

Soil Terpenoid Storage and Emissions Are Shaped by Litter Chemistry and Soil Depth

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Abstract

Aims

Soil VOCs, particularly reactive terpenoids, play a crucial role in atmospheric chemistry but remain poorly quantified. This study investigates how soil terpenoid storage and emissions vary with depth and tree species in a mixed temperate forest and evaluates the role of litter chemistry in shaping these patterns.

Methods

Soil samples were collected from Douglas fir (*Pseudotsuga menziesii*) and European beech (*Fagus sylvatica*) plots under identical climatic and edaphic conditions. Soil terpenoid storage was assessed via solvent extraction, while emissions were captured using dynamic headspace sampling; both were analyzed by gas chromatography-mass spectrometry.

Results

Soil terpenoid storage and emissions were significantly higher in Douglas fir soils ($627,386 \pm 650,060 \text{ ng g}^{-1}$; $4,718 \pm 5,978 \text{ ng g}^{-1} \text{ h}^{-1}$) than in European beech soils ($17,868 \pm 19,981 \text{ ng g}^{-1}$; $234 \pm 123 \text{ ng g}^{-1} \text{ h}^{-1}$) ($p < 0.01$). In the Douglas fir plot, storage peaked in the Oi horizon, whereas in the European beech plot, it was highest in the Oe horizon, likely due to differences in litter chemistry, decomposition rates, and soil adsorption. Emissions were highest in the Oi horizon of Douglas fir soils, reflecting direct volatilization from resin-rich litter, while European beech soils showed consistently low emissions. Terpenoid composition differed between the two plots, further suggesting that litter chemistry influences VOC transformation and release.

Conclusions

These findings highlight the importance of integrating soil VOC fluxes, litter characteristics, and vegetation-specific influences into forest VOC models to improve atmospheric VOC budget prediction.

Introduction

Soils play a crucial role in the global carbon (C) cycle as both major carbon reservoirs and sources of emissions. While soils store approximately 1,500–2,500 Gt C, exceeding carbon stocks in vegetation and the atmosphere (Lal et al., 2021; Smith et al., 2020), they also release large amounts of carbon annually through microbial decomposition of soil organic matter (SOM) and root respiration (Hirano et al., 2023; Jian et al., 2021). Although numerous studies have focused on soil CO₂ fluxes and carbon pools, other key processes such as the emission and storage of volatile organic compounds (VOCs) in soils remain

comparatively understudied, despite their potential to influence atmospheric chemistry, radiative forcing, and global climate.

Volatile organic compounds (VOCs) are highly reactive carbon-based chemicals that influence atmospheric chemistry and climate through processes such as ozone formation and secondary organic aerosol (SOA) production (Atkinson, 2000; Fry et al., 2014). Globally, biogenic VOCs (BVOCs) account for the majority of emissions, with annual fluxes ranging from 0.42 to 0.59 Gt C yr⁻¹, significantly higher than anthropogenic VOCs (0.14–0.16 Gt C yr⁻¹) (Duan et al., 2023). Among BVOCs, isoprene and terpenoids (e.g., monoterpenoids and sesquiterpenoids) are the dominant contributors to global emissions. For example, monoterpenes and sesquiterpenes collectively account for approximately 17–18% of global BVOC emissions, with α -pinene, a key monoterpene, contributing 27–32% of this fraction (Sindelarova et al., 2022). Despite significant advances in BVOC research, the magnitude and variability of soil-derived VOC fluxes remain highly uncertain. This is partly due to the predominant focus of VOC emission models on plant sources, often neglecting or underrepresenting soil contributions (Isidorov & Zaitsev, 2022; Sindelarova et al., 2022). Unlike plant emissions, which have been well quantified, soil VOC production and emission are shaped by multiple interacting factors, including microbial activity, soil organic matter (SOM) decomposition, and soil and litter physicochemical properties (Insam & Seewald, 2010; Isidorov & Zaitsev, 2022; Meischner et al., 2022; Peñuelas et al., 2014; Yang et al., 2024). Understanding these processes is essential for refining VOC budget estimates and predicting their impact on biogeochemical cycles and global climate.

Beyond production and emission, VOC storage within soils can play a crucial role in determining soil-atmosphere fluxes of VOCs. VOCs generated through microbial metabolism and decomposition of SOM can either be emitted into the atmosphere or retained within the soil matrix through adsorption onto soil particles and organic matter (Hui et al., 2023; Schmidt et al., 2015; Xu et al., 2015). As this adsorption capacity depends on SOM content and particle size (Hui et al., 2023), the extent of VOC storage could vary with soil depth (Mäki et al., 2019). Additionally, litterfall itself act as a direct VOC source (Leff & Fierer, 2008). Hence, vegetation type can play a crucial role in shaping soil VOC dynamics, primarily through differences in litter chemistry (Meischner et al., 2022). Coniferous trees produce litter rich in resins containing various terpenoids, resulting in higher VOC fluxes and potentially greater VOC accumulation in soils compared to deciduous trees (Geron et al., 2000; Isidorov & Zaitsev, 2022; Uusitalo et al., 2008; Wang et al., 2024). In contrast, deciduous litter, which generally lacks resins, could be associated with lower VOC emissions and storage (Isidorov & Zaitsev, 2022; Smolander et al., 2006). These differences further influence microbial communities, favoring taxa specialized in terpene metabolism, which in turn can alter VOC decomposition rates and storage patterns across soil depths (Asensio et al., 2012; Hui et al., 2023; Schmidt et al., 2015; Xu et al., 2015). However, the extent to which these factors regulate soil VOC storage and emissions remains largely unexplored, highlighting the need for further research.

To address these gaps, this study investigates how soil VOC storage and emissions vary across soil depths and different tree species. Specifically, we focus on forest soils dominated by Douglas fir

(coniferous) and European beech (deciduous), which differ significantly in litter chemistry and terpenoid content. The measurements were conducted in the ECOSENSE forest (Werner et al. 2024), a mixed forest at the foothills of the Black forest in South-West Germany. Coniferous litter, enriched in resin-derived terpenoids, is expected to contribute to higher VOC storage and emissions compared to deciduous litter, which lacks resin components. This study aims to (i) quantify the vertical distribution of VOC storage and emissions across different soil horizons, and (ii) evaluate how vegetation-driven litter chemistry influences soil VOC dynamics in forest ecosystems. We hypothesize that VOC storage and emissions will decrease with depth, with the O horizon exhibiting the highest VOC storage and emissions due to abundant decomposing litter and active microbial metabolism. Furthermore, we predict that Douglas fir soils, which receive terpenoid-rich litter, will exhibit significantly higher VOC storage and emissions compared to European beech soils. By elucidating these patterns, this study provides essential insights into how vegetation composition affects soil VOC fluxes, with implications for biogeochemical cycling, atmospheric chemistry, and regional VOC budgets.

Materials and Methods

Field site and soil sampling

The soil samples were collected at the ECOSENSE forest site (48°16.07'N, 7°52.67'W, 480 m asl) which is located at the western border of the Black Forest near the city of Ettenheim (Werner et al., 2024) on January 12, 2024. This mixed forest mainly consists of Douglas fir (*Pseudotsuga menziesii*) and European beech (*Fagus sylvatica* L.), with a lower share of Norway spruce (*Picea abies* L.), European larch (*Larix decidua* Mill.) and Silver fir (*Abies alba* Mill.). The soil parent material is lower shell limestone with the soil type luvisol (IUSS Working Group WRB, 2015). The soils studied in this forest were dominated by either 55 years old Douglas fir (tree height ca. 36 m) or 110 years old European beech (tree height ca. 30 m), respectively. We collected twelve intact soil cores using Plexiglas cylinders (12.7 cm ID, 24.0 cm height), six cylinders from each plot. For this purpose, cylinders were carefully driven 16.5 cm into the soil without destroying the natural layering. The sampled soil cores were then transferred to the laboratory and sectioned into distinct horizons: litter layer (Oi, 0–2 cm), organic layer (Oe, 2–4 cm), upper soil layer (A, 4–7 cm), and mineral soil layer (B, 7–16.5 cm).

Soil VOC storage

For VOC extraction from soil horizons, aliquots of 5 g from the Oi and Oe horizons were mixed in 30 ml methanol and homogenized with mortar and pestle. All other samples (A and B horizons) were extracted with 20 ml methanol. In addition, blank samples for background determination without soil were prepared with 20 ml methanol. Soil/methanol suspensions were transferred into glass bottles and shaken at RT for 60 min. After shaking, 1 ml aliquots of each extract were centrifuged (14,000 rpm, 5 minutes) and 150 µl supernatants were transferred into 1.5 ml reaction tubes containing five passive samplers and 1.3 ml H₂O_{demin}. For A and B horizon samples, 350 µl supernatant was added to 1.1 ml H₂O_{demin}. Passive samplers were prepared in advance according to the protocol of (Kallenbach et al.,

2014). Non-polar compounds such as many volatiles adsorb to the passive samplers and are thus extracted from the liquid phase. The samples were shaken on a thermomixer (Eppendorf, Germany) at 30°C and 1,200 rpm for 1 hour. Thereafter, the passive samplers were carefully dried using a precision wipe (Kimtech Science, USA), and placed into 2 ml screw top vials (Agilent Technologies, USA) and stored at -22°C until gas chromatography-mass spectrometry (GC-MS) analysis.

Soil VOC emissions

Aliquots of 5 g soil from each horizon were weighed into 100 ml glass cuvettes equipped with an inlet (for synthetic air) and an outlet (for VOC sampling). The cuvettes were temperature-controlled to 25°C. Before starting the air sampling, the cuvettes were flushed with synthetic air at a flow rate of 100 ml min⁻¹ for 10 minutes ensuring removal of any pre-existing VOCs. Thereafter, the inlet was closed allowing the VOCs naturally emitted from the soil to accumulate within the headspace of the cuvette for 3 hours. After this period, the cuvette air was channeled through air sampling cartridges (Thermodesorption tubes, Gerstel, Germany) filled with 50 mg Tenax TA (Sigma-Aldrich, Germany) to capture the emitted soil VOCs. Air was drawn through the cuvette at a flow rate of 100 ml min⁻¹ for 15 minutes using an air sampling pump (SKC Inc., USA). During this phase, synthetic air was supplied to the cuvette inlet to avoid low pressure in the system. The air sampling tubes were stored at 4°C until analysis which usually took place within one week.

VOC analysis

For analysis of soil stored VOCs (Chap. 2.2), the passive samplers were transferred into empty thermodesorption tubes (Gerstel, Germany). For emitted VOCs (Chap. 2.3), air sampling tubes were used directly. We used an Agilent 7820A GC system (Agilent Technologies, USA) equipped with mass selective detector (MSD 5975C, Agilent, USA), a thermodesorption unit and cold injection system (TDU-CIS, Gerstel, Germany). Thermodesorption tubes containing passive samplers and Tenax filled air sampling tubes were placed into the TDU with a MultiPurpose Sampler (MPS2, Gerstel, Germany). Thereafter, the TDU was heated to 240°C at a rate of 60°C min⁻¹, with a final hold time of 4 minutes. The desorbed VOCs were cryofocused at -70°C in the CIS before being rapidly heated to 240°C for 3 minutes. During this time, the analytes were transferred into the GC separation column (DB-5MS UI capillary column, 30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA). Helium (5.0, Messer Griesheim, Germany) was used as the carrier gas at a constant flow of 1 ml min⁻¹. Oven temperature was initially set to 45°C for 2 minutes, ramped up to 80°C at 2°C min⁻¹, then to 140°C at 4°C min⁻¹, and finally to 280°C at 9°C min⁻¹, where it was held for 5 minutes. The MS was operated in electron ionization (EI) mode at 70 eV, scanning a mass range of 35–350 m/z. The ion source temperature was set to 230°C, and the quadrupole temperature was maintained at 150°C.

Analysis of raw data including peak identification, re-integration and peak alignment was done with the MassHunter Qualitative (Vers.10.0, Agilent Technologies, USA) and Quantitative Analysis Software (Vers.12.0, Agilent Technologies, USA). For peak identification mass spectra measured were compared

to those of the NIST mass spectral library. Quantification was conducted using calibration curves from authentic standards (α -pinene, β -pinene, limonene, caryophyllene). Monoterpenes and sesquiterpenes not available as standards were quantified using the calibration factor of α -pinene and caryophyllene, respectively. Non-terpenoid compounds were not further considered due to lack of standards for quantification. VOC emission rates were determined based on the compound abundance on the thermodesorption tubes, normalized to the dry weight of the soil sample and the incubation time. The emission rate was calculated using the following equation:

$$\text{VOC emission} = \frac{\text{VOC mass (ng)}}{\text{Soil dry weight (g)} \times \text{Incubation time (h)}}$$

Statistical analysis

To compare VOC storage and emissions across different soil layers and between plots (Douglas fir vs. European beech), a series of non-parametric statistical tests was performed in RStudio (R Core Team, 2024). Since normality is a prerequisite for parametric tests, we first assessed whether the data followed a normal distribution using the Shapiro-Wilk test (*shapiro.test()* function). As the data did not meet the normality assumption, non-parametric tests were applied.

To assess differences in VOC storage and emissions across soil depth groups, the Kruskal-Wallis test (*kruskal.test()* function) was conducted. When significant differences were detected, the Dunn's test (*dunn.test()* function) was used as a post-hoc analysis to determine pairwise differences between depth groups. Additionally, to compare overall differences in VOC storage and emissions between Douglas fir and European beech soils, the Wilcoxon rank-sum test (*wilcox.test()* function) was performed. All statistical analyses were conducted at a significance level of $\alpha = 0.05$ using R (version 4.2.2).

Results

Contrasting soil terpenoid dynamics between Douglas fir and European beech plots

Douglas fir soils exhibited significantly higher terpenoid storage and emissions compared to European beech soils across all horizons (Fig. 1a). Total terpenoid storage in Douglas fir soils was $627,386 \pm 650,060 \text{ ng g}^{-1}$, approximately 35 times higher than that in European beech soils ($17,868 \pm 19,981 \text{ ng g}^{-1}$) ($p < 0.01$). This disparity was most pronounced in the surface Oi horizon and gradually diminished with increasing depth (Fig. 2). Similarly, total terpenoid emissions in Douglas fir soils ($4,718 \pm 5,978 \text{ ng g}^{-1} \text{ h}^{-1}$) were nearly 20 times higher than those in European beech soils ($234 \pm 123 \text{ ng g}^{-1} \text{ h}^{-1}$) ($p < 0.01$) (Fig. 1b). The Oi horizon dominated these emissions, with Douglas fir soils exhibiting emissions nearly 50 times greater than European beech soils ($4,519 \pm 5,627 \text{ ng g}^{-1} \text{ h}^{-1}$ vs. $87 \pm 94 \text{ ng g}^{-1} \text{ h}^{-1}$) (Fig. 3).

However, as soil depth increased, this difference in emissions decreased, with both species showing comparably low emission rates in the A and B horizons.

Douglas fir soils also exhibited greater variability in both total terpenoid storage and emissions, with storage ranging from 124,648 to 1,870,405 ng g⁻¹ and emissions from 403 to 15,684 ng g⁻¹ h⁻¹ (Fig. 1). In comparison, European beech soils showed a narrower range for both total storage (3,957–57,598 ng g⁻¹) and total emissions (75–384 ng g⁻¹ h⁻¹). This pronounced variability in Douglas fir soils likely reflects significant spatial heterogeneity in litter composition, microbial activity, or microenvironmental conditions.

Vertical distribution and compositional dynamics of soil terpenoid storage and emissions

In Douglas fir soils, the O horizons exhibited the highest terpenoid storage ($333,315 \pm 690,628$ ng g⁻¹ in Oi; $229,392 \pm 180,085$ ng g⁻¹ in Oe), which accounted for over 80% of the total storage across all horizons (Fig. 2a). Storage decreased significantly with depth, with the B horizon containing only $16,333 \pm 22,609$ ng g⁻¹, less than 5% of the total storage ($p = 0.019$). A total of over 60 terpenoids were identified in the storage profile of Douglas fir soils, exhibiting a diverse terpenoid composition. Among different terpenoid groups, monoterpenoids dominated in the Oi horizon, contributing over 90% of the total terpenoid content. Although monoterpenoids continued to constitute a significant portion of storage in the deeper horizons (Oe, A, and B), sesquiterpenoids became more prevalent, accounting for over 30% of the total storage in the deeper horizons. Similarly, in European beech soils, the O horizons ($5,289 \pm 2,467$ ng g⁻¹ in Oi; $9,882 \pm 17,638$ ng g⁻¹ in Oe) accounted for over 80% of the total storage, while the B horizon exhibited the lowest storage (735 ± 777 ng g⁻¹) ($p = 0.002$) (Fig. 2b). Terpenoid profiles revealed a less diverse composition compared to Douglas fir soils, with up to 25 terpenoids detected. Monoterpenoids dominated storage in the Oi horizon, contributing over 70%, while sesquiterpenoids became more prominent in the deeper horizons, comprising nearly 50% of the Oe and A horizon storage.

Terpenoid emissions in Douglas fir soils decreased significantly with depth, with the Oi horizon contributing over 90% of the total emissions ($4,519 \pm 5,627$ ng g⁻¹ h⁻¹) (Fig. 3a). Emissions were substantially lower in the A (9.9 ± 8.3 ng g⁻¹ h⁻¹) and B horizons (10.6 ± 19.2 ng g⁻¹ h⁻¹) compared to the Oi horizon ($p < 0.018$). Across all depths, Douglas fir soils exhibited high terpenoid diversity, with nearly 60 distinct terpenoids detected in emission profiles. Among different terpenoid groups, monoterpenoids dominated emissions across all horizons, while sesquiterpenoids contributed minimally to the total emissions. European beech soils exhibited consistently low emission rates across all horizons. Emissions ranged from 28.1 ± 8.3 ng g⁻¹ h⁻¹ to 88.1 ± 124 ng g⁻¹ h⁻¹, with slightly higher values in the Oi horizon compared to deeper layers, although these differences were not statistically significant (Fig. 3b). Terpenoid diversity in European beech soils was markedly lower, with < 15 distinct terpenoids identified in emission profiles. Emissions were primarily dominated by monoterpenoids, while sesquiterpenoids made negligible contributions to total emissions.

Compound-specific characteristics of dominant terpenoids across depths

The dominant compounds within the monoterpene and sesquiterpene groups varied between plots, and each compound exhibited distinct depth-dependent patterns. In Douglas fir soils, monoterpenes such as α -pinene, β -pinene, p-cymene, limonene, and isobornyl acetate dominated storage in the Oi horizon, with concentrations ranging from 10,175 ng g⁻¹ to 224,930 ng g⁻¹ (Fig. 4). Sabinene and γ -terpinene were also present, although their contributions to storage were relatively minor (< 10,000 ng g⁻¹). In the deeper soil horizons, α -pinene and β -pinene remained dominant, but other monoterpenes showed significant declines. In contrast, sesquiterpenes such as δ -cadinene and α -longipinene, which were present in low amounts in the Oi horizon (< 2,000 ng g⁻¹), became more abundant in the Oe, A, and B horizons, with storage concentrations ranging from 13,024 to 23,104 ng g⁻¹. Similarly, emissions in the Oi horizon were dominated by α -pinene, p-cymene, and limonene, which exhibited higher emission-to-storage ratios compared to other compounds (0.8% for α -pinene, 9.9% for p-cymene, and 3.3% for limonene). In contrast, emissions of β -pinene, sabinene, γ -terpinene, longifolene, and α -longipinene were negligible (< 92 ng g⁻¹ h⁻¹). Emissions of isobornyl acetate and sesquiterpenes including δ -cadinene, junipercamphor, and caryophyllene oxide were below detection limits. This suggests limited volatilization, stronger adsorption to soil particles, or reduced microbial activity for these compounds. While α -thujene was not detected in storage, it was observed in emissions at very low levels. As soil depth increased from Oi to B, most emissions became undetectable. However, limonene emissions remained relatively high compared to those of other compounds, particularly in the Oe horizon.

In European beech soils, geranylacetone dominated storage in the Oi horizon, with concentrations of 2,519 ng g⁻¹, but its levels declined sharply to below 100 ng g⁻¹ in the deeper horizons (Oe, A, and B) (Fig. 5). Other monoterpenes including limonene, sabinene, and p-cymene were also detected in the Oi horizon at lower concentrations, with their storage remaining relatively stable across horizons (65–545 ng g⁻¹). In the deeper horizons, α -pinene and sesquiterpenes such as δ -cadinene and germacrene D showed greater contributions, particularly in the Oe and A horizons (~ 3,360 ng g⁻¹). Emissions in European beech soils were consistently low across all horizons, with limonene and p-cymene showing negligible emission levels (< 10 ng g⁻¹ h⁻¹) and emission-to-storage ratios of 2.0% and 1.1%, respectively. The emission rate of α -pinene was greater than limonene and p-cymene, and the emission-to-storage ratio was 3.9%. Unlike Douglas fir soils, eucalyptol was exclusively detected in European beech soils, appearing only in emissions (Fig. 5). Emissions of sesquiterpenes were below detection limits, suggesting limited microbial activity or abiotic processes driving their volatilization in these soils.

Discussion

This study demonstrates that soil terpene storage and emissions are strongly influenced by species specific litter chemistry and soil depth. Douglas fir soils exhibited significantly higher terpene concentrations and emissions than European beech soils, even though both were studied within the same forest stand under identical soil and microclimate conditions. Additionally, depth-dependent

variations in terpenoid storage and emissions were observed, with the highest storage occurring in the Oi or Oe horizons, depending on species. These differences likely result from interactions between litter composition, microbial metabolism, and soil adsorption dynamics, which vary across depths and tree species, leading to strong spatial variability within the mixed forest stand. In the following sections, we discuss how these factors collectively regulate soil VOC storage and emissions.

Why do soil terpenoid storage and emissions vary with depth?

The depth-dependent variations in soil terpenoid storage and emissions differed between Douglas fir and European beech soils (Figs. 2–3). In Douglas fir soils, terpenoid storage tended to be highest in the Oi horizon and gradually decreased with depth, whereas in European beech soils, the Oe horizon tended to have the highest storage (Fig. 2). Although these trends were not always statistically significant, likely due to large standard deviations, they align with previous findings by Komprdová et al. (2016). The discrepancy between the two plots might result from differences in litter decomposition rates and soil physicochemical properties.

In general, soil particles and humus have strong VOC adsorption capacities, which can be further enhanced by low pH and high SOM content (Hui et al., 2023; Insam & Seewald, 2010; Peñuelas et al., 2014). The Oe horizon, which likely contains partially decomposed SOM with finer particle sizes and increased humification, can retain more VOCs compared to the Oi horizon. This pattern is expected to be more pronounced in European beech soils where faster litter decomposition could result in a greater proportion of highly humified organic matter in the Oe horizon (Berger & Berger, 2014), thereby potentially enhancing VOC adsorption. In contrast, the slow decomposition rates in Douglas fir soils driven by more acidic conditions and lower microbial activity (Berger & Berger, 2014), could contribute to the accumulation of terpenoid-rich litter in the Oi horizon, resulting in higher terpenoid storage in this layer. Another possible explanation for this slower decomposition in Douglas fir soils is the high abundance of terpenoids in Douglas fir needles. Coniferous litter contains substantial amounts of resin-derived terpenoids (Geron et al., 2000; Isidorov & Zaitsev, 2022; Uusitalo et al., 2008), and terpenoids are known to have antimicrobial and decomposition-inhibiting properties (Adamczyk et al., 2015; Šimpraga et al., 2019). These compounds can suppress microbial activity or alter microbial community composition, ultimately slowing down litter breakdown and promoting the accumulation of organic material. The persistence of less decomposed litter in the Oi horizon may further enhance terpenoid storage in Douglas fir soils, as the stored terpenoids in litter itself were extracted and quantified in this study. In contrast, European beech litter, which lacks resins, has lower terpenoid concentrations and decomposes more rapidly. This leads to faster incorporation of organic material into the soil, allowing VOCs to adsorb more effectively on the finer SOM particles present in the Oe horizon. The higher VOC retention in the Oe horizon of European beech soils may therefore be a consequence of both lower initial terpenoid input and the more advanced decomposition state of the organic layer, which enhances VOC adsorption capacity.

Terpenoid emission patterns also varied between the two forest types (Fig. 3). In both Douglas fir and European beech soils, terpenoid emissions were highest in the Oi horizon. However, this trend was statistically significant only in Douglas fir soils, whereas European beech soils exhibited no significant depth-dependent differences. This suggests that in Douglas fir soils, emissions are largely driven by direct volatilization from litter, whereas in European beech soils, emissions are more uniform across depths. The sharp decline in emissions from the Oi to Oe horizon in Douglas fir soils suggests that highly volatile terpenoids are primarily released from fresh litter, while in deeper layers, remaining terpenoids are either strongly adsorbed to soil particles or decomposed by microbial activity (Hui et al., 2023; McBride et al., 2020; Peñuelas et al., 2014; Ramirez et al., 2010). The lack of depth-dependent emission differences in European beech soils is likely due to the inherently low terpenoid content of European beech litter, which results in consistently low VOC fluxes regardless of depth. This finding is consistent with the previous study showing that monoterpene emissions from other deciduous trees like red maple were over 100 times lower than those from loblolly pine (Ramirez et al., 2010).

Although microbial activity is generally higher in surface soils as also suggested by our data, the low abundance of terpenoid-metabolizing microbial taxa in European beech soils may further explain the absence of significant emission differences across horizons. Since litter VOCs can shape microbial activity or composition (Asensio et al., 2012; McBride et al., 2020), the lower terpenoid input in European beech soils might lead to a reduced presence of microbes specializing in terpenoid degradation, thereby limiting emissions.

How do litter chemistry and microbial activity shape soil VOC dynamics?

The composition and abundance of terpenoids varied significantly between Douglas fir and European beech soils, with Douglas fir soils exhibiting greater diversity and higher concentrations of terpenoids in both storage and emissions (Figs. 2–3). Consistent with previous studies, α -pinene and β -pinene were the dominant compounds in Douglas fir litter (Geron et al., 2000), whereas European beech soils contained fewer terpenoid species and lower concentrations of these key compounds (Isidorov & Zaitsev, 2022). These differences highlight the strong influence of litter chemistry on soil terpenoid profiles. Such patterns are in very good agreement with the terpenoid content in intact leaves and needles collected from the trees nearby the soil plots investigated (data not shown).

The disparity between soil stored and emitted terpenoids further underscores the role of microbial activity in VOC dynamics. Certain terpenoids such as isobornyl acetate, geranylacetone, or δ -cadinene detected in storage were absent in emissions (Figs. 4–5), suggesting that they were either strongly adsorbed to soil particles, had low volatility, or were subject to microbial degradation before volatilization (Hui et al., 2023). Conversely, some terpenoids such as eucalyptol detected in emissions but absent in storage likely resulted from microbial transformation of stored compounds (McBride et al., 2020; Zhao et al., 2015). The presence of unique terpenoids in emissions suggests that microbial metabolism generates novel terpenoids, supporting the notion that microbial community composition plays a crucial role in shaping soil VOC fluxes (Honeker et al., 2023; Isidorov & Zaitsev, 2022; Jiao et al., 2023; Peñuelas

et al., 2014; Pugliese et al., 2023). Additionally, differences in microbial assemblages between Douglas fir and European beech soils may further contribute to variations in VOC decomposition and emission patterns (Asensio et al., 2012; Hui et al., 2023).

What are the implications of forest composition for regional and global VOC fluxes?

These findings indicate that the combined effects of litter chemistry and soil depth play a pivotal role in determining soil VOC dynamics. In mixed forests, the spatial distribution of tree species can lead to localized hotspots of VOC storage and emissions, with Douglas fir-dominated areas acting as major contributors. The observed differences in terpenoid storage and emissions between Douglas fir and European beech soils suggest that forest composition, alongside the spatial heterogeneity of tree species distribution within mixed forests, significantly influences regional VOC budgets. Since Douglas fir soils exhibit higher terpenoid emissions, coniferous forests may contribute more substantially to atmospheric terpenoid concentrations than deciduous forests (Albers et al., 2018; Ramirez et al., 2010). Given that terpenoids contribute to ozone formation and secondary organic aerosol (SOA) production, changes in forest composition can have broader implications for air quality and climate regulation (Atkinson, 2000; Bonn et al., 2020; Fry et al., 2014). Currently, most VOC emission models focus primarily on plant emissions, with limited consideration of soil-derived VOCs (Sindelarova et al., 2022). Incorporating soil VOC fluxes into atmospheric models could also improve the accuracy of VOC budget estimates, particularly in forest ecosystems where soil contributions might be substantial.

How can future research address the limitations of this study?

This study provides valuable insights into how litter chemistry influences soil VOC storage and emissions, but several limitations should be addressed in future research. First, the study was conducted at a single site within the Black Forest (ECOSENSE site) and focused solely on Douglas fir and European beech plots. Expanding the study to encompass diverse forest types across different climatic regions would improve our understanding of species-specific VOC fluxes and enable the development of a broader VOC database (Insam & Seewald, 2010).

Second, this study was based on soil samples collected from the ECOSENSE site and analyzed through laboratory-based VOC extractions. While this approach offers crucial insights into soil VOC storage and emissions, it did not determine the relative contribution of soil VOCs within the overall forest VOC budget. To better understand the role of soils within the broader forest VOC budget, future studies will integrate direct field measurements using techniques such as relaxed eddy accumulation (REA) or flux chambers to compare soil VOC fluxes with canopy-level emissions (Werner et al., 2024). This could provide a more comprehensive assessment of the proportional contribution of soil-derived VOCs to total forest VOC fluxes.

Furthermore, VOC storage and emissions are highly sensitive to seasonal changes in temperature, moisture, and microbial activity (Isidorov & Zaitsev, 2022; Jiao et al., 2023; Peñuelas et al., 2014). Year-round field measurements under diverse climatic conditions may offer a more complete understanding

of how environmental variability regulates soil VOC fluxes. Incorporating additional environmental parameters, such as soil temperature, moisture, and precipitation, could further improve our ability to model VOC flux responses to changing climatic conditions (Honeker et al., 2023; Mäki et al., 2017; Meischner et al., 2022; Pugliese et al., 2023; Werner et al., 2021; Yang et al., 2024).

By addressing these research gaps, future work could enhance our ability to quantify soil VOC contributions to atmospheric VOC pools and refine our predictions of how forest composition, climate change, and land-use shifts influence VOC fluxes at regional and global scales.

Conclusion

This study demonstrates that litter chemistry and soil depth play key roles in shaping soil terpenoid storage and emissions, with Douglas fir soils exhibiting significantly higher terpenoid concentrations and fluxes than European beech soils. Depth-dependent differences in storage and emissions suggest that decomposition rates and soil adsorption capacity influence terpenoid retention across soil horizons. Furthermore, variations in terpenoid composition and the presence of microbially transformed compounds highlight the importance of microbial activity in regulating VOC dynamics. These findings underscore the need to integrate soil VOC fluxes into broader forest VOC models, particularly as forest composition shifts under climate change and land-use changes. Future research should incorporate year-round field measurements, explore a wider range of forest types, and assess environmental factors driving VOC variability. A deeper understanding of soil VOC dynamics will not only enhance global VOC budget estimations but also refine predictions of how forest ecosystems regulate atmospheric chemistry and influence climate at regional and global scales.

Declarations

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Competing Interests

The authors declare that they have no competing financial or non-financial interests.

Author Contributions

Hojin Lee: Formal analysis and investigation; Writing - original draft preparation, Writing - review and editing; Sofie Katlewski: Formal analysis and investigation; Writing - original draft preparation; Pia Carolin Weber: Formal analysis and investigation; Writing - original draft preparation; Christiane Werner: Funding acquisition, Supervision, Writing - review and editing; Jürgen Kreuzwieser: Conceptualization, Methodology, Supervision, Writing - original draft preparation, Writing - review and editing

References

1. Adamczyk, S., Adamczyk, B., Kitunen, V., & Smolander, A. (2015). Monoterpenes and higher terpenes may inhibit enzyme activities in boreal forest soil. *Soil Biology and Biochemistry*, *87*, 59–66. <https://doi.org/10.1016/j.soilbio.2015.04.006>
2. Albers, C. N., Kramshøj, M., & Rinnan, R. (2018). Rapid mineralization of biogenic volatile organic compounds in temperate and Arctic soils. *Biogeosciences*, *15*(11), 3591–3601. <https://doi.org/10.5194/bg-15-3591-2018>
3. Asensio, D., Yuste, J. C., Mattana, S., Ribas, À., Llusà, J., & Peñuelas, J. (2012). Litter VOCs induce changes in soil microbial biomass C and N and largely increase soil CO₂ efflux. *Plant and Soil*, *360*(1–2), 163–174. <https://doi.org/10.1007/s11104-012-1220-9>
4. Atkinson, R. (2000). Atmospheric chemistry of VOCs and NO_x. *Atmospheric Environment*, *34*, 2063–2101. [https://doi.org/10.1016/S1352-2310\(99\)00460-4](https://doi.org/10.1016/S1352-2310(99)00460-4)
5. Berger, T. W., & Berger, P. (2014). Does mixing of beech (*Fagus sylvatica*) and spruce (*Picea abies*) litter hasten decomposition? *Plant and Soil*, *377*(1–2), 217–234. <https://doi.org/10.1007/s11104-013-2001-9>
6. Bonn, B., Kreuzwieser, J., Magh, R. K., Rennenberg, H., Schindler, D., Sperlich, D., Trautmann, R., Yousefpour, R., & Grote, R. (2020). Expected impacts of mixing european beech with silver fir on regional air quality and radiation balance. *Climate*, *8*(10), 1–20. <https://doi.org/10.3390/cli8100105>
7. Duan, C., Liao, H., Wang, K., & Ren, Y. (2023). The research hotspots and trends of volatile organic compound emissions from anthropogenic and natural sources: A systematic quantitative review. *Environmental Research*, *216*. <https://doi.org/10.1016/j.envres.2022.114386>
8. Fry, M. M., Schwarzkopf, M. D., Adelman, Z., & West, J. J. (2014). Air quality and radiative forcing impacts of anthropogenic volatile organic compound emissions from ten world regions. *Atmospheric Chemistry and Physics*, *14*(2), 523–535. <https://doi.org/10.5194/acp-14-523-2014>
9. Geron, C., Rasmussen, R., Arnts, R. R., & Guenther, A. (2000). A review and synthesis of monoterpene speciation from forests in the United States. *Atmospheric Environment*, *34*, 1761–1781. [https://doi.org/10.1016/S1352-2310\(99\)00364-7](https://doi.org/10.1016/S1352-2310(99)00364-7)
10. Hirano, T., Cui, R., Sun, L., Teramoto, M., & Liang, N. (2023). Partitioning of root respiration into growth, maintenance, and ion uptake components in a young larch-dominated forest. *Plant and Soil*, *482*(1–2), 57–72. <https://doi.org/10.1007/s11104-022-05674-0>
11. Honeker, L. K., Pugliese, G., Ingris, J., Fudyma, J., Gil-Loaiza, J., Carpenter, E., Singer, E., Hildebrand, G., Shi, L., Hoyt, D. W., Chu, R. K., Toyoda, J., Krechmer, J. E., Claflin, M. S., Ayala-Ortiz, C., Freire-

- Zapata, V., Pfannerstill, E. Y., Daber, L. E., Meeran, K., ... Meredith, L. K. (2023). Drought re-routes soil microbial carbon metabolism towards emission of volatile metabolites in an artificial tropical rainforest. *Nature Microbiology*, 8(8), 1480–1494. <https://doi.org/10.1038/s41564-023-01432-9>
12. Hui, K., Yuan, Y., Xi, B., & Tan, W. (2023). A review of the factors affecting the emission of the ozone chemical precursors VOCs and NO_x from the soil. *Environment International*, 172. <https://doi.org/10.1016/j.envint.2023.107799>
 13. Insam, H., & Seewald, M. S. A. (2010). Volatile organic compounds (VOCs) in soils. *Biology and Fertility of Soils*, 46(3), 199–213. <https://doi.org/10.1007/s00374-010-0442-3>
 14. Isidorov, V. A., & Zaitsev, A. A. (2022). Reviews and syntheses: VOC emissions from soil cover in boreal and temperate natural ecosystems of the Northern Hemisphere. *Biogeosciences*, 19(19), 4715–4746. <https://doi.org/10.5194/bg-19-4715-2022>
 15. IUSS Working Group WRB. (2015). *World Reference Base for Soil Resources 2014*.
 16. Jian, J., Vargas, R., Anderson-Teixeira, K., Stell, E., Herrmann, V., Horn, M., Kholod, N., Manzon, J., Marchesi, R., Paredes, D., & Bond-Lamberty, B. (2021). A restructured and updated global soil respiration database (SRDB-V5). *Earth System Science Data*, 13(2), 255–267. <https://doi.org/10.5194/essd-13-255-2021>
 17. Jiao, Y., Kramshøj, M., Davie-Martin, C. L., Albers, C. N., & Rinnan, R. (2023). Soil uptake of VOCs exceeds production when VOCs are readily available. *Soil Biology and Biochemistry*, 185. <https://doi.org/10.1016/j.soilbio.2023.109153>
 18. Kallenbach, M., Oh, Y., Eilers, E. J., Veit, D., Baldwin, I. T., & Schuman, M. C. (2014). A robust, simple, high-throughput technique for time-resolved plant volatile analysis in field experiments. *Plant Journal*, 78(6), 1060–1072. <https://doi.org/10.1111/tpj.12523>
 19. Komprdová, K., Komprda, J., Menšík, L., Vaňková, L., Kulhavý, J., & Nizzetto, L. (2016). The influence of tree species composition on the storage and mobility of semivolatile organic compounds in forest soils. *Science of the Total Environment*, 553, 532–540. <https://doi.org/10.1016/j.scitotenv.2016.02.132>
 20. Lal, R., Monger, C., Nave, L., & Smith, P. (2021). The role of soil in regulation of climate. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 376(1834). <https://doi.org/10.1098/rstb.2021.0084>
 21. Leff, J. W., & Fierer, N. (2008). Volatile organic compound (VOC) emissions from soil and litter samples. *Soil Biology and Biochemistry*, 40(7), 1629–1636. <https://doi.org/10.1016/j.soilbio.2008.01.018>
 22. Mäki, M., Aaltonen, H., Heinonsalo, J., Hellén, H., Pumpanen, J., & Bäck, J. (2019). Boreal forest soil is a significant and diverse source of volatile organic compounds. *Plant and Soil*, 441(1–2), 89–110. <https://doi.org/10.1007/s11104-019-04092-z>
 23. Mäki, M., Heinonsalo, J., Hellén, H., & Bäck, J. (2017). Contribution of understory vegetation and soil processes to boreal forest isoprenoid exchange. *Biogeosciences*, 14(5), 1055–1073. <https://doi.org/10.5194/bg-14-1055-2017>

24. McBride, S. G., Choudoir, M., Fierer, N., & Strickland, M. S. (2020). Volatile organic compounds from leaf litter decomposition alter soil microbial communities and carbon dynamics. *Ecology*, *101*(10). <https://doi.org/10.1002/ecy.3130>
25. Meischner, M., Haberstroh, S., Daber, L. E., Kreuzwieser, J., Caldeira, M. C., Schnitzler, J. P., & Werner, C. (2022). Soil VOC emissions of a Mediterranean woodland are sensitive to shrub invasion. *Plant Biology*, *24*(6), 967–978. <https://doi.org/10.1111/plb.13445>
26. Peñuelas, J., Asensio, D., Tholl, D., Wenke, K., Rosenkranz, M., Piechulla, B., & Schnitzler, J. P. (2014). Biogenic volatile emissions from the soil. *Plant, Cell and Environment*, *37*(8), 1866–1891. <https://doi.org/10.1111/pce.12340>
27. Pugliese, G., Ingrisch, J., Meredith, L. K., Pfannerstill, E. Y., Klüpfel, T., Meeran, K., Byron, J., Purser, G., Gil-Loaiza, J., van Haren, J., Dontsova, K., Kreuzwieser, J., Ladd, S. N., Werner, C., & Williams, J. (2023). Effects of drought and recovery on soil volatile organic compound fluxes in an experimental rainforest. *Nature Communications*, *14*(1). <https://doi.org/10.1038/s41467-023-40661-8>
28. Ramirez, K. S., Lauber, C. L., & Fierer, N. (2010). Microbial consumption and production of volatile organic compounds at the soil-litter interface. *Biogeochemistry*, *99*(1), 97–107. <https://doi.org/10.1007/s10533-009-9393-x>
29. Schmidt, R., Cordovez, V., De Boer, W., Raaijmakers, J., & Garbeva, P. (2015). Volatile affairs in microbial interactions. *ISME Journal*, *9*(11), 2329–2335. <https://doi.org/10.1038/ismej.2015.42>
30. Šimpraga, M., Ghimire, R. P., Van Der Straeten, D., Blande, J. D., Kasurinen, A., Sorvari, J., Holopainen, T., Adriaenssens, S., Holopainen, J. K., & Kivimäenpää, M. (2019). Unravelling the functions of biogenic volatiles in boreal and temperate forest ecosystems. *European Journal of Forest Research*, *138*(5), 763–787. <https://doi.org/10.1007/s10342-019-01213-2>
31. Sindelarova, K., Markova, J., Simpson, D., Huszar, P., Karlicky, J., Darras, S., & Granier, C. (2022). High-resolution biogenic global emission inventory for the time period 2000-2019 for air quality modelling. *Earth System Science Data*, *14*(1), 251–270. <https://doi.org/10.5194/essd-14-251-2022>
32. Smith, P., Soussana, J. F., Angers, D., Schipper, L., Chenu, C., Rasse, D. P., Batjes, N. H., van Egmond, F., McNeill, S., Kuhnert, M., Arias-Navarro, C., Olesen, J. E., Chirinda, N., Fornara, D., Wollenberg, E., Álvaro-Fuentes, J., Sanz-Cobena, A., & Klumpp, K. (2020). How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. *Global Change Biology*, *26*(1), 219–241. <https://doi.org/10.1111/gcb.14815>
33. Smolander, A., Ketola, R. A., Kotiaho, T., Kanerva, S., Suominen, K., & Kitunen, V. (2006). Volatile monoterpenes in soil atmosphere under birch and conifers: Effects on soil N transformations. *Soil Biology and Biochemistry*, *38*(12), 3436–3442. <https://doi.org/10.1016/j.soilbio.2006.05.019>
34. Uusitalo, M., Kitunen, V., & Smolander, A. (2008). Response of C and N transformations in birch soil to coniferous resin volatiles. *Soil Biology and Biochemistry*, *40*(10), 2643–2649. <https://doi.org/10.1016/j.soilbio.2008.07.009>
35. Wang, L., Lun, X., Wang, Q., & Wu, J. (2024). Biogenic volatile organic compounds emissions, atmospheric chemistry, and environmental implications: a review. *Environmental Chemistry Letters*.

<https://doi.org/10.1007/s10311-024-01785-5>

36. Werner, C., Meredith, L. K., Nemiah Ladd, S., Ingris, J., Kübert, A., van Haren, J., Bahn, M., Bailey, K., Bamberger, I., Beyer, M., Blomdahl, D., Byron, J., Daber, E., Deleeuw, J., Dippold, M. A., Fudyma, J., Gil-Loaiza, J., Honeker, L. K., Hu, J., ... Williams, J. (2021). Ecosystem fluxes during drought and recovery in an experimental forest. *Science*, 374(6574). <https://www.science.org>
37. Werner, C., Wallrabe, U., Christen, A., Comella, L., Dormann, C., Göritz, A., Grote, R., Haberstroh, S., Jouda, M., Kiese, R., Koch, B., Korvink, J., Kreuzwieser, J., Lang, F., Müller, J., Prucker, O., Reiterer, A., Rühle, J., Rupitsch, S., ... Wöllenstein, J. (2024). ECOSENSE - Multi-scale quantification and modelling of spatio-temporal dynamics of ecosystem processes by smart autonomous sensor networks. *Research Ideas and Outcomes*, 10. <https://doi.org/10.3897/rio.10.e129357>
38. Xu, Y. Y., Lu, H., Wang, X., Zhang, K. Q., & Li, G. H. (2015). Effect of Volatile Organic Compounds from Bacteria on Nematodes. *Chemistry and Biodiversity*, 12(9), 1415–1421. <https://doi.org/10.1002/cbdv.201400342>
39. Yang, K., Llusà, J., Preece, C., Tan, Y., & Peñuelas, J. (2024). Exchange of volatile organic compounds between the atmosphere and the soil. *Plant and Soil*, 501(1–2), 509–535. <https://doi.org/10.1007/s11104-024-06524-x>
40. Zhao, J., Wang, Z., Wu, T., Wang, X., Dai, W., Zhang, Y., Wang, R., Zhang, Y., & Shi, C. (2015). Volatile organic compound emissions from straw-amended agricultural soils and their relations to bacterial communities: A laboratory study. *Journal of Environmental Sciences*, 45, 257–269. <https://doi.org/10.1016/j.jes.2015.12.036>

Figures

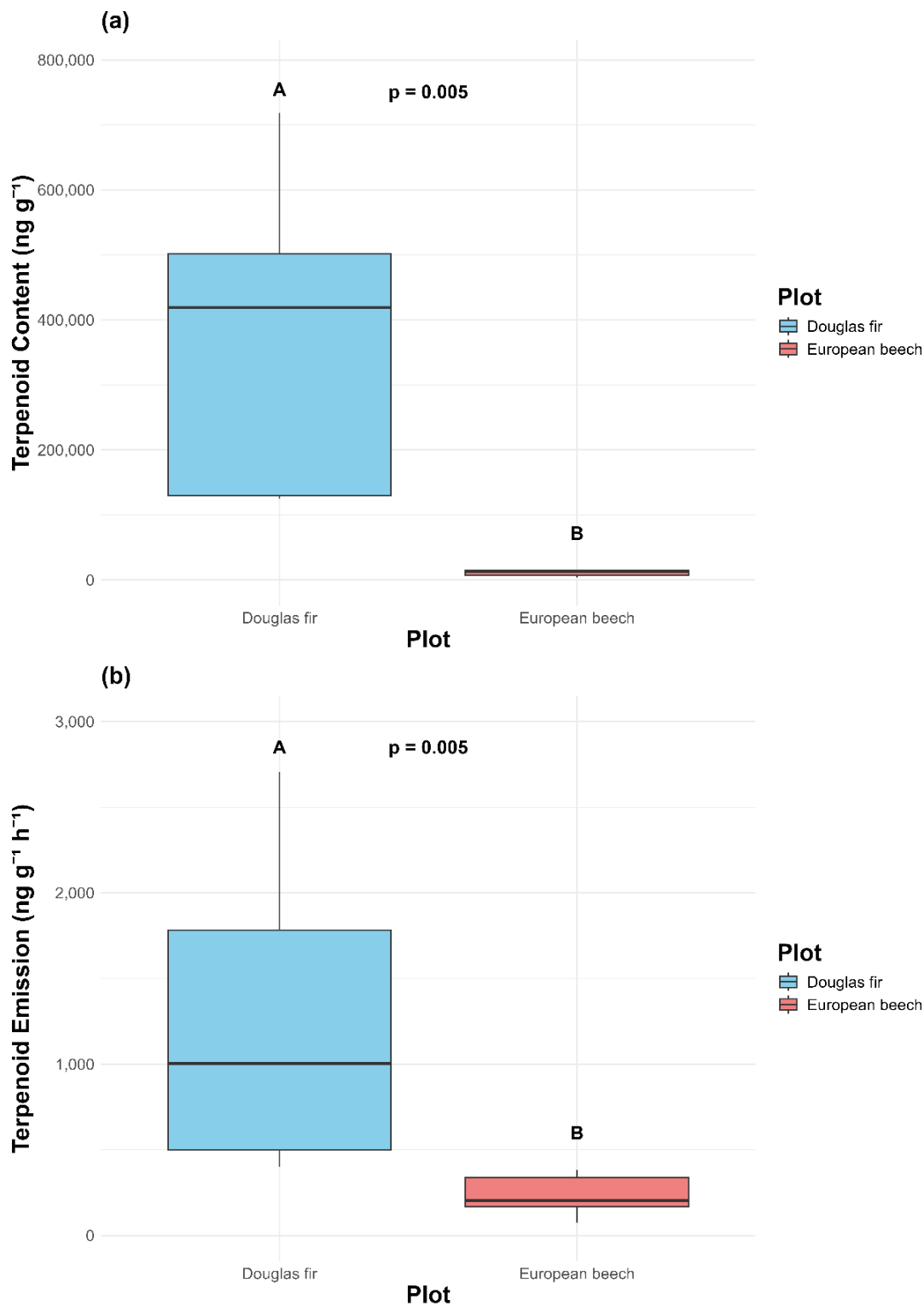


Figure 1

Comparison of terpenoid storage and emissions between Douglas fir and European beech soils. (a) Terpenoid content in soil samples from the Douglas fir and European beech plots. (b) Terpenoid emission rates from soil samples in the two plots. Note that the scales in (a) and (b) are different.

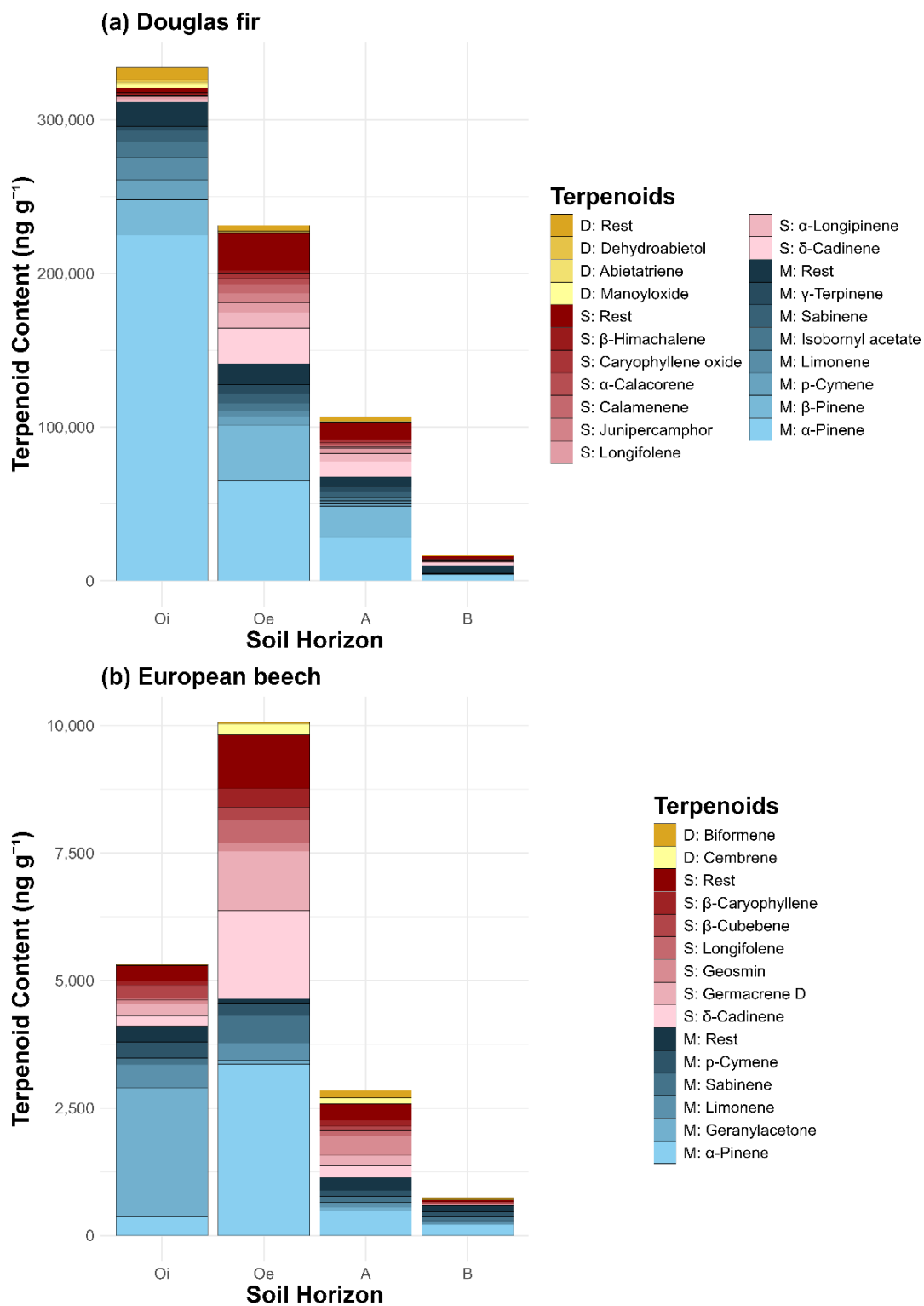


Figure 2

Terpenoid composition and total content across soil horizons in the Douglas fir plot (a) and the European beech plot (b). "Rest" refers to the sum of all minor terpenoid compounds that were detected but not individually classified in the legend due to their relatively low abundance. Note that the scales in (a) and (b) are different.

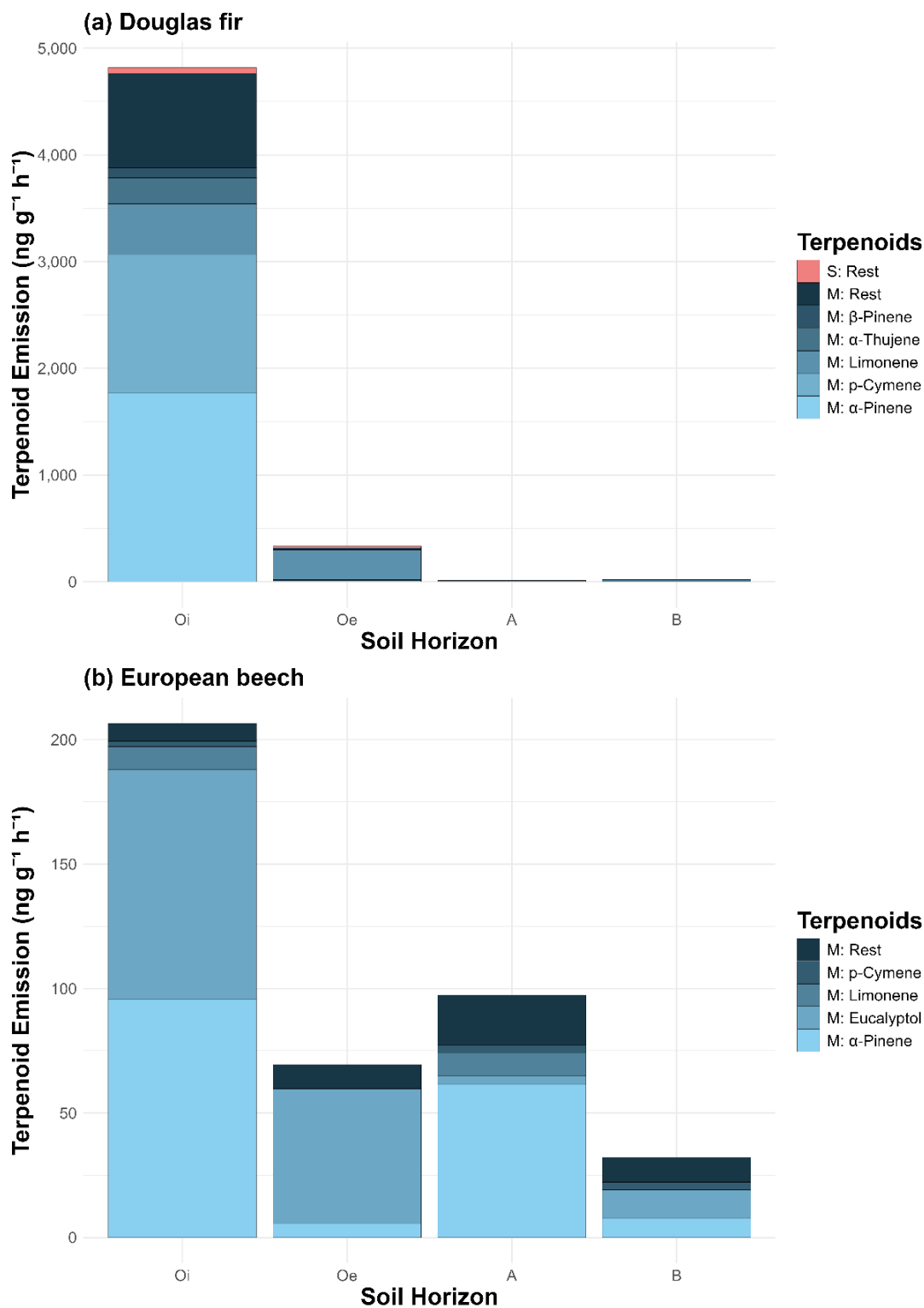


Figure 3

Terpenoid composition and total emissions across soil horizons in the Douglas fir plot (a) and the European beech plot (b). "Rest" refers to the sum of all minor terpenoid compounds that were detected but not individually classified in the legend due to their relatively low abundance. Note that the scales in (a) and (b) are different.

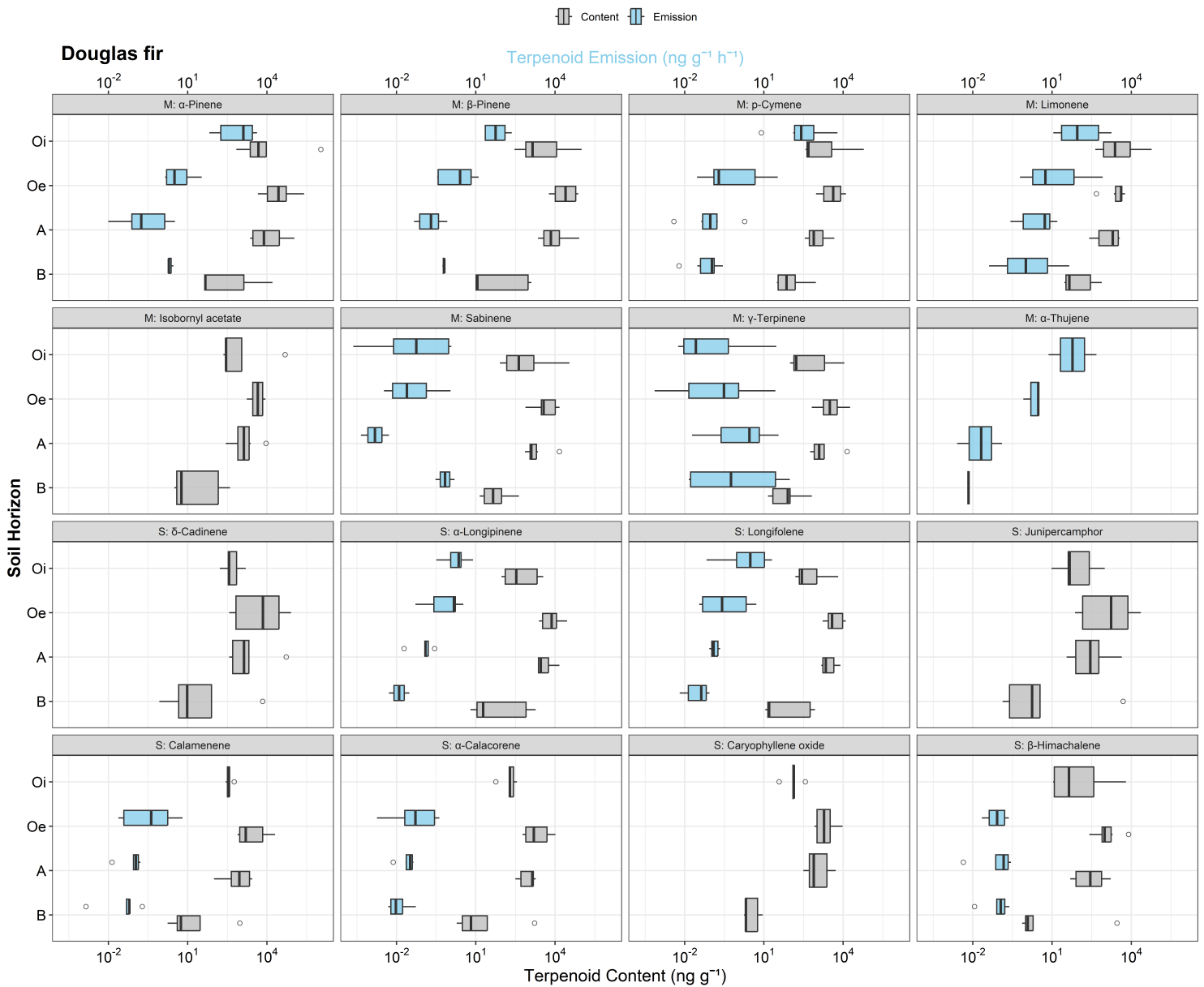


Figure 4

Terpenoid content and emissions across soil horizons in the Douglas fir plot. Boxplots show the depth distribution of terpenoid storage (gray) and emissions (blue) across different soil horizons (Oi, Oe, A, B).

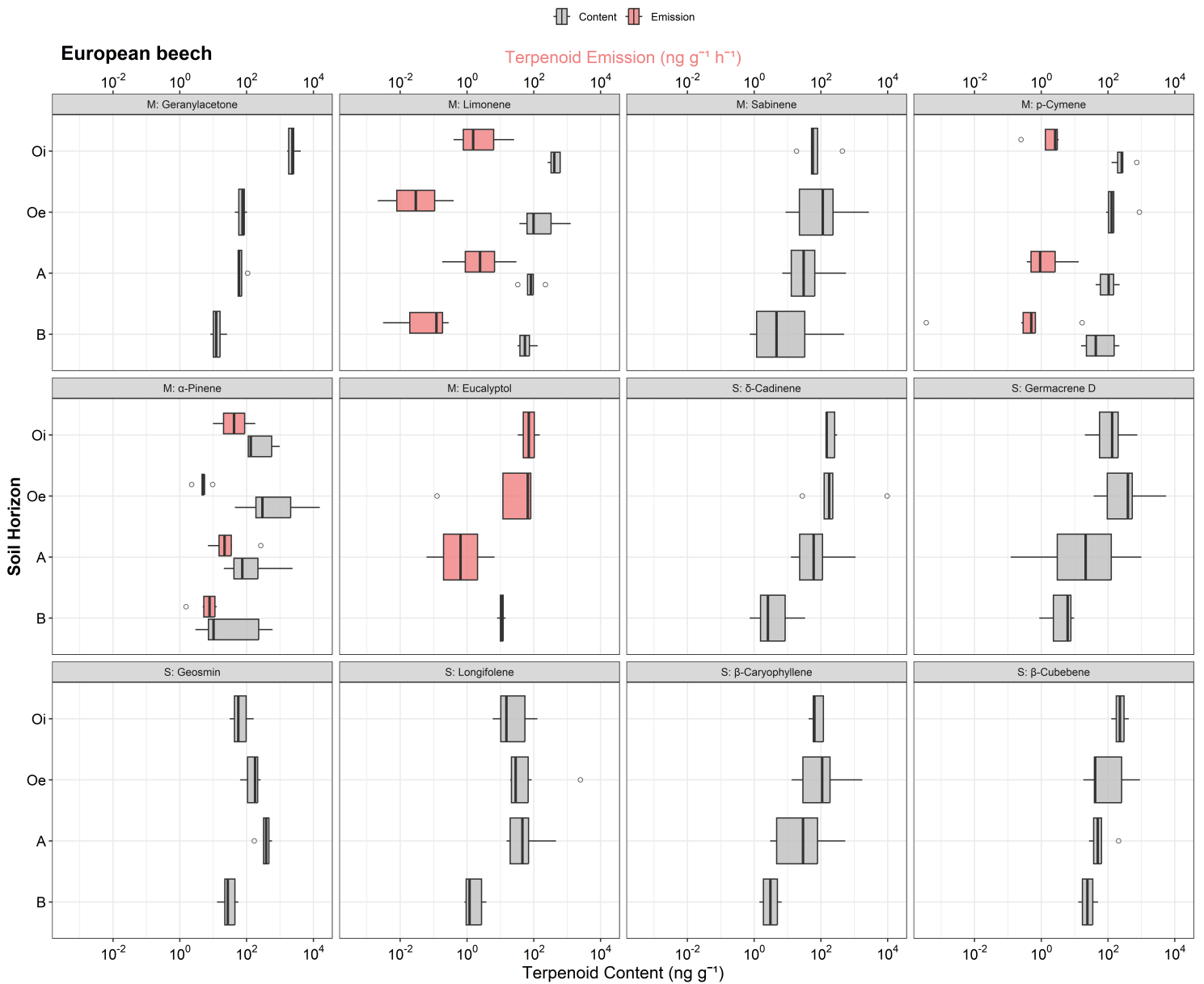


Figure 5

Terpenoid content and emissions across soil horizons in the European beech plot. Boxplots show the depth distribution of terpenoid storage (gray) and emissions (red) across different soil horizons (Oi, Oe, A, B).