

Article

Decoupled Leaf Physiology and Branch-Level BVOC Emissions in Two Tree Species Under Water and Nitrogen Treatments

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Abstract

Soil water availability and nitrogen (N) deposition critically influence biogenic volatile organic compound (BVOC) emissions, thereby affecting atmospheric chemistry. However, their differential short- and long-term effects remain unclear. Here, *Ormospira pinnata* and *Pinus massoniana* seedlings were exposed to three water regimes (moderate drought, MD; normal irrigation, NI; near-saturated irrigation, NSI) and two nitrogen (N0; 0 kg N ha^{−1} yr^{−1}; N80; 80 kg N ha^{−1} yr^{−1}) treatments for 20 months. Branch-level BVOC emissions and leaf physiological and biochemical traits were examined after 8 months (short term) and 16 months (long term). In the short term, *P. massoniana* predominantly emitted α-pinene, β-pinene, and γ-terpinene, whereas *O. pinnata* emitted isoprene (ISO). After prolonged exposure, ISO became the dominant in both species. Short-term MD and NSI conditions stimulated ISO emissions in *O. pinnata*, with N80 addition further amplifying this effect. In contrast, long-term treatments tended to suppress ISO emissions in *O. pinnata*, particularly under N80. Short-term water treatments had no significant effect on monoterpene (MT) emissions in *P. massoniana*. Under long-term water treatments, N80 suppressed ISO emissions; nevertheless, ISO emission rates (ISO rate) progressively increased with increasing soil water availability. Although leaf intercellular CO₂ concentration (C_i), stomatal conductance (g_s), and photosynthesis-related enzymes exhibited partial correlations with BVOC emissions, an overall decoupling between leaf traits and emission patterns was evident. Our findings demonstrate the significant changes in both BVOC composition and emission magnitudes under the joint effects of water availability and nitrogen deposition, providing important implications for improving regional air quality modeling and BVOC emission predictions.

Keywords: isoprene; monoterpenes; soil moisture; nitrogen deposition; long-term exposure



Academic Editors: Yunjian Luo, Hesong Wang, Xiaolei Sun and Hongwei Xu

Received: 14 October 2025

Revised: 5 November 2025

Accepted: 7 November 2025

Published: 9 November 2025

Citation: Li, S.; Yan, D.; Liu, X.; Lin, M.; Yi, Z. Decoupled Leaf Physiology and Branch-Level BVOC Emissions in Two Tree Species Under Water and Nitrogen Treatments. *Forests* **2025**, *16*, 1708. <https://doi.org/10.3390/f16111708>

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1. Introduction

Biogenic volatile organic compounds (BVOCs) are emitted in substantial quantities by terrestrial vegetation and profoundly influence tropospheric photochemistry owing to their high chemical reactivity [1,2]. Among them, isoprene (ISO) and monoterpenes (MTs) dominate the global BVOCs budget [3,4], accounting for approximately 49% and 15% of the total emission, respectively [5]. Though rapid reactions with atmospheric oxidants (e.g., ·OH), these compounds promote ozone and the biogenic secondary organic aerosol (BSOA) formation, significantly affecting air quality and climate forcing [6–8]. However, global change drivers are expected to modify BVOC fluxes by regulating photosynthetic capacity

and enzymatic activity [9–11], thereby complicating the accurate quantification of BVOC emissions and their atmospheric impacts. Thus, reliable quantification and an improved mechanistic understanding of the environmental controls on BVOC emissions are urgently needed to strengthen atmospheric modeling and climate assessments.

Soil moisture, a key determinant of BVOC emissions, tends to exhibit increasing intensity and frequency of dry–wet anomalies under warming [12,13]. Predicting its dynamics is further shaped by changing precipitation, evapotranspiration, and snowmelt [14,15]. Existing studies indicate that BVOC responses to soil water availability are highly dependent on the severity and duration of stress [11,16]. Moderate water limitation can stimulate isoprenoid emissions [14,17–19], whereas severe drought often suppresses emissions through declines in photosynthetic rate, isoprene synthase (IspS) and monoterpene synthases (MtpS) activities, and substrate supply [2,9,20,21]. Simultaneously, increasing nitrogen availability in many regions driven by atmospheric deposition and fertilization can further modulate photosynthesis, enzyme synthesis, and carbon allocation. However, its interactive effects with water availability on BVOC fluxes and composition have received little attention, except for a few in situ studies conducted under controlled conditions [22,23].

ISO and MTs are synthesized from photosynthetic intermediates via the methylerythritol 4-phosphate (MEP) pathway, catalyzed by IspS and MtpS, respectively [24–26]. Most process-based interpretations of BVOC variability have relied on leaf-level gas exchange metrics and enzyme activity to explain their emission rates [27,28]. However, the reliability of these leaf-level proxies for predicting branch- or canopy-level emissions, particularly under combined water and nitrogen manipulations, remains uncertain. Increasing evidence also suggests that BVOC responses to short- and long-term stress may differ in both magnitude and chemical composition [29–31]. Given the orders-of-magnitude variation in reactivity of different BVOCs toward atmospheric oxidants, elucidating how water and nitrogen availability alter both emission rates and compound profiles across temporal scales is crucial for accurate air-quality modeling and predicting BVOC-mediated climate feedbacks.

In this study, we examined branch-level emissions of ISO and MTs from two native, strongly emitting tree species in southern China—*Ormosia pinnata* and *Pinus massoniana* [32,33]—grown under factorial soil-moisture (three levels) and nitrogen (two levels) treatments in a greenhouse. Emissions were monitored at both short- and long-term timescales, alongside concurrent measurements of leaf gas exchange and key enzymes involved in BVOC biosynthesis and photosynthesis. Our objectives were to: (1) explore how soil moisture and nitrogen availability shape branch-level ISO and MTs emissions, and whether responses differ between short- and long-term exposure; and (2) evaluate the extent to which leaf-level physiological and biochemical traits (photosynthetic parameters and activities of IspS, MtpS, and major photosynthetic enzymes) can explain branch-level emission patterns. By integrating compound-specific emissions data with physiological measurements across temporal scales, this study aims to reveal the mechanistic basis of BVOC responses to water and nitrogen availability, thereby contributing to improved predictions of BVOC emissions under global change scenarios.

2. Materials and Methods

2.1. Experimental Treatments

The experiment was conducted in a greenhouse (approximately 60 m²) at Fujian Agriculture and Forestry University, located in Fujian Province, southern China (26°05' N, 116°15' E). The greenhouse contained 18 cement containers, each with a volume of 0.6 m³ (1 m × 1 m × 0.6 m, length × width × height). In November 2017, well-mixed soil (pH 3.99 ± 0.01, NH₄⁺-N 6.01 ± 0.69 mg kg^{−1}, and NO₃[−]-N 17.39 ± 1.34 mg kg^{−1}) was placed into cement containers. After a one-month stabilization period, two one-year-old seedlings

of *Ormosia pinnata* (Lour.) Merr. (*O. pinnata*) and *Pinus massoniana* Lamb. (*P. massoniana*) were carefully transplanted into each container, with full protection of the root systems during planting. The four seedlings in each cement container were randomly arranged with equal spacing between them. Water and nitrogen treatments began after a four-month adaptation in April 2018. Specifically, two nitrogen treatments were applied: no nitrogen addition (N0, 0 kg N ha⁻¹ yr⁻¹) and nitrogen addition (N80, 80 kg N ha⁻¹ yr⁻¹, applied as ammonium nitrate) across a total of 18 cement containers, with nine containers assigned to each nitrogen treatment. The N80 level was selected to simulate the reported nitrogen deposition rate in forest ecosystems of southern China [32,34], and no other commercial fertilizers were used in this study. Within each nitrogen level, three water treatments were imposed, with three container replicates per treatment, namely moderate drought (MD, 15.43 ± 1.22% v/v, approximately 50% of field capacity, FC), normal irrigation (NI, 22.42 ± 1.39% v/v, approximately 70% FC) and near-saturated irrigation (NSI, 28.29 ± 1.27% v/v, approximately 90% FC) were imposed (Figure 1). Thus, the experimental design consisted of three water treatments and two nitrogen levels, with three container replicates per treatment combination, giving a total of 18 containers. Two seedlings per species were placed in each container, resulting in a total of 72 experimental seedlings (3 water treatments × 2 nitrogen levels × 2 species × 2 seedlings × 3 cement containers). To maintain the soil moisture, all treatments were irrigated every three days, with the irrigation volume adjusted based on ambient temperature and seasonal variations. Soil volumetric water content was regularly measured to estimate water loss, and additional water was supplied to keep moisture at the expected level.

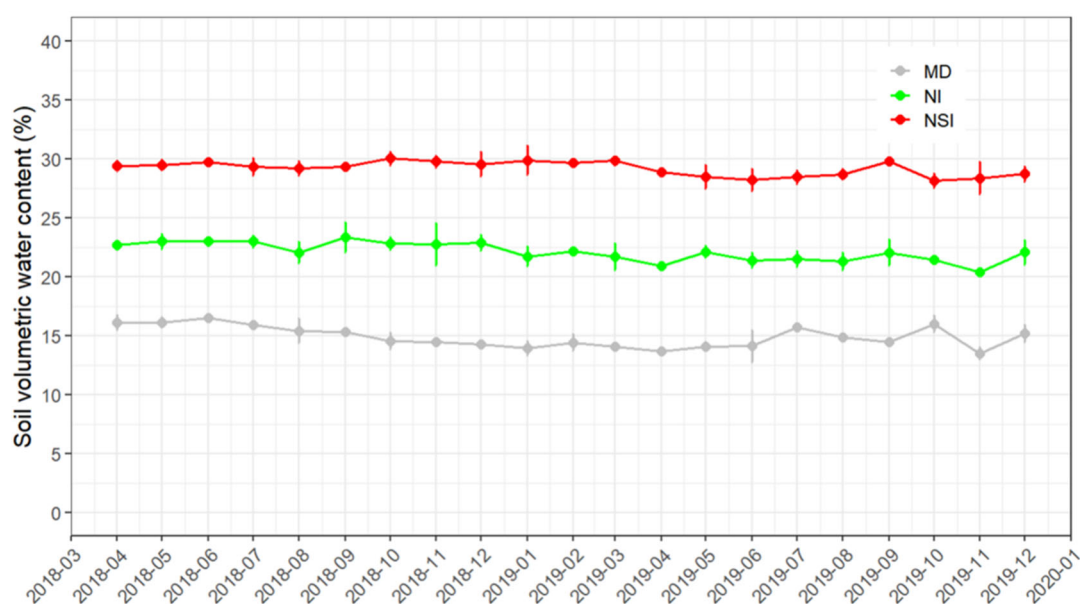


Figure 1. Mean monthly soil volumetric water content during the experimental period. Values are mean ± SD ($n = 3$). MD, moderate drought (14.87 ± 1.00% v/v); NI, normal irrigation (22.09 ± 1.01% v/v); NSI, near-saturated irrigation (29.18 ± 0.81% v/v).

2.2. BVOC Emissions and Gas Exchange Measurements

Biogenic volatile organic compound emissions were measured from the sun-exposed branches of each seedling between 09:00 and 16:00 using a dynamic branch-enclosure technique, following the principle and configuration described by Huang et al. (2020) [33]. Short-term sampling was conducted in December 2018, and long-term sampling in August 2019. Briefly, during each sampling event, an intact, south-facing branch of similar size from each seedling was enclosed in a 7-L polytetrafluoroethylene (PTFE) bag without

damaging leaves. VOC-free air, generated by passing ambient air sequentially through silica gel, activated carbon, and potassium iodide scrubbers, was supplied at 3.5 L min^{-1} by a flow-controlled sampling pump (10L-D, Delin, Dalian, China). A PTFE fan and a perforated PTFE ring manifold inside the bag ensured thorough mixing. After the enclosure reached steady state ($\sim 30 \text{ min}$), 0.5 L of air was drawn into Tedlar bags (SKC Inc., Covington, GA, USA) at 200 mL min^{-1} . Samples were kept dark and analyzed within three days. Enclosure air temperature and relative humidity were continuously recorded with a hygrothermograph (RC-4HC, Elitech, Xuzhou, China). Throughout the sampling period, the measured ranges were $20.82\text{--}34.40^\circ\text{C}$ for air temperature and $44.68\%\text{--}67.40\%$ for relative humidity. Bag blanks (empty PTFE bags operated identically) were collected for background correction. Net emission rates were derived from blank-subtracted values.

After BVOC sampling, the same enclosed branches were removed from the chamber and allowed to stabilize for 1 h before gas exchange measurements. Measurements were performed using a portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) equipped with an LED light source and a $2 \times 3 \text{ cm}^2$ leaf cuvette (LI-6400-02B, LI-COR Biosciences, Lincoln, NE, USA). For *P. massoniana*, twelve needles were arranged side by side to fully cover the cuvette window for each measurement. The measured parameters included net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (Tr). During the measurements, the leaf temperature was maintained at 30°C , with the reference CO_2 concentration set at $380 \mu\text{mol mol}^{-1}$, photosynthetically active radiation (PAR) at $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and the air flow rate at 670 mL min^{-1} . To further reduce measurement error, sampling was alternated among the two nitrogen treatments and three water treatments.

2.3. BVOCs Quantification and Emission Determination

Plant-emitted BVOCs were analyzed using a gas chromatograph-mass spectrometer (GC-MS, 7890B-5977A, Agilent Technologies, Santa Clara, CA, USA) coupled with a three-stage cryogenic preconcentrator (7200, Entech Instruments, Simi Valley, CA, USA). The analytical procedure was based on Wang and Wu (2008) [35] with minor modifications. Briefly, 300 mL of air sample was drawn through the first cryogenic trap (module 1, -170°C) to retain BVOCs while removing major air constituents (N_2 , O_2). The trapped compounds were subsequently desorbed at 10°C and refocused in the second trap (module 2, -60°C) to remove most of CO_2 and H_2O , then transferred to a third trap (module 3, -170°C) for cryogenic focusing to improve the separation in the GC column. Finally, BVOCs were desorbed from the third trap by rapid heating to 80°C and injected into the GC-MS system. Chromatographic separation was performed on a HP-1 capillary column ($60 \text{ m} \times 0.32 \text{ mm} \times 1.0 \mu\text{m}$, Agilent Technologies, Santa Clara, CA, USA) with helium as the carrier gas at a constant flow of 4 mL min^{-1} . The GC oven was programmed as follows: initial temperature at 10°C for 3 min , ramped to 120°C at 5°C min^{-1} , and then increased to 250°C at $10^\circ\text{C min}^{-1}$ with a final hold of 7 min . The MS was operated in selected ion monitoring (SIM) mode, targeting $m/z = 67$ (ISO), 93 and 137 (MT fragments). Ion assignments were verified against authentic standards and the NIST library. Quantification of ISO and total MTs (α -pinene, β -pinene, limonene, α -terpinene, γ -terpinene, and 3-carene) was achieved using calibration curves prepared from authentic standards (diluted to gas phase). Instrument sensitivity was routinely checked before sample analysis using standard injections, and blank runs were conducted regularly to verify background levels and minimize analytical bias.

ISO and MTs emission from plant branches were calculated as:

$$E = \frac{\Delta C \times F}{A} \quad (1)$$

where E is the emission of ISO and MTs ($\text{nmol m}^{-2} \text{s}^{-1}$); ΔC is the concentration difference between air samples and blank (nmol m^{-3}); F is the inlet flow of zero air into the enclosure system ($\text{m}^3 \text{s}^{-1}$); A is the total leaf area of the measured branch (m^2). For comparability across treatments, all ISO and MT emissions were normalized to standard conditions ($T_{\text{leaf}} = 30^\circ\text{C}$ and $\text{PAR} = 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) according to the algorithm proposed by Guenther (1993) [36].

2.4. Enzyme Activity Assays of BVOC Biosynthetic and Photosynthetic Enzymes

The activities of key enzymes were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (Youxuan Biotechnology Co., Ltd., Shanghai, China). The assays targeted two BVOC biosynthetic enzymes—IspS and MtpS—and three photosynthesis-related enzymes—Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), Phosphoenolpyruvate Carboxykinase (PEPCK) and Carbonic Anhydrase (CA). Briefly, 100 mg of fresh leaf tissue was homogenized in 5 mL of extraction buffer and centrifuged at $10000 \times g$ for 20 min at 4°C . The supernatant was collected, diluted five-fold with extraction buffer, and used for subsequent assays. For the ELISA procedure, 50 μL of diluted supernatant and 100 μL of horseradish peroxidase (HRP)-conjugate reagent were sequentially added to each well of a 96-well microtiter plate pre-coated with specific antibodies against IspS, MtpS, RuBisCO, PEPCK, or CA. The plates were covered with adhesive strips and incubated at 37°C for 60 min. After incubation, the wells were washed five times with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T), with complete removal of wash buffer after the final rinse. Subsequently, each well was filled with 50 μL of chromogenic substrate solution A and 50 μL of chromogenic substrate solution B. Thereafter, the wells were gently mixed and incubated at 37°C in the dark for 15 min. The reaction was terminated by adding 50 μL of stop solution per well, and the absorbance was measured at 450 nm within 15 min using a microplate reader (EnSpire, PerkinElmer, Shelton, CT, USA). Blank controls were processed in parallel with the leaf extracts under identical conditions. Calibration curves were generated using authentic standards for each enzyme provided by the manufacturer, and sample activities were quantified accordingly. To minimize the random error of the assays, each sample and standard was analyzed in duplicate.

2.5. Statistical Analysis

The single cement container was treated as the statistical unit ($n = 3$). Prior to analysis, all data were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. When necessary, data were log-transformed to meet the assumptions of normality and homogeneity. Statistical analyses were performed in R version 4.1.1 (R Core Team, Vienna, Austria, 2024), with significance set at $p \leq 0.05$. A three-way mixed-effects analysis of variance (ANOVA) was conducted using the *lme* function from the *nlme* package to evaluate the main and interactive effects of sampling period, water treatment, and nitrogen treatment, with container included as a random factor to account for variation among replicates. Tukey's Honestly Significant Difference (HSD) test was applied for post hoc multiple comparisons. Principal component analysis (PCA) was performed on centered and standardized ISO and MTs emission data using *FactoMineR* to distinguish BVOC compositional profiles across different sampling periods and treatment combinations. Finally, Pearson's correlation coefficients between BVOC emissions and physiological traits (e.g., photosynthetic parameters and enzyme activities) were calculated using *Hmisc* and visualized with *corrplot*. All data are presented as means $\pm$ standard deviation (SD).

3. Results

3.1. Effects of Water and Nitrogen Treatments on Isoprene and Total Monoterpene Emission Across Two Sampling Periods

O. pinnata was identified as a strict ISO-emitting tree species (MTs were not detected), whereas *P. massoniana* exhibited different BVOC emission profiles across sampling periods. In *O. pinnata*, short-term MD and NSI conditions both stimulated ISO emissions, and nitrogen addition (N80) further amplified these stimulatory effects. In contrast, without nitrogen addition (N0), long-term MD and NSI conditions did not significantly enhance ISO emissions; with nitrogen addition, however, the same treatments significantly suppressed ISO emissions compared with normal irrigation. For *P. massoniana*, MTs were emitted under short-term water and nitrogen treatments, but emissions shifted to ISO under long-term treatments. Specifically, under short-term exposure, MTs emission rates did not differ significantly among water and nitrogen treatments. Under long-term exposure, however, the response of ISO emissions to water treatments varied depending on nitrogen availability (Figure 2).

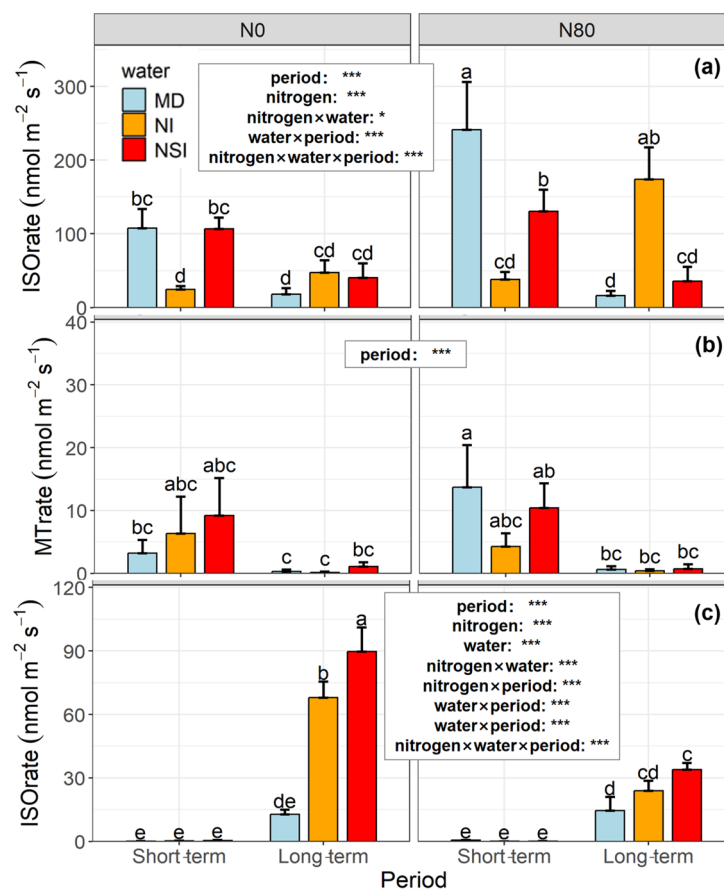


Figure 2. Standardized isoprene emission rate (ISO rate) and monoterpene emission rate (MT rate) of *O. pinnata* (a) and *P. massoniana* (b,c) under three water levels (MD, NI, and NSI) and two nitrogen levels (N0, N80) across two sampling periods (short-term and long-term). Values are means $\pm$ SD ($n = 3$). Different letters for a given species indicate significant differences among all bars (Tukey's HSD test, $p < 0.05$). Asterisks (*, ***) indicate significant effects for $p < 0.05$ and $p < 0.001$, respectively (non-significant effects are not displayed).

3.2. Differences in BVOC Composition Under Short- and Long-Term Water and Nitrogen Treatments

In both sampling periods, *O. pinnata* emitted only ISO and was therefore identified as a strict ISO-emitting tree species. In contrast, *P. massoniana* emitted multiple compounds. The ISO and MT emissions were subjected to multivariate analysis (i.e., PCA) based on

the sampling periods. The PC1 and PC2 components together explained 63.24% of the total variation (Figure 3a). PCA revealed that the BVOC emission profiles from short-term and long-term sampling periods were well-separated in multivariate space, with minimal overlap in the 95% confidence ellipses. The direction and magnitude of the loading vectors (arrows) indicated that MTs were predominantly associated with the short-term sampling group. Specifically, α -pinene, β -pinene, and α -terpinene made significant contributions to PC1 at 25.76%, 21.05%, and 12.09%, respectively, while β -pinene, α -terpinene, limonene, and 3-carene contributed notably to PC2 at 10.11%, 13.36%, 33.78%, and 41.28%, respectively. In contrast, ISO was more strongly associated with the long-term sampling group, contributing 12.22% to PC1 and 1.07% to PC2. This distribution pattern reflects a systematic difference in emission profiles and composition between the two groups, a result further supported by statistical testing (Figure 3b). Specifically, when averaged across all water and nitrogen treatments, α -pinene, β -pinene, and γ -terpinene dominated the emissions in the short-term sampling period, accounting for 78.69%, 10.30% and 4.83% of the total emissions, respectively. In contrast, ISO was the predominant compound during the long-term sampling period, accounting for 97.75% of the total emissions.

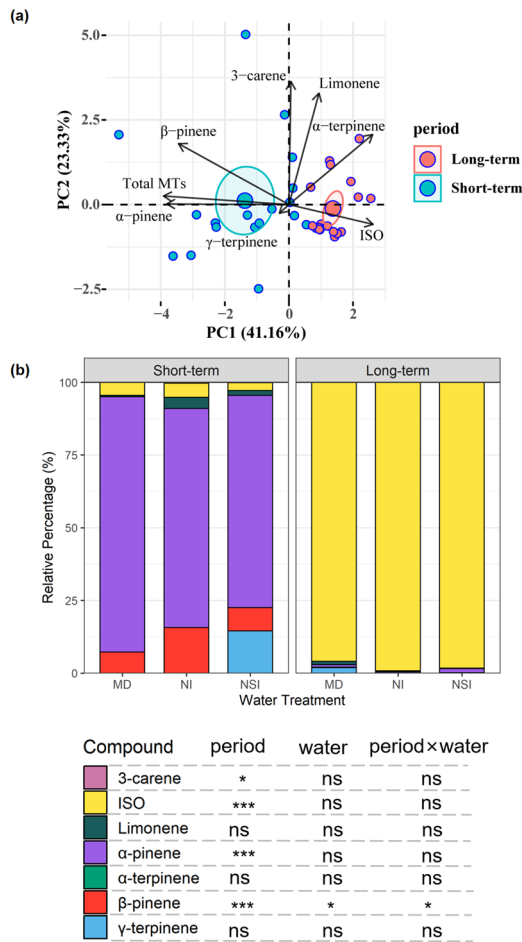


Figure 3. Principal component analysis (PCA) of BVOC emissions from *P. massoniana*. (a) PCA score plot of different BVOCs across sampling periods. Red and blue symbols represent short-term and long-term sampling groups, respectively. The percentage of variance explained by each principal component (PC) is indicated. (b) Contribution of ISO and individual monoterpenes to total BVOC emissions after short-term and long-term water and nitrogen treatments. Total MTs denote the sum of the six monoterpenes listed. The asterisks * and *** indicate significance at $p < 0.05$ and $p < 0.001$, respectively, and “ns” indicates non-significance.

3.3. Effects of Water and Nitrogen Treatments on Leaf Physiological Parameters

At the leaf level, nitrogen treatment had no significant effect on Pn and g_s of *O. pinnata* and *P. massoniana*. However, nitrogen addition (N80) significantly decreased Ci of *O. pinnata* (−9.56%, $p < 0.05$). Similarly, moderate drought tended to reduce g_s and Ci of both *O. pinnata* ($p < 0.05$) and *P. massoniana* ($p < 0.001$) (Table 1). In the long-term sampling stage, *O. pinnata* showed higher Pn (+33.57%), g_s (+95.08%), Ci (+12.91%), and Tr (+211.05%) than in the short-term stage (Table S1), as indicated by the significant main effect of period (Table 1). For *P. massoniana*, although multiple comparisons showed no significant differences in IspS among treatments, its value was still significantly higher in the long-term ($2.84 \pm 0.56 \text{ UI g}^{-1}$) than in the short-term sampling period ($2.25 \pm 0.39 \text{ UI g}^{-1}$). Likewise, MtpS, RuBisCO, and CA activities were all significantly higher in the long-term than in the short-term stage, as shown by the significant main effect of period (Table 1). When averaged within the same sampling period, PEPC activity of *O. pinnata* was higher in the short-term ($0.87 \pm 0.20 \text{ UI g}^{-1}$) than in the long-term sampling period ($0.68 \pm 0.18 \text{ UI g}^{-1}$).

Table 1. Results of analysis of variance (ANOVA) (p value) for the effects of water, nitrogen and sampling period of *O. pinnata* and *P. massoniana* and their interaction on the net photosynthetic rate (Pn), stomatal conductance (g_s), intercellular CO₂ concentration (Ci), transpiration rate (Tr), isoprene synthase (IspS), monoterpene synthase (MtpS), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), carbonic anhydrase (CA), and phosphoenolpyruvate carboxykinase (PEPCK) activities.

Species		Pn	g_s	Ci	Tr	IspS	MtpS	RuBisCO	CA	PEPCK
<i>O. pinnata</i>	P	<0.01	<0.001	<0.01	<0.001	0.890	/	0.411	0.981	<0.01
	W	0.260	<0.05	<0.05	0.075	0.918	/	0.075	0.139	0.379
	N	0.423	0.899	<0.05	0.837	0.205	/	0.006	0.582	<0.05
	W × N	0.09	0.455	0.789	0.437	<0.01	/	0.571	0.910	0.569
	P × N	0.518	0.826	0.597	0.745	0.397	/	0.998	0.890	0.653
	W × P	0.394	0.210	0.122	0.292	0.774	/	0.987	0.687	0.373
	P × W × N	0.297	0.252	0.957	0.165	0.392	/	0.388	0.257	0.781
<i>P. massoniana</i>	P	0.182	0.123	0.233	0.608	<0.01	<0.001	<0.001	<0.01	0.296
	W	0.285	<0.001	<0.001	0.065	0.555	0.707	0.152	0.697	0.028
	N	0.560	0.734	0.713	0.705	0.923	0.340	0.901	0.146	0.564
	W × N	0.975	0.780	0.698	0.662	0.163	0.256	0.053	0.514	<0.01
	P × N	0.910	0.803	<0.05	0.972	0.679	0.117	0.622	0.655	0.928
	W × P	0.824	0.308	0.068	0.877	0.441	0.911	0.673	0.251	<0.01
	P × W × N	0.752	0.972	0.670	0.510	0.406	0.558	0.385	0.538	0.319

Significant effects ($p < 0.05$) are shown in bold; sampling period, P; water, W; nitrogen, N. The slash (/) indicates that data are not available because *O. pinnata* was a non-monoterpene-emitting species.

3.4. Correlations Between BVOC Emissions and Leaf Physiological and Biochemical Parameters

To elucidate the regulation mechanism of ISO and MT emissions after short-term and long-term water and nitrogen treatments, correlations between branch-level BVOC emissions and associated leaf physiological and biochemical parameters were examined for each tree species separately (Figure 4). In *O. pinnata*, Ci was negatively correlated with ISO rate after short-term treatments, whereas no clear relationship was detected during the long-term sampling period. In *P. massoniana*, g_s displayed a slight positive correlation with limonene emission rate in the short-term, while other BVOCs emission showed no significant relationships with measured parameters. After long-term treatment, the ISO emission rate in *P. massoniana* was weakly correlated with Ci. Meanwhile, several photosynthesis-related enzymes displayed weak negative correlations with γ -terpinene and 3-carene emission rates, and IspS activity was negatively correlated with total MT emission rate. No significant correlations were observed between BVOC emissions and Pn, g_s , Tr or MtpS activities in either species.

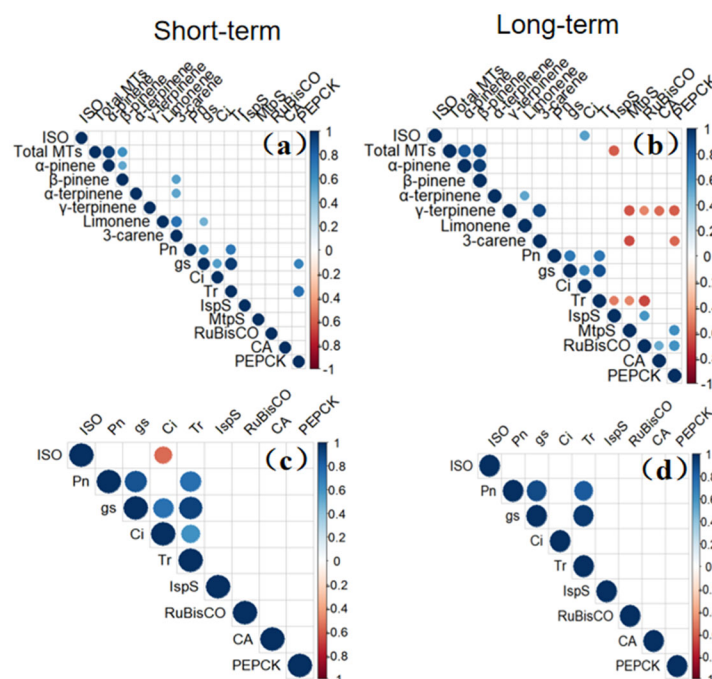


Figure 4. Correlations between different BVOC emissions and leaf physiological and biochemical parameters of *P. massoniana* (a,b) and *O. pinnata* (c,d) after short-term and long-term water and nitrogen treatments. The colors represent the coefficient of correlation between each pair of variables, where blue represents significant positive correlation and red represents significant negative correlation. Statistical significance was set at $p < 0.05$. ISO, isoprene; Total MTs, total monoterpenes; Pn, net photosynthetic rate; g_s , stomatal conductance; Ci, intercellular CO₂ concentration; Tr, transpiration rate; IspS, isoprene synthase; MtpS, monoterpene synthase; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; CA, carbonic anhydrase; PEPCK, phosphoenolpyruvate carboxykinase.

4. Discussion

In this study, the ISO and MTs emissions were explored for the effects of three soil water and two N levels in *O. pinnata* and *P. massoniana* over short- and long-term exposures. Our results revealed distinct species-specific characteristics: *O. pinnata* was characterized as a strict ISO emitter with no detectable MTs, whereas the BVOC profile of *P. massoniana* shifted dynamically from MTs dominance under short-term treatments to ISO emission under long-term treatments (Figures 2 and 3a). Although earlier work by Klinger et al. (2002) [37] reported emissions of both ISO and MTs from these species, our findings are consistent with the broader pattern that most broad-leaved species are primarily ISO emitters, whereas coniferous species prioritize MTs [20,38]—a tendency also observed in *P. massoniana* by Huang et al. (2020) [33]. These emission patterns underscore potential differences in carbon investment strategies and ecological adaptation. Specifically, the strict ISO emission in *O. pinnata* implies a high-turnover metabolic strategy, relying on the rapid de novo synthesis of a highly volatile compound to protect photosynthetic function under environmental change [25]. In contrast to the constitutive defense strategy seen in its initial reliance on stored MTs [39], *P. massoniana*'s shift toward ISO under prolonged stress likely represents a carbon-saving acclimation strategy, highlighting the dynamic plasticity of its BVOC emissions [40].

4.1. Responses of BVOC Emissions to Short-Term Water and Nitrogen Treatments

ISO and MTs, synthesized via the methylerythritol 4-phosphate (MEP) pathway, act as membrane stabilizers and antioxidants, and play an important role in plant tolerance of abiotic stress [11,17,41]. Under short-term drought conditions, branch-level ISO emis-

sions of *O. pinnata* significantly increased in both MD and NSI treatments compared with NI (Figure 2). Similar stimulation of leaf-level ISO emission and photosynthetic carbon assimilation under moderate drought has been reported in hybrid poplar, interpreted as a mechanism to stabilize thylakoid membranes [17,22,41]. In the present study, ISO emission rates of *O. pinnata* were negatively correlated with C_i , suggesting that elevated C_i may reduce dimethylallyl diphosphate (DMADP) availability [42] or limit the supply of phosphoenolpyruvate (PEP) for DMADP synthesis [19,43], ultimately constraining ISO emission (Figure 4). Consistent with these findings, Haworth et al. (2017) [44] observed that moderate drought stimulated ISO emissions in *Arundo donax*, accompanied by enhanced foliar dimethylsulphoniopropionate concentrations, both serving to mitigate excess energy and oxidative stress [45,46]. Nevertheless, in our study, physiological and biochemical factors, including photosynthesis-related enzymes such as IspS and MtpS, could not fully explain BVOC emissions at the branch level (Table S1, Figure 4). Considering that ISO and MT emission capacities differ across leaf ages and positions [19,26], variations in leaf physiological status, potential compensatory effects at the branch scale under different water treatments, and even shading interactions among leaves within a branch may collectively contribute to this discrepancy [27,47]. On the other hand, no significant difference in MTs emissions was detected among different water treatments for *P. massoniana* ($p > 0.05$, Figure 2). This may reflect the differential sensitivity of BVOC classes to water stress, as numerous studies have shown that the availability of primary carbon substrates necessary for MT biosynthesis only declines under severe drought, thereby leading to reduced emission rates [23,48].

With the intensified combustion of fossil fuels and the expanding production and application of nitrogen fertilizers, atmospheric N deposition has emerged as a serious environmental issue in subtropical China. The N load applied in this study ($80 \text{ kg N ha}^{-1} \text{ year}^{-1}$) was comparable to the peak deposition levels reported for some forests in southern China ($30\text{--}73 \text{ kg N ha}^{-1} \text{ year}^{-1}$) [32,34]. Similar to the water treatments, N addition significantly stimulated the ISO emission in *O. pinnata*. This result is consistent with previous findings in poplar, where N fertilization increased ISO emission by 19.6% under N50 ($50 \text{ kg N ha}^{-1} \text{ year}^{-1}$) and by 33.4% under N100 ($100 \text{ kg N ha}^{-1} \text{ year}^{-1}$) compared with the no N load [49]. In contrast, when averaged across all water treatments, N addition had no significant effect on the MTs emission in *P. massoniana* (Figure 2), further supporting the notion that the stimulatory effect of N on ISO emission is generally stronger and more frequent than on MTs [50]. Moreover, several studies have reported that N addition can even suppress MT emission in some tree species [14,51,52]. Beyond compound-specific differences, the contrasting N-fixation traits of the two species may also potentially explain their divergent responses to N addition, as *O. pinnata* is an N_2 -fixing species, whereas *P. massoniana* is a non- N_2 -fixing species, despite limited direct evidence [32].

4.2. Responses of BVOC Emissions to Long-Term Water and Nitrogen Treatments

Under long-term water treatment, *O. pinnata* maintained ISO emissions, whereas *P. massoniana* shifted its emission profile from MTs to ISO. Such changes in BVOC spectra may be attributed to stress-induced selective expression of genes regulating BVOC biosynthesis [53,54]. For example, evidence indicates that abiotic stress commonly shifts BVOC emissions from cyclic to acyclic MTs [55–59]. Both ISO and MTs exert antioxidant functions by quenching ROS to stabilize cellular membranes [53]; however, MTs are often considered more effective antioxidants than ISO in scavenging ROS [54,59]. Thus, the observed transition from MTs to ISO emission under long-term water treatment may represent an adaptive strategy, allowing *P. massoniana* to cope with moderate, chronic abiotic stress by relying on the smaller antioxidant molecule ISO to sustain a low-intensity defense against ROS.

Meanwhile, moderate drought significantly reduced the ISO emission rate of *P. massoniana* (Figure 2).

Although leaf g_s showed a decreasing trend, the large Henry's law constant of ISO and its rapid gas–liquid phase conversion indicate that the increased diffusion resistance caused by stomatal closure is insufficient to fully explain the reduced emission rate. Instead, decreased soil moisture may limit substrate availability for isoprenoid biosynthesis [11,21,60]. Moreover, the declining leaf emission patterns are often linked to inhibition of IspS [9,11,25]. However, in this study, IspS activity did not differ significantly across treatments, and only weak negative correlations were observed between several photosynthesis-related enzymes and γ -terpinene and 3-carene emissions. No significant associations were detected between BVOC emissions and Pn, g_s , Tr, or MtpS activities in either species. Similarly, Otu-Larbi et al. (2020) [16] applied a canopy exchange model to examine the response of ISO emission to heatwave and drought in Wytham Woods, and observed that ISO emission became uncoupled from photosynthesis during periods of drought stress. These findings further support our inference that leaf-level physiological and biochemical traits are insufficient to explain the branch-level ISO and MTs emission patterns. Under long-term water treatments, N addition increased ISO emissions in *O. pinnata* at NI but not at MD or NSI. In contrast, it had no effect at MD but suppressed ISO emissions in *P. massoniana* under NI and NSI. On the one hand, the carbon–nutrient balance hypothesis (CNBH) may partly account for the inhibitory effect of N addition on MTs, as greater N availability could favor carbon allocation to primary metabolism associated with plant growth rather than to carbon-based secondary products such as BVOCs. On the other hand, these divergent responses may also arise from the regulation of terpene synthases or their gene expression [61]. Supporting this, Centritto et al. (2011) [62] showed that ISO-related metabolism in *Populus nigra* can be up- or down-regulated under chronic drought and combined abiotic stresses. However, as our findings are based on an analysis specifically targeted at ISO and MTs, the potential responses of a broader array of BVOCs remain unexplored. Consequently, future research employing untargeted methodologies is encouraged to elucidate the interactive effects of soil water availability and N addition on the full spectrum of BVOC emissions.

5. Conclusions

Our study identified and quantitatively characterized the combined responses of *O. pinnata* and *P. massoniana* to short- and long-term water treatments and simulated nitrogen deposition. Long-term water treatment markedly altered the branch-level BVOC emission profiles of *P. massoniana*, shifting emissions from MTs to ISO, while *O. pinnata* consistently emitted ISO across all treatments and sampling periods. Divergent isoprene emission rates between the two species under varying water and nitrogen conditions were largely driven by differences in N-fixation capacity and the regulation of terpene synthase gene expression. Neither leaf-level physiological nor biochemical traits could fully explain the branch-level BVOC emission characteristics. These findings highlight the importance of paying close attention to potential shifts in plant BVOC emission profiles under long-term water and nitrogen treatments, which is crucial for precise air pollution control and effective climate risk management, given the orders-of-magnitude differences in OH radicals and ozone reactivity among BVOC classes [60]. Furthermore, we emphasize that branch-level emissions cannot be reliably inferred from leaf-level traits alone, and call for large-scale studies across tree species and age classes to clarify scaling relationships from leaves to branches and beyond.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f16111708/s1>, Table S1: physiological parameters of *O. pinnata*

and *P. massoniana* leaves under different water and nitrogen treatments after short-term and long-term exposure.

Author Contributions: Conceptualization, S.L. and Z.Y.; Methodology, S.L., X.L. and Z.Y.; Investigation, Z.Y.; Data curation, S.L.; Writing—original draft, S.L. and Z.Y.; Writing—review and editing, D.Y., X.L., M.L. and Z.Y.; Supervision, M.L. and Z.Y.; Funding acquisition, S.L. and Z.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (grant numbers. 42577271, 32401386, 41877326); Fujian Agriculture and Forestry University Special Fund for Scientific and Technological Innovation (grant numbers. KFB23115; KFB24129) and the Natural Science Foundation of Fujian Province, China (grant number. 2025J08252).

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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