Article

Volatile Organic Compounds (VOCs) in Mediterranean Oak Forests of Hungarian Oak (*Quercus frainetto* Ten) Affected by Dieback Phenomena

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Abstract: In recent years, long periods of drought and heat waves have become increasingly frequent, causing forest dieback phenomena that make stands more sensitive to biotic stressors. How trees may respond to extreme climatic events and which metabolites are involved under stress conditions is still not clear. In this study, using Solid Phase Micro-Extraction (SPME)-GC/MS, we analysed how dieback (D) and non-dieback (ND) Hungarian oak trees from the San Paolo Albanese site respond to these climatic dynamics, focusing on volatile organic compounds (VOCs). For each group of trees, three wood samples were taken, and each was divided into four sub-samples with five growth rings and subjected to SPME and increase in basal area (BAI) analysis of the last 20 years. Dieback trees had a lower number of leaves, and this condition may translate into less photosynthesis, less organic matter production, and lower reserves of carbohydrates being available for growth. Indeed, D trees showed lower radial increases and a lower content of aldehydes, terpenes, and fatty acids than ND trees, indicating a better health of ND trees compared to D trees. Meanwhile, D trees showed a reduction in terpenes, such as α-pinene, γ-eudesmol, and cyperene (with significant insecticidal activity), a reduction in aromatic aldehydes, such as furfural and 5-methylfurfural, and an increase in silanols (with antimicrobial function). Considering the different compounds' contents between D and ND trees, our study could be useful for detecting bio-indicators to identify an early warning signal of dieback phenomena.

Keywords: climate change; drought; forest dieback; Hungarian oak; growth ring; SPME; VOC

1. Introduction

Anthropogenic emissions of greenhouse gases are significantly changing the global climate (IPCC, 2022). The increased frequency of climatic anomalies, such as long periods of drought and heatwaves, cause physiological stress in vegetation, making forests more susceptible to dieback phenomena [1–3].

The increase in forest mortality associated with global climate change is of great concern [4,5]. Forest decline has been attributed to interconnected abiotic and biotic factors. Triggers include extreme weather (drought, temperature extremes, high irradiance), often followed by attacks of defoliating insects, fungi, and pathogens, but air pollution with tropospheric ozone and sulphur dioxide can also affect forest health, as these substances can be phytotoxic, causing injuries to photosynthetic apparatus. In a recent study exploring

the interactive effects of [CO_2], nitrogen supply, and water availability on physiological (gas exchange and chlorophyll fluorescence), morphological, and stoichiometric traits of Norway spruce (*Picea abies*), it was shown that an elevated CO_2 concentration (which is currently increasing every year) could help trees cope with drought conditions and a high nitrogen supply [6]. The response of forests to such phenomena is very complex and can also be affected by local conditions, vegetation type, and general site characteristics (elevation, slope, and exposure) [7]. The combination of drought and excess nitrogen reduces the concentration of allelochemicals in plant leaves [6], which are secondary metabolites produced by plants that can have a defensive function thanks to their repellent and/or toxic action against harmful defoliators and pathogens. These substances include phenolic compounds and their derivatives (e.g., tannins against defoliating or herbivorous insects), whose reduction in the leaves can make trees more susceptible to insect attacks. Thus, biotic and abiotic factors are interconnected: defoliation, favoured by drought and reduced allelochemicals, causes a decrease in leaf area and a consequent reduction in photosynthetic activity and in the supply of carbohydrates to the plants [8,9]. In order to understand the mechanisms that cause carbon depletion in underground structures, some authors have studied the dynamics of metabolites with progressive drought in forest species: tree carbon fluxes revealed that in roots, fructose, glucose, sucrose, non-structural carbohydrates (NSC), starch, and the osmoprotectant proline increased during drought, while in the phloem, there was only an accumulation of sucrose, and in leaves, no accumulation occurred [10–12]. Based on this, it seems that trees that are initially damaged by drought give priority to root assimilates, probably to promote root function after the drought event [10]. The reduction in carbon in the belowground sink is caused by the persistence and frequency of drought conditions over time, like a prolonged stress that does not allow for the storage of additional reserve substances, thus causing the phenomenon of carbon starvation [11–13].

The content of carbon, aquaporins (which facilitate water flow in the roots), glucose, sucrose, and other reserve substances could also be associated with a further response of vegetation to extreme weather, namely xylem filling after embolism. Some forest species die because of embolism, while others tolerate high levels of embolism and subsequently recover their hydraulic function during rehydration [14]. The current knowledge about this physiological response suggests that sugars and ions released into the ducts cause osmotic gradients by channelling the flow of water through parenchyma, phloem, and xylem. However, frequent drought events with embolism phenomena can cause chronic stress and thus an inability to reverse embolism [14]. These processes are still not very clear and require further studies to better understand how plants can respond to harsh conditions and which substances/metabolites are involved in forest resilience or recovery after periods of stress.

Therefore, in order to have more accurate information on the dynamics that are affecting a dieback forest within the Pollino National Park area (Basilicata Region, Southern Italy), we analysed whether there are differences in volatile organic compounds (VOC) and plant secondary compounds (PSCs) between decaying (D) and non-decaying (ND) trees, linked to environmental and climatic stresses, in particular to severe drought conditions. Several papers about this forest have already been published in recent years by the same authors, proving that trees died because of severe drought stress occurred during the beginning of the 2000s [15–17]. We evaluated the differences in VOCs and PSCs by using for the first time Solid Phase Micro-Extraction (SPME) on wood rings in order to evaluate the effects of climate change on the last 20 years of tree growth.

We hypothesize that declining trees, due to extreme drought conditions occurring during the last two decades [15,16], led to considerable variations in VOCs and PSCs that have made the trees more susceptible to the actions of pests. Here, we assessed the presence or absence of metabolites (e.g., phenolic compounds and their derivatives) that are useful for warding off biotic attacks within growth rings or the presence or absence of substances that are useful for enabling the vegetation to cope with stress conditions. Furthermore,

by observing variations in these substances, this study could be useful in identifying a signal of dieback phenomena and guide us towards a deeper analysis of forest dynamics in response to climate change.

2. Materials and Methods

2.1. Study Site

This study was carried out in a *Q. frainetto* Ten. high forest that manifested dieback phenomena. The stand was an even-aged high forest with an average breast height diameter of 40 cm and a mean age of about 145 years [17]. This stand is located in the Pollino National Park in the municipality of San Paolo Albanese within the Basilicata region in southern Italy (40°1′16.90″ N; 16°20′27.57″ E), with an elevation of 1030 m a.s.l. and an average slope of 25%–30%. According to Pavari's phyto-climatic classification, the forest belongs to the *Castanetum* belt, which is typical of mesophilic oak woods. The site has a sandy–clayey soil and a Mediterranean climate, with hot, dry summers and wet, mild winters; the average annual temperature is 16.4 °C, and the annual rainfall is 742 mm. The *Q. frainetto* stand has shown symptoms of dieback since the early 2000s (shoot desiccation, significant growth decline, high mortality rate), with 80% of the trees showing crown transparency levels > 50% [17,18].

2.2. Field Sampling and Dendrochronological Data

At the analysed site, two groups of trees were identified through visual assessment (Figure S1): decaying plants (hereafter, D trees) with crown transparency >50% and non-decaying plants (hereafter, ND trees) with crown transparency <50% [17,18]. For each group (D and ND), three plants were sampled by extracting wood cores at breast height (1.30 m) using a 1 cm Pressler drill, and the diameter of each plant was measured using a tree caliper. The samples taken were coded and stored in a refrigerator at 4 °C in order to avoid alterations that could affect subsequent analyses. To assess the growth increments of the woody rings, we used dendrochronology [19]. Each sample was set up on special supports and smoothed so that the annual growth rings were visible [20] (Figure S2). Subsequently, the tree rings were visually crossed under a binocular microscope [19].

The analysis involved the study of the last 20 years of growth by using a dendro-cronograph (*LINTAB6*, Rinntech). Each growth ring was measured with an accuracy of 1/1000 mm using the TSAPWin Scientific version 4.81 (Rinntech, Heidelberg, Germany). Using the measured tree ring widths (TRWs), growth histories from 2002 to 2021 were obtained for each tree group (D and ND). These chronologies were used to calculate the basal area increment (BAI) by applying the following formula:

$$BAI_n = (d^2_n \times \pi)/4 - (d^2_{n-1} \times \pi)/4 = (d^2_n - d^2_{n-1}) \times \pi/4,$$

$$BAI_{n-1} = (d^2_{n-1} - d^2_{n-2}) \times \pi/4,$$

where d^2_n is the diameter squared of the year n, while d^2_{n-1} is the diameter squared of the previous year n − 1, and so on $(d^2_{n-1} - d^2_{n-2})$.

The calculation of the BAI was performed without detrending the radial increments, as the analysed trees were adults, with an age of approximately 145 years [17] and therefore had a fairly parallel growth to the *x*-axis, which was not influenced by age trends [21]. Using the raw data, we stayed as close as possible to the source data and thus made fewer assumptions. Calculating the BAI relative to the TRW allowed us to reduce the geometric effect of radial growth increments of cores taken at 1.30 m [22]. The average BAI was calculated for each ND and D tree class, and the average BAI increment was obtained for each analysed period.

2.3. Sample Preparation

The last 20 rings (20 years, from 2021 to 2002) of each sample were divided into 4 sections of 5 growth rings per each. For each sample, 4 Eppendorf tubes were used, each

containing 5 growth rings, i.e., 2002/2006–2007/2011–2012/2016–2017/2021. Thus, a total of 24 sections were obtained from the 6 samples analysed (3 D and 3 ND), and their wood material was placed in sterile Eppendorf tubes. The samples were then dried at 50 °C overnight, and each sample had 5 mL of distilled water added to it and was analysed by SPME-GC/MS [23].

2.4. Statistical Analyses

With the aid of the SPSS software version 11.0 (SPSS, Chicago, IL, USA), analysis of variance and comparison of treatment means (one-way ANOVA) were conducted in order to investigate the impact of trees (D and ND) on the peak area of various volatile chemicals detected in the cores of oak trees. All series of data sets (D and ND) were statistically analysed to evaluate the significance of the results.

2.5. Solid Phase Micro-Extraction–GC/MS Analyses

Solid Phase Micro-Extraction (SPME)–GC/MS was used to determine the VOCs' chemical composition in the 24 samples. An aliquot of 200 mg of sample was placed in vials and incubated at 50 °C for 15 min to promote volatile compounds in the headspace. Extractions were achieved by immersing the fibre in the headspace at 36 °C for 30 min and then withdrawing it into the needle and transferring it to a GC/MS system [24]. A 50/30-μm DVB/CAR/PDMS module (57328-U, Supelco, Milan, Italy) was employed to determine VOCs, conditioned for 1 h at 250 °C in a stream of helium. A blank run was performed after the analysis in order to confirm that no residual compound was polluting the fibre or the column.

The fibre was then introduced into the injection port of an HP 6890 Plus gas chromatograph (Agilent) equipped with a Phenomenex Zebron ZB-5 MS capillary-fused silica column (30 m × 0.25 mm I.D., film thickness 0.25 μm FT coated with non-polar 5% poly(dimethyl siloxane) stationary phase) (Agilent, Milan, Italy). An HP 5973 mass-selective detector (mass range: 15–800 amu; scan rate: 1.9 scans/s) (Agilent) was utilized with helium at 0.8 mL min^{-1} as the carrier gas. Ionization was performed by electronic impact (EI), setting the electron multiplier to 1435 eV. A splitless injector was maintained at 250 °C and the detector at 230 °C. The oven was held at 40 °C for 2 min, then gradually warmed, by 8 °C min^{-1}, up to 250 °C and held for 10 min. The chromatograms obtained from the total ion current (TIC) were integrated without any correction for coelutions, and the identification of components was tentatively based on mass spectra and NIST 11 library comparison. A single VOC peak was considered to be identified when its experimental spectrum matched with the one in the library with a score over 90% and if the retention time was in agreement with the reported retention index [24]. The analyses were performed in triplicate, and the percent area (% ±0.03), proportional to the amount of each identified compound present in the sample mixture, was given. The Kovats retention index (RI) was used to identify the VOCs.

3. Results and Discussion

3.1. Dendrochronological Analysis

The differences between the growth rates of ND and D trees were evident (Figures 1 and S2, Table 1). The BAI of ND plants for each 5-year period analysed showed increases of about 7–8 cm^2. In contrast, in D plants, the increases in BAI over the 20 years analysed were always below 5 cm^2, except for the period of 2007/2011, when the value was almost 5 cm^2. These observations further confirm the difference in the health status of the two groups observed by the visual inspection carried out by Colangelo et al. (2017) (D trees with crown transparency > 50% and ND trees with crown transparency < 50%) [17]. Certainly, the increases in BAI were very low for both groups over the analysed period, but the lower growth rate in D trees compared to ND trees is an unequivocal sign of decay phenomena [15]. Indeed, periods of drought can favour embolism, leading to physiological stress and consequent canopy self-thinning. A very low BAI rate could be attributable to differ-

ences in the amount and number of available crown/leaves between D and ND trees. In fact, dieback trees have a lower number of leaves, and this condition may translate into less photosynthesis, less organic matter production, and lower reserves of carbohydrates being available for their growth [25]. In recent decades, there have been many years characterized by drought and heat waves in the Mediterranean environment [7,17,26], such as 2001, 2008, 2012, 2013, and 2017, which may have eroded the carbohydrate reserves of plants, causing a greater influence of recent climatic conditions on decaying plants [27].
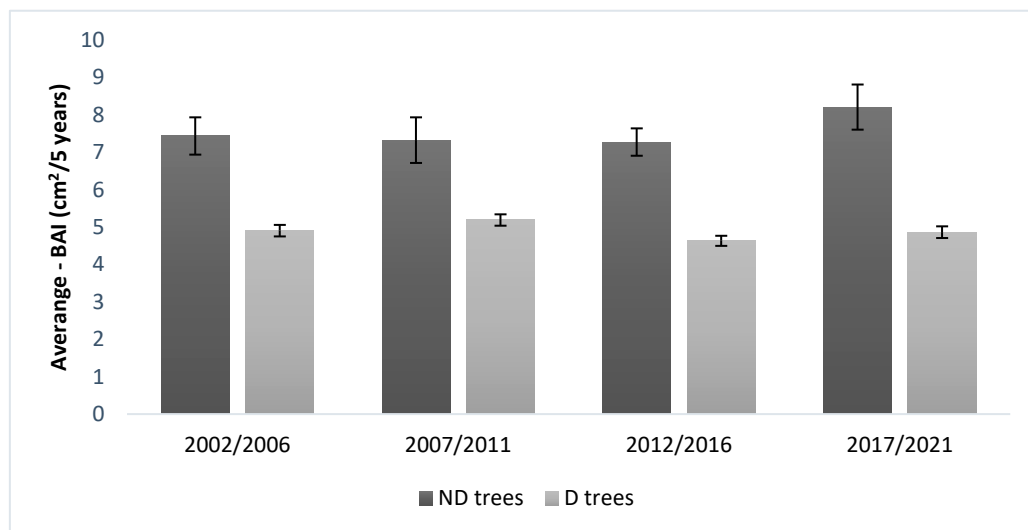
Figure 1. Average of basal area increment (BAI) in (n = 12) dieback (D) and (n = 12) non-dieback (ND) trees over five-year periods (overbars indicate the standard error).

Table 1. Averages of the BAI growth increments (related to Figure 1) for ND trees and D trees. SD indicates the standard deviation and SE the standard error.

Years	Average BAI (cm^2/5 Years) ND Trees	SD	SE	Average BAI (cm^2/5 Years) D Trees	SD	SE
2002/2006	7.44	1.12	0.50	4.90	0.34	0.15
2007/2011	7.33	1.36	0.61	5.19	0.34	0.15
2012/2016	7.27	0.82	0.36	4.63	0.30	0.14
2017/2021	8.21	1.35	0.60	4.87	0.35	0.16

3.2. SPME-GC/MS Analysis

Plant secondary compounds (PSCs) have a high chemical and structural diversity and include non-volatile or volatile organic compounds. These compounds have specific physiological and ecological functions in plants' adaptation [28,29]. PSCs can help trees to adapt to climate change and to the attacks of invasive insects and pathogens. In higher plants, terpenoids (30,000), alkaloids (21,000), and phenolic compounds (8000) are the most diverse PSC groups [30–32]. Table 2 shows the total percentage areas of VOCs, both for decaying (1D, 2D, 3D) and non-decaying (1ND, 2ND, 3ND) wood material in the four five-year periods of 2002/2006–2007/2011–2012/2016–2017/2021. The compounds were separated into different classes of organic compounds to better evaluate the variations: acids, aldehydes, alkanes, nitrogen compounds, aromatic compounds, fatty acids (FAs), ketones, silanols, and terpenes.

As shown in Figure 2 (Table 3), over the years, a notable reduction in aliphatic and aromatic aldehydes, alkanes, aromatic compounds, terpenes, and fatty acids was observed in D plants compared to ND plants, while the same plants showed an increase in acids, nitrogen compounds, ketones, and silanols.

Table 2. Kovats retention index (RI) and relative peak area (% ±0.03) of main compounds found in SPME analysis of dieback (D) and non-dieback (ND) *Q. frainetto* Ten. trees every 5 years, for the past 20 years (I: 2002–2006; II: 2007–2011; III: 2012–2016; IV: 2017–2021).

Class of Compounds	Compound	RI	1D				2D				3D			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
Acids	Acetic acid	655	2.75	1.35	1.37	1.27	1.37	1.83	4.15	3.42	2.83	1.35	1.38	1.28
Aromatic Aldehydes	Vanillin	1407	6.88		0.92	10.95	2.95			18.90	6.53		2.68	8.08
	Syringaldehyde	1662	4.15	3.15	2.05	1.90	3.95	4.00	2.60	2.43	1.90	1.82	1.72	1.63
	Coniferaldehyde	1741												
	Sinapaldehyde	1909		1.27			4.17						1.42	
Aliphatic Aldehydes	3-methylbutanal	659												
	Heptanal	1176												
	Decanal	1182												
	Octanal	1282												
	Nonanal	1387												
	Furfural	1462												
Alkanes	Eicosane	2000												
Nitrogen Compounds	Diethyltoluamide	1571		6.30	4.75	4.48			20.70	20.10	6.52		11.58	11.38
	N-*p*-bromophenylselenoacetamide	1724						1.05						
	Dimethyl palmitamine	1894												
Aromatics	Xylene	1137												
	1,1,3-trimethyl-3-phenyl-2H-indene	1714												
	Methyl n-hydroxybenzimidate	1814	1.05	0.83			2.45	1.27			1.35	2.88	4.70	
	2,6-dimethoxyphenol	2269			2.05		3.25							
	2,6-di-tert-butylphenol	2327												
Fatty acids	Nonanoic acid	1265	0.37											
	Pentanoic acid	1685											0.60	
	Tetradecanoic acid	1768												
	Hexadecanoic acid	1968				2.62				2.75			2.15	2.45
	Hexanedioic acid	2137				2.22				2.03				1.98
	Octadecanoic acid	2182	1.55		1.32									
Ketones	4-oxo-β-dihydroionone	1659									12.37		3.75	
	3-oxo-7,8-dihydro-α-ionone	1731			1.52	1.70			5.75	6.35	12.22		2.10	2.35

Table 2. *Cont.*

Class of Compounds	Compound	RI	1D I	1D II	1D III	1D IV	2D I	2D II	2D III	2D IV	3D I	3D II	3D III	3D IV
Silanols	Dimethylsilanediol	649	8.22	9.33	7.43	7.05	3.43	3.72	7.03	5.48	5.50	7.02	7.63	7.28
Terpenes	α-pinene	936												
	Pinocarvone	1138												
	Cyperene	1398												
	α-bergamotene	1407												
	β-panasinsene	1527												
	γ-eudesmol	1632												

Class of Compounds	Compound	RI	1ND I	1ND II	1ND III	1ND IV	2ND I	2ND II	2ND III	2ND IV	3ND I	3ND II	3ND III	3ND IV
Acids	Acetic acid	655												
Aromatic Aldehydes	Vanillin	1407	0.43				11.73	18.20	16.05	13.20	2.32			2.13
	Syringaldehyde	1662					14.45	13.20	12.20	6.40				
	Coniferaldehyde	1741					17.13	20.38	12.40	8.35				
	Sinapaldehyde	1909												
Aliphatic Aldehydes	3-methylbutanal	659				0.93								
	Heptanal	1176	1.02	2.05	2.65	2.45	4.40			0.97	4.25		2.43	1.88
	Decanal	1182	0.58	16.45	3.27	4.77		4.27		2.13	1.77	7.92		5.57
	Octanal	1282	0.62	8.10	3.17	3.32								
	Nonanal	1387		1.25	7.90	9.63						4.82	7.08	
	Furfural	1462					0.52	2.07	0.70					
Alkanes	Eicosane	2000	2.67							7.20			4.28	
Nitrogen Compounds	Diethyltoluamide	1571					5.87			8.47				
	N-*p*-bromophenylselenoacetamide	1724												
	Dimethyl palmitamine	1894	5.93											

Table 2. *Cont.*

Class of Compounds	Compound		1ND				2ND				3ND			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
Aromatics	Xylene	1137			0.53	4.72								1.93
	1,1,3-trimethyl-3-phenyl-2H-indene	1714			0.52					0.43			0.48	
	Methyl n-hydroxybenzimidate	1814	1.03				1.60	0.45	2.72		1.30	0.72		
	2,6-dimethoxyphenol	2269					5.93	4.70	1.35					
	2,6-di-tert-butylphenol	2327	2.27							1.73				
Fatty acids	Nonanoic acid	1265							1.88	4.90				
	Pentanoic acid	1685	0.30	4.05	2.87	2.87				1.93		3.55		2.40
	Tetradecanoic acid	1768	1.37					1.10		0.30		1.23		
	Hexadecanoic acid	1968	20.75			0.98	1.92	1.55		2.08	2.12			1.63
	Hexanedioic acid	2137	1.87		2.10	1.77				5.07				3.88
	Octadecanoic acid	2182	17.57				1.40			1.60	9.63			
Ketones	4-oxo-β-dihydroionone	1659												
	3-oxo-7,8-dihydro-α-ionone	1731												
Silanols	Dimethylsilanediol	649	0.17		2.30			0.28	2.70	3.43			2.52	
Terpenes	α-pinene	936			3.62								2.87	
	Pinocarvone	1138			1.02								1.40	
	Cyperene	1398		1.18		1.37						1.13		
	α-bergamotene	1407	0.60			1.08								0.98
	β-panasinsene	1527			1.80	0.43							1.62	
	γ-eudesmol	1632			1.27					1.70				

Table 3. Average peak area of volatile compounds from dieback (D) and non-dieback (ND) oak wood, determined by SPME.

	Wood Type	n	Acids	Aromatic Aldehydes	Aliphatic Aldehydes	Alkanes	Nitrogen Compounds	Aromatics	Fatty Acids	Ketones	Silanols	Terpenes
average	D	12	2.0	4.5	n.d.	n.d.	9.7	2.2	1.8	5.3	6.6	n.d.
st. dev			1.0	4.1			6.5	1.2	0.7	4.1	1.7	
average	ND	12	n.d.	10.6	3.8	4.7	6.8	1.9	3.6	n.d.	1.9	1.5
st. dev				5.9	3.4	1.9	1.2	1.6	4.6		1.2	0.8
t Test (prob) significance				0.008 **	n.d.	n.d.	0.502 n.s.	0.649 n.s.	0.220 n.s.	n.d.	0.000 ***	n.d.

Data given as area units of chromatograph peaks, mean of three analyses. n, sample size. n.d., not detected. Significance: ** $p < 0.01$, *** $p < 0.001$; n.s., not significant.
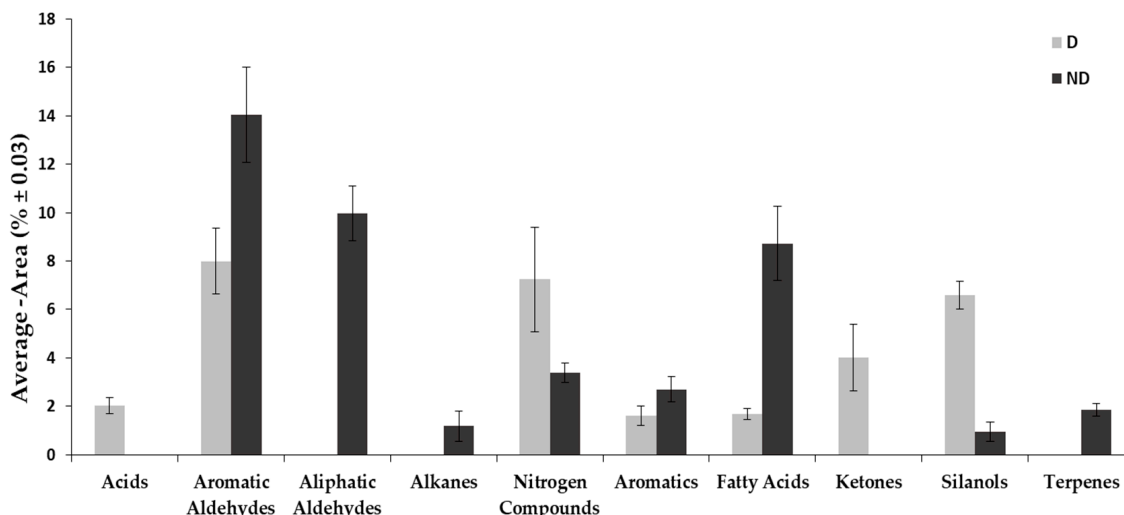
Figure 2. Average of the total areas (% ±0.03) of the classes of compounds of dieback (D) and non-dieback (ND) trees (overbars indicate the standard error).

Aldehydes can be involved in signalling pathways and are often associated with stress responses in plants. The higher content of aldehydes in ND trees, especially aromatic ones like vanillin, furfural, and 5-methylfurfural, suggests a more effective stress response mechanism, aiding these trees in coping with adverse conditions [25]. In D trees, however, the presence of Sinapaldehyde was found in the years 2007–2011. In contrast, aliphatic aldehydes, such as nonanal, heptanal, decanal, and octanal, disappeared in D trees, indicating that they are more affected by stress. Alkanes are typically part of the waxy cuticle, serving as a protective barrier against environmental stressors. The absence or presence of alkanes might reflect changes in the composition of the protective surface, potentially impacting the tree's ability to resist drought and pathogens. For example, eicosane, detected in ND trees, is completely absent in all samples of D trees.

Aromatic compounds can serve as defence mechanisms against herbivores and pathogens. The reduction in aromatic compounds in D trees (e.g., xylene, 2,6-di-tert-butylphenol, 2,6-dimethoxyphenol, etc.) may indicate a weakened defence mechanism, making them more susceptible to stressors like pests and diseases [33].

Terpenes are a diverse class of compounds with various functions, including defence against herbivores and pathogens. The reduction in terpenes in D trees indicates a reduced capacity to defend against stressors, such as pests and diseases [34]. In fact, α-pinene, pinocarvone, cyperene, α-bergamotene, β-panasinsene, and γ-eudesmol were completely absent in all samples of D trees, while they were detected in the ND tree rings, especially in the 2012–2016 period.

Fatty acids (FAs) are fundamental components of lipids and are involved in energy storage and membrane structure. The higher content of fatty acids in ND trees (e.g., hexadecanoic acid, octadecanoic acid, and Pentanoic acid) suggests that they have more energy reserves for growth and stress responses, which is essential for maintaining good physiological status; they are essential for cell membranes' stability and fluidity and plant metabolism and indicate a better health status of the plants. In fact, among the multiple roles of FAs, they have structural functions such as being constituents of phospholipids, which are the "building blocks" of cell membranes. As part of the neutral lipids, FAs serve as storage materials in cells, and FA derivatives are involved in cell signalling [35]. At the moment, their role in the regulation of the cellular membrane fluidity and their participation in complex processes, such as proliferation, differentiation, secretion, migration, invasion, and phagocytosis, highlight the importance of these molecules for the maintenance and adaptation of plants in the forests [35].

Acids can play multiple roles in plants' metabolism. Organic acids are involved in various biochemical pathways, such as the citric acid cycle (Krebs cycle), and as components

of amino acids. A higher presence of acids in D trees, such as acetic acid, could indicate a response to metabolic stress to compensate for the potential impact on energy production and nutrient availability [36]; in fact, this was detected in all the analysed D trees samples of the last 20 years, while on the other hand, it was completely absent in the ND tree samples.

Nitrogen compounds are essential for plant growth and are typically found in amino acids, proteins, and nucleic acids. The presence or absence of certain nitrogen compounds could indicate changes in the nitrogen availability and utilization, which are critical for plants' development and response to stress [37]. In particular, as shown in Table 2, it is possible to notice the absence of N-p-bromophenylselenoacetamide in the ND trees, as well as the absence of dimethyl palmitamine in the D trees; even more interesting is diethyltoluamide, which increases markedly in all D trees, especially in the years from 2007 to 2021.

Ketones can be involved in the energy metabolism. The presence of ketones like 4-oxo-β-dihydroionone and 3-oxo-7,8-dihydro-α-ionone—which are absent in the ND trees and, in contrast, present in the D, ones especially in the years 2012–2016—may indicate changes in energy metabolism due to stress, but the specific role in this context would require further investigation.

Silanols are organic compounds containing silicon, and they can have antimicrobial properties. An increase in silanols in D trees, particularly dimethylsilanediol, may be part of their defence mechanism against pathogens due to the absence of healthier alternatives [38].

Previous studies explain how the embolism of xylem vessels, caused by drought, which leads to a discontinuity of hydraulic flows, induces branch desiccation, leaves yellowing, and generalized necrosis, causing a decrease in the leaf area and a consequent reduction in photosynthetic activity [14]. Plants, to limit the loss of water through transpiration, close the stomata during drought, resulting in a poor supply of CO_2. The main consequence of carbon starvation and the reduction in photosynthesis is a reduction in carbohydrate synthesis and supply to the plants [8,39]. This would explain why in D plants, a reduction in compounds deriving from carbohydrates, such as aldehydes, terpenoids, terpenes, and phenolic compounds, occurred. In fact, terpenoids and some terpenes originate directly from the glycolysis of glucose through the mevalonate pathway or the methyleritritol phosphate (MEP) pathway [40], while phenolic compounds originate from the shikimic acid pathway, which is related to the metabolism of carbohydrates and aromatic amino acids [31].

Climate stress could have led to a reduction in compounds such as oxime methoxyphenyl and benzaldehyde 4-hydroxy-3,5-dimethoxy (or syringaldehyde) with important antimicrobial and antifungal activities [41,42], and in terpenes, such as α-pinene, γ-eudesmol, and cyperene, which have significant insecticidal activity [43–45] (Tables 1 and 2). Such decreases in compounds thus explain part of the deterioration of D plants compared to ND plants, because the reduction in these substances in the leaves could have made trees more sensitive to insect attacks and parasites. To compensate, D plants defended themselves by increasing compounds such as silanediol dimethyl, an important antimicrobial, and diethyltoluamide, which has marked insecticidal activity [46,47].

According to Martinez et al. (1996) [48], the decrease in these compounds is a consequence of the uncontrolled decomposition of several compounds (for example, lignin), which causes the formation of much less reactive compounds. At the same time, under stress conditions, plants—especially those progressing towards dieback—may release mixtures of volatile organic compounds (like silanols) that indirectly protect the plant by attracting natural enemies of the herbivore [49]. Recent studies suggest that these VOCs can also prime nearby crop plants for enhanced induction of defence upon future insect attacks [50]. Thus, these differences could be bio-indicators of early warning signals of an onset of the dieback state [51].

4. Conclusions

This study offers valuable insights into how *Q. frainetto* trees respond to the increasing climatic challenges of drought and heat waves. The lower growth rate in D trees compared to ND trees is an unequivocal sign of decay phenomena. In fact, D trees have a lower number of leaves, and this condition may translate into less photosynthesis, less organic matter production, and lower reserves of carbohydrates being produced by the trees and available for growth. The observed differences in VOCs and plant secondary compounds (PSCs) between dieback (D) and non-dieback (ND) trees suggest that these compounds could serve as indicators of tree health and susceptibility to dieback phenomena. ND trees showed a higher content of aldehydes, terpenes, and fatty acids, indicators of a better state of health, than D trees did. On the other hand, D trees contained less terpenes, like α-pinene, γ-eudesmol, and cyperene (which exhibits insecticidal activity), and had low levels of aromatic aldehydes (i.e., furfural and 5-methylfurfural) and high levels of silanols (these latter have an antimicrobial function). From a practical point of view, these findings have the potential to inform future forest management strategies, enabling the early detection of declining tree health. However, the study also underscores the complexity of tree responses to environmental stress, leaving room for future investigations to delve deeper into the precise roles of these compounds, explore underlying genetic and physiological mechanisms, fully understand which metabolites are involved in the response to dieback phenomena, and and expand the scope to encompass multiple tree species and diverse regions. As climate change continues to pose threats to forests, this research paves the way for further studies and practical tools aimed at safeguarding our valuable tree populations and ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f15061072/s1: Figure S1: The location of the San Poalo Albanese site in the Basilicata Region; (a) an example of an ND tree with crown transparency < 50%; (b) an example of a D tree with crown transparency > 50%; Figure S2: An example of cross-sections of *Q. frainetto* wood. The arrows indicate the last 3 growth rings of (a) an ND tree and (b) a D tree. The difference in annular growth between the two samples is evident.

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