

## Postprint: Quantity and Spectroscopic Characteristics of Leaf Soluble Organic Matter in Competitive and Stress-Tolerant Tree Species in Subtropical Regions

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### Abstract

Under climate change, tree species with different ecological strategies exhibit varying response capabilities to environmental changes, affecting the quantity and quality of DOM (Dissolved Organic Matter) produced through leaf leaching, which in turn influences soil nutrient cycling. By investigating the differences in the quantity and spectroscopic characteristics of leaf DOM among tree species with different ecological strategies in subtropical regions, we evaluated the impacts of DOM inputs with varying quantities and structural features on soil nutrient cycling. This study selected fresh leaves of six tree species for extraction, including three Competitive (C) species and three Stress-tolerant (S) species: *Dendropanax dentiger*, *Machilus grijsii*, *Symplocos cochinchinensis* (Lour.), *Altingia gracilipes*, *Castanopsis fargesii*, and *Castanopsis fabri*. The quantity characteristics of DOM were characterized by Dissolved Organic Carbon (DOC) and Dissolved Organic Nitrogen (DON), while the quality characteristics were assessed through spectroscopic indices including Special Ultraviolet-Visible Absorption (SUVA), Humification Index (HIX), and Fourier-Transform Infrared (FTIR) spectroscopy. The results showed that there was no significant difference in Dissolved Organic Carbon concentrations among leaf leachates from tree species with different ecological strategies; however, the Dissolved Organic Nitrogen concentration in leachates from C-strategy species was higher than that of DON in S-strategy species. Furthermore, the Aromatic Index (AI) and Humification Index (HIX) of S-strategy were both higher than those of C-strategy. The emission fluorescence intensity of C-strategy species was also higher than that of S-strategy, indicating that DOM from C-strategy species had a lower degree of humification and higher content of readily decomposable substances, whereas S-strategy contained more recalcitrant substances with a higher degree of humi-

fication. FTIR spectroscopy results revealed that DOM extracted from leaves of all tree species exhibited similar absorption peaks, with the H-bonded -OH stretching vibration being the strongest, and the spectra of C-strategy species were relatively simpler, which corroborated the findings from fluorescence spectroscopy. Overall, compared with C-strategy, DOM extracted from leaves of S-strategy species had a more complex structure and higher nutrient content. This may be because S-strategy species possess higher adaptability to environmental changes. Due to its relatively complex DOM structure, which slows down soil carbon turnover rates after input into the soil, S-strategy species may be beneficial for soil carbon sequestration under future climate change scenarios.

## Full Text

### Preamble

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### Spectral Characteristics and Quantities of Dissolved Organic Matter Released from Leaves with Competitive and Stress-Tolerant Ecological Strategies in a Subtropical Region

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### Abstract

Plants employ different ecological strategies to respond to environmental stress under global climate change, and the spectral characteristics and quantities of dissolved organic matter (DOM) leaching from leaves depend on the plant's ecological strategy. DOM leaching resulting from different strategies will influence soil nutrient cycling. The objective of this study was to investigate the differences in the spectral characteristics and quantities of DOM derived from leaves of plants with different ecological strategies in a subtropical region.

We sampled fresh leaves from six plant species, including three competitive-type plants (C-strategy) and three stress-tolerant-type plants (S-strategy). The competitive species were *Dendropanax dentiger*, *Machilus grijsii*, and *Castanopsis fargesii*; the stress-tolerant species were *Castanopsis fabri*, *Symplocos cochinchinensis* (Lour.), and *Altingia gracilipes*. We examined dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) to determine the quantities of DOM, and employed Special Ultraviolet-Visible Absorption (SUVA), Humification Index (HIX), and Fourier-Transform Infrared (FTIR) spectroscopy to analyze the spectral characteristics of DOM.

The results showed that there were no significant differences in DOC concentrations between C-type and S-type plants. However, DON was higher in C-type plants compared to S-type plants. In addition, we examined the structural characteristics of DOM between the C- and S-strategies using SUVA, fluorescence, and FTIR absorption spectrometry. The aromatic index and HIX were in the order:  $S > C$ . In addition, the synchronous fluorescence spectra were stronger in C-type compared to S-type plants, which suggests that DOM leaching from the leaves of C-type plants contained more labile materials. The degree of humification was lower in C-type plants, while it was higher in the S-type plants.

FTIR spectroscopy indicated that DOM from different types of leaves exhibited similar absorption peaks, with the strongest absorption arising from the prolonged vibration of H-bonding hydroxyl groups in all cases. This result shows that DOM from C-type plants has a simple structure that is in accord with fluorescence spectra. Overall, DOM from S-type plants was richer in nutrient composition and had a more complex structure compared to that from C-type plants. Our results suggested that S-type plants have better adaptation under environmental stress. Owing to the complex structure of DOM from S-type plants, it would reduce soil carbon turnover after leaching into the soil. Therefore, S-type plants are more effective in the formation of soil carbon sink under climate change.

**Keywords:** CSR theory; dissolved organic matter; fluorescence spectra; aromatic index; humification index

## Introduction

Dissolved organic matter (DOM) is a mixture of organic compounds that can dissolve in water, acid, and alkaline solutions, mainly including dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP) [1-3]. DOM can regulate the balance among various nutrient pools within terrestrial ecosystems and affect material cycling and energy flow between ecosystems [4-5]. In forest ecosystems, DOM originates from litter decomposition, root exudates, and microbial metabolic processes [6-9]. Leaf leaching by rainfall is particularly important, and both the quantity and quality of leached DOM vary according to canopy structure and composition, which in turn differentially stimulates soil organic matter mineralization [10]. Consequently, DOM plays a significant role in terrestrial biogeochemical cycles.

China's humid subtropical region contains the world's largest and most typical evergreen broad-leaved forests, with an average annual rainfall of 1552-2141 mm. The forest coverage area accounts for 45.56% of the national total forest area, making it one of China's most important forest bases [11]. Compared with temperate regions, this region is characterized by high temperatures, high soil weathering degree, and weak erosion resistance. The region's forest tree species are diverse and exhibit different traits [12], resulting in forest ecosystems with higher DOM fluxes than temperate forest ecosystems [13]. Therefore, DOM

may play a more important role in the ecosystem carbon and nitrogen cycles of this region [14].

Climate change, characterized by global warming, is altering global precipitation patterns, with increased precipitation in high-latitude regions and decreased precipitation in subtropical regions, leading to frequent drought and extreme precipitation events [15]. Extreme precipitation changes hydrological processes, soil evaporation, infiltration, and runoff, which subsequently alters plant population and community structure, plant physiological characteristics [16-17], and thus the quantity and quality of DOM leached from leaves. Different chemical compositions and molecular weights of DOM from various plant types can have different effects on soil biogeochemical cycles [18-20].

With advances in chemical analysis techniques, spectroscopic methods such as ultraviolet-visible absorption, fluorescence spectroscopy, and nuclear magnetic resonance have been introduced for DOM chemical composition analysis, becoming a research hotspot [21-22]. For example, Kang et al. [23] studied *Castanopsis carlesii* and Chinese fir by measuring SUVA, humification index, and infrared spectra, finding that *C. carlesii* had higher nutrient content and more complex structure, which was more conducive to organic matter accumulation. Bischoff et al. [24] compared DOM from European birch and spruce, finding that birch-derived DOM contained more labile substances and was more easily decomposed. These studies indicate that differences among tree species affect the content of recalcitrant substances, quantity characteristics, and spectral features of DOM, which may indirectly influence soil nutrient cycling.

Grime [25-26] proposed that vegetation ecological strategies can be divided into three primary types: Competitive (C), Stress-tolerant (S), and Ruderal (R). Different ecological strategies exhibit different response capacities to environmental changes [27]. Previous research has mainly focused on comparing differences between coniferous and broad-leaved tree species [18-19], with few studies investigating DOM leaching from different ecological strategy vegetation types. Under intensifying climate change, studying the quantity and spectroscopic characteristics of DOM leached from different ecological strategy tree species in subtropical regions has important guiding significance for understanding soil biogeochemical cycling.

This study was conducted in the Geshikao Nature Reserve in Sanming, Fujian. Through principal component analysis of leaf traits including specific leaf area and leaf dry matter content, we classified common tree species and selected three competitive strategy species and three stress-tolerant strategy species with large differences. We analyzed the leaching characteristics and spectroscopic features of DOM from leaves to explore how different ecological strategies affect DOM quantity and structure, and their influence on soil nutrient cycling. This research helps reveal how soil biogeochemical cycling in this region responds to climate change.

## 1. Study Site Overview

The study site is located in the Geshikao Nature Reserve in Sanming, Fujian (117°24′-117°27′E; 26°7′-26°10′N), situated in the eastern extension of the Wuyi Mountain range. The area features low mountains and hills with a subtropical monsoon climate, an average annual temperature of 19.5°C, and average annual precipitation of 1700 mm. The reserve contains 1700 species of vascular plants and is divided into twelve associations, including *Castanopsis carlesii* associations and *Castanopsis kawakamii*-*Pinus massoniana*-*Schima superba* associations. Fagaceae (beech family), Lauraceae (laurel family), Theaceae (tea family), and Aquifoliaceae (holly family) are widely distributed in this area [29].

Through principal component analysis of leaf traits from different tree species in our study area, we determined the study species. We selected three competitive strategy species (*Dendropanax dentiger*, *Machilus grisea*, *Castanopsis fargesii*) and three stress-tolerant strategy species (*Castanopsis fabri*, *Symplocos cochinchinensis* (Lour.), *Altingia gracilipes*) with large differences. Ruderal strategy species are herbaceous plants and were not included in this study. The CSR ecological strategies of each species are shown in [Figure 1: see original paper].

## 2. Sample Collection

In August, we surveyed six common tree species in the Geshikao Nature Reserve. We randomly selected healthy mature trees with diameter at breast height (DBH)  $\geq 10$  cm. We climbed to the tree tops and used pruning shears to cut sun-exposed branches. We randomly selected 30-50 healthy, undamaged leaves from each tree, weighed them to obtain leaf fresh weight (LFW, g), then measured leaf area (LA, cm<sup>2</sup>) using a WinRHIZO/WinFOLIA scanner (Régent Instruments, Quebec, Canada). After oven-drying at 70°C for 48 hours, we obtained leaf dry weight (LDW, g) and calculated specific leaf area (SLA, cm<sup>2</sup>/g) = LA/LDW and leaf dry matter content (LDMC) = LDW/LFW [30].

## 3. Laboratory Experiments

We accurately weighed 5.0 g of fresh leaf samples, mixed them with 500 mL of ultrapure water, and extracted DOM following the method of water extraction at room temperature [30-32]. The organic matter in the filtered solution was defined as DOM. DOC concentration was measured using a total organic carbon analyzer (TOC-L CPH/CPN, Shimadzu), and DON concentration was measured using a continuous flow analyzer (Skalar san+++). All filtered solutions were stored at 4°C for analysis.

Aromatic compound content was determined using UV-Vis spectrophotometry. We measured absorbance at 254 nm using a UV-2450 spectrophotometer. Specific UV absorbance (SUVA) was calculated as  $SUVA_{254} = A_{254}/(\text{mg/L})$ , which reflects aromatic compound content and correlates positively with aromaticity

determined by liquid-state  $^{13}\text{C}$  NMR spectroscopy [34]. The aromatic index was calculated following established protocols.

Humification index (HIX) was determined by fluorescence spectroscopy. We used an F-7000 fluorescence spectrophotometer to measure synchronous and emission fluorescence spectra of samples [36]. The humification index characterizes the degree of humification; higher HIX values indicate greater content of humic substances such as condensed aromatic rings and macromolecular compounds. Emission humification index (HIX<sub>em</sub>) was calculated as the ratio of fluorescence intensity at 460 nm to that at 345 nm, while synchronous humification index (HIX<sub>syn</sub>) was the ratio of fluorescence intensity at 435–480 nm to that at 300–345 nm. To improve sensitivity and eliminate carbonate interference, all solutions were adjusted to pH 2 with 2 mol/L HCl before measurement. Since fluorescent substances undergo quenching at high concentrations, resulting in fewer peaks and lower intensity, all samples were diluted to 10 mg/L before spectral analysis.

Fourier-transform infrared (FTIR) spectroscopy was performed by mixing 1 mg of freeze-dried sample with 400 mg of KBr and pressing into pellets at 10 t/cm<sup>2</sup> pressure for 10 minutes. Spectra were recorded using a Nicolet Magna FTIR 550 spectrometer with a scanning range of 4000–400 cm<sup>-1</sup>, with each sample scanned 32 times [37]. The main attributions of infrared absorption peaks are shown in .

Statistical analysis was performed using SPSS 20.0 software. One-way ANOVA was used to test for significant differences in 各项指标 among different tree species, followed by multiple comparisons. Origin 9.0 and SigmaPlot were used for data visualization.

## 4. Results

### 4.1 DOM Concentrations in Different Ecological Strategy Tree Species

The mean DOC concentrations of leaf extracts from C-strategy and S-strategy tree species were 579.01 mg/L and 453.36 mg/L, respectively, with no significant difference between strategies. However, significant differences existed among species: *Machilus grijsii* had the highest DOC concentration, significantly greater than *Dendropanax dentiger* and *Castanopsis faberi*.

The mean DON concentrations of C-strategy and S-strategy tree species were 2.07 mg/L and 1.17 mg/L, respectively, with C-strategy significantly higher than S-strategy. Among species, *Castanopsis faberi* had the highest DON concentration, which was 2.25 times that of *Dendropanax dentiger* [Figure 2: see original paper].

## 4.2 Spectral Characteristics of Different Ecological Strategy Tree Species

The SUVA values (aromatic index) for C-strategy and S-strategy tree species were  $0.94 \text{ L mg}^{-1} \text{ m}^{-1}$  and  $2.25 \text{ L mg}^{-1} \text{ m}^{-1}$ , respectively, with S-strategy significantly higher than C-strategy. *Castanopsis faberi* had the highest SUVA value, while *Machilus grijsii* had the lowest [Figure 3: see original paper].

From the emission humification index perspective, S-strategy tree species showed higher overall humification degree than C-strategy species. *Castanopsis fargesii* had the highest emission humification index value, while *Machilus grijsii* had the lowest. Synchronous humification index results showed S-strategy values generally lower than C-strategy, but the difference was not significant [Figure 4: see original paper].

In emission fluorescence spectra, both strategy types showed absorption peaks at 330 nm, except *Machilus grijsii* which showed a peak at 506 nm. The spectral characteristics were generally consistent across species, but C-strategy tree species showed higher emission fluorescence intensity than S-strategy species, with *Castanopsis faberi* having the highest peak intensity and *Castanopsis fargesii* the lowest [Figure 5: see original paper].

In synchronous fluorescence spectra, both strategy types showed absorption peaks at 286 nm. C-strategy species showed slightly higher fluorescence intensity than S-strategy species, with *Machilus grijsii* having the highest peak intensity [Figure 6: see original paper].

FTIR spectroscopy can accurately monitor vibrations of phenolic and polysaccharide substances in DOM, providing important structural information. The infrared absorption peaks of DOM from different tree species were mainly distributed in the range of  $3300\text{--}3600 \text{ cm}^{-1}$ , with similar peak positions but different absorption rates. For example, *Castanopsis fargesii* showed the highest peak values across all bands in the infrared spectrum, while *Castanopsis faberi* showed the lowest. All six tree species showed a strong, broad absorption peak in the  $3400\text{--}3550 \text{ cm}^{-1}$  range. Although infrared spectra were similar across species, S-strategy tree species showed more peaks in the short-wave region than C-strategy species [Figure 7: see original paper].

## 5. Discussion

### 5.1 Differences in DOM Concentrations Among Different Ecological Strategy Tree Species

Leaf leaching through precipitation is one of the primary sources of DOM in forest ecosystems, and the quality and quantity of leached DOM play important roles in forest soil nutrient cycling [19]. Compared with temperate forest systems, the DOC concentrations of all tree species in this study were higher than those in temperate forests ( $3\text{--}35 \text{ mg/L}$ ) [10]. Lü et al. [10] showed that *Castanopsis carlesii* had higher DOM content than Chinese fir fresh leaves, which

is inconsistent with our results. The discrepancy may be due to differences in leaf quality and structure, as DOM content is related not only to plant growth strategy but also to these physical characteristics.

The average DON concentration of C-strategy tree species was higher than that of S-strategy species. C-strategy species are fast investment-return types [41-43] that invest more nutrients into soil through leaching during growth, accelerating nutrient cycling. In contrast, S-strategy species are slow investment types [41-43] that primarily use nutrients for self-growth and organ construction, resulting in less DOM leaching. Kang et al. [21] found that *C. carlesii* plantations were more conducive to soil nitrogen accumulation. From an ecological strategy perspective, C-strategy species may increase soil nitrogen availability more than S-strategy species.

## 5.2 Spectral Characteristics of Different Ecological Strategy Tree Species

Wieder et al. [13] showed that DOM leached from different vegetation types interacts differently with soil minerals due to its chemical properties, significantly affecting soil labile carbon pools. Although DOM quantity showed no significant difference between strategies in our experiment, DOM quality may be the key factor affecting its participation in soil biogeochemical cycling.

Higher aromatic and humification indices indicate that DOM contains more condensed aromatic rings and recalcitrant components [38]. S-strategy leaf extracts had higher aromatic and humification indices than C-strategy extracts, indicating more unsaturated bonds and aromatic compounds that may be unfavorable for microbial activity and metabolism [45]. However, this characteristic is beneficial for soil fertility maintenance and carbon sink formation [44].

Synchronous and emission fluorescence spectra revealed that characteristic peaks of all six tree species mainly appeared at  $\lambda_{ex}$  278-285 nm (representing protein-like groups) [46], indicating that DOM was rich in protein groups but contained fewer lignin or aliphatic groups. C-strategy species showed stronger synchronous fluorescence intensity than S-strategy species, suggesting more protein groups and labile substances in C-strategy DOM. Higher fluorescence intensity indicates simpler molecular structures and more easily decomposable organic matter. Kaiser et al. [47] found that shorter wavelength peaks in emission fluorescence spectra represent simple, low-condensation substances, while longer wavelength peaks represent the opposite. This further demonstrates that S-strategy species in our study area had higher humification degrees with more condensed aromatic ring substances and macromolecules than C-strategy species, while C-strategy species had lower humification degrees with more abundant labile substances.

These results are consistent with aromatic index, humification index, and synchronous fluorescence spectra. Lü et al. [10] also found that *C. carlesii* leaf extract DOM had a more complex structure than Chinese fir. The more com-

plex DOM structure from S-strategy species may be more conducive to soil humus formation, as these species tend to store nutrients for long-term growth. In contrast, C-strategy species are fast investment-return types with more labile components that can be easily utilized by soil microorganisms and plant roots, promoting rapid tree growth. This aligns with Grime' s definition of C-strategy species having relatively high resource acquisition capabilities [48]. According to CSR theory, S-strategy species have stronger resistance capabilities when facing environmental changes, which may have important impacts on soil ecosystem carbon cycling processes under future altered hydrothermal conditions.

FTIR spectroscopy can accurately monitor vibrations of phenolic and polysaccharide substances in DOM. Our results showed similar FTIR spectra across tree species, with the strongest absorption in the 3400-3550  $\text{cm}^{-1}$  range, attributed to O-H stretching vibrations of hydroxyl groups in structural and non-structural carbohydrates. The absorption peak at 1629-1700  $\text{cm}^{-1}$  corresponds to C=O stretching vibrations in organic carboxylates and lignin aromatic rings. Absorption peaks at 1000-1260  $\text{cm}^{-1}$ , 1145-1149  $\text{cm}^{-1}$ , and 600-870  $\text{cm}^{-1}$  indicate the presence of alcohols and phenols in leaf leachate DOM. Stronger vibrations in the low-frequency region of S-strategy species suggest more macromolecular functional groups, consistent with results from aromatic index, humification index, and fluorescence spectra. Similar results were reported by Zhou et al. [37] and Bu et al. [38], who found that DOM with high aromaticity and humification had high saturation degrees, fewer aromatic unsaturated substances, and was rich in carbohydrates, carboxyl, or hydroxyl substances.

The structural differences in DOM from different tree species are substantial, and complex physical and chemical reactions occur after DOM enters the soil system. S-strategy species have better adaptation capabilities in subtropical regions experiencing frequent drought and heavy rainfall [28]. Due to their complex DOM structure, S-strategy species may play important roles in forest carbon sinks under future climate scenarios. However, since FTIR spectroscopy only provides semi-quantitative analysis [49] and our results showed differences in absorption values but similar peak positions among species, more precise spectroscopic techniques should be applied in future research to determine the chemical structure of functional groups in leaf leachate DOM from different tree species.

## 6. Conclusion

This study compared the quantity and spectral characteristics of DOM from different ecological strategy tree species. C-strategy species had higher DON concentrations, while S-strategy species showed higher aromatic and humification indices, more complex DOM structures, and stronger resistance to environmental change. The complex structure of DOM from S-strategy species would reduce soil carbon turnover rates after leaching into soil, making them more effective for soil carbon sink formation under climate change scenarios. In contrast, C-strategy species provide larger inputs of labile DOM that can be more

quickly utilized by soil microorganisms, accelerating nutrient cycling and promoting plant growth, which may be beneficial for maintaining soil fertility in the short term.

Under the background of dramatic global climate change, different ecological strategy vegetation types have different response capacities to environmental changes, leading to changes in the proportion of DOM input to soil, which may ultimately affect soil biogeochemical cycling. When facing disturbances, S-strategy species show higher adaptability than C-strategy species. Due to their relatively complex structure, DOM from S-strategy species slows soil carbon turnover after input. Therefore, under future climate change scenarios, S-strategy species may be more favorable for forest carbon sequestration.

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