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Aerial drone as a carrier for miniaturized air sampling systems

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Highlights

- Aerial drone was used as a carrier for miniaturized air sampling systems
- Simultaneous passive SPME Arrow and active ITEX air sampling
- Sampling allows use of different SPME Arrow coating and ITEX sorbent materials
- Direct comparison of simultaneously collected multiple samples can be made
- The system enables low-cost vertical profile studies

Abstract

The applicability of an aerial drone as a carrier for new passive and active miniaturized air sampling systems, including solid phase microextraction Arrow (SPME Arrow) and in-tube extraction (ITEX), was studied in this research. Thermal desorption, gas chromatography and mass spectrometry were used for the determination of volatile organic compounds (VOCs) collected by the sampling systems. The direct comparison of the profiles of VOCs, simultaneously sampled in air by SPME Arrow system including four different coatings, allowed the elucidation of their adsorption selectivity. A more complex experimental design, involving 20 samples (10 flights) and non-supervised pattern recognition techniques, was needed for the clarification of the same sampling parameters in the case of five ITEX sorbent materials. In addition, ITEX sampling accessories, such as particle, water and
ozone traps, were evaluated by comparing the results obtained for air samples simultaneously collected by two ITEX systems, packed with the same sorbent and furnished or not with sampling accessories. The effect of the aerial drone horizontal displacement (HD) on the sampling efficiency was clear in the case of SPME Arrow. The number of detected compounds and their relative peak area values (RPA) revealed a clear increase (4 and 43 %, respectively) in comparison with samples collected without drone HD. However, just minor differences were observed in the case of ITEX (2 compounds and 9 % of the \( \Sigma \)RPA). In addition, the system was able to provide almost simultaneous passive (SPME Arrow) and active (ITEX) samplings at different altitudes (5 and 50 m), being a good tool for low cost vertical profiling studies (\( \Sigma \)RPA decreased over 35% for the samples collected at 50 m). Finally, the successful simultaneous air sampling by SPME Arrow and ITEX systems in two difficult access places, such as boreal forest and wetlands, was demonstrated, resulting in 21 and 31 detected compounds in forest and wetlands by SPME Arrow, and 27 and 39 compounds by ITEX.

**Keywords:** Aerial drone; Miniaturized air sampling systems; SPME Arrow; ITEX; Sampler; Sampling accessories; Air sampling
1. Introduction

Aerial drones, also called unmanned aerial vehicles, remotely piloted aircraft systems or simply drones, have become very popular over the last decade [1]. The original military use of these devices has expanded to civil and scientific applications [2]. Among others, aerial drones can be used as carriers for air sampling systems, analyzers and sensors, replacing or complementing the traditional ways to sampling at high altitudes such as airships, aircrafts, tethered balloons and meteorological towers [3-6].

Aerial drones can be classified into two different categories according with their design: fixed wing, mainly used for sampling over long distances, and; rotary-wing, used on localized studies [7-8]. The later has clear advantages in terms of maneuverability, cost and cleanliness. The use of electrically powered engines limits the potential contamination sources [8]. The effect of the number of rotors on the aerial drone payload capacity, in-flight stability and maneuverability have been widely described in the literature without clear conclusions. Old aerial drone models show a clear improvement of flight performance with the number of rotors [9]. However, the opposite trend was observed for new aerial drone models furnished with a relative low number of rotors. More clear conclusions were drawn from the design of rotary-wing aerial drones. The use of multiple rotors at the periphery, equidistant around a central core, allows the allocation of sensors and sampling systems on the center of the craft, far away from potential turbulences and interferences [10].

A wide variety of sensors have been attached to aerial drones to provide continuous monitoring of different parameters such as temperature, humidity, pressure, black carbon, CO₂, CO, NO₂, NO, the number of particles, particle size distribution or even total volatile organic compounds (VOC) concentration [8-9, 11]. However, the use Tedlar bags, stainless steel canisters and a wide variety of active sampling systems including filters, adsorption tubes is still mandatory for the analysis of
individual compounds in the samples [8, 12-14]. In addition, to our best knowledge, SPME Arrow systems have never been used for the collection of air in combination with aerial drones.

Solid phase microextraction (SPME) have been widely used in extraction of different sample matrices and also as air sampling systems. Despite its popularity, some drawbacks related with the SPME fibers such as, easy breakage, limited lifetime, restricted coating thickness, limited sorption capacity and instability have been observed. Different microextraction techniques, including SPME Arrow and ITEX, have been developed in order to overcome these problems and to improve sampling performance [15-17].

SPME Arrow consists of a steel rod coated with a larger volume of extraction sorbent than traditional SPME fibers. The use of high sorbent volumes improves the detection limits. The design of the SPME Arrow device with the arrow-shaped tip is compatible with the GC injector port with some minor modifications. Furthermore, design of this device protects the sorbent, minimizing unfavorable influences and loss of analytes during desorption [15-20].

In-tube extraction (ITEX) system was developed to provide high performance. It was designed as a two-part stainless steel needle attached to a pumping system. The lower part consists of a conical tip needle with the side hole for septum penetration. The upper part is the larger diameter tube filled with a sorbent material, allowing higher amount of extraction sorbent in comparison with other approaches such as needle trap devices (NTD). However, unlike SPME Arrow, NTD and ITEX need a pump for the collection of the analytes on the sorbent and an external heater to facilitate thermal desorption to the GC system [17, 21].

The potential applicability of an aerial drone as a carrier for new miniaturized air sampling systems, such as SPME Arrow and ITEX, was evaluated in this research. The use of these sampling techniques could overcome problems found in traditional sampling techniques such as SPME fiber or thermal desorption tube (TDT). In addition, this is the first time when a SPME Arrow was used in combination
with aerial drone. Different experimental approaches and configurations, based on simultaneous collection of air samples were developed for the characterization of the sampling systems and the evaluation of the sampler performance. The effect of horizontal and vertical displacements of the drone during the sampling was also evaluated. The latter will allow the almost simultaneous air sampling at different altitudes, making possible the development of low-cost vertical profile studies. Finally, an aerial drone, furnished with both, ITEX and SPME Arrow sampling systems, was used for the simultaneous collection of air samples in two difficult access places, such as boreal forest and wetland.

2. Experimental

2.1. Materials

Decafluorobiphenyl (99.99 %, Fisher Chemical) was used as internal standard (IS). The stock standard solution was prepared dissolving 0.1 g of IS in 100 ml of methanol. Standard working solution (1µg/ml) was prepared by subsequent dilution in methanol. Liquid and gas phase solutions were used for ITEX and SPME Arrow including IS addition, respectively. Diffusion vials were prepared by addition of 1µl IS working solution to a 20 mL pre-heated (40 °C) vial.

A 0.45 µm Nylon filter from Nalgene (Rochester, NY, USA) was used as aerosol particles trap. Potassium iodine from Merck (Darmstadt, Germany), glycerol from Sigma–Aldrich (St. Louis, MO, USA), methanol from J.T. Baker (Deventer, The Netherlands) and ultrapure water (DirectQ-UV, Millipore Corp., Billerica, USA) were used for ozone trap coating. Finally, magnesium perchlorate from Aldrich (Steinheim, Germany) and deactivated glass wool were used for water trap preparation. Altogether seven ITEX and six SPME Arrows were used for air sampling. In detail: three ITEX packed with 50 mg of three lab-made polyacrylonitrile (PAN) nanofibers (10% PAN, 8.75% PAN, 5% PAN), two commercial Tenax GR and two commercial Tenax TA (CTC Analytics AG, Zwingen, Switzerland) [22]. Two SPME Arrows coated with carbon (Carbon wide range (WR) and Carbon
WR/Poly(dimethylsiloxane) (PDMS), two with polymeric (PDMS/Divinylbenzene (DVB)) (CTC Analytics AG, Zwingen, Switzerland) and two with self-made functionalized mesoporous silica materials (MCM-41-Ti) [23].

2.2. Sampling device

Air samples were collected by a sampler, consisting of a remote controlled drone Geodrone X4L, with dimensions 58 x 58 x 37 cm and a total weight of 2 kg, (Videodrone, Finland) furnished with a drone box, which allows the simultaneous sampling on multiple ITEX and SPME Arrow systems. Detailed scheme and pictures of aerial drone--based SPME sampler can be seen in Figure 1. Additional information of the aerial drone can be found from electronic supplementary information (ESI-1). Drone box device involved a 3G remote-controlled circuit, two SPME Arrow holders with opening/closing system, two on/off valves for gas flow control and tubes for ITEX connections with sampling pump. The total weight of drone box including the pump was about 1.4 kg, which allowed a total flight time close to 35 min. The smartphone app Juice SSH mobile had the access to the operating system Raspbian to control the hardware Raspberry Pi (Raspberry Pi Foundation) in the drone box. The system was used via the 3G modem (Huawei) to control the ITEX pump, valves, SPME holders and to their opening/closing system.

2.3. Instruments

An Agilent 6890N gas chromatograph (Agilent Technologies, Pittsburg, PA, USA), equipped with a split/splitless injector and a lab-made ITEX heater for thermal desorption, was coupled to an Agilent 5973 mass spectrometer for the separation and detection of VOCs present in the total air samples. The chromatograph was furnished with a GC Sciences (Eindhoven, The Netherlands) InertCap for Amines (30 m x 0.25 mm i.d., no information was provided for the film thickness) fused silica column coupled with a 3-m deactivated retention gap (i.d. 0.53 mm, Agilent). Additional information about the gas chromatographic analysis, including sample storage, thermal desorption conditions, GC oven temperature program and SPME Arrow and ITEX preconditioning, can be found in ESI-2.
2.4. Measurement sites

The applicability of aerial drone as a carrier for miniaturized air sampling systems was evaluated from the 22nd to 26th of October 2018 at the SMEAR II station (Station For Measuring Ecosystem–Atmosphere Relations), Hyytiälä. The soccer field area (61.844400° N - 24.287033° E) was selected as test field due to good visible conditions and the scarce number of obstacles in the zone [24]. In addition, 8 samples were collected on difficult access places, such as the forest nearby next to the SMEAR II tower (61.846350° N - 24.295333° E – 23 October 2018) and Siikaneva wetland area (26 October 2018). The latter was located in Ruovesi (61.833266° N, 24.192550° E), 8 km from SMEAR II station, a well-known site for carbon and energy studies and soil processes [25]. In both cases, two ITEX and two SPME Arrows, the first without any sampling accessories, were simultaneously sampled during a single flight. Control valves and SPME Arrow actuators were in close position up to when aerial drone was placed in the sampling position, avoiding the sampling while the drone was moving.

2.5. Adsorption selectivity studies

The versatility of aerial drone-based SPME sampler allowed the evaluation of the sorbent selectivity of both passive and active sampling by using two different approaches. The first approach, included four SPME Arrows with Carbon WR, Carbon WR/PDMS, PDMS/DVB and MCM-41-Ti coatings, was based on the simultaneous collection of multiple samples during a single flight (30 min at 5 m), followed by the analysis of the samples and the direct comparison of the results. The second approach, included five ITEXs with Tenax GR, Tenax TA, PAN 5 %, PAN 8.75 % and PAN 10% sorbents, was more demanding due to the pump needed for active air sampling, limiting the number of simultaneous ITEX system to two during each flight. An experimental design comprising 10 flights within three days, VOC analysis and statistical evaluation of the results were then developed. Additional information about the sampling procedures and the experimental design is found from ESI-3.
2.6. Evaluation of the effect of sampling accessories for in-tube extraction

The effect of three different accessories, such as particle, water and ozone traps, on ITEX (Tenax GR) sampling was evaluated by comparing the results obtained for analysis of air samples, simultaneously collected for 30 min at 5 m, with and without sampling accessories. Description of the different sampling accessories is found elsewhere [8, 26].

2.7. Effect of drone horizontal and vertical displacements on the sampling

The effect of the drone horizontal displacement (HD) zone sampling on the samples, collected by two ITEXs with Tenax GR as sorbent and two SPME Arrow systems with DVB-PDMS as coatings was evaluated during one single flight at 5m by sequential sampling with and without HD. The effect of the drone vertical displacement (VD) on the samples collected by two ITEXs with Tenax TA sorbent and two SPME Arrow systems with MCM-41-Ti coating was evaluated during a single flight by alternative sampling at different altitudes. No sampling accessories were used in the case of ITEX. In both cases, simultaneous sampling was developed for SPME Arrow and ITEX systems. A detailed description of the flying programs for HD and VD is seen in ESI-4.

2.8. Data handling and statistical analysis

ADAP-GC (Automated Data Analysis Pipeline) for pre-processing untargeted mass spectrometry-based omics data, an algorithm included in Mzmine2 software, was used for the detection, deconvolution and alignment of the chromatographic peaks following the workflow described in Figure S1 [27-28]. The identification of the aligned peaks was developed by comparing their mass spectra and retention indices to NIST2014 mass spectral database. Finally, results of the zero samples (conditioned SPME Arrows and ITEXs) were subtracted from all the samples. R 3.5.1 was used for the development of ANOVA, linear regression, principal component analysis (PCA) and cluster analysis (CA) [29].

3. Results and discussion
VOC compounds sampled by microextraction based sampling techniques and detected by gas chromatography with a polar column and low resolution mass spectrometry were classified into the main functional chemical groups. Considering the limitations of methodology used for the determination of the VOCs, which does not allow reliable identification of the peak without using standard compounds, just main functional group present in the different compounds was considered in the discussion of the results. In addition, due to the potential low concentration of the target analytes in the samples, 30 min, close to the maximum flight time of the aerial drone, was selected for sampling time. When different sampling conditions were evaluated in a single flight, sampling time was adjusted to 15 min. More detailed optimization of the sampling time should be developed in future studies under specific conditions. For comparison of active ITEX and passive SPME Arrow sampling systems, the results were evaluated in terms of the number of compounds and peak areas (PA). Peak areas were replaced with relative peak area (RPA) values (Eq.1) when samples collected within the same sampling system were compared.

\[
\text{RPA} = \frac{A_{ci}}{A_{IS}} 
\]

where \(A_{ci}\) is the peak area of the compound; and \(A_{IS}\) is the peak area of decafluorobiphenyl, which was used as an internal standard for injection.

### 3.1. Adsorption selectivity

#### 3.1.1. Solid phase microextraction Arrow

Commercial SPME Arrow coatings, such as Carbon WR, Carbon WR/PDMS and PDMS/DVB allowed to collect a large number of compounds (ranged from 27 to 31) within a wide variety of functional groups, from hydrocarbons to oxygenated compounds. Specifically, results were very close to those obtained with Carbon WR based SPME Arrow (Fig S2A). However, some differences were observed when the results were compared with those achieved by DVBcoatings (Fig. S2 B-C).
Anyway, the $\sum RPA$ for compounds found just in one of the commercial SPME Arrow, was $\leq 13\%$ in all the cases.

The number of compounds, adsorbed on lab-made functionalized mesoporous silica material MCM-41-Ti (13) was two to three times smaller, in comparison with commercial sorbents. Similar trend was also found for $\sum RPA$ which was also from 3 to 10 times smaller in comparison with commercial Arrows (Fig. S2 D-F). As a positive thing, it is good to mention that opposite to commercial coatings, MCM-41-Ti allowed the adsorption of alkylamines, which were not detected when collected by the commercial SPME Arrow coatings. This can be explained by considering that MCM–41 silica has ordered two dimensional pore–channel structures and alterable mesopore sizes to afford size–exclusion selectivity. Furthermore, additional Lewis and Brønsted acid sites were introduced into MCM-41 after grafting with titanium groups (–Ti), increasing the surface acidity of MCM-41 which provide additional chemical selectivity towards the basic amines [23].

Finally, Figure S2 G shows the comparison of SPME Arrow coating materials as a function of their adsorption selectivity for the compounds present natural air samples.

### 3.1.2. In-tube extraction

The contribution of the sample composition to the results was partially avoided by the use of non-supervised pattern recognition techniques for data analysis, allowing the comparison of samples collected during different flights or during even days. The use of a randomized experimental design avoids the correlation between samples. As can be seen in Figure S3A, cluster analysis reveals the presence of three different clusters which can be easily associated to the PCA results. The first cluster (A) includes all the samples collected on Tenax based sorbents GR and TA (10 samples). Selectivity for those materials follows the sequence shown in Figure S3B: hydrocarbons $>$ alcohols $>$ aldehydes $>$ nitrogen compounds $>$ acids $>$ ketones. The second cluster (B) includes the samples obtained by ITEX PAN 5% and 8.5% sorbents. The selectivity changes to acids $>$ alcohols $>$ aldehydes $>$ ketones.
> nitrogen compounds > hydrocarbons. Finally, in the third group (C) it was possible to find the samples collected by PAN 10 % sorbent. The selectivity is similar to other PAN based sorbents. However, $\Sigma$RPA values observed were bigger for all the groups of compounds under study. These results can be explained considering that PAN 10% has larger absorbent volume, compared with PAN 5% and 8.5%, thus providing the highest extraction affinity for a wide range of compounds. [22].

3.2. In-tube extraction sampling accessories

As can be seen from the results (Figure S4B), the total number of compounds (58) detected in the samples without accessories decreased at least three times, when a trap for aerosol particles was used (18). Similar trend was found for $\Sigma$RPA. In addition, only six compounds (2 aldehydes, one alcohol and 3 hydrocarbons) were detected at relatively lower concentrations (3.5 % $\Sigma$RPA) a only in samples collected with this accessory. Filters, used as traps for aerosol particles, can also remove gas phase compounds from air samples by adsorption on the filter surface or on the particles retained on the filters. In addition, the presence of these particles and compounds on the trap could help to protect the VOCs collected on the ITEX system from potential oxidation agents such as ozone [30], thus explaining the presence of new compounds in samples collected by filter.

Similar results, in terms of number of compounds and $\Sigma$RPA values, were obtained in the case of water traps (Figure S4A). However, the comparison between them revealed that most of the compounds (70%) present in the samples were different (Figure S4D). No significant differences, evaluated by linear regression methodology (slope and intercept equal to 1 and 0, respectively), were found for those 6 compounds present in both samples. The low number of compounds in the sample can be explained by the design of the water trap, similar to a filter. In addition, differences in the composition of the samples collected with water trap or filter could be explained by the drying capacity of the Mg(ClO$_4$)$_2$, which is able to retain water molecules on its surface and therefore most of the water soluble compounds, such as acids and nitrogen containing compounds [8, 31-32].
In comparison the results obtained with particle and water traps, the additional ozone trap should produce a relative small effect on the sample composition considering that gas phase and aerosol particles were collected by the ITEX system (Figure S4C). This was observed for a high number of compounds (40) found in the sample. However, this number is far away from the total number of compounds (53) detected in the samples collected without accessories. Thirteen compounds, including alcohol, aldehydes, acids, hydrocarbons and nitrogen compounds were found at relative low concentrations (14.1 % of ΣRPA) just in samples collected with ozone trap. These numbers are small in comparison with the sample collected without accessories in terms of number of compounds (26) but similar in terms ΣRPA (16.9%). However, it is clear that ozone trap is able to remove ozone from the air sample, thus protecting compounds from oxidation during sampling. More and different compounds, most of them oxidized, were found in the case of samples collected without ozone trap [26].

3.3. Effect of drone horizontal and vertical displacements on the sampling

Drone HDs with ITEX as sampling system affected only slightly the VOC composition in total air samples. Similar variations in terms of number of compounds and ΣRPA smaller than 10%, were achieved for drone static and zone samplings (Figure S4). These differences can be explained by the changes in the total air composition during the sequential sampling with 15 min difference between samples. However, clear differences were found for SPME Arrow samples. The number of VOCs detected in samples and the ΣRPA values revealed a clear increase, up to three times, for zone sampling. This can be explained by the airflow and the turbulences produced by the propellers during drone HD. Based on the recent studies the latter might affect due to the location of SPME Arrows in the drone [1, 19, 33]. The use of a dynamic air sampling system for SPME Arrow would help to minimize these problems [19].
Similar trend was seen in the case of drone VD for ITEX and SPME Arrow sampling systems. Namely the number of compounds detected in the samples and their relative peak area values decreased with the sampling altitude. Some compounds, 11 by ITEX and 8 by SPME Arrow, were found only in samples at height of 5 m. However, most of the compounds (13 by ITEX and 10 by SPME Arrow) were detected at both altitudes. In addition, just a few compounds, three by passive SPME Arrow sampling and four by active ITEX sampling systems, were detected at relatively low concentrations (≤ 6.5%, in all the cases) in samples collected at higher altitude 50 m. These results are in good agreement with the values found in the literature for VOCs vertical profile distribution [34].

3.4. Analysis of natural samples

Good reproducibility, in terms of number of compounds ≥ 84% and ΣRPA ≥ 99%, was achieved by SPME Arrow and ITEX sampling systems for two samples collected simultaneously with both systems. In all the cases, linear regression studies did not reveal any statistical differences (slope and intercept equal to 1 and 0, respectively) for the RPA values of VOCs found in both replicates (ESI-5). However, these numbers should be considered just as preliminary results considering the very limited number of samples analyzed.

The number of compounds and ΣRPA values are found in Figure 2, and additional numerical information is available in ESI-6. Similar trend was seen by ITEX and SPME Arrow sampling systems. However, wetland samples were the most complex in terms of total number of compounds and ΣRPA. Some compounds (14 by SPME Arrow and 17 by ITEX), most of them oxygenated (aldehydes, ketones and alcohols), were detected simultaneously in both sampling places. However, a quite high number of compounds was found just in one sampling site. It should be emphasized that the concentrations of the number of compounds (22), detected in wetland sampling site by ITEX were relatively low (22.8% of the ΣRPA). Sample composition differences can be explained also by sampling time (2 days). However, the detection of several characteristic compounds invites to
consider the contribution of the sampling location to the results. A clear example is the detection of sulphur containing compounds in wetlands samples (the same compounds in SPME Arrow and ITEX samples) which can be explained by incomplete humification of the organic matter in waterlogged conditions (anoxic conditions) [35].

Main differences within the samples collected in the same location (higher number of compounds and ΣPA in ITEX samples) can be easily explained by different sampling techniques (passive SPME Arrow and active ITEX). Results suggest that VOCs, collected almost under passive sampling conditions by SPME Arrow, were almost unaffected by turbulences produced by propellers. However, additional studies, based on simultaneous total air sampling by ITEX and SPME Arrow systems located in the aerial drone-based SPME sampler, are needed.

4. Conclusions

The potential of an aerial drone as a carrier for new, miniaturized and environmentally friendly air sampling systems accessible also difficult places, such as forest or wetlands, was demonstrated in this research. - Aerial drone-based SPME sampler allowed the simultaneous collection of multiple air samples by different sampling systems such as ITEX or SPME Arrow, up to two SPME Arrow (four in manual mode) and two ITEX systems. Flight time was 30 min under current payload, but more efficient battery can increase the total flight time, if needed. Remote control of the sampling procedures avoids the effect of the battery changes during the sampling. Different parameters such as the sample matrix, the concentration of the analytes in the samples and the instrumental setup used for the determination of the target analytes must be considered before the selection of the most suitable sampling system.

Different approaches enabled the direct comparison of the multiple samples simultaneously collected during a single flight or the comparison of the samples collected during different flights. The latter demonstrated to be useful for ITEX sampling system, due to the sampler limitations. The use of a
more powerful air pump, which allows the simultaneous collection of air samples by a higher number of ITEX systems, would simplify their analysis.

Accessories such as, filters and traps for ITEX system, allow also the collection of gas phase compounds and/or prevent the samples from undesirable chemical reactions. This approach was not feasible for SPME Arrow using the configuration developed in this research. However, the use of a dynamic air sampling system by SPME Arrow would help to control its sampling variables.

Finally, the use of fast vertical drone movements and the remote control of the multiple sampling systems allowed the almost simultaneous air sampling at different altitudes, making the development of low-cost vertical profile studies possible. No substantial effect of the drone vertical displacement on the samples collected by passive SPME Arrow and active ITEX sampling systems was noticed. However, the use of horizontal displacement during the sampling, useful for the evaluation of zone effects on natural samples, had a clear influence on the results achieved by SPME Arrow system.

Proper quality assurance/quality control (QA/QC) procedures are certainly vital to assure reliable analytical information, and this Concept Paper will be followed by full future validation study.

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References


Figure Captions

Figure 1. Aerial drone as a carrier for new miniaturized air sampling systems (A) Front schematic view and pictures from details (B) Side schematic view and pictures from details.

Figure 2. Results obtained for the analysis of air samples collected in difficult access locations by aerial drone-based SPME sampler using simultaneous active and passive sampling systems. Venn diagrams compares samples collected in different places using the same sampling system. Doughnut charts show the results in terms of VOC composition. Number of compounds are noted.
as black numbers. $\Sigma$RPA values are noted as black numbers in brackets. Forest samples (■); Wetland samples (■); Aldehydes (■); Ketones (■); Alcohols (■); Hydrocarbons (■); Acids and derivatives (■); Nitrogen containing compounds (■); Sulphur containing compounds (■).