain types of aerosol, how that composition compares with other aerosols, and the overall impact it has on the atmosphere. Even though several studies discussed chemical composition of pollen, especially bee pollen for nutritional purposes, little is known about the chemical composition of atmospheric pollen and its contribution to atmospheric organic compounds (including saccharides, proteins, organic acids, and lipids) and total aerosol concentrations on a global scale. Thus, the purpose of this research is to determine species-specific chemical composition of several types of pollen species, including store-bought pollens of common trees and shrubs from several continents, as well as pollens of plants endemic to the Great Basin Desert and Eastern Sierra Nevadas in northern Nevada and California, USA. Compound groups focused on are saccharides, amino acids, anhydrosugars, and resin acids.

2. Experimental section

2.1. Standards and materials

Resin acid, anhydrosugar, and saccharide standards used for GC–MS and UPLC–MS analysis were purchased from Sigma Aldrich (Burlington, MA, USA), Accustandard (New Haven, CT, USA), and Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Internal standards and calibration standards are listed in Table S1. Solvents utilized included high-performance liquid chromatography-grade water, acetonitrile, and

toluene, purchased from Fisher Scientific (Waltham, MA, USA). For derivatization, pyridine (Sigma-Aldrich) and *N*,*O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMSC) (Thermo Scientific, Waltham, MA, USA) were used.

Amino acid standards used for UPLC–MS analysis were purchased from Sigma Aldrich (Burlington, MA, USA), as well as Toronto Research Chemicals (North York, ON, Canada), and are listed in Table S1. Standards were dissolved in HPLC-grade acetonitrile with 0.1 N hydrochloric acid from Sigma Life Science (Burlington, MA, USA). Eluents used were HPLC-grade acetonitrile and toluene from Fisher Scientific, and eluent additives included formic acid and ammonium formate, purchased from Fisher Scientific and Sigma Aldrich, respectively.

2.2. Pollen species

Six pollen species used were purchased from online chemical and laboratory suppliers (Table 1). These pollens included pecan, ragweed, paper mulberry, and corn pollen purchased from Polysciences, Inc. (Warrington, PA, USA), and aspen and eastern cottonwood pollen purchased from Sigma-Aldrich (Burlington, MA, USA).

The experimentally collected pollens included species in several locations across the area of Lake Tahoe and Northern Nevada, USA. Surface-deposited aerosols were also collected off of patio surfaces and aluminum foil in June and July of 2020 in Reno, NV, Truckee, CA, and the Tahoe Regional Planning Agency air quality sampling site LTCC1 in South Lake Tahoe, CA. Locations and species are given in Fig. 1 and Table 1.

2.3. Pollen sample extract preparation

Water-soluble extracts of pollen were prepared to analyze saccharide and other organic species (e.g., amino acids and resins) composition. Extracts were produced at a concentration of 3 mg of pollen per mL of solvent (ultra-high purity water, Elga-Veolia PURELAB Chorus, UK). Pollen samples were weighed out and mixed with an amount of water that requires to prepare three mg-pollen per mL solution. Then, in order to enhance dissolution of water-soluble compounds, the pollen suspension was run on the Bertin Minilys tissue homogenizer (Rockville MD, USA) at 3 intervals of 20 s at high speed, icing each sample vial for 2 min in between each lysing interval to prevent the sample from overheating from friction caused by lysing. Then, the solution was centrifuged and the supernatant was collected and filtered through 0.22 μm pore size hydrophilic PTFE syringe filters (Foxx Life Sciences, Salem, NH, USA). After extraction, the solutions were stored at 4 °C overnight.

2.4. GC–MS analysis

To make saccharide and polar compounds in pollen extract samples viable for chromatographic separation and detection, a chemical derivatization procedure based on silylation of the polar groups of compounds in the sample was performed (Schummer et al., 2009). Filtered pollen extracts were placed in individual deactivated vials (Waters, Milford, MA, USA) in volumes of 50 μL spiked with the water-dissolved internal standard glucose-d6 intended for quantification of saccharides. Then, the solvent (water) from each sample was evaporated via a gentle nitrogen gas flow using the Pierce Model 18,780 Reacti-Vap Evaporating Unit (Rockford, IL, USA) and ultra-high purity nitrogen gas (Airgas, Radnor, PA, USA). After evaporation of all solvent, 50 μL acetonitrile was added, as well as internal standards for polar compound quantification (Table S1). Samples were then sonicated in their vials for 10 min. 50 μL of the pyridine were added to each sample vial, followed by 150 μL of BSTFA/TMCS reagent. The samples were heated at 65 °C for 2 h, 300 μL of toluene were added to each sample, and the samples were analyzed via GC–MS.

The GC–MS system used was the Varian CP-3800 gas chromatograph in tandem with the Varian 4000 Ion Trap Mass Spectrometer (Varian, Inc., Walnut Creek, CA, USA). The column installed in the gas chromatograph was a 30-m 5% phenylmethylsilicone fused silica capillary column (model DB-5MS, Agilent Technologies, Santa Clara, CA, USA). Samples were run for both quantitative analysis of saccharides and other polar compounds. The polar compounds quantified included various resin acids and anhydrosugars.

2.5. UPLC-MS analysis

For analysis of free amino acid composition of pollen extracts, a UPLC-MS method based on hydrophilic interaction liquid chromatography, adopted from Prinsen et al. (2016) was used. Samples were prepared in the same way as in Section 2.3, but not evaporated, spiked with internal standard, or derivatized. They were instead run on

Table 1
Pollen species purchased and collected for the present study. Two aerosol samples do not have species identifications as they were collected from deposition surfaces, unlike the other samples which were collected directly from plant anthers.

Sample type	Species (common name)[a]	Botanical name	Source or collection location (date)[b]
Purchased species	Aspen	*Populous tremuloides*	Sigma-Aldrich
	Eastern cottonwood	*Populus deltoids*	Sigma-Aldrich
	Ragweed	*Ambrosia artemisiifolia*	Polysciences Inc.
	Paper mulberry	*Broussonetia papyrifera*	Polysciences Inc.
	Pecan	*Carya illinoensis*	Polysciences Inc.
	Corn	*Zea mays*	Polysciences Inc.
Locally-collected species	Jeffrey pine	*Pinus jeffreyi*	**1** (6/29/2020)
	Lodgepole pine	*Pinus contorta*	**2** (6/30/2020)
	Lodgepole pine	*Pinus contorta*	**2** (7/5/2020)
	Lodgepole pine	*Pinus contorta*	**2** (7/6/2020)
	Lodgepole pine	*Pinus contorta*	**3** (6/30/2020)
	Lodgepole pine	*Pinus contorta*	**3** (7/5/2020)
	Lodgepole pine	*Pinus contorta*	**3** (7/6/2020)
	Bitterbrush	*Purshia tridentata*	**4** (5/21/2020)
	Bitterbrush	*Purshia tridentata*	**4** (5/25/2020)
	Rabbitbrush	*Ericameria nauseosa*	**5** (10/18/2019)
	Rabbitbrush	*Ericameria nauseosa*	**6** (10/5/2020)[c]
Locally-collected surface deposition aerosols	South Lake Tahoe, CA		**7** (July 2020)
			1 (June 2020)
	Truckee, CA		

[a] Common names are given for known pollen species. Surface deposition aerosols, the species of which are not definitively known, are referred to by their collection location throughout this paper.
[b] The supplier is listed for purchased pollens, while the location and collection dates are listed for locally-collected pollen species or surface deposition aerosol.
[c] The rabbitbrush collected on October 5, 2020 was only analyzed in the methods of Section 2.5 and results of Section 3.2, due to unavailability of sample during other analyses.

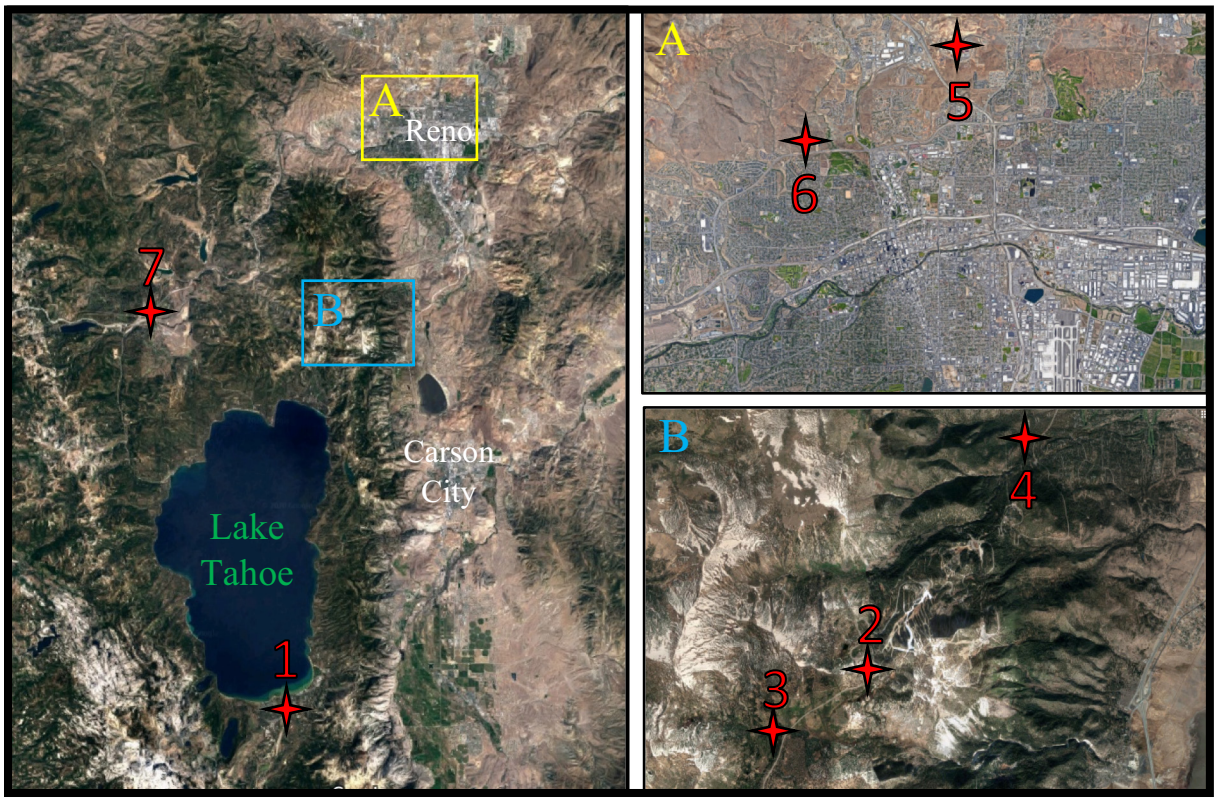


Fig. 1. Locations in northern Nevada and California of pollen/aerosol collection. Images from Google Satellite on 11/13/2020.

UPLC-MS (Prinsen et al., 2016). The UPLC system used was the Waters Acquity UPLC coupled with the Waters MicroMass Quattro Micro API mass spectrometer (Waters Co., Milford, MA, USA). The UPLC instrument had the Waters Acquity UPLC BEH Amide 1.7 μm 2.1 × 150 mm column installed (Waters Co., Milford, MA, USA). Samples were run for quantitative analysis using an external standard quantification method (see Table S1 for standards).

During data analysis, errors for results were calculated by taking the standard deviation of multiple extractions of a single species or sample. If multiple extractions were not performed on a species, the percent error of the most similar species on which multiple extractions were performed was used. The most similar species was determined via matching source (purchased, or field collected) and matching plant type (tree, or other). Explicitly, eastern cottonwood, ragweed, bitterbrush, Jeffrey pine, lodgepole pine, rabbitbrush from 2019, and rabbitbrush from 2020 all were extracted and analyzed multiple times (2-6 times), and thus had their own errors assigned to them. Aspen, paper mulberry, and pecan were assigned the percent error of eastern cottonwood, corn was assigned the percent error of ragweed, and the surface deposition aerosols were assigned the percent error of Jeffrey pine.

3. Results and discussion

3.1. Saccharides and other polar compounds

The results of the GC–MS analysis for saccharides and various other polar compounds in each sample are given in Figs. 2 and 3, respectively. Concentrations of eight saccharides in commercially available and collected pollen species are presented in Fig. 2, in units of micrograms per milligram of dry pollen mass (μg/mg). Though concentrations vary widely among the different pollen species, α-D-glucose and β-D-glucose were found in all pollen samples tested, which is in a good agreement with previous studies on honeybee pollens (Kalaycioglu et al., 2017) and fresh pollens (Fu et al., 2012; Nakamura and Suzuki, 1981; Zhu et al., 2015), though these studies used different pollen species. β-D-fructose was found in all tested pollen samples, with its highest concentration being in the bitterbrush pollen sample (Fig. 2b, 93 ± 9 μg/mg). α-D-glucose was at high concentrations in aspen (Fig. 2c, 67 ± 4 μg/mg), corn (Fig. 2b, 66 ± 1 μg/mg), and bitterbrush (Fig. 2b, 67 ± 2 μg/mg). β-D-glucose was at its highest concentrations in aspen (71 ± 4 μg/mg), corn (65 ± 2 μg/mg), and bitterbrush (74 ± 2 μg/mg) as well.

β-D-fructose, α-D-glucose, and β-D-glucose together comprise the most abundant occurrences across all analyzed pollen species, with all pollen species having a quantifiable concentration of each of these three sugars. However, the percent total of these three combined sugars varies greatly among species (29% of total in aspen, 4.0% of total in eastern cottonwood). Overall, concentrations of β-D-fructose, α-D-glucose, and β-D-glucose across all pollen species correlate well with each other, with Pearson correlation coefficients of 0.97, 0.98, and 0.99 between ß-D-fructose – α-D-glucose, ß-D-fructose - ß-D-glucose, and α-D-glucose - ß-D-glucose, respectively. Fructose and glucose have previously been identified as common constituents of primary biological aerosols, including pollen, decomposed plant materials, airborne fungi and biogenic constituents of suspended topsoil (Medeiros et al., 2006; Samaké et al., 2019; Xiao et al., 2018; Zhu et al., 2015). The strong correlation between these saccharides across the samples tested in our study indicate that the ratios between the concentrations of these saccharides are consistent across these different pollen species. This suggests that these compounds could be useful for identifying pollen contribution to ambient aerosol, though they alone do not help distinguish pollen species from one another.

The disaccharide sucrose was found in high concentrations (64-80 μg/mg) in five of the ten analyzed pollen species: aspen, pecan, paper mulberry, lodgepole pine, and Jeffrey pine, while other species showed little to no sucrose. It was also found in high concentrations (79-93 μg/mg) in the surface deposition aerosol samples. Overall, the presence of sucrose is in agreement with previous studies that assert pollen as an important contributor to sucrose levels in the atmosphere (Fu et al.,

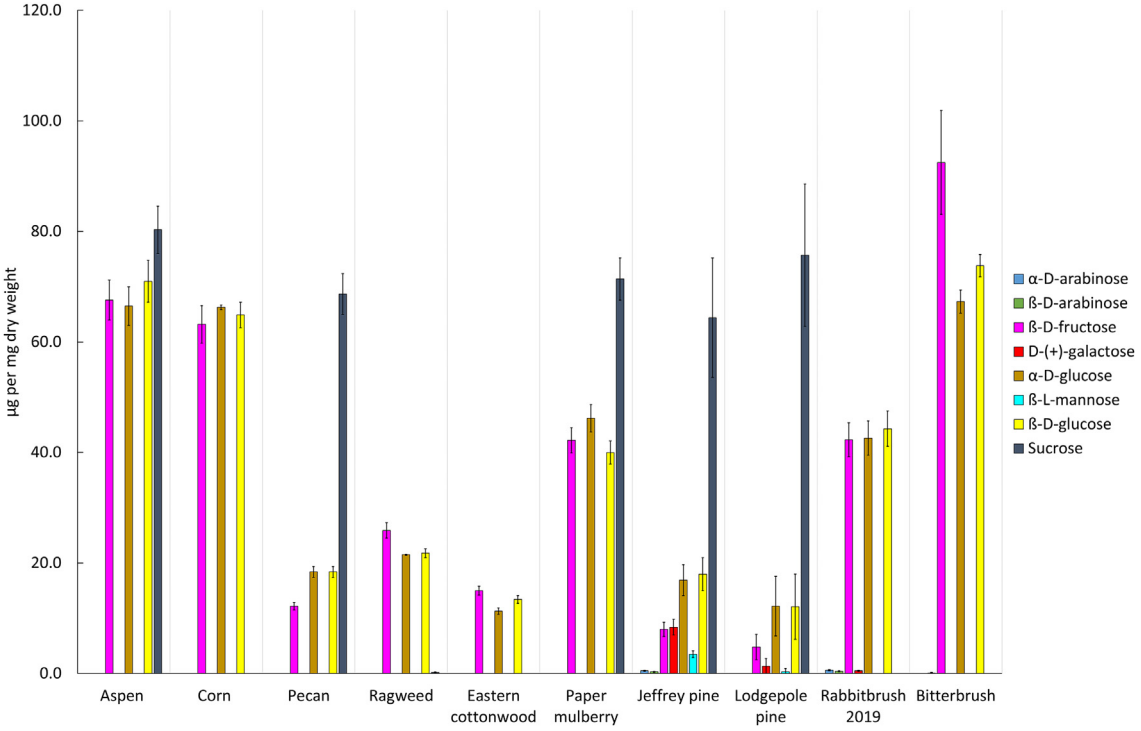


Fig. 2. Species-specific concentrations of saccharides in pollen. Among all compounds tested, only compounds that had a quantifiable concentration in at least one sample are depicted.

2012; Zhu et al., 2015). Fu et al. (2012) identified sucrose as a tracer for pollen grains, being the most common saccharide (80%) among other analyzed saccharides (which included glucose, fructose, levoglucosan, trehalose, and sugar alcohols) in their study. However, contrasting to Fu et al. (2012), our results showed that not all pollen species can contribute to atmospheric sucrose concentrations. This disparity could be explained by differences between analyzed pollen species in our study and Fu et al. (2012). Thus, using certain saccharides as tracers in the atmosphere may be more useful on a species-by-species basis. For example, because the aspen pollen showed high concentrations of sucrose, β-D-fructose, α-D-glucose, and β-D-glucose, aspen pollen may have a particularly high contribution among pollen species to saccharide mass in the atmosphere, while eastern cottonwood pollen may not.

D-galactose was found in low amounts in Jeffrey pine pollen (Fig. 2a, 8.4 ± 1.4 µg/mg), and in negligible amounts in all other species tested (<1.5 µg/mg in lodgepole pine and rabbitbrush, none in all others). While atmospheric galactose sources are currently not well identified, it is theorized that major contributors are primary bioaerosols which may be enhanced by biomass burning emissions (Medeiros et al., 2006; Santos et al., 2021). However, levels are still relatively low (2-7 times) in comparison to glucose, fructose, and sucrose. β-L-mannose (Fig. 2a, 3.5 ± 0.6 µg/mg) was also found in Jeffrey pine (negligible in other species, <0.5 µg/mg), though once again in very low levels compared to saccharides previously discussed. In addition, arabinoses were found in very low quantities relative to saccharides previously discussed (>1.0 µg/mg) in rabbitbrush and Jeffrey pine).

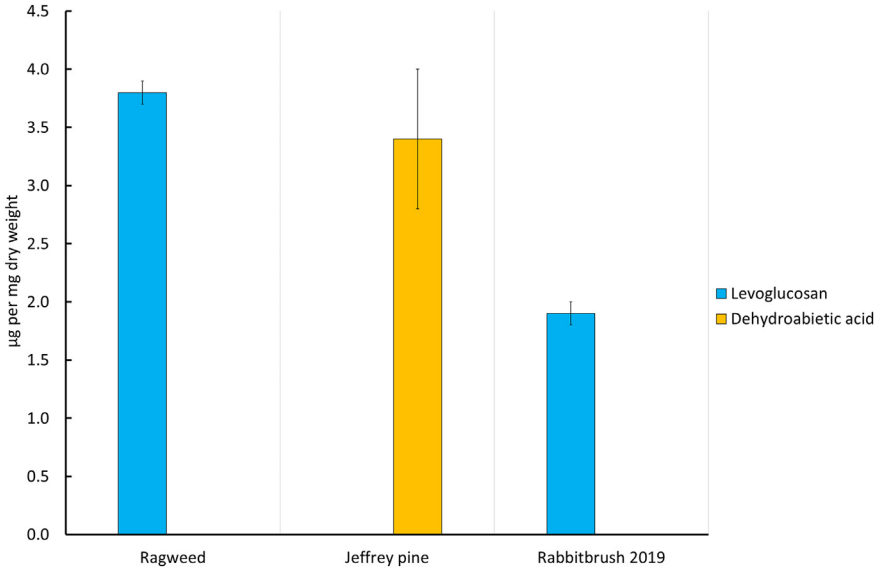


Fig. 3. GC–MS results of anhydrosugars and resin acid analysis. Among all compounds tested, only compounds that had a quantifiable concentration in at least one sample are depicted.

Results of the quantitative analysis of anhydrosugars and resin acids (Table S1) are given in Fig. 3 (units: μg/mg). Overall, only two compounds, levoglucosan and dehydroabietic acid, were found in any sample. Concentrations of these compounds were significantly lower (~ 10-100 times) than those of analyzed saccharides (Fig. 2). The total concentration of these compounds (Table S1) varied between 0 and 3.8 μg/mg for all pollen species, while total concentrations of analyzed saccharides were between 6.4 and 290 μg/mg.

Regarding individual compounds, dehydroabietic acid was found at a quantity of 3.4 ± 0.6 μg/mg in Jeffrey pine, while not present in any other pollen species. This compound is usually associated with biomass-burning emissions (Leithead et al., 2006; Sengupta et al., 2020; Simoneit, 1999), since it is a product of conifer resin pyrolysis (Lai et al., 2015). This discovery has implications on total contribution of dehydroabietic acid in the atmosphere, as well as source apportionment studies that assume biomass burning to be the sole source of this compound, and thus implications on how biomass burning is traced, not just in the atmosphere but in other areas like ice core records (Kawamura et al., 2012) and snow deposition (Gao et al., 2015). Dehydroabietic acid has also been suggested to have a role in atmospheric chemistry, experimentally shown to react with atmospheric oxidants such as the hydroxyl radical (Sengupta et al., 2020).

Levoglucosan was found in small quantities in ragweed and rabbitbrush pollens (3.8 ± 0.1 μg/mg and 1.9 ± 0.1 μg/mg respectively). Levoglucosan is a well-known product of the pyrolysis of cellulose and hemicelluloses in biomass-burning emissions (Hennigan et al., 2010; Simoneit et al., 2004; Zhang et al., 2013). To our knowledge it has not been found in extracts of fresh pollen, and it may be due to contamination in these two samples. Ragweed pollen was purchased, and thus we cannot speculate as to sources of contamination. Rabbitbrush was field-collected in October 2019 after plumes from several major forest fires had been impacting the collection sites (around Reno, NV) throughout the summer and early fall of the year (Kelly et al., 2020). It is thus possible that substances present in forest fire smoke were deposited and/or adsorbed on rabbitbrush pollen. Further investigation is needed to explain this finding, as the presence of levoglucosan in these pollen species has implications on tracing of biomass burning emissions.

3.2. Free amino acids

Results of the quantitative analysis of free amino acids in the analyzed pollen extract samples are given in Fig. 4. Full quantification data is given in Fig. S2. Across all samples (purchased, field-collected, and aerosol deposition), the free amino acid most commonly present is proline (Fig. 4e), varying between 0.4 μg/mg (ragweed) to 24 μg/mg (bitterbrush). Ragweed (Fig. 4b and e, 2.9 ± 0.6 μg/mg), lodgepole pine (Fig. 4c and e, 9.5 ± 4.3 μg/mg), pecan (Fig. 4a and e, 13 ± 1 μg/mg) and Jeffrey pine (Fig. 4c and e, 15 ± 3 μg/mg) had the lowest concentrations of total free amino acids among all samples. Meanwhile, eastern cottonwood is the sample with the greatest concentration of free amino acids, at 150 ± 2 μg/mg (15% of total dry weight). With eastern cottonwood also having the lowest concentration of saccharides among all samples, at 40 μg/mg (4.0% of total dry weight), this may indicate that eastern cottonwood pollen, per unit aerosol concentration, may be a larger contributor of amino acids (notably asparagine, 102 ± 2 μg/mg) and a smaller of a contributor of saccharides than other pollens in this study. More generally, it demonstrates that pollens from different plant species can have different chemical profiles.

Aside from eastern cottonwood pollen, aspen (105 ± 4 μg/mg) and bitterbrush (94 ± 18 μg/mg) pollens showed the next highest levels of total free amino acids. Aspen showed higher concentrations of glutamine (16 ± 1 μg/mg), arginine (9.5 ± 0.3 μg/mg), and serine (8.0 ± 0.5 μg/mg) than all other samples tested. This may indicate that aspen is a much higher contributor of these amino acids in the atmosphere (per unit aerosol) than other analyzed pollen species. Bitterbrush, meanwhile, showed higher levels of leucine (2.9 ± 0.6 μg/mg) than any other sample (second-highest was aspen at 1.7 ± 0.1 μg/mg), as well as the second-highest level of asparagine (43 ± 9 μg/mg) after eastern cottonwood. But, unlike eastern cottonwood, these pollens had higher levels of saccharides identified. Nonetheless, the identification of these pollen species with high total free amino acid concentration is important, as aerosols with free amino acids have been shown to be good CCN (Kristensson et al., 2010) (though that study did not investigate all amino acids measured in this study) and IN (Szyrmer and Zawadzki, 1997).

Rabbitbrush samples collected in the year of 2019 and 2020 (Fig. 4d) have similar total concentrations of free amino acids (22 ± 2 μg/mg in 2019, 18 ± 1 μg/mg in 2020), with the two samples having a Pearson correlation coefficient of 0.99 across all amino acids (based on normalized concentrations, see Table S3). In these samples, the primary amino acids are proline (17 ± 1 μg/mg in 2019, 15 ± 1 μg/mg in 2020), hydroxyproline (0.7 ± 0.1 μg/mg in 2019, 0.8 ± 0.1 μg/mg in 2020), and histidine (2.3 ± 0.1 μg/mg in 2019, 1.5 ± 0.1 μg/mg in 2020). This indicates that free amino acid composition is repeatable within the same species across different years, which would allow for reliable source apportionment of these compounds.

Jeffrey pine and lodgepole pine pollen had similar overall chemical compositions, with total free amino acids being (15 ± 3 μg/mg) and (9.5 ± 4.3 μg/mg) respectively, and the highest concentrations being of proline (2.9 ± 0.4 μg/mg for Jeffrey pine, 3.7 ± 1.8 μg/mg for lodgepole pine), arginine (4.7 ± 1.0 μg/mg for Jeffrey pine, 2.5 ± 1.2 μg/mg for lodgepole pine), and γ-aminobutyric acid (GABA) (1.3 ± 0.1 μg/mg for Jeffrey pine, 1.1 ± 0.4 μg/mg for lodgepole pine). We thus speculate that these pollen species may be a contributor of GABA in the atmosphere. It did not appear in any other species in this study, though it is a common free amino acid in many biological processes across many organisms including plants, animals, and microorganisms and is not incorporated into long-chain proteins (Dhakal et al., 2012). Further investigation is needed to determine the extent of GABA in other species of pollen aerosol, as it has been identified in in particulate matter and fog waters (Zhang and Anastasio, 2003). In addition, bacterial sources are theorized to be contributors of GABA in the atmosphere (Zhu et al., 2021), indicating that GABA is by no means unique to these specific species of pollen. However, this finding nonetheless indicates that different species of pollen contribute to the total amount of amino acid types in the atmosphere disproportionately.

A notable omission from our findings was the free amino acid glycine, which was found to be a very common amino acid in both free form and peptide form in a variety of sources, including biogenic sources, soil, and especially biomass burning emissions (Zhu et al., 2020). We speculate that glycine may reside more in protein/peptide form as opposed to free form in pollen, as there has been glycine discovered in atmospheric aerosol via analysis with hydrolysis methods (Zhu et al., 2021), and it has also been noted in bee pollen (DeGrandi-Hoffman et al., 2018; Taha et al., 2019). More research on glycine in peptide form in pollen, like quantification of amino acids in proteins/peptides via hydrolysis (Zhang and Anastasio, 2003), is necessary. Further, it is also noteworthy that the proteinogenic amino acid aspartic acid was not quantified in this experiment due to a high detection limit in our UPLC-based method. As such, we cannot speculate as to its presence in fresh pollen, though it has been observed in bee pollen samples as described earlier (Taha et al., 2019).

Overall, our results indicate that free amino acids are common in these types of bioaerosols, though their chemical stability in the atmosphere has not been well characterized and needs further investigation. These results also demonstrate that bioaerosols can play a role in the global nitrogen cycle via atmospheric nitrogen transport and deposition (Kanakidou et al., 2018).

3.3. Analysis of surface-deposited aerosols

Chemical composition of two surface-deposited aerosols collected in the area of Truckee and South Lake Tahoe in California (USA) are given

in Fig. 5. The most abundant compound in both samples (Fig. 5a) was sucrose (79 ± 13 μg/mg in Truckee and 93 ± 16 μg/mg in South Lake Tahoe samples), while α-D-glucose and β-D-glucose were the second most abundant saccharides (α-D-glucose: 4.4 ± 0.7 μg/mg for Truckee and 9.2 ± 1.5 μg/mg for South Lake Tahoe samples; β-D-glucose: 4.9 ± 0.8 μg/mg for Truckee and 10.0 ± 1.7 μg/mg for South Lake Tahoe samples). In addition, β-D-fructose was abundant in South Lake Tahoe aerosol samples (4.5 ± 0.8 μg/mg). D-galactose was also found in the South Lake Tahoe surface deposition aerosol, though in lower concentration (2.4 ± 0.4 μg/mg). The most common amino acids in these samples (Fig. 5b) were proline and arginine (proline: 2.7 ± 0.3 μg/mg for Truckee and 3.3 ± 0.4 μg/mg for South Lake Tahoe samples; arginine: 2.8 ± 0.6 μg/mg for Truckee and 3.9 ± 0.9 μg/mg for South Lake Tahoe samples). This aerosol is suspected to be comprised primarily of pine pollen, as the area is characterized by pine forests that were pollinating during the collection of the aerosol.

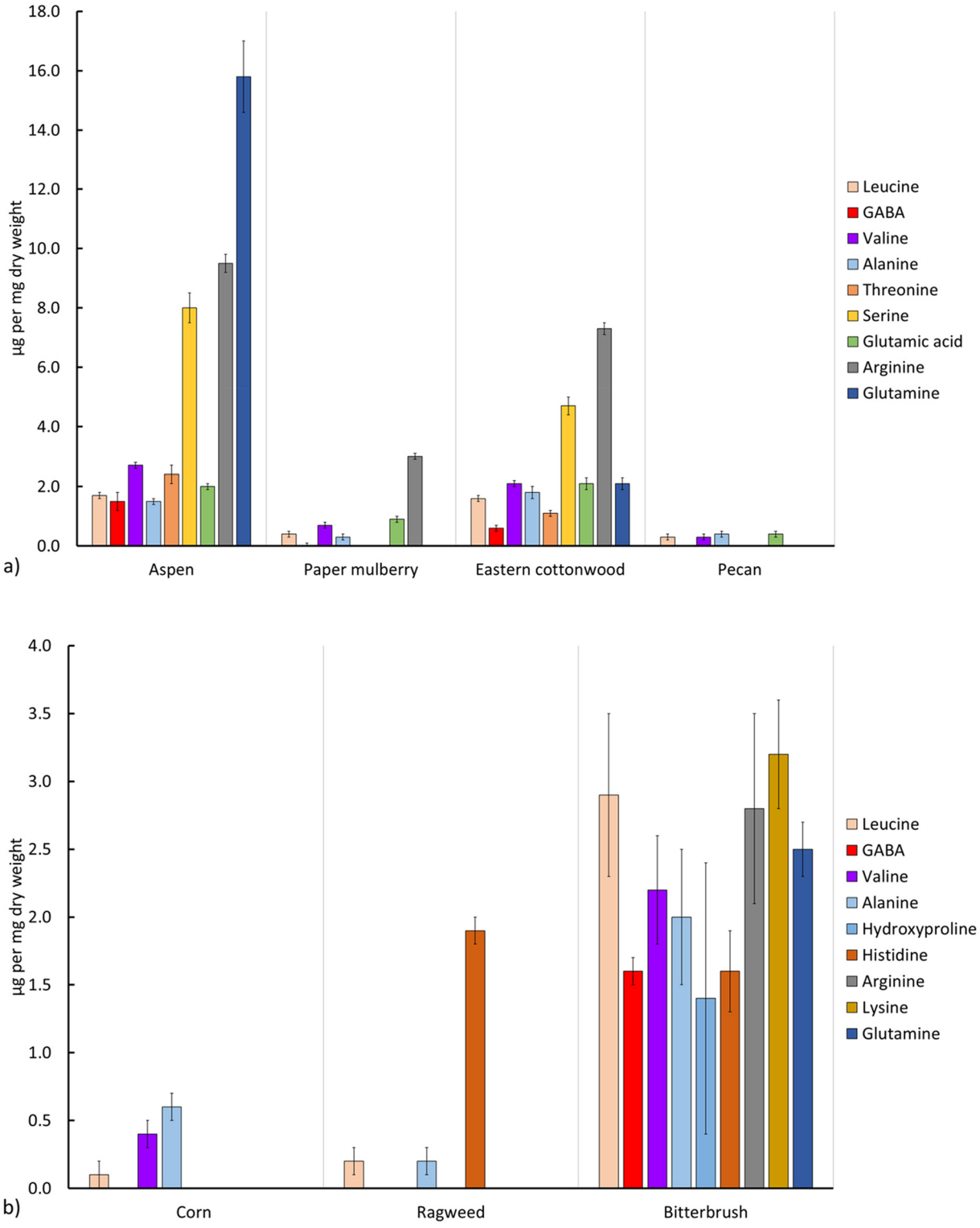


Fig. 4. Quantification of free amino acids in pollen and surface-deposited samples, with units in μg/mg dry sample weight. Proline and asparagine are separated in their own subfigure 4e due to their higher concentrations. Subfigures 4a-d depict different pollen species and their major free amino acid constituents.

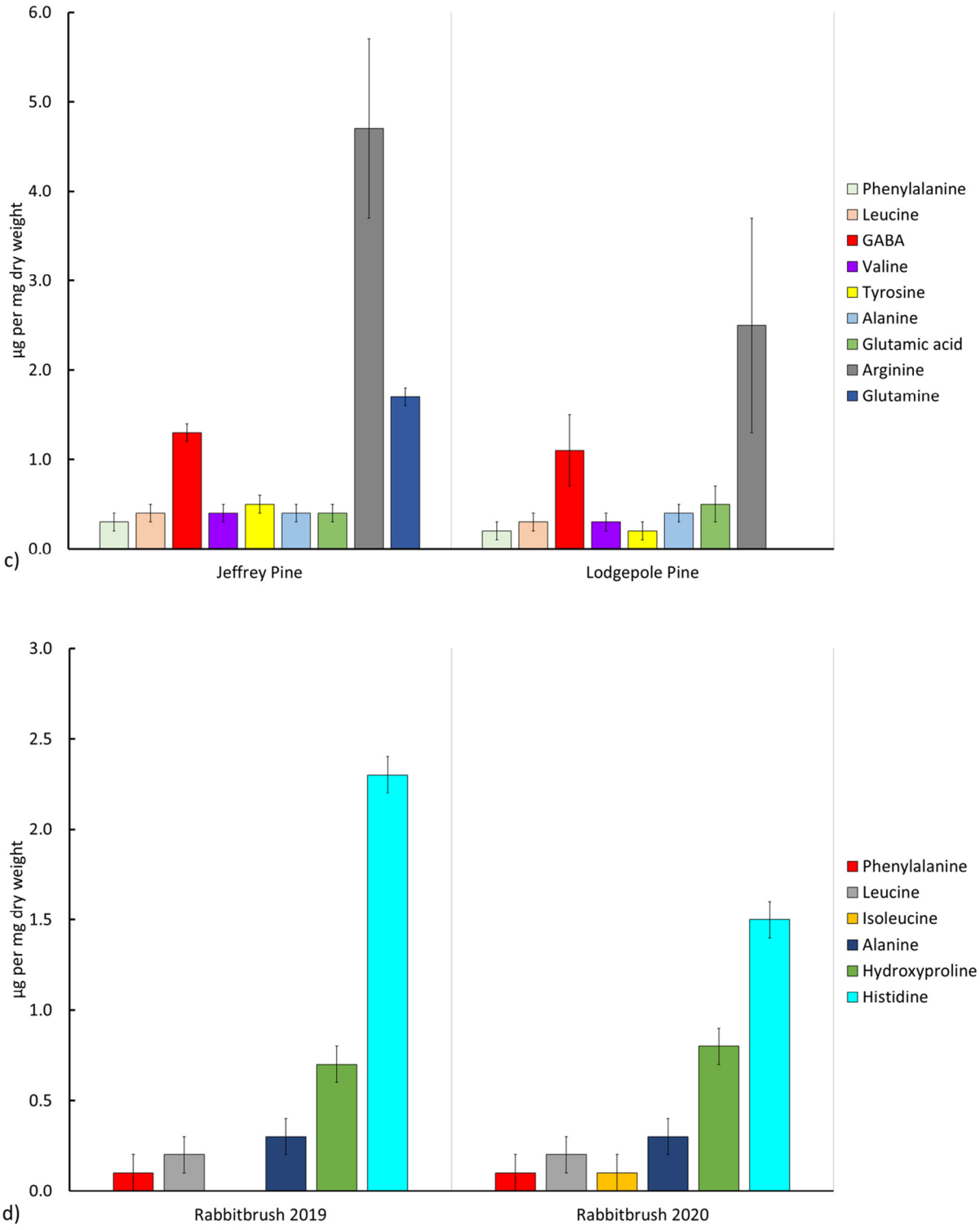


Fig. 4 (*continued*).

Fig. 6 provides a comparison of each sample based on their saccharide fractions and amino acid fractions, with each sample having their total individual compound concentrations normalized by the total concentration of either saccharides or amino acids analyzed in this experiment. In addition, Table S3 provides Pearson correlation coefficients of these normalized values across all samples. The normalized amino acid concentrations (Table S3b) of the South Lake Tahoe surface-deposited aerosol correlate well with those of Jeffrey pine (correlation coefficient of 0.90) and lodgepole pine (0.89) pollens. In addition, the normalized saccharide concentrations (Table S3a) of the South Lake Tahoe surface-deposited aerosol also have good correlation with Jeffrey pine (0.98) and lodgepole pine (0.99). The absolute concentrations of all compounds, including anhydrosugars and resin acids, also correlate well (Table S3c) between these samples (Jeffrey pine – South Lake Tahoe: 0.97; Lodgepole pine – South Lake Tahoe: 0.99), indicating that this surface deposition aerosol is very likely one of these two species. The Truckee surface deposition aerosol correlates almost as well with these two pollen samples (Jeffrey pine: 0.97 for saccharides and 0.80

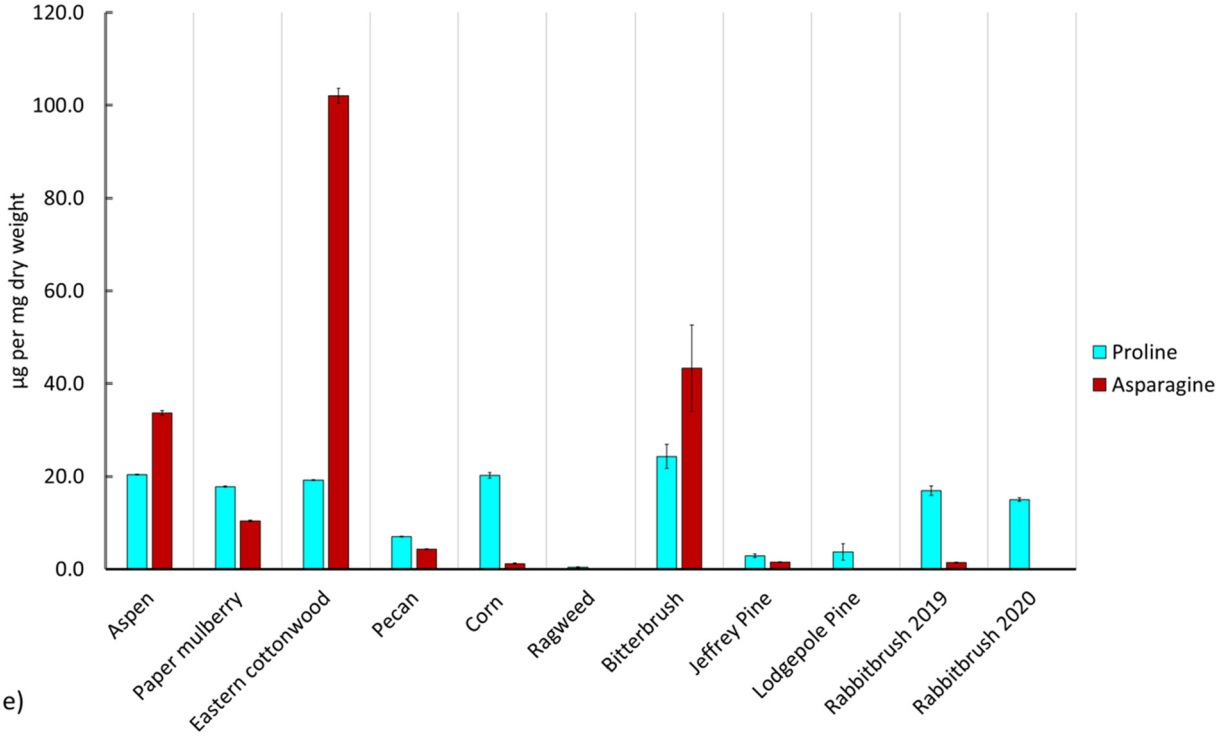


Fig. 4 (*continued*).

for amino acids: Lodgepole pine: 0.99 for saccharides and 0.90 for amino acids) as the South Lake Tahoe sample does. The absolute concentrations of chemicals in Jeffrey and lodgepole pine pollen samples, taking into account anhydrosugars and resin acids, also correlate as well with concentrations of the Truckee surface deposition aerosol (Pearson correlation coefficients of 0.95 and 0.99 for Jeffrey and lodgepole, respectively). The good correlation of the Truckee aerosol with the Jeffrey and lodgepole pines with the normalized amino acid and saccharides concentrations is expected, as Jeffrey pine, like lodgepole, is also very common in the collection areas of the surface deposition aerosol. This diagnosis helps illustrate the usefulness of the quantitative analysis of saccharides and amino acids in the identification of aerosol sources.

Notably, pecan also demonstrated a significant correlation with the deposition aerosols, especially with the South Lake Tahoe aerosol (correlation coefficients of 0.95 and 0.97 for Truckee and South Lake Tahoe, respectively). Pecan also had correlation coefficients of 0.96 and 0.97 with regards to normalized saccharide concentration, but just 0.49 and 0.45 with regards to normalized amino acid concentration. This demonstrates that considering both saccharide and amino acid profiles can significantly improve source identification. For example, it is very difficult to distinguish pecan and pine pollen using only their saccharide profiles, but together with the amino acid profiles these species can be distinguished. Likewise, pecan correlates in amino acid composition much stronger with rabbitbrush, bitterbrush, and paper mulberry, but less so with their saccharide profiles. Overall, this analysis demonstrates that the chemical profiles of pollen samples collected can be very useful in the identification of ambient aerosol, and that free amino acid composition may prove necessary for identification of species that are indistinguishable using saccharide profiles alone.

3.4. Relative contribution of the measured compound classes

Total quantified chemical composition of all samples, including purchased pollen, field-collected pollen samples (lodgepole pine, Jeffrey pine, rabbitbrush, and bitterbrush), and surface-deposited aerosols collected in Northern Nevada and California are given in Fig. 7. Throughout the samples, saccharides were frequently the most abundant identified group in terms of total mass percentage (4.0% to 29%, as described previously). Only eastern cottonwood had less total identified mass of saccharides than other compounds. Other compounds such as levoglucosan and dehydroabietic acid were the least abundant, only appearing in ragweed, rabbitbrush, and Jeffrey pine, in minute quantities. This indicates that most pollen species tested are not contributors of the resin acids or anhydrosugars analyzed in this experiment, or are not significant contributors relative to their contribution of saccharides and free amino acids.

Aspen and bitterbrush had the highest percentages of total dry weight identified (39% and 33% respectively), with significant percentages being saccharides (29% and 23% of total) and free amino acids (11% and 9.4% of total). In addition, eastern cottonwood shows a high percentage of free amino acid composition (15% of total mass). This may indicate that these three pollens can be larger contributors of free amino acids per unit mass to atmospheric aerosol than the other pollen species tested in this experiment, further illustrating the differences in chemical contributions to the atmosphere based on species.

4. Conclusions

Fresh pollen species, including purchased, field-collected, and surface deposited aerosol collected during various tree and shrub pollen seasons in the areas surrounding Lake Tahoe and Reno in Northern Nevada and California were extracted for detailed chemical analysis of free organic compounds such as saccharides, free amino acids, resin acids, and anhydrosugars. We found that 4 analyzed saccharides comprise a larger portion of pollen dry weight (4.0-29% of the total dry pollen mass) than the other groups of analyzed compounds. The most common saccharides found were α-D-glucose, β-D-glucose, and β-D-fructose, with sucrose also appearing in aspen, pecan, paper mulberry, Jeffrey pine, lodgepole pine, and surface-deposited aerosol. In addition, D-galactose was found in smaller quantities in Jeffrey and lodgepole

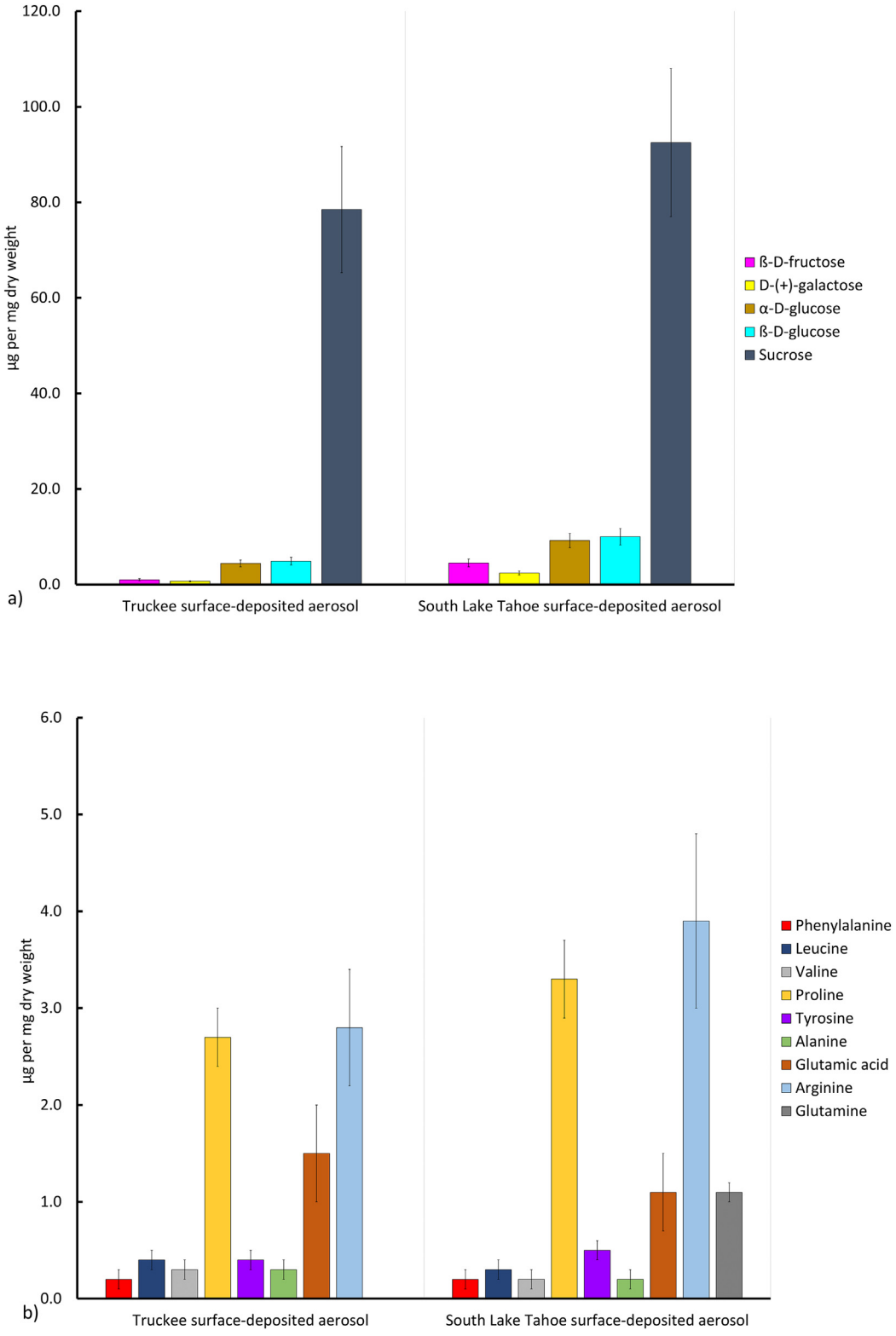


Fig. 5. Chemical composition of surface-deposited aerosol collected in two different locations. Subfigure 5a depicts saccharides and subfigure 5b depicts free amino acids.

pines and surface-deposited aerosol collected in the area of these species during their pollen season. Free amino acid quantification of samples yielded many different compounds identified, with and eastern cottonwood, bitterbrush, and aspen pollens having the highest total quantity. An especially high concentration of asparagine was found in eastern cottonwood, at more than 10% of total dry pollen weight. The amino acid proline was found in substantial concentrations (as much as 2.4% of dry pollen weight) across all analyzed species. Composition of rabbitbrush samples collected in two separate years also yielded similar free amino acid compositions, indicating repeatability of free amino acid composition within the same species of pollen. In addition, Jeffrey and lodgepole pine pollens were determined to have a similar chemical composition as the two surface-deposited aerosols collected, illustrating the usefulness of these chemical profiles in ambient aerosol identification. It was found

that saccharide composition alone could not identify the source of the surface-deposited aerosol, but identification was more plausible when free amino acid composition was accounted for. This indicates that free amino acid and saccharide composition, when used in tandem, may be more able to identify possible sources of bioaerosol. However, further research is necessary in order to determine the full extent of contributions of free amino acids and saccharides in the atmosphere from other sources (e.g., biomass burning).

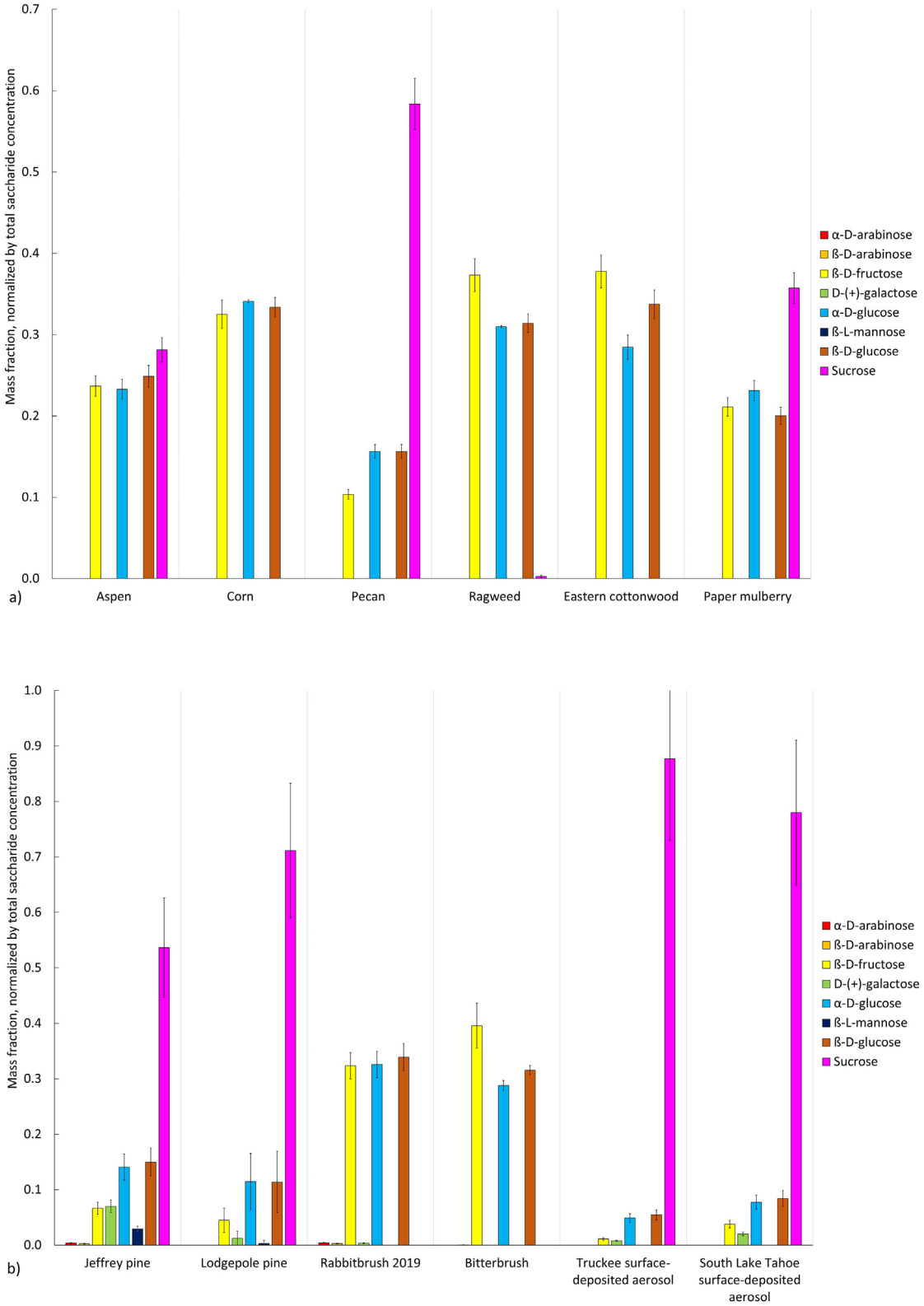


Fig. 6. Normalized comparison of pollen samples with surface-deposited aerosol. The figure is normalized by total saccharide concentration per sample (6a, b) and total amino acid concentration per sample (6c, d).

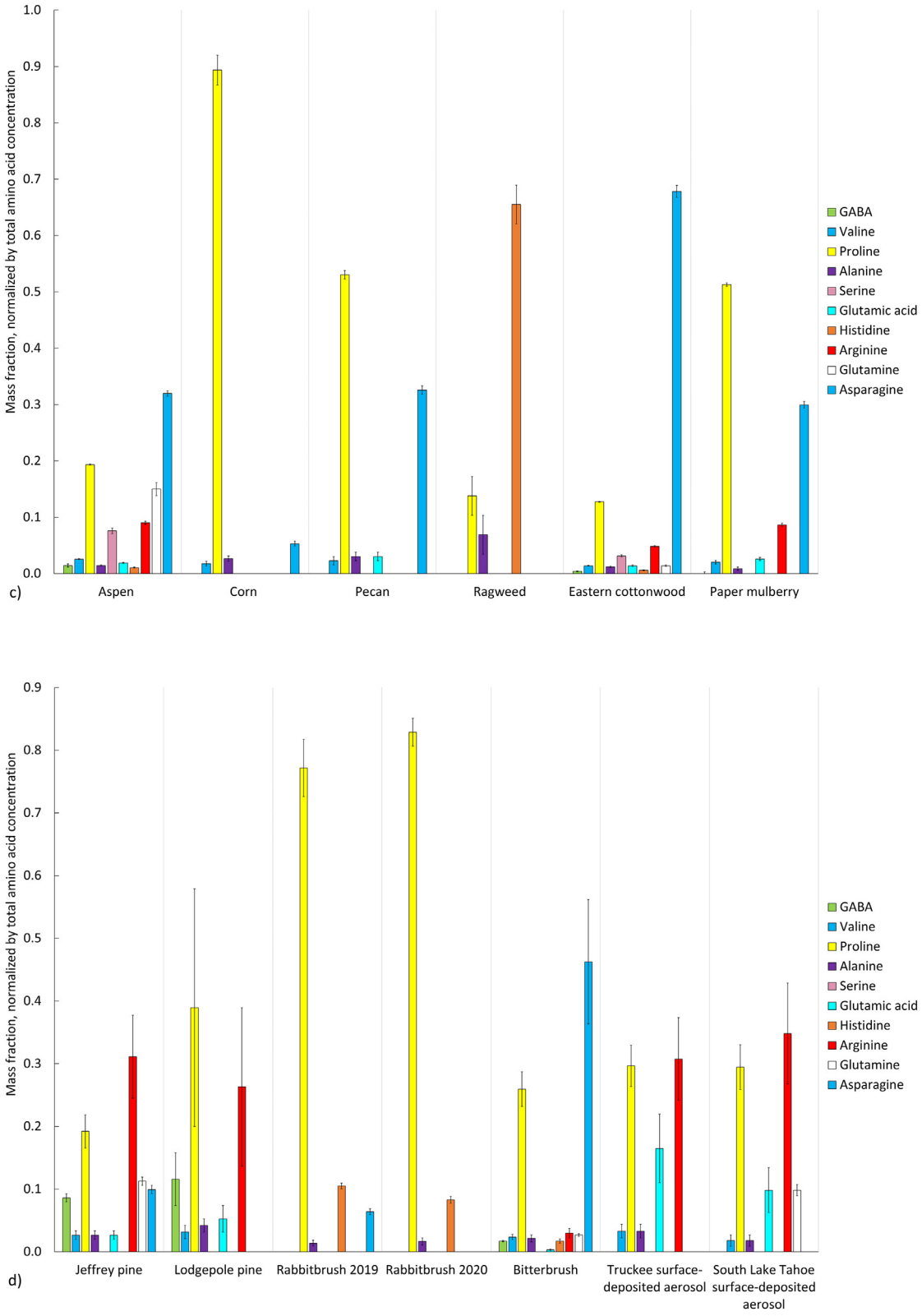


Fig. 6 (*continued*).

CRediT authorship contribution statement

Kevin Axelrod – conceptualization, design, experimentation, data analysis, writing.

Dr. Vera Samburova - conceptualization, design, experimentation, data analysis, writing, editing, funding acquisition.

Dr. Andrey Khlystov - conceptualization, design, experimentation, data analysis, editing, funding acquisition.

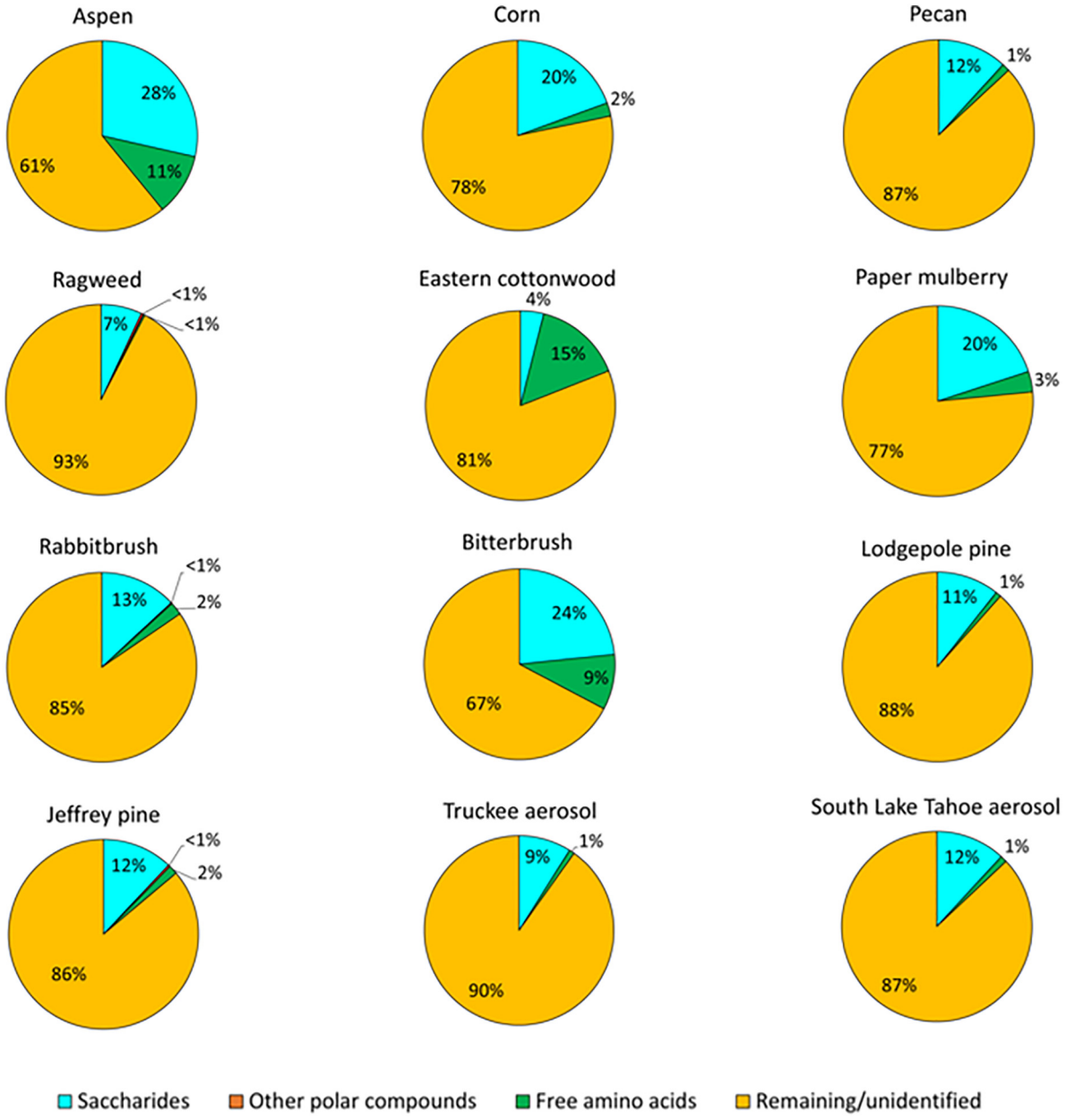


Fig. 7. Total percentage of pollen dry weight quantified across all analyses performed and percent remaining unidentified.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. (AGS-1760328). The authors thank Dr. Alison Murray, whose expertise was very useful in the design of experiments conducted in this research. The authors also thank Chiranjivi Bhattarai, Dr. Yeongkwon Son, and Dr. Deep Sengupta, who provided expertise in analytical methods.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149254.

References

Anderson, B.D., Ma, M., Xia, Y., Wang, T., Shu, B., Lednicky, J.A., Ma, M.J., Lu, J., Gray, G.C., 2016. Bioaerosol sampling in modern agriculture: a novel approach for emerging pathogen surveillance? J. Infect. Dis. 214, 537–545. https://doi.org/10.1093/infdis/jiw180.

Andreae, M.O., Rosenfeld, D., 2008. Aerosol-cloud-precipitation interactions. Part 1. The nature and sources of cloud-active aerosols. Earth Sci. Rev. 89, 13–41. https://doi.org/10.1016/j.earscirev.2008.03.001.

Bonifait, L., Veillette, M., Létourneau, V., Grenier, D., Duchainea, C., 2014. Detection of Streptococcus suis in bioaerosols of swine confinement buildings. Appl. Environ. Microbiol. 80, 3296–3304. https://doi.org/10.1128/AEM.04167-13.

Carrizo García, C., Guarnieri, M., Pacini, E., 2010. Soluble carbohydrates content in tomato pollen and its variations along and between blooming periods. Sci. Hortic. (Amsterdam) 125, 524–527. https://doi.org/10.1016/j.scienta.2010.04.026.

Chen, F., Ran, L., Mi, J., Yan, Y., Lu, L., Jin, B., Li, X., Cao, Y., 2018. Isolation, characterization and antitumor effect on du145 cells of a main polysaccharide in pollen of chinese wolfberry. Molecules 23. https://doi.org/10.3390/molecules23102430.

D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., Van Cauwenberge, P., 2007. Allergenic pollen and pollen allergy in Europe. Allergy Eur. J. Allergy Clin. Immunol. 62, 976–990. https://doi.org/10.1111/j.1398-9995.2007.01393.x.

Dedic, A., Gadermaier, G., Vogel, L., Ebner, C., Vieths, S., Ferreira, F., Egger, M., 2009. Immune recognition of novel isoforms and domains of the mugwort pollen major allergen art v 1. Mol. Immunol. 46, 416–421. https://doi.org/10.1016/j.molimm.2008.10.012.

DeGrandi-Hoffman, G., Gage, S.L., Corby-Harris, V., Carroll, M., Chambers, M., Graham, H., Watkins deJong, E., Hidalgo, G., Calle, S., Azzouz-Olden, F., Meador, C., Snyder, L., Ziolkowski, N., 2018. Connecting the nutrient composition of seasonal pollens with changing nutritional needs of honey bee (Apis mellifera L.) colonies. J. Insect Physiol. 109, 114–124. https://doi.org/10.1016/j.jinsphys.2018.07.002.

Denisow, B., Denisow-Pietrzyk, M., 2016. Biological and therapeutic properties of bee pollen: a review. J. Sci. Food Agric. 96, 4303–4309. https://doi.org/10.1002/jsfa.7729.

Dhakal, R., Bajpai, V.K., Baek, K.H., 2012. Production of GABA (?-aminobutyric acid) by microorganisms: a review. Braz. J. Microbiol. 43, 1230–1241. https://doi.org/10.1590/S1517-83822012000400001.

Douglas, P., Robertson, S., Gay, R., Hansell, A.L., Gant, T.W., 2018. A systematic review of the public health risks of bioaerosols from intensive farming. Int. J. Hyg. Environ. Health 221, 134–173. https://doi.org/10.1016/j.ijheh.2017.10.019.

Estillore, A.D., Trueblood, J.V., Grassian, V.H., 2016. Atmospheric chemistry of bioaerosols: heterogeneous and multiphase reactions with atmospheric oxidants and other trace gases. Chem. Sci. 7, 6604–6616. https://doi.org/10.1039/c6sc02353c.

Fröhlich-Nowoisky, J., Kampf, C.J., Weber, B., Huffman, J.A., Pöhlker, C., Andreae, M.O., Lang-Yona, N., Burrows, S.M., Gunthe, S.S., Elbert, W., Su, H., Hoor, P., Thines, E., Hoffmann, T., Després, V.R., Pöschl, U., 2016. Bioaerosols in the earth system: climate, health, and ecosystem interactions. Atmos. Res. 182, 346–376. https://doi.org/10.1016/j.atmosres.2016.07.018.

Fu, P., Kawamura, K., Kobayashi, M., Simoneit, B.R.T., 2012. Seasonal variations of sugars in atmospheric particulate matter from gosan, Jeju Island: significant contributions of airborne pollen and asian dust in spring. Atmos. Environ. 55, 234–239. https://doi.org/10.1016/j.atmosenv.2012.02.061.

Gao, S., Liu, D., Kang, S., Kawamura, K., Wu, G., Zhang, G., Cong, Z., 2015. A new isolation method for biomass-burning tracers in snow: measurements of p-hydroxybenzoic, vanillic, and dehydroabietic acids. Atmos. Environ. 122, 142–147. https://doi.org/10.1016/j.atmosenv.2015.09.049.

Gong, F., Wu, X., Wang, W., 2015. Diversity and function of maize pollen coat proteins: from biochemistry to proteomics. Front. Plant Sci. 6, 1–7. https://doi.org/10.3389/fpls.2015.00199.

Graham, K.E., Prussin, A.J., Marr, L.C., Sassoubre, L.M., Boehm, A.B., 2018. Microbial community structure of sea spray aerosols at three California beaches. FEMS Microbiol. Ecol. 94, 1–11. https://doi.org/10.1093/femsec/fiy005.

Gute, E., David, R.O., Kanji, Z.A., Abbatt, J.P.D., 2020. Ice nucleation ability of tree pollen altered by atmospheric processing. ACS Earth Sp. Chem. 4, 2312–2319. https://doi.org/10.1021/acsearthspacechem.0c00218.

Hansen, V.M., Meyling, N.V., Winding, A., Eilenberg, J., Madsen, A.M., 2012. Factors affecting vegetable growers' exposure to fungal bioaerosols and airborne dust. Ann. Occup. Hyg. 56, 170–181. https://doi.org/10.1093/annhyg/mer090.

Hennigan, C.J., Sullivan, A.P., Collett, J.L., Robinson, A.L., 2010. Levoglucosan stability in biomass burning particles exposed to hydroxyl radicals. Geophys. Res. Lett. 37, 1–5. https://doi.org/10.1029/2010GL043088.

Hirsche, J., Fernández, J.M.G., Stabentheiner, E., Großkinsky, D.K., Roitsch, T., 2017. Differential effects of carbohydrates on arabidopsis pollen germination. Plant Cell Physiol. 58, 691–701. https://doi.org/10.1093/pcp/pcx020.

Ischebeck, T., 2016. Lipids in pollen — they are different. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1861, 1315–1328. https://doi.org/10.1016/j.bbalip.2016.03.023.

Jiang, Y., Lahlali, R., Karunakaran, C., Kumar, S., Davis, A.R., Bueckert, R.A., 2015. Seed set, pollen morphology and pollen surface composition response to heat stress in field pea. Plant Cell Environ. 38, 2387–2397. https://doi.org/10.1111/pce.12589.

Joung, Y.S., Ge, Z., Buie, C.R., 2017. Bioaerosol generation by raindrops on soil. Nat. Commun. 8, 1–10. https://doi.org/10.1038/ncomms14668.

Kalaycioglu, Z., Kaygusuz, H., Döker, S., Kolayli, S., Erim, F.B., 2017. Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. LWT Food Sci. Technol. 84, 402–408. https://doi.org/10.1016/j.lwt.2017.06.003.

Kanakidou, M., Myriokefalitakis, S., Tsigaridis, K., 2018. Aerosols in atmospheric chemistry and biogeochemical cycles of nutrients. Environ. Res. Lett. 13. https://doi.org/10.1088/1748-9326/aabcdb.

Kawamura, K., Izawa, Y., Mochida, M., Shiraiwa, T., 2012. Ice core records of biomass burning tracers (levoglucosan and dehydroabietic, vanillic and p-hydroxybenzoic acids) and total organic carbon for past 300years in the Kamchatka peninsula, Northeast Asia. Geochim. Cosmochim. Acta 99, 317–329. https://doi.org/10.1016/j.gca.2012.08.006.

Kelly, H., Samenow, J., Freedman, A., 2020. California wildfires burn 771, 000 acres in one week, killing 5 and degrading air quality. The Washington Post.

Komosinska-vassev, K., Olczyk, P., Ka, J., Mencner, L., Olczyk, K., 2015. NAFSA: study abroad participation in the US by state and demographics data for the academic year 2014–2015. Evid. Based Complement. Alternat. Med. 2015, 6. https://doi.org/10.1155/2015/297425.

Kristensson, A., Rosenørn, T., Bilde, M., 2010. Cloud droplet activation of amino acid aerosol particles. J. Phys. Chem. A 114, 379–386. https://doi.org/10.1021/jp9055329.

Lai, C., Liu, Y., Ma, J., Ma, Q., He, H., 2015. Laboratory study on OH-initiated degradation kinetics of dehydroabietic acid. Phys. Chem. Chem. Phys. 17, 10953–10962. https://doi.org/10.1039/c5cp00268k.

Lawson, S.P., Kennedy, K.B., Rehan, S.M., 2021. Pollen composition significantly impacts the development and survival of the native small carpenter bee, Ceratina calcarata. Ecol. Entomol. 46, 232–239. https://doi.org/10.1111/een.12955.

Lee, S.A., Adhikari, A., Grinshpun, S.A., McKay, R., Shukla, R., Reponen, T., 2006. Personal exposure to airborne dust and microorganisms in agricultural environments. J. Occup. Environ. Hyg. 3, 118–130. https://doi.org/10.1080/15459620500524607.

Leithead, A., Li, S.M., Hoff, R., Cheng, Y., Brook, J., 2006. Levoglucosan and dehydroabietic acid: evidence of biomass burning impact on aerosols in the lower Fraser Valley. Atmos. Environ. 40, 2721–2734. https://doi.org/10.1016/j.atmosenv.2005.09.084.

Marghitas, L.A., Stanciu, O.G., Dezmirean, D.S., Bobis, O., Popescu, O., Bogdanov, S., Campos, M.G., 2009. In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. Food Chem. 115, 878–883. https://doi.org/10.1016/j.foodchem.2009.01.014.

Medeiros, P.M., Conte, M.H., Weber, J.C., Simoneit, B.R.T., 2006. Sugars as source indicators of biogenic organic carbon in aerosols collected above the Howland experimental Forest, Maine. Atmos. Environ. 40, 1694–1705. https://doi.org/10.1016/j.atmosenv.2005.11.001.

Millner, P.D., 2009. Bioaerosols associated with animal production operations. Bioresour. Technol. 100, 5379–5385. https://doi.org/10.1016/j.biortech.2009.03.026.

Monks, P.S., Granier, C., Fuzzi, S., Stohl, A., Williams, M.L., Akimoto, H., Amann, M., Baklanov, A., Baltensperger, U., Bey, I., Blake, N., Blake, R.S., Carslaw, K., Cooper, O.R., Dentener, F., Fowler, D., Fragkou, E., Frost, G.J., Generoso, S., Ginoux, P., Grewe, V., Guenther, A., Hansson, H.C., Henne, S., Hjorth, J., Hofzumahaus, A., Huntrieser, H., Isaksen, I.S.A., Jenkin, M.E., Kaiser, J., Kanakidou, M., Klimont, Z., Kulmala, M., Laj, P., Lawrence, M.G., Lee, J.D., Liousse, C., Maione, M., McFiggans, G., Metzger, A., Mieville, A., Moussiopoulos, N., Orlando, J.J., O'Dowd, C.D., Palmer, P.I., Parrish, D.D., Petzold, A., Platt, U., Pöschl, U., Prévôt, A.S.H., Reeves, C.E., Reimann, S., Rudich, Y., Sellegri, K., Steinbrecher, R., Simpson, D., ten Brink, H., Theloke, J., van der Werf, G.R., Vautard, R., Vestreng, V., Vlachokostas, C., von Glasow, R., 2009. Atmospheric composition change - global and regional air quality. Atmos. Environ. 43, 5268–5350. https://doi.org/10.1016/j.atmosenv.2009.08.021.

Nakamura, N., Suzuki, H., 1981. Sugar composition of pollen grain and pollen tube cell walls. Phytochemistry 20, 981–984. https://doi.org/10.1016/0031-9422(81)83012-9.

Nogueira, C., Iglesias, A., Feás, X., Estevinho, L.M., 2012. Commercial bee pollen with different geographical origins: a comprehensive approach. Int. J. Mol. Sci. 13, 11173–11187. https://doi.org/10.3390/ijms130911173.

Pacini, E., Guarnieri, M., Nepi, M., 2006. Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma 228, 73–77. https://doi.org/10.1007/s00709-006-0169-z.

Pan, Y.Le, Kalume, A., Wang, C., Santarpia, J., 2021. Atmospheric aging processes of bioaerosols under laboratory-controlled conditions: a review. J. Aerosol Sci. 155, 105767. https://doi.org/10.1016/j.jaerosci.2021.105767.

Pope, F.D., 2010. Pollen grains are efficient cloud condensation nuclei. Environ. Res. Lett. 5. https://doi.org/10.1088/1748-9326/5/4/044015.

Prinsen, H.C.M.T., Schiebergen-Bronkhorst, B.G.M., Roeleveld, M.W., Jans, J.J.M., de Sain-van der Velden, M.G.M., Visser, G., van Hasselt, P.M., Verhoeven-Duif, N.M., 2016. Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass-spectrometry. J. Inherit. Metab. Dis. 39, 651–660. https://doi.org/10.1007/s10545-016-9935-z.

Ravishankara, A.R., 1997. Heterogeneous and multiphase chemistry in the troposphere. Science 276, 1058–1065. https://doi.org/10.1126/science.276.5315.1058.

Samaké, A., Jaffrezo, J.-L., Favez, O., Weber, S., Jacob, V., Canete, T., Albinet, A., Charron, A., Riffault, V., Perdrix, E., Waked, A., Golly, B., Salameh, D., Chevrier, F., Oliveira, D.M., Besombes, J.-L., Martins, J.M.F., Bonnaire, N., Conil, S., Guillaud, G., Mesbah, B., Rocq, B., Robic, P.-Y., Hulin, A., Le Meur, S., Descheemaecker, M., Chretien, E., Marchand, N., Uzu, G., 2019. Arabitol, mannitol and glucose as tracers of primary biogenic organic aerosol: influence of environmental factors on ambient air concentrations and spatial distribution over France. Atmos. Chem. Phys. Discuss. 1–24. https://doi.org/10.5194/acp-2019-434.

Santos, P.S.M., Santos, G.T.A.D., Cachada, A., Patinha, C., Coimbra, M.A., Coelho, E., Duarte, A.C., 2021. Sources of carbohydrates on bulk deposition in South-Western of Europe. Chemosphere 263, 127982. https://doi.org/10.1016/j.chemosphere.2020.127982.

Schummer, C., Delhomme, O., Appenzeller, B.M.R., Wennig, R., Millet, M., 2009. Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. Talanta 77, 1473–1482. https://doi.org/10.1016/j.talanta.2008.09.043.

Schwacke, R., Grallath, S., Breitkreuz, K.E., Stransky, E., Frommer, W.B., Rentsch, D., The, S., Cell, P., Mar, N., Schwacke, R., Grallath, S., Breitkreuz, K.E., Stransky, E., Stransky, H., Frommer, W.B., Rentsch, D., 2020. Pollen Published by : American Society of Plant Biologists (ASPB) Linked References are Available on JSTOR for This Article : LeProTI, a Transporter for Proline, Glycine Betaine, and Y-Amino Butyric Acid in Tomato Pollen. 11, pp. 377–391.

Sénéchal, H., Visez, N., Charpin, D., Shahali, Y., Peltre, G., Biolley, J.P., Lhuissier, F., Couderc, R., Yamada, O., Malrat-Domenge, A., Pham-Thi, N., Poncet, P., Sutra, J.P., 2015. A review of the effects of major atmospheric pollutants on pollen grains, pollen content, and allergenicity. Sci. World J. 2015. https://doi.org/10.1155/2015/940243.

Sengupta, D., Samburova, V., Bhattarai, C., Watts, A.C., Moosmüller, H., Khlystov, A.Y., 2020. Polar semivolatile organic compounds in biomass-burning emissions and their chemical transformations during aging in an oxidation flow reactor. Atmos. Chem. Phys. 20, 8227–8250. https://doi.org/10.5194/acp-20-8227-2020.

Simoneit, B.R.T., 1999. A review of biomarker compounds as source indicators and tracers for air pollution. Environ. Sci. Pollut. Res. 6, 159–169. https://doi.org/10.1007/BF02987621.

Simoneit, B.R.T., Elias, V.O., Kobayashi, M., Kawamura, K., Rushdi, A.I., Medeiros, P.M., Rogge, W.F., Didyk, B.M., 2004. Sugars - dominant water-soluble organic compounds in soils and characterization as tracers in atmospheric participate matter. Environ. Sci. Technol. 38, 5939–5949. https://doi.org/10.1021/es0403099.

Stabler, D., Power, E.F., Borland, A.M., Barnes, J.D., Wright, G.A., 2018. A method for analysing small samples of floral pollen for free and protein-bound amino acids. Methods Ecol. Evol. 9, 430–438. https://doi.org/10.1111/2041-210X.12867.
Stanic, D., Burazer, L., Gavrovic-Jankulovic, M., Jankov, R.M., Velickovic, T.C., 2009. Russian source. J. Serb. Chem. Soc. 74, 359–366. https://doi.org/10.2298/JSC0904359S.
Steiner, A.L., Brooks, S.D., Deng, C., Thornton, D.C.O., Pendleton, M.W., Bryant, V., 2015. Pollen as atmospheric cloud condensation nuclei. Geophys. Res. Lett. 42, 3596–3602. https://doi.org/10.1002/2015GL064060.
Steiner, A.L., Solmon, F., 2018. Pollen rupture and its impact on precipitation in clean continental conditions. Geophys. Res. Lett. 45, 7156–7164. https://doi.org/10.1029/2018GL077692.
Szyrmer, W., Zawadzki, I., 1997. Biogenic and anthropogenic sources of ice-forming nuclei: a review. Bull. Am. Meteorol. Soc. 78, 209–228. https://doi.org/10.1175/1520-0477(1997)078<0209:BAASOI>2.0.CO;2.
Taha, E.K.A., Al-Kahtani, S., Taha, R., 2019. Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia. Saudi J. Biol. Sci. 26, 232–237. https://doi.org/10.1016/j.sjbs.2017.06.003.
Tang, M., Gu, W., Ma, Q., Jie Li, Y., Zhong, C., Li, S., Yin, X., Huang, R.J., He, H., Wang, X., 2019. Water adsorption and hygroscopic growth of six anemophilous pollen species: the effect of temperature. Atmos. Chem. Phys. 19, 2247–2258. https://doi.org/10.5194/acp-19-2247-2019.
Taylor, P.E., Jacobson, K.W., House, J.M., Glovsky, M.M., 2007. Links between pollen, atopy and the asthma epidemic. Int. Arch. Allergy Immunol. 144, 162–170. https://doi.org/10.1159/000103230.
Tobo, Y., Prenni, A.J., Demott, P.J., Huffman, J.A., McCluskey, C.S., Tian, G., Pöhlker, C., Pöschl, U., Kreidenweis, S.M., 2013. Biological aerosol particles as a key determinant of ice nuclei populations in a forest ecosystem. J. Geophys. Res. Atmos. 118, 10100–10110. https://doi.org/10.1002/jgrd.50801.
Triadó-Margarit, X., Veillette, M., Duchaine, C., Talbot, M., Amato, F., Minguillón, M.C., Martins, V., de Miguel, E., Casamayor, E.O., Moreno, T., 2017. Bioaerosols in the Barcelona subway system. Indoor Air 27, 564–575. https://doi.org/10.1111/ina.12343.
Wang, C.H., Chen, B.T., Han, B.C., Liu, A.C.Y., Hung, P.C., Chen, C.Y., Chao, H.J., 2015. Field evaluation of personal sampling methods for multiple bioaerosols. PLoS One 10, 1–20. https://doi.org/10.1371/journal.pone.0120308.
Wéry, N., 2014. Bioaerosols from composting facilities-a review. Front. Cell. Infect. Microbiol. 4, 1–9. https://doi.org/10.3389/fcimb.2014.00042.
Whitehead, J.D., Darbyshire, E., Brito, J., Barbosa, H.M.J., Crawford, I., Stern, R., Gallagher, M.W., Kaye, P.H., Allan, J.D., Coe, H., Artaxo, P., McFiggans, G., 2016. Biogenic cloud nuclei in the Central Amazon during the transition from wet to dry season. Atmos. Chem. Phys. 16, 9727–9743. https://doi.org/10.5194/acp-16-9727-2016.
Wilson, T.W., Ladino, L.A., Alpert, P.A., Breckels, M.N., Brooks, I.M., Browse, J., Burrows, S.M., Carslaw, K.S., Huffman, J.A., Judd, C., Kilthau, W.P., Mason, R.H., McFiggans, G., Miller, L.A., Najera, J.J., Polishchuk, E., Rae, S., Schiller, C.L., Si, M., Temprado, J.V., Whale, T.F., Wong, J.P.S., Wurl, O., Yakobi-Hancock, J.D., Abbatt, J.P.D., Aller, J.Y., Bertram, A.K., Knopf, D.A., Murray, B.J., 2015. A marine biogenic source of atmospheric ice-nucleating particles. Nature 525, 234–238. https://doi.org/10.1038/nature14986.
Xiao, M., Wang, Q., Qin, X., Yu, G., Deng, C., 2018. Composition, sources, and distribution of PM2.5 saccharides in a coastal urban site of China. Atmosphere (Basel) 9. https://doi.org/10.3390/atmos9070274.
Yang, K., Wu, D., Ye, X., Liu, D., Chen, J., Sun, P., 2013. Characterization of chemical composition of bee pollen in China. J. Agric. Food Chem. 61, 708–718. https://doi.org/10.1021/jf304056b.
Yang, X., Guo, D., Zhang, J., Wu, M., 2007. Characterization and antitumor activity of pollen polysaccharide. Int. Immunopharmacol. 7, 427–434. https://doi.org/10.1016/j.intimp.2006.10.003.
Zhang, M., Khaled, A., Amato, P., Delort, A.M., Ervens, B., 2021. Sensitivities to biological aerosol particle properties and ageing processes: potential implications for aerosol-cloud interactions and optical properties. Atmos. Chem. Phys. 21, 3699–3724. https://doi.org/10.5194/acp-21-3699-2021.
Zhang, Q., Anastasio, C., 2003. Free and combined amino compounds in atmospheric fine particles (PM2.5) and fog waters from northern California. Atmos. Environ. 37, 2247–2258. https://doi.org/10.1016/S1352-2310(03)00127-4.
Zhang, X., Yang, W., Dong, C., 2013. Levoglucosan formation mechanisms during cellulose pyrolysis. J. Anal. Appl. Pyrolysis 104, 19–27. https://doi.org/10.1016/j.jaap.2013.09.015.
Zhu, C., Kawamura, K., Kunwar, B., 2015. Organic tracers of primary biological aerosol particles at subtropical Okinawa Island in the western North Pacific rim. J. Geophys. Res. Atmos. 175, 238. https://doi.org/10.1038/175238c0.
Zhu, R.Guo, Xiao, H.Y., Zhu, Y., Wen, Z., Fang, X., Pan, Y., 2020. Sources and transformation processes of proteinaceous matter and free amino acids in PM2.5. J. Geophys. Res. Atmos. 125, 1–16. https://doi.org/10.1029/2020JD032375.
Zhu, R.G., Xiao, H.Y., Luo, L., Xiao, H., Wen, Z., Zhu, Y., Fang, X., Pan, Y., Chen, Z., 2021. Measurement report: hydrolyzed amino acids in fine and coarse atmospheric aerosol in Nanchang, China: concentrations, compositions, sources and possible bacterial degradation state. Atmos. Chem. Phys. 21, 2585–2600. https://doi.org/10.5194/acp-21-2585-2021.