Profiles of Volatile Compounds in Blackcurrant (*Ribes nigrum*) Cultivars with Special Focus on Influence of Growth Latitude and Weather Conditions

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Abstract

The volatile profile of three blackcurrant (*Ribes nigrum* L.) cultivars grown in Finland and their response to growth latitude and weather conditions were studied over an eight-year period by headspace solid-phase microextraction (HS-SPME) followed by gas chromatographic-mass spectrometric (GC-MS) analysis. Monoterpene hydrocarbons and oxygenated monoterpenes were the major classes of volatiles. The cultivar 'Melalahti' presented lower content of volatiles compared with 'Ola' and 'Mortti', the two latter showing a very similar composition. Higher contents of volatiles were found in berries cultivated at higher latitude (66° 34' N) than in those from the southern location (60° 23' N). Among the meteorological variables, radiation and temperature during the last month before harvest were negatively linked with the volatile content. Storage time had a negative impact on the amount of blackcurrant volatiles.

Keywords: Blackcurrant; Cultivar; HS-SPME-GC-MS; Latitude; Meteorological data; *Ribes nigrum*; Volatile compounds; Weather conditions.

INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is widely cultivated across the temperate zone in Europe with the total annual production of blackcurrant close to 160,000 tons.[1] In countries outside Europe, there has been an increasing interest in cultivation and consumption of blackcurrant and other berries of *Ribes* species with New Zealand being among the leading countries in cultivation and processing of blackcurrants.[2] Various health promoting properties of blackcurrant have been shown by both traditional use and modern research likely due to the high content of phytochemicals such as phenolic compounds and vitamin C.[3-5] Blackcurrant berries are highly popular in the Nordic countries where they are appreciated for their flavor and nutritional properties. According to the Natural Resources Institute Finland (LUKE), the Finnish production of berries accounted almost 15,000 tons in 2016, blackcurrant berries being in the third position of the most produced berries (950 tons) after strawberry and raspberry (http://statdb.luke.fi). The composition of blackcurrants has been widely studied in regard to several phytochemicals such as phenolic compounds,[6, 7] carotenoids and phytosterols,[8] and vitamin, C[9] both in fresh berries and in berry-derived products such as juices.[10, 11]

Blackcurrant berries are consumed as fresh berries in households and they are industrially processed into a wide range of products such as juices, jam, jelly, yoghurt, and fruit bars. The unique aroma profile is an essential element of the blackcurrant flavor. Several studies on blackcurrant berries,[7-9] and more recently the one published by Jung et al.[12] have been focused on volatile compounds. These studies have been devoted to characterization of the aroma compounds[13] as well as the impact of cultivars,[14] ripening stage,[9] thermal[15] and enzymatic treatments,[16] and freezing.[12] Altogether, these studies have characterized a vast number of compounds as constituents of the volatilome of blackcurrants including compounds of various chemical classes such as alcohols, aldehydes, esters, and terpenes. However, to the best knowledge of the authors, no research has been reported on the contribution of harsh Nordic environment and the associated meteorological data to the volatile content and composition in blackcurrant cultivars.

The influence of environmental conditions on the emitted volatile compounds in plants has been highly examined from a biochemical point of view, especially regarding terpene biosynthesis. Methyl erythritol phosphate pathway (MEP) is known to be responsible for the formation of the basic C5 units of isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP). In addition to the above mentioned MEP, in plants, such basic structures are formed via the mevalonic acid pathway (MVA).[17] Although the environmental stress-induced emission of volatiles has been widely reported, it is not clear which are the ecological actions that should be attributed to volatile terpenes. However, in fruits grown under conventional agricultural practices, the influence of environmental conditions on the volatiles has been scarcely studied. It has been reported that sunlight and UV exposure in grapes affect terpene alcohols, C_{13}-norisprenoids and other volatiles of wine, depending on the compound.[18] The volatile composition of strawberry cultivars was found to be more dependent on the genotype than the environmental conditions.[19] UV-C pre-harvest treatment of strawberry showed no significant effects on any of the volatiles,[20] and, finally, it has been reported that the volatile composition of essential oil from aromatic plants is extremely dependent on the weather conditions although the effects were not clearly stated.[21]

In the current research we aim to study the volatile composition of three commercial, Nordic blackcurrant berry cultivars 'Mortti', 'Ola', and 'Melalahti', and the variations according to the growth latitude and weather conditions in open test fields in southern and northern Finland. The study samples included berries harvested annually during 2010-2017 from two latitudes.

Headspace (HS) solid-phase micro-extraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS), was used as a reliable technique to sample the volatile fraction of complex matrices due to high-throughput and possibility of automation. A large dataset was produced from GC-MS analyses and used for identification and quantitation of the volatile compounds. Further data analysis was carried out with multivariate techniques to classify the samples and to detect the association between cultivars, growth latitudes and environmental factors with specific metabolite profiles.

This study is a subproject of an on-going large study, where we investigate the impact of growth latitude and environmental factors on the metabolomic profile of berry crops using blackcurrant as one of the model species. Hence, the research will produce new information on volatile compounds to our previous research on the impact of growth environment on composition and quality of blackcurrants.

MATERIALS AND METHODS

Blackcurrant samples.

Three cultivars of blackcurrant (*Ribes nigrum* L.), 'Mortti', 'Ola', and 'Melalahti', were cultivated by applying identical farming practices in Piikkiö, Kaarina, southern Finland (latitude 60° 23' N, longitude 22° 33' E, altitude 5−15 m) and Apukka, Rovaniemi, northern Finland (66° 34' N, 26° 01' E, 100−105 m) by MTT Agrifood Research Finland / Natural Resources Institute Finland (Luke). 'Melalahti' is an old local cultivar from Paltamo, northern Finland.[22] 'Mortti' is a crossing 'Öjebyn' (from Sweden) x 'Wellington XXX' (from Great Britain)[23] and 'Ola' is a crossing

'Wellington XXX' x 'Lepaan Musta' (from Finland),[24] both cultivars developed in Finland. Twelve bushes of each cultivar were planted in four field blocks in May in 2002. Little irrigation was applied during the study period, and fertigation and other growing methods were carried out according to Finnish standard guidelines.[22] The berries were harvested in quadruplicate, one sample (*ca.* 500 g) from each of the four field blocks, from both southern and northern Finland in consecutive years from 2010 to 2017. No berries were collected in 2015 from either location, and in 2014 and 2016 no berries were collected from Apukka (N). The berries were picked optimally ripe for harvesting as defined by experienced horticulturists. This was based on sensory evaluation of intensity of surface color, tasted flavor with typical sweetness-acidity ratio and aroma, and firmness of the berries. The berries were frozen and stored at −20 °C immediately after harvesting until being analyzed.

Chemicals and reagents.

Hexanal, nonanal, undecane, α-pinene, camphene, β-pinene, myrcene, α-phellandrene, δ-3-carene, α-terpinene, *p*-cymene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, γ-terpinene, terpinolene, eucalyptol, terpinen-4-ol, bornyl acetate, terpinyl acetate, β-caryophyllene, *n*-nonane, neryl acetate and a homologous series of *n*-alkanes (C9–C30) of analytical purity were purchased from Sigma–Aldrich (St. Louis, MO). Glucose, fructose and sucrose were obtained from Merck (Darmstadt, Germany), citric acid from J.T.Baker (Deventer, the Netherlands), and pectin from Herbstreith & Fox KG (Neuenburg, Germany). The aforementioned sugars, organic acids, and pectin were employed to prepare a synthetic blackcurrant juice containing no volatiles following the composition detailed in Food Composition and Food Nutrition Tables. A ditch, 10 cm deep and 20 cm wide, was plowed through every block. The ditches were filled with white Sphagnum peat (pH 6) mixed with 8 kg dolomite lime and 1.5 kg·m^{-3} of NPK basic fertilizer. The seedlings were planted and the peat was covered with the local fine sand soil. Sodium chloride (99% purity) was from Sigma–Aldrich. Methanol and acetone (HPLC grade) were purchased from J.T.Baker.

HS-SPME-GC-MS profiling.

Frozen berries were thawed overnight at 4 °C. Next, 50 g were homogenized in 50 mL of H_2O saturated with sodium chloride with a Bamix mixer (Bamix M13, Mettlen, Switzerland). Water was added to help the homogenization process while sodium chloride had an effect in reducing enzymatic activity thus helping in preserving samples from enzymatic degradation. Furthermore, salts have an enhancing effect on the extraction efficiency of volatile compounds due to the salting-out effect. From the slurry, 2 g were transferred to a 20-mL headspace vial and spiked with 10 µL of the internal standard mixture containing 250 µg·mL^{-1} *n*-nonane and 100 µg·mL^{-1} neryl acetate. The internal standards fulfilled 3 points: 1) they were not initially present in the samples; 2) their retention time was free of possible coelutions; 3) they proved to be robust and stable to be used in a long sample sequence.

Collection of the volatiles by HS-SPME was carried out with a 2-cm SPME fiber CAR/PDMS/DVB (Carboxen/Polydimethylsiloxane/Divinylbenzene; 50/30 µm) from Supelco (Bellefonte, PA) at 45 °C for 30 min under agitation employing a TriPlus RSH multipurpose autosampler (Thermo Scientific, Reinach, Switzerland).

After extraction, GC-MS analyses were performed with a Trace 1310 (Thermo Scientific) gas chromatograph coupled to a TSQ 8000 EVO mass spectrometer (Thermo Scientific). Volatiles were desorbed from the fiber into the injection port equipped with and SPME liner at 240 °C for

3 min. Compounds were separated with a DB-5MS column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) from Agilent (Palo Alto, CA) using helium as carrier gas (1.2 mL·min^{-1}). The oven was temperature programmed from 40 °C (held for 1 min) to 160 °C at 5 °C·min^{-1}, then to 240 °C at 12 °C·min^{-1} (held for 1 min). Mass spectra were recorded in electron impact (EI) mode at 70 eV within the mass range *m*/*z* 40–300. The transfer line and the ionization source were thermostated at 250 and 220 °C, respectively. The system was operated using Xcalibur 4.0 (Thermo Scientific). All analyses were carried out in triplicate.

Identification of volatile compounds was based on authentic reference compounds, when available. Tentative identifications were based on the comparison of experimental mass spectra with those of the Wiley 7 and Essential Oils mass spectral libraries (Wiley, New York, NY). The identifications were then further confirmed by linear retention indices (RI) calculated using an *n*-alkane mixture (C9–C30),[26] which were compared to those reported in Adams' database[27] and Nist WebBook.[28]

TraceFinder™ 4.1. (Thermo Scientific) was used to carry out peak detection and integration by using an extracted ion for each detected compound (Table 1). Areas obtained were then normalized using *n*-nonane from the internal standard mixture to correct any possible analytical deviation produced caused by variations in the performance of fiber and instrumentation. Normalized area values were further used for statistical and multivariate data analysis. On the other hand, neryl acetate was used to check repeatability and response of *n*-nonane in consecutive analyses. The results showed a relatively constant ratio between *n*-nonane and neryl acetate with a relative standard deviation (% RSD) of 13% when calculating inter-day repeatability (n = 12).

Quantitation of main volatiles in the 2017 samples was carried out by response factors (RF) (Supplementary Table 1), which were calculated by spiking the individual standard reference compounds together with the internal standards, at the same concentration. To simulate real blackcurrant samples, a synthetic blackcurrant juice with no initial content of volatiles was prepared following the composition detailed elsewhere (2.4 g glucose, 3.2 g fructose, 0.6 g sucrose, 2.35 g citric acid, 2.0 g cellulose, and 1.7 g pectin in 100 mL of water).[25] This was used to calculate the response factors of commercial standard volatiles respective to the internal standard in a volatile-free matrix.

Meteorological data.

Meteorological data from meteorological stations in Piikkiö, Kaarina (latitude 60° 23' N, longitude 22° 33' E, altitude 6 m) and Rovaniemi Airport (66° 33' N, 25° 50' E, altitude 195 m) from 2010 to 2017 were provided by the Finnish Meteorological Institute (Helsinki, Finland). Data provided included the following weather parameters: daily values of maximum, minimum and average temperature (°C), precipitation (mm), relative humidity (%), and global radiation ($kJ\cdot m^2$). The weather variables and the corresponding abbreviations used in this study are shown in Table 2. Complete weather data can be found in Supplementary Table 2.

Statistical analyses.

Univariate analyses were carried out by using SPSS 16.0.1 (SPSS Inc., Chicago, IL). Differences between groups were assessed with one-way analysis of variance (ANOVA) in normal distributed variables and Tukey’s HSD test or Kruskal–Wallis test with multiple comparisons for non-parametric variables. Statistical significance was set at $p < 0.01$. For comparisons between samples grown at two latitudes, t-test (or Mann–Whitney for non-parametric variables) at a confidence interval of 99% were considered as being statistically different.

Multivariate analyses were performed by using SIMCA-P$^+$ version 15.0 software package (Umetrics, Umeå, Sweden). The datasets were scaled (unit variance (UV) or Pareto) prior to multivariate analysis by principal component analysis (PCA) or partial least square discriminant analysis (PLS-DA). PCA is an unsupervised technique that reduces the dimensionality of the data set but retains the maximum amount of variability.[29] PLS-DA is a supervised method that focuses on class separation. The VIP (Variable Influence on Projection) values indicate the major compounds contributing to the separation of each sample in PLS-DA scores plots. The VIP value is a weighted sum of squares of the PLS-DA weights that takes the explained *Y* variance in each dimension into account.[30] The PLS-DA models were validated with permutation tests.

RESULTS AND DISCUSSION

HS-SPME-GC-MS analyses of volatile profiles.

HS-SPME conditions were optimized to achieve optimum analytical performance. In this regard, sample amount (0.5–4 g), pre-equilibrium time (5–20 min), extraction time (20–50 min) and temperature (35–60 °C), and desorption time (1–3 min) were assessed (data not shown) as done in a previous work.[31] Optimum HS-SPME conditions were selected on the basis of the total area of detected volatiles leading to a 2 g of sample amount, 10 min pre-equilibrium, 30 min extraction time, 45 °C extraction temperature, and a desorption time of 3 min.

Volatile composition of berries of blackcurrant cultivars was determined by sampling the compounds on a 2-cm CAR/PDMS/DVB fiber followed by GC-MS analysis. The chromatographic profiles obtained from berries of all the three blackcurrant cultivars picked in 2017 in Piikkiö (S) and Apukka (N) are shown as an example in Supplementary Figure 1. In total, 41 compounds were detected and quantified in the samples. A list of the detected compounds and the basis for the identification are given in Table 1. The relative proportions of the 41 detected compounds in berries of the three cultivars grown in the southern and northern locations for all the study years, are listed in the Supplementary Table 3.

Initial inspection of the volatile headspace composition revealed terpenoids clearly dominating the chromatographic profile. Monoterpenoids were the most abundant compounds. Non-oxygenated monoterpenes accounted for 19 compounds, and the oxygenated ones for 15 compounds, although the relative abundance of the latter group was much lower than the former. The so-called oxygenated monoterpenes included several volatiles not previously detected in black currant samples such as campholenal, *p*-cymen-9-ol, cumaldehyde, and two degradation products of the α-pinene degradation pathway, namely pinocarvone and myrtenol. This quantitative difference was significantly reinforced by the higher distribution of the hydrophobic monoterpene hydrocarbons in the gas phase compared to the oxygenated counterparts. The only sesquiterpenes found in the headspace, existing in each of the blackcurrant samples analyzed, were α- and β-caryophyllene. This does, however, not exclude the commonly known presence of other sesquiterpenes in blackcurrant berries.

Regarding the non-terpenoid compounds, four esters, two aldehydes (hexanal and nonanal), and one alkane (undecane) were detected. The compositional differences among the samples highlighted the different abundance of volatile compounds rather than the presence of different compounds. These results are in agreement with other studies in which frozen blackcurrant berries were analyzed and stated that proportions of terpenes are not significantly affected by freezing at −20 °C from picking until analysis.[14] It has been reported that terpenes are the most representative group of compounds in the volatile profile of blackcurrant berries.[16] Terpenoids are reported to be reliable indicators of the fruit freshness, maturity, botanical and geographical origin as well as quality and authenticity.[32] On the other hand, a recently published study by Jung et al.[12] reported a high abundance of C_6-compounds and esters in blackcurrant samples grown in southern Germany and Austria when samples were freshly analyzed and a trend to decrease in favor of terpenoids upon storage at −20 °C for three months.[12] This might explain the low number of aldehydes detected in our samples as can be seen in Table 1. However, it needs to be taken into consideration that the presence and abundance of these compounds may also be significantly dependent on the cultivar, the growth site, and the stage of ripeness. It is important to notice that the storage of several years may have affected significantly the composition of the volatiles. However, all the berries of same age were treated the same way, which makes the statistical comparison relevant.

Comparison of volatile profiles of 'Mortti', 'Ola', and 'Melalahti' cultivars.

The whole data set of profiles obtained was submitted to principal component analysis (PCA) to explore possible compositional differences between the three Finnish cultivars under study. The data set was mean-centered, Pareto-scaled, and standardized with the standard deviation. The PCA model showed excellent goodness-of-fit ($R^2X_{(\mathrm{cum})} = 0.90$) and predictive ability ($Q^2_{(\mathrm{cum})} = 0.85$). The scores plot of PC1 and PC2 in Figure 1A shows a clear separation between samples of the 'Melalahti' cultivar and those of 'Mortti' and 'Ola', the latter two being grouped together in the plot. A similar phenomenon was already described by Zheng et al. when analyzing the phenolic compounds, acids and sugars of the same cultivars.[7] That previous work pointed out that the phenolic composition did not differ significantly between the cultivars 'Mortti' and 'Ola', whereas 'Melalahti' presented significantly lower content of phenolics. The loading plot (Figure 1B) indicates that the cultivars ′Mortti′ and 'Ola' were richer in volatiles compared with the cultivar 'Melalahti'. In addition, multiple comparisons were carried out by means of ANOVA and Tukey's HSD test, or by Kruskal–Wallis test when variables showed no normal distribution. The obtained results revealed that most of the compounds showed statistically different abundance ($p < 0.01$) between 'Melalahti' and 'Mortti', being in most cases higher for ′Mortti′, with the only exceptions of ethyl butanoate, α-thujene and γ-terpinene. Oppositely, the abundances of a few compounds were not found to be statistically different ($p > 0.01$): hexanal and nonanal (i.e. autoxidation products of linoleic acid and oleic acid), undecane, ethyl isovalerate, eucalyptol, verbenene, and α-terpinene. Similar results were observed when comparing 'Melalahti' and 'Ola' with the only addition of methyl 2-methylbutanoate to the group of compounds not significantly differing ($p > 0.01$) in abundance between the cultivars. On the other hand, no statistically significant differences were found when comparing 'Ola' and ′Mortti′ cultivars.

Another trend observed in the PCA scores plot (Figure 1A) was the influence of the storage time on the volatile composition. In this regard, the samples of 2017, although analyzed after frozen storage, presented a higher content of volatiles compared with the samples collected in the previous years. The impact of freezing on the volatile composition of blackcurrant was shown by Jung et al. to be especially remarkable during the first 3-months of storage, whereas the composition kept close to constant from this point onwards.[12] Figure 2 depicts the total volatile content in regards of storage time, i.e. total volatiles (Figure 2A), hydrocarbons (Figure 2B), and oxygenated monoterpenes (Figure 2C). This effect is more apparent in ′Mortti′ and ′Ola′ than in ′Melalahti′. In addition, berries grown in the North present this effect in a higher extend. Compared with monoterpene hydrocarbons, the relative changes in oxygenated monoterpenes were less and more random, evidently due to their lower volatility and lower permeability through the cuticular membrane. This gradual decrease of volatiles during storage is one of the sources of deviation when calculating the effects of weather conditions which makes the differences between samples smaller. The present results and those released by Zheng et al.[7] showed the compositional similarities of 'Ola' and 'Mortti'. Both of them have 'Wellington XXX' background.

The weight of individual berries was not measured in this research. In the earlier studies, however, the berry weight of the studied cultivars have been shown to be rather alike: Lehmushovi[24] reported the berry weight of ‘Ola’ to be only slightly lower than that of the standard cultivar ‘Öjebyn’, whereas Mattila et al.[33] did not find difference between the average berry weight of the cultivars ‘Öjebyn’, ‘Mortti’ and ‘Melalahti’.

Regarding the comparison of southern and northern samples, it was not feasible to draw any conclusions from the PCA plot as samples were not separated on this basis in the scatter plot. For this purpose a supervised multivariate technique such as PLS-DA was of utmost importance.

Effect of growth latitude on volatiles composition.

The qualitative composition of volatiles was found to be the same in berries from the northern (Apukka) and southern (Piikkiö) orchards. A possible explanation to this fact is that the biosynthesis pathways of the volatiles are primarily determined by the genotype while the quantity of these compounds show a certain dependency on the environmental factors intimately linked with the growth location.

PLS-DA was applied to classify samples between different growth latitudes. Three separate models, one for each cultivar, were created to investigate which were the volatile compounds responsible of the compositional differences between southern and northern locations. The PLS-DA scores plot (t[3] vs. t[2]) showed an excellent discrimination between the berries grown in the northern and southern locations for 'Mortti' (Figure 3A) and 'Ola' (Figure 3C) cultivars. For 'Mortti' cultivars model parameters were: $R^2X_{(cum)} = 0.98$, $R^2Y_{(cum)} = 0.91$, and $Q^2_{(cum)} = 0.72$ while for 'Ola' the corresponding parameters were as the following: $R^2X_{(cum)} = 0.97$, $R^2Y_{(cum)} = 0.86$, and $Q^2_{(cum)} = 0.75$. In both cases, the obtained values of $R^2X_{(cum)}$ and $R^2Y_{(cum)}$ represented and excellent goodness-of-fit and $Q^2_{(cum)}$ a high predictive ability. The model was validated with 20 permutations resulting in an R^2Y-intercept of 0.21 and 0.31 and a Q^2Y-intercept of -0.57 and -0.59 for 'Mortti' and 'Ola', respectively. The intercept values of the permutation plots are shown in Supplementary Figure 2. According to Eriksson et al., R^2Y-intercept <0.3–0.4 and Q^2Y-Intercept <0.05 prove model validity.[30] Contrarily, 'Melalahti' showed a less good discrimination between northern and southern samples with: $R^2X_{(cum)}$, $R^2Y_{(cum)}$, and $Q^2_{(cum)}$ of 0.54, 0.37, and 0.28, respectively (Supplementary Figure 3). Hence, it can be stated that composition in 'Melalahti' cultivars was on average weakly affected by the growth location for the years under study. These findings are in accordance with Zheng et al. describing little association of phenolic compounds in 'Melalahti' with the growth latitudes[7]. Regardless of the unfitting in the PLS-DA model for 'Melalahti', when performing univariate tests significant differences were found for some terpenoids i.e. α-pinene, myrcene, α-phellandrene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, γ-terpinene, borneol, and campholenal being the content, in all cases, higher for the samples grown in the northern location with the only exception of campholenal which was found in a higher content in the South. Loading plot (Figure 3B) and variable importance in the projection (VIP) showed that the most important compounds in the PLS-DA model for 'Mortti' resulted from ethyl isovalerate, methyl benzoate, verbenene, β-pinene, eucalyptol, and β-caryophyllene in northern samples and α-terpinene, limonene, and terpinolene in the samples from the southern location. Similarly, the most important variables in the loading plot for 'Ola' were ethyl isovalerate, methyl benzoate, α-pinene, verbenene, β-pinene, eucalyptol, and β-caryophyllene and α-terpinene and terpinolene for the samples grown in the North and the South, respectively (Figure 3D). The similarity of PLS-DA models for 'Mortti' and 'Ola' is in accordance with the results previously obtained in the PCA analysis, where both cultivars were grouped together indicating a similar behavior of these cultivars.

Figure 4 shows the content of most abundant compounds, representing altogether above 94% of the total volatile profile obtained from the headspace. The plotted samples include the samples of all three cultivars harvested from southern and northern Finland in 2017, which is the last year under study. The total content of quantified volatiles in the headspace ranged from 550 $\mu g \cdot kg^{-1}$ fresh weight in southern samples of 'Ola' and 'Mortti' to 1000 $\mu g \cdot kg^{-1}$ in the northern samples, while for 'Melalahti' the values were between 6- and 4-fold lower being 86 and 250 $\mu g \cdot kg^{-1}$, respectively. In addition, it can easily be observed that in all cases the contents of volatiles found in the samples from the North were higher than in the corresponding samples from the South as previously anticipated in the PLS-DA analysis of northern and southern samples of the same cultivar. Regarding the individual compounds, limonene was the most abundant compound in all samples (25–375 $\mu g \cdot kg^{-1}$) followed by δ-3-carene in 'Ola' and 'Mortti' cultivars while for 'Melalahti' the second most abundant compound was γ-terpinene (10–24 μg·kg^{-1}). Δ-3-carene was the least abundant of the quantified compounds in 'Melalahti' with a content below 1 μg·kg^{-1}. Plots for 'Mortti' and 'Ola' cultivars for 2017 show a high similarity in regards not only of the qualitative composition but also the quantitative results. Oppositely, 'Melalahti' showed a higher abundance of α-terpinene, eucalyptol, and γ-terpinene.

Effect of meteorological variables on volatile composition.

With the aim of assessing the response of blackcurrant berries to weather conditions a PLS-DA model was constructed using 'Mortti' and 'Ola' cultivars, which already showed a similar behavior and differentiation upon change of growth location. The variables included in the model together with the abbreviations used are shown in Table 2. Before constructing the model data was submitted to UV scaling. The PLS-DA model yielded $R^2X_{(\mathrm{cum})}$, $R^2Y_{(\mathrm{cum})}$, and $Q^2_{(\mathrm{cum})}$ of 0.77, 0.96, and 0.93, respectively; representing good values in regards of its goodness-of-fit and prediction ability. The model was validated with 20 permutations giving a R^2Y-intercept of 0.18 and Q^2Y-Intercept of −0.59, as shown in Supplementary Figure 3. In addition to the PLS-DA model shown, a PCA model constructed using the same variables and samples can be found in Supplementary Figure 4. This model reinforces the results discussed in the current section.

The PLS-DA bi-plot (Figure 5), showing simultaneously the scores and the correlation coefficients, presents clear separation between samples from the North and the samples from the South when meteorological variables were added to the dataset. In this regard, the variables that most influenced the separation between northern and southern cultivars were the ones associated with temperature, especially from the last month before harvest. As shown in Figure

5B as an expansion of the circled area in Figure 5A, these weather variables included the following variables during the last month before harvest: highest daily average temperature, lowest daily average temperature, highest temperature, and temperature sum. Also the average temperature in the last week before harvest is among the important temperature variables separating the samples of the North from those of the South. Radiation, especially the total radiation from the last month and week until harvest, also played an important role in the discrimination between southern and northern cultivars of 'Mortti' and 'Ola'. These meteorological variables were positively associated with the first component which is the main responsible for the separation between northern and southern samples. Compared with the northern location, radiation and temperature are generally higher in the southern location during the growth season and during the month and week before harvest. Hence, low temperature and radiation values during the last month before harvest could be linked with a higher abundance of volatile compounds in these blackcurrant cultivars. The effects of radiation on the volatiles in fruits are still to be further studied in detail as a report from Xu et al. revealed that pre-harvest radiation of strawberry with UV-C had no significant impact on the volatile on the volatile composition, while the content of sugars and ascorbic acid were increased.[20] On the other hand, Severo et al. stated that UV-C promoted increase in total polyphenolic and volatile organic content, mostly in proanthocyanidins, anthocyanins and esters in external tissues.[34] The difference in light quality between the South and the North may also have played a role in the difference in the abundance of volatiles observed in this study, although we did not investigate the impact of these factors in detail. Our previous research with the same blackcurrant cultivars used in the present study has shown a positive impact of temperature and radiation variables, i.e. the southern location, on phenolic compounds reporting higher contents of dephinidin-3-O-glucoside, delphinidin-3-O-rutenoside, and mirycetin-3-O-glucoside[22] and on sugars, acids, and ascorbic acid.[7] Meteorological variables related with precipitation had a less remarkable effect on the separation of northern and southern cultivars. In this regard, humidity from the start of the growth season until the day of harvest together with the percentage of days with high humidity were higher in the southern cultivars while humidity during the last month and week before harvest were higher in the northern cultivars. In previous research with the same cultivars of the same test fields, we found that the content of sugars and acids were negatively associated with low humidity variables, whereas the content of vitamin C was positively correlated with these variables.[35] However, in Nordic environments water-related variables are not expected to be the limiting factor as the values between the southern and northern location do not differ in a big extend compared with radiation and temperature-related variables which are highly affected by the change of latitude.

Previous studies focused on grape berry samples have reported that precipitation and humidity variables might have a certain effect on carotenoid and terpenoid biosynthesis when they were exposed to a moderate water stress.[36] Moreover, it has been reported by several authors that different abiotic stress factors have an impact on the production of volatiles un plants,[37-39] although the exact signaling mechanisms for regulation of volatile emission by the environmental or physiological factors still await further examination.[40] In addition, existing reports on fruits state that the effects of environmental conditions vary among compounds[18] thus making it difficult to draw conclusions on how the environment affects individual volatile compounds. These findings, together with the results of the current research, indicate that the regulating role of environmental variables may vary depending on the biosynthetic and metabolic pathways in plants.

Considering volatile compounds, as already pointed out in the previous section, they affected the negative side of the first component i.e. higher contents of volatiles were found in the cultivars from the northern location. Moreover, they also presented a certain scattering along the second component, meaning that the volatile composition may also vary depending on the year of harvest which implies other variables in addition to those related with weather.

This study demonstrated for the first time that northern latitudes may increase the content of volatile compounds in blackcurrant berries. The fact that this study was carried out in an 8-year time period adds robustness to the obtained results as it is evident that volatile composition of blackcurrants can suffer important year-to-year variations. According to the reported results, this variations would be more related to the abundance of volatiles rather than their qualitative composition for the cultivars and locations reported here as year-to-year compounds present in the samples remained constant. The meteorological variables studied included a selection of the most remarkable among them. Here, we were able to link the most decisive weather variables in the differentiation of the blackcurrant cultivars. In this regard, temperature and radiation which are constantly lower in the norther location played a key role. In this work we focused on the variations of the volatiles in a long time period. However, it remained outside of the scope the relationship of both Nordic locations with the aroma composition of such blackcurrant cultivars. For this purpose, further studies are needed and justified to investigate the significance and the influence on the sensory properties of these berries.

SUPPORTING INFORMATION

Table S1: Response factors used for quantification of 2017 samples; **Table S2:** Weather variables employed in the study; **Figure S1:** HS-SPME-GC-MS chromatograms (TIC) obtained from 2017 samples; **Figure S2:** The validation test plots of 20 permutations for the PLS-DA models; **Figure S3:** PLS-DA of 'Melalahti' cultivar; **Figure S4:** PCA of 'Mortti' and 'Ola' cultivars including weather variables.

ACKNOWLEDGMENT

The research staff, especially Professor Emeritus Risto Tahvonen, M.Sc. Kati Hoppula, research technicians Alpo Heinonen, Jorma Hellsten and Kaisa Soppela, laboratorian Sari Välitalo and trainee Juho Haveri-Heikkilä at Natural Resources Institute Finland (Luke) are warmly acknowledged for providing the samples from the test fields in Apukka and Piikkiö.

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Table 1: Volatiles detected by HS-SPME-GC-MS profiling of blackcurrant samples.

Peak num.	Compound	Code	RI_{Cal}[a]	RI_{Lit}[b]	Quantification ion (*m/z*)	Identification criteria
Esters						
1	methyl 2-methylbutanoate	E1	793	780	88	MS, RI
3	ethyl butanoate	E2	808	805	88	MS, RI
4	ethyl isovalerate	E3	860	858	88	MS, RI
26	methyl benzoate	E4	1102	1102	105	STD
Aldehydes						
2	hexanal	Ad1	804	800	82	STD
27	nonanal	Ad2	1108	1102	98	STD
Alkanes						
25	undecane	H1	1100	1100	156	STD
Internal standards						
5	*n*-nonane	IS1	900	900	128	
42	neryl acetate	IS2	1369	1362	121	
Non-oxygenated monoterpenes						
6	α-thujene	MT1	926	930	93	MS, RI
7	α-pinene	MT2	931	939	93	STD
8	camphene	MT3	946	953	93	STD
9	verbenene	MT4	970	968	119	MS, RI
10	β-pinene[c]	MT5	975	979	93	STD
11	myrcene	MT6	993	991	93	STD
12	*pseudo*-limonene	MT7	1001	1004	93	MS, RI
13	α-phellandrene	MT8	1006	1003	93	STD
14	δ-3-carene	MT9	1010	1010	93	STD
15	α-terpinene	MT10	1017	1017	121	STD
16	*o*-cymene	MT11	1022	1020	119	MS, RI
17	*p*-cymene	MT12	1025	1026	119	STD
18	limonene	MT13	1028	1029	93	STD
20	*cis*-β-ocimene	MT14	1039	1040	93	STD
21	*trans*-β-ocimene	MT15	1050	1050	93	STD
22	terpenoid (MW=136)	MT16	1057	-	93	MS
23	γ-terpinene	MT17	1064	1060	93	STD
24	terpinolene	MT18	1089	1089	136	STD
Oxygenated monoterpenes						
19	eucalyptol	OMT1	1034	1031	154	STD
28	*cis*-rose oxide	OMT2	1112	1108	139	MS, RI
29	campholenal	OMT3	1132	1126	108	MS, RI
30	borneol	OMT4	1162	1169	95	MS, RI
31	pinocarvone	OMT5	1169	1164	108	MS, RI
32	terpinen-4-ol	OMT6	1183	1182	136	STD

33	*p*-cymen-8-ol	OMT7	1188	1183	135	MS, RI
34	*p*-cymen-9-ol	OMT8	1193	1200	135	MS, RI
35	α-terpineol	OMT9	1198	1192	136	MS, RI
36	myrtenol	OMT10	1200	1196	107	MS, RI
37	cumaldehyde	OMT11	1248	1242	133	MS, RI
38	bornyl acetate	OMT12	1290	1289	136	STD
39	terpinen-4-ol acetate	OMT13	1338	1340	93	MS, RI
40	terpinyl acetate	OMT14	1355	1350	121	STD
41	citronellyl acetate	OMT15	1358	1353	123	MS, RI
Sesquiterpenes						
43	β-caryophyllene	ST1	1422	1419	133	STD
44	α-caryophyllene	ST2	1456	1455	93	MS, RI

[a] Calculated retention index (RI) according to Van den Dool & Kratz equation.[41]

[b] RI from the literature.

[c] In the cultivar 'Melalahti' coelutes with sabinene.

STD: Identification based on comparison of GC and mass spectra with those of reference compounds.

MS, RI: Tentatively identified by comparison of mass spectral and retention index data with those from databases.

Table 2: Weather variables and the corresponding abbreviations used in the study.

abbreviation	weather variable	abbreviation	weather variable
ΣT_{gh}	temperature sum over 5 °C in growth season (°C)	DHu20to30gh	percentage of the days with relative humidity 20−30% from the start of growth season until the day of harvest (%)
ΣT_{mon}	temperature sum over 5 °C last month in growth season (°C)	DHu30to40gh	percentage of the days with relative humidity 30−40% from the start of growth season until the day of harvest (%)
HD_{gh}	hot days (temperature >25 °C) from the start of growth season until the day of harvest (day)	DHu40to50gh	percentage of the days with relative humidity 40−50% from the start of growth season until the day of harvest (%)
HD_{mon}	hot days (temperature >25 °C) in the last month before harvest (day)	DHu50to60gh	percentage of the days with relative humidity 50−60% from the start of growth season until the day of harvest (%)
T_{mon}	average temperature in the last month before harvest	DHu60to70gh	percentage of the days with relative humidity 60−70% from the start of growth season until the day of harvest (%)
T_{w}	average temperature in the last week before harvest (°C)	DHu70to80gh	percentage of the days with relative humidity 70−80% from the start of growth season until the day of harvest (%)
ΔT_{mon}	mean daily temperature difference in the last month	DHu80to90gh	percentage of the days with relative humidity 80−90% from the start of growth season until the day of harvest (%)
$MinT_{mon}$	minimum temperature of the last month	DHu90to100gh	percentage of the days with relative humidity 90−100% from the start of growth season until the day of harvest (%)
LoT_{mon}	lowest daily temperature average last month	DHu<70gh	percentage of the days with relative humidity below 70% from the start of growth season until the day of harvest (%)
$MaxT_{mon}$	highest temperature of last month	DHu>70gh	percentage of the days with relative humidity above 70% from the start of growth season until the day of harvest (%)
HiT_{mon}	highest daily average temperature last month	DHu20to30m	percentage of the days with relative humidity 20−300% in the last month before harvest (%)
ΣR_{gh}	radiation sum from the start of growth season until the day of harvest	DHu30to40m	percentage of the days with relative humidity 30−40% in the last month before harvest (%)
ΣR_{mon}	radiation sum from the last month until the day of harvest	DHu40to50m	percentage of the days with relative humidity 40−50% in the last month before harvest (%)
ΣR_{w}	radiation sum from the last week until the day of harvest	DHu50to60m	percentage of the days with relative humidity 50−60% in the last month before harvest (%)

Pre_{gh}	precipitation sum from the start of growth season until the day of harvest	DHu60to70m	percentage of the days with relative humidity 60−70% in the last month before harvest (%)
Pre_{mon}	precipitation sum from the last month until the day of harvest	DHu70to80m	percentage of the days with relative humidity 70−80% in the last month before harvest (%)
Pre_{W}	precipitation sum from the last week until the day of harvest	DHu80to90m	percentage of the days with relative humidity 80−90% in the last month before harvest (%)
Hu_{gh}	average humidity from the start of growth season until the day of harvest	DHu90to100m	percentage of the days with relative humidity 90−100% in the last month before harvest (%)
Hu_{mon}	average humidity from the last month until harvest		
Hu_{w}	average humidity from the last week until harvest		

FIGURE CAPTIONS

Figure 1. PCA of blackcurrant samples: A) Scores plot of 'Melalahti' (🟦), 'Mortti' (🔴), and 'Ola' (▲); 1,2,3, and 4, sample collected from field block 1,2,3, and 4, respectively; S, southern Finland (Piikkiö); N, northern Finland (Apukka); 10, 11, 12, 13, 14, 16, and 17 represents samples collected in year 2010, 2011, 2012, 2013, 2014, 2016, and 2017, respectively. B) Loadings plot. Compounds coded according to Table 1.

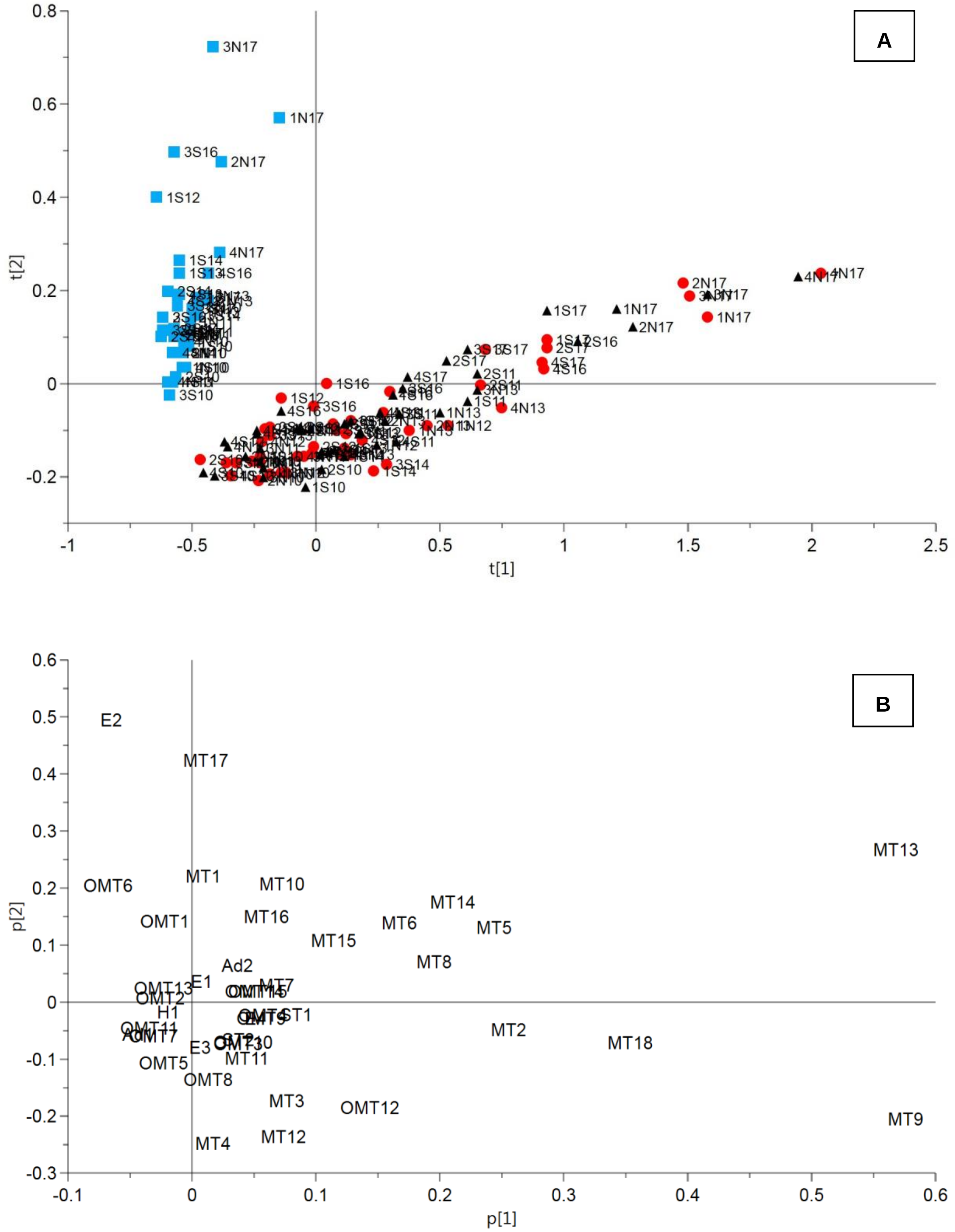
Figure 2: Volatiles content in respect to the storage time. A) Total volatiles; B) Non-oxygenated monoterpenes; C) Oxygenated monoterpenes for 'Melalahti' (N) (🟥), 'Melalahti '(S) (🟦), 'Mortti' (N) (🔴), 'Mortti' (S) (🔵), 'Ola' (N) (🔺), and 'Ola' (S) (▲).

Figure 3: PLS-DA of 'Mortti' and 'Ola' cultivars showing: A) 'Mortti' scores plot; B) 'Mortti' loadings plot C) 'Ola' scores plot; D) 'Ola' loadings plot for samples grown in Apukka (N) (🔴) and Piikkiö (S) (▲).

Figure 4: Composition of 2017 samples expressed as mean μg·kg^{-1} of fresh weight. Error bars indicate standard deviation (n = 3).

Figure 5: A) PLS-DA model biplot of 'Mortti' and 'Ola' samples grown in Apukka (N) (🔴) and Piikkiö (S) (▲). B) Expanded areas circled in Figure 4 A. Compounds coded according to Table 1. Weather variable abbreviations represented according to Table 2.

FIGURE 1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t[1]
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0.2
0
-0.2
-1
-0.5
0
0.5
1
1.5
2
2.5
3N17
1N17
3S16
2N17
1S12
4N17
1S14
1S13
4S16
3S10
2N17
3N17
1N17
4N17
1S17
1N17
2N17
1S17
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4S16
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1S16
3S16
4S16
1S12
3S16
4N13
1S11
1N13
2N13
1N12
1N15
4S11
3S14
1S14
2S10
2N10
1S10
B
p[2]
p[1]
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
E2
MT17
MT13
OMT6
MT1
MT10
MT14
OMT1
MT16
MT6
MT5
MT15
MT8
Ad2
OMT13
E1
MT7
OMT2
OMT15
H1
OMT4
ST1
OMT9
OMT11
MT2
MT18
Ad1
OMT7
E3
ST2
OMT3
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OMT10
OMT5
OMT8
MT3
OMT12
MT9
MT12
MT4

FIGURE 2

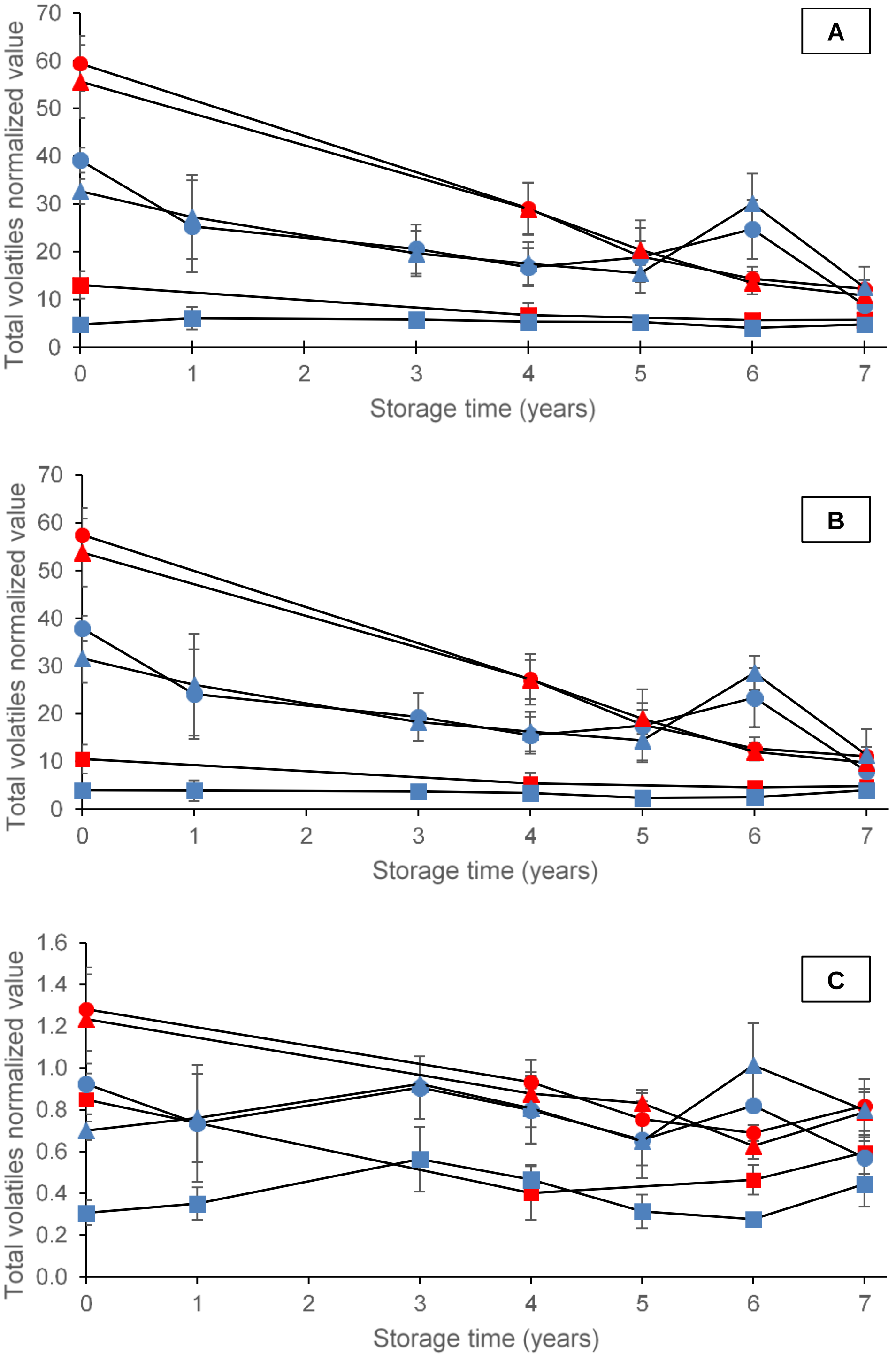
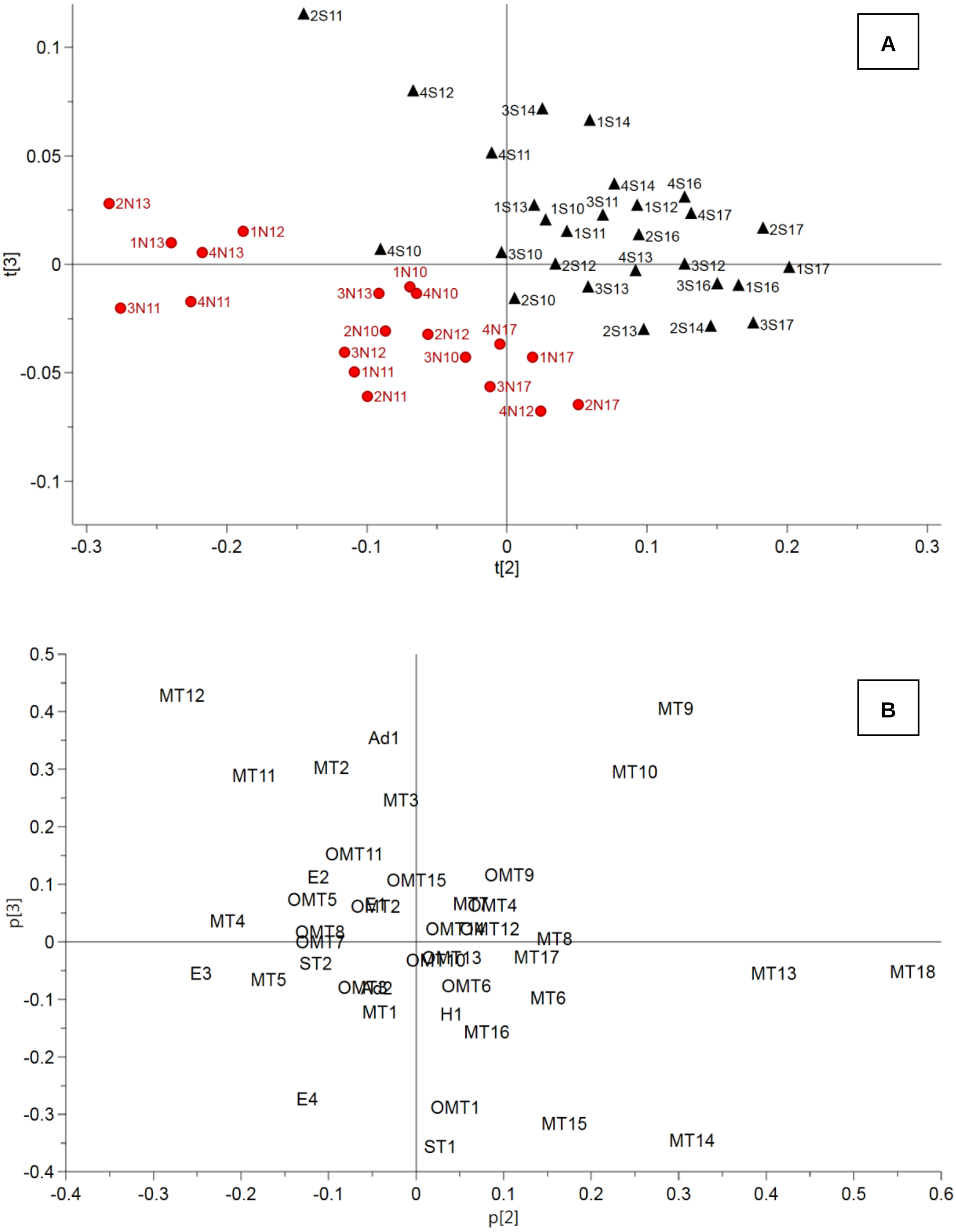

A
B
C
Total volatiles normalized value
Storage time (years)
0
10
20
30
40
50
60
70
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
1
2
3
4
5
6
7

FIGURE 3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2S11
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3N12
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2N17
4N12
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0
-0.05
-0.1
t[3]
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-0.2
-0.1
0
0.1
0.2
0.3
t[2]
B
MT12
MT9
Ad1
MT11
MT2
MT10
MT3
OMT11
E2
OMT15
OMT9
OMT5
OMT2
E1
MT7
OMT4
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OMT14
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MT8
ST2
OMT10
OMT13
MT17
E3
MT5
MT13
MT18
OMT3
Ad2
OMT6
MT6
MT1
H1
MT16
E4
OMT1
MT15
MT14
ST1
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0.4
0.3
0.2
0.1
0
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-0.2
-0.3
-0.4
p[3]
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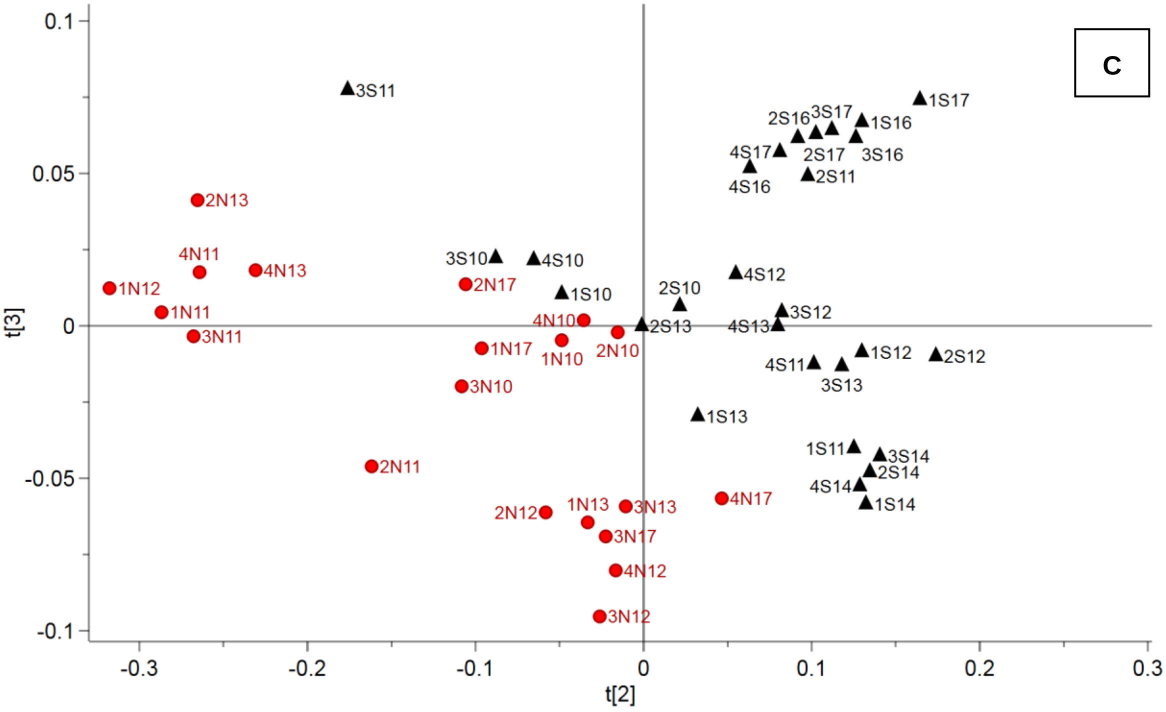
0.6
p[2]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0
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t[3]
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-0.1
0
0.1
0.2
0.3
t[2]
3S11
1S17
2S16
3S17
1S16
4S17
2S17
3S16
4S16
2S11
2N13
4N11
4N13
1N12
1N11
3N11
3S10
4S10
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4S12
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2S13
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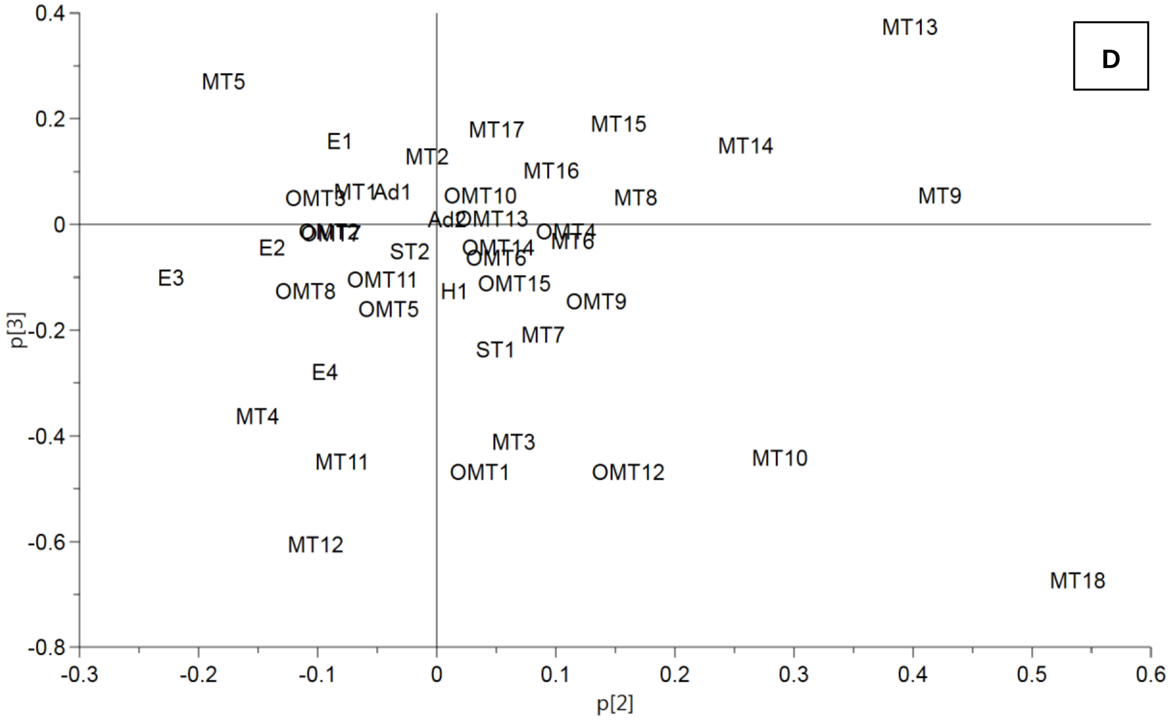
4N12
3N12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0.2
0
-0.2
-0.4
-0.6
-0.8
p[3]
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
p[2]
MT13
MT5
MT17
MT15
E1
MT2
MT14
MT16
OMT3
MT1
Ad1
OMT10
MT8
MT9
Ad2
OMT13
OMT2
OMT7
OMT4
E2
ST2
OMT14
MT6
OMT6
E3
OMT8
OMT11
H1
OMT15
OMT9
OMT5
MT7
ST1
E4
MT4
MT3
MT11
OMT1
OMT12
MT10
MT12
MT18

FIGURE 4

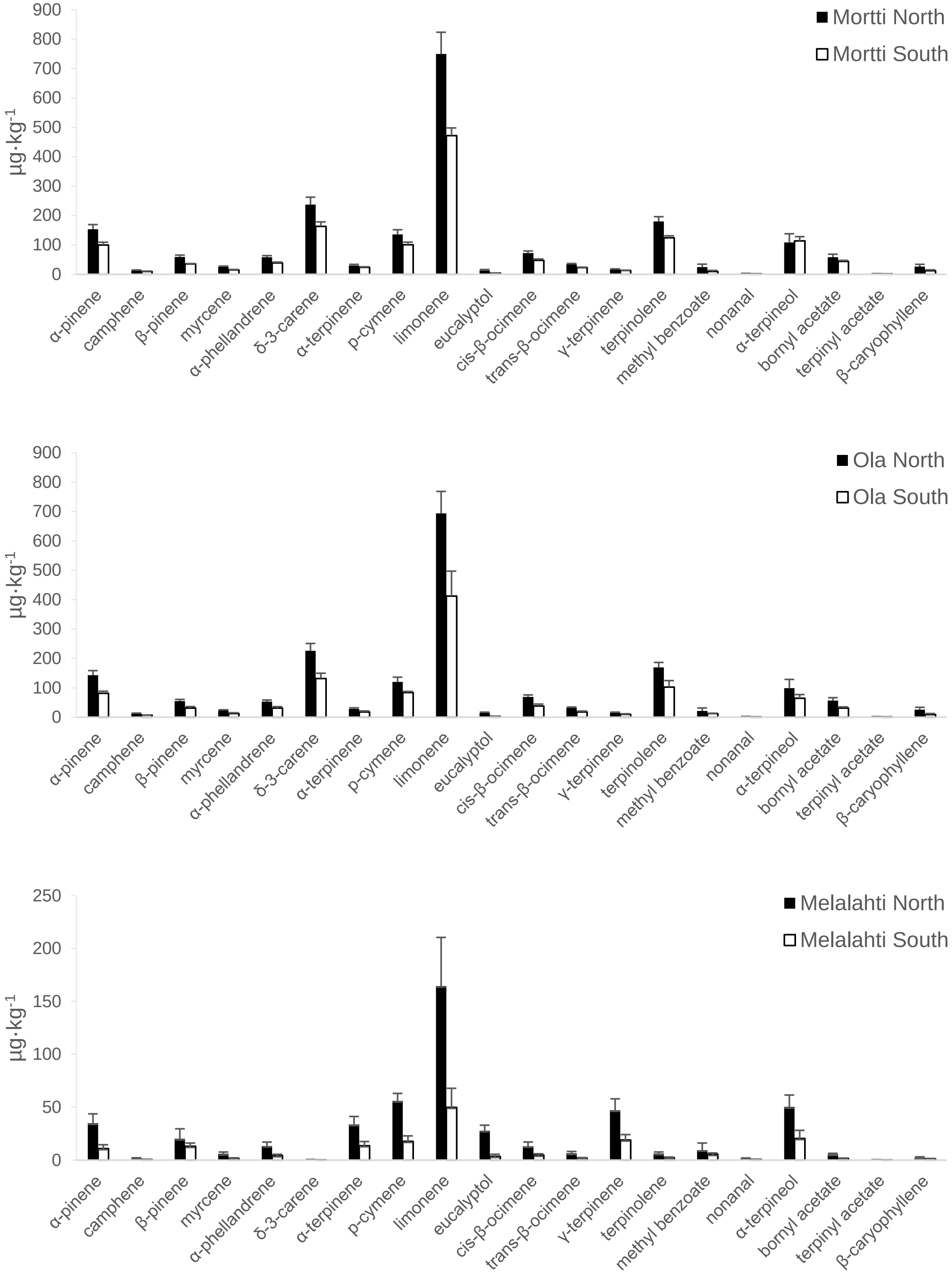
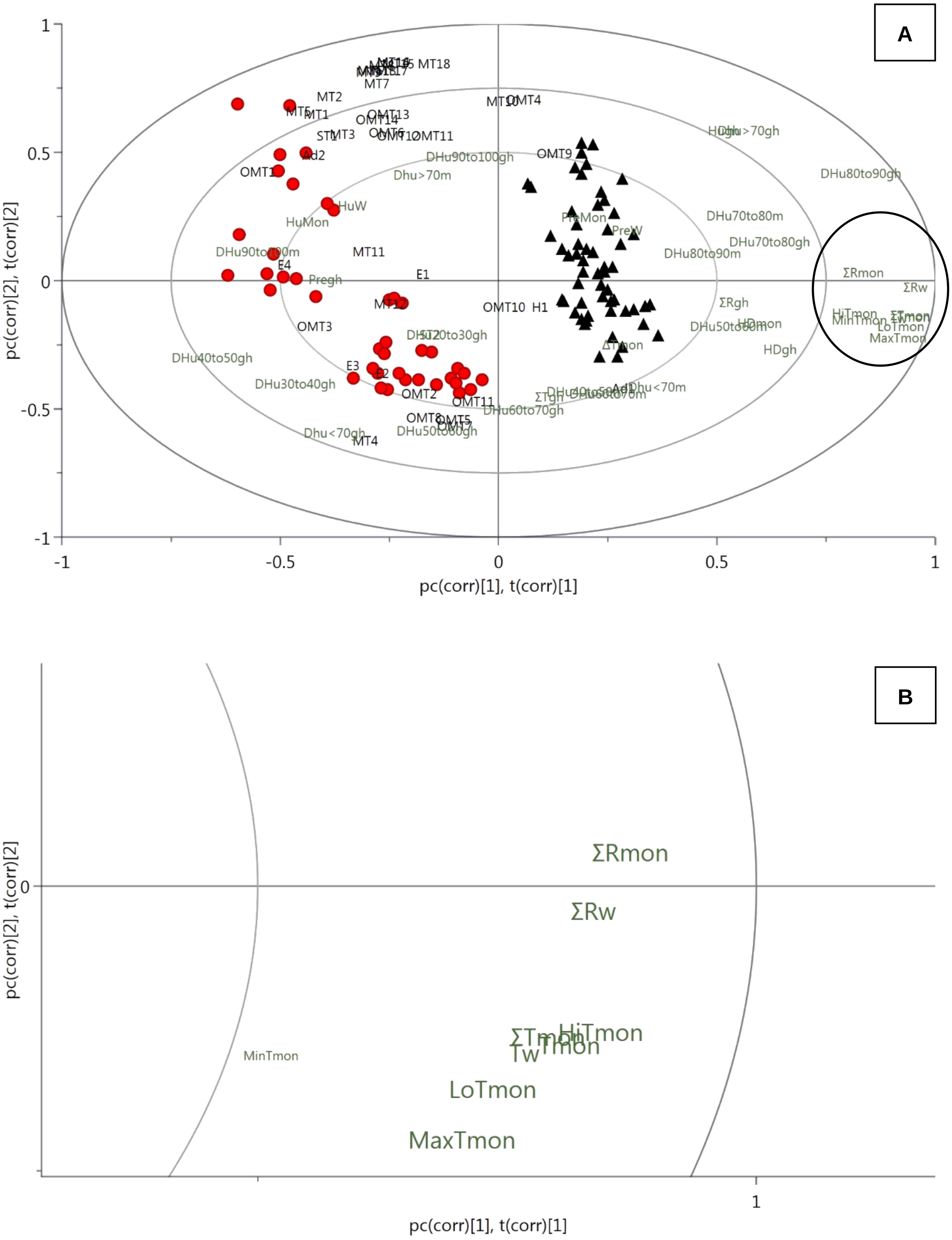

900
800
700
600
500
400
300
200
100
0
µg·kg-1
■ Mortti North
□ Mortti South
α-pinene
camphene
β-pinene
myrcene
α-phellandrene
δ-3-carene
α-terpinene
p-cymene
limonene
eucalyptol
cis-β-ocimene
trans-β-ocimene
γ-terpinene
terpinolene
methyl benzoate
nonanal
α-terpineol
bornyl acetate
terpinyl acetate
β-caryophyllene
900
800
700
600
500
400
300
200
100
0
µg·kg-1
■ Ola North
□ Ola South
α-pinene
camphene
β-pinene
myrcene
α-phellandrene
δ-3-carene
α-terpinene
p-cymene
limonene
eucalyptol
cis-β-ocimene
trans-β-ocimene
γ-terpinene
terpinolene
methyl benzoate
nonanal
α-terpineol
bornyl acetate
terpinyl acetate
β-caryophyllene
250
200
150
100
50
0
µg·kg-1
■ Melalahti North
□ Melalahti South
α-pinene
camphene
β-pinene
myrcene
α-phellandrene
δ-3-carene
α-terpinene
p-cymene
limonene
eucalyptol
cis-β-ocimene
trans-β-ocimene
γ-terpinene
terpinolene
methyl benzoate
nonanal
α-terpineol
bornyl acetate
terpinyl acetate
β-caryophyllene

FIGURE 5


A
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
pc(corr)[2], t(corr)[2]
pc(corr)[1], t(corr)[1]
MT18
MT7
MT2
MT5
MT1
OMT13
OMT14
ST1
MT3
OMT6
OMT12
OMT11
MT10
OMT4
Ad2
OMT1
HuW
HuMon
DHu90to100m
E4
MT11
E1
Pregh
MT12
OMT10
H1
OMT3
DHu20to30gh
DHu40to50gh
E3
E2
DHu30to40gh
OMT2
OMT11
OMT8
OMT5
OMT7
DHu60to70gh
DHu50to60gh
Dhu<70gh
MT4
DHu90to100gh
Dhu>70m
OMT9
PreMon
PreW
Dhu>70gh
DHu80to90gh
DHu70to80m
DHu70to80gh
DHu80to90m
ΣRmon
ΣRw
ΣRgh
HiTmon
MinTmon
ΣTmon
Twmon
LoTmon
MaxTmon
HDmon
DHu50to60m
HDgh
ΔTmon
Ad1
Dhu<70m
ΣTgh
DHu40to50m
DHu60to70m
B
ΣRmon
ΣRw
ΣTmon
HiTmon
Twmon
MinTmon
LoTmon
MaxTmon
0
1
pc(corr)[2], t(corr)[2]
pc(corr)[1], t(corr)[1]

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