Do long-term high nitrogen inputs change the composition of soil dissolved organic matter in a primary tropical forest?

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Keywords: soil carbon sequestration, nitrogen addition, water-extractable organic matter, FT-ICR MS, tropical forest, dissolved organic carbon

Abstract
Dissolved organic matter (DOM) plays a key role in forest carbon biogeochemistry by linking soil organic carbon (SOC) sequestration and water fluxes, which is further shaped by elevated atmospheric nitrogen (N) deposition. Although enhanced SOC sequestration was evidenced in tropical forests due to rising N deposition, it remains unclear how long-term N inputs affect soil DOM composition, which regulates SOC sequestration capability due to its mobility and biological instability. Here, the quantity, optical properties, and molecular-level characteristics of soil DOM based on a simulative N deposition experiment with four N addition levels (0, 5, 10, and 15 g m⁻² yr⁻¹) were studied in a primary tropical forest in south China. Results showed that 18 year N additions significantly altered soil DOM composition, with an increasing trend in soil dissolved organic carbon content. Medium- (10 g m⁻² yr⁻¹) and high-N addition (15 g m⁻² yr⁻¹) markedly elevated DOM average molecular weight by 12% and aromaticity, with specific ultraviolet absorbance at 254 nm increasing by 17%, modified aromatic index by 35%, and condensed aromatics by 67%. Medium- and high-N addition also increased recalcitrant DOM components but decreased other DOM components, with increasing percentages of lignin-like, tannin-like, and carboxylic-rich alicyclic molecule-like compounds, and decreasing percentage of more bioavailable contributions with H/C ratio > 1.5. Importantly, significant correlations of the SOC content of the heavy fraction with optical properties and with recalcitrant DOM components were observed. These findings suggest that long-term N additions may alter soil DOM composition in a way to benefit soil OC storage in the primary tropical forests. It merits focusing on the mechanisms to association of soil DOM dynamics with SOC sequestration.

1. Introduction

Forest soils, as the largest terrestrial soil organic carbon (SOC) reservoir, contain 70% of carbon (C) in forest ecosystems and even a small change could cause vast perturbation in atmospheric CO₂ concentration (Pan et al 2011, Cremer et al 2016, Pugh et al 2019). Concurrent global changes, such as increasing nitrogen (N) deposition caused by human activities, remain weighty stressors to forest ecosystems worldwide (Canfield et al 2010, Clark et al 2019, Stevens 2019). In forests, the total N deposition amount ranges from 1.5 to 3.1 g m⁻² yr⁻¹ at a global scale, and the value could increase 3–4 fold in hot spot regions of N deposition, such as southern China (Schwede et al 2018, Wen et al 2020). Low-N deposition could increase soil N availability and potentially increase primary production, but high-N deposition might threaten forest structure and function, such as a decrease in species diversity and soil
pH (De Vries et al. 2014, Du and de Vries 2018, Borer and Stevens 2022). Several studies have been done concerning the impacts of increased N deposition on SOC stocks, as well as dissolved organic matter (DOM), but these estimates remain largely uncertain (Tian et al. 2015, Wiesmeier et al. 2019, Nottingham et al. 2020, Xu et al. 2021).

Studies on soil DOM composition might help better understand SOC formation because DOM is the most reactive and bioavailable fraction of soil organic matter (SOM), whose dynamics are related to the potential stability of SOC (Kramer and Chadwick 2018, Hasegawa et al. 2021, McDonough et al. 2022, Mo et al. 2022). Although soil DOM accounts for only a small fraction of SOM, it consists of various of reactive and mobile organic molecules (Kramer and Chadwick 2018). These mixed components regulate plant growth, soil respiration, and biogeochemical and energy fluxes in forest ecosystems (Wu et al. 2018, Huang et al. 2020). For example, labile components (e.g. simple carbohydrates) of soil DOM could fuel microbial activity and increase mineralization by microbes, whereas antimicrobial agents (e.g. tannins) might cause opposite effects (Pace et al. 2004, Daglià et al. 2012). Moreover, soil dissolved organic carbon (DOC) held by mineral colloids is considered as an important mechanism for long-term SOC sequestration in wet forested biomes (Lu et al. 2013, Kramer and Chadwick 2018). Therefore, identifying soil DOM storage and composition parameters, especially under global change scenarios, is necessary.

Studies have increasingly explored the impacts of N deposition on soil DOM quantity and composition in forest ecosystems in recent decades (Monteith et al. 2007, Evans et al. 2008, Pisci et al. 2015, Bowden et al. 2019, Wang et al. 2019, 2022). Specifically, increased effects of N addition on soil DOC and dissolved organic nitrogen (DON) concentrations have often been reported, while DOC/DON ratio could be further decreased with N addition (Fang et al. 2009, Pisci et al. 2015). Advanced techniques such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) have been used to characterize the detailed soil DOM composition in recent years (Cai et al. 2020, Wang et al. 2020, McDonough et al. 2022). For example, Wang et al. (2020) reported that N addition increased DOM molecular size and aromaticity, and differentially affected the abundance of soluble and recalcitrant DOM components in temperate forest soils. Nevertheless, previous studies had three limitations. (a) Most previous studies were usually of much shorter N treatment duration (<10 years), and the results could not reflect the long-term responses of soil DOM. For example, increased and no obvious effects of 1 year and 7 year N addition on soil DOC concentration were observed in similar forested ecosystems of the upper Great Lakes Region (Currie et al. 1996, Sinsabaugh et al. 2004). (b) Past studies did not distinguish the effects between low-N addition rate and high-N addition rate because they generally selected only one N addition rate (Monteith et al. 2007, Pisci et al. 2015, Wang et al. 2019, Wilcots et al. 2022). Notably, there has not been enough field-based data using FT-ICR MS and spectroscopic analyses to reveal soil DOM composition dynamic as these advanced techniques have been mainly used in aquatic systems—until now (Wang et al. 2020, McDonough et al. 2022). (c) Current studies of soil DOM with N addition mainly focused on boreal or temperate climatic regions, where forest growth was usually constrained by N supply. Tropical forests, covering less than 10% of the Earth’s land area, store about 40% of terrestrial vegetation carbon (Baccini et al. 2017). Therefore, there is an urgent requirement to understand the dynamics of soil DOM with N addition and the underlying mechanism in tropical forests, where most ecosystems are N-saturated or N-rich.

Tropical forests in south China have experienced pronounced high-N deposition over the past decades (Wen et al. 2020). High N deposition accelerates SOC sequestration in tropical forests, but the patterns and composition of soil DOM with N addition remain unclear (Lu et al. 2014, Wang et al. 2018, Lu et al. 2021). Here, the effects of 18 year N addition on soil DOM quantity and its component characteristics using optical spectroscopy and FT-ICR MS analysis in an intact tropical forest have been reported. It was hypothesized that long-term high N inputs would alter soil DOM composition, which may benefit SOC sequestration in the primary tropical forests, considering that long-term N addition can increase DOM molecular size and aromaticity and affect the recalcitrant components (Wang et al. 2020).

2. Materials and methods

2.1. Experimental site information and treatments

The experiment was performed at the Dinghushan Forest Ecosystem Research Station (DFERS), which is located in Guangdong Province of southeast China (112°10’E, 23°10’N). The topographic relief illustrates little variation, with elevation ranging from 250 to 300 m. A tropical humid monsoon climate dominates this region, and the growing season is from April to early October. Mean annual temperature and mean annual precipitation were 22.2 °C and 1896 mm (data of DFERS from 2002 to 2019), respectively. The soil is lateritic red soil (FAO soil classification system), and content of clay, silt, and sand in the 0–10 cm soil layer is 18%, 61%, and 19%, respectively (Su et al. 2021).

In 2002, three random blocks were established in a monsoon evengreen broad-leaved forest, and N addition treatments were initiated in July 2003. Each block consists of one 10 × 20 m control plot (0 g N m⁻² yr⁻¹) and three 10 m × 20 m N-added plots with Low-N (5 g N m⁻² yr⁻¹), medium-N (10 g N m⁻² yr⁻¹) and high-N addition
(15 g N m\(^{-2}\) yr\(^{-1}\)) treatments, respectively, above the background N deposition. These N addition rates were based on the current atmospheric N deposition rate (about 4.9 g N m\(^{-2}\) yr\(^{-1}\) for total N deposition), and the expected increase caused by human activities (Clark \textit{et al.} 2019, Lu \textit{et al.} 2021). NH\(_4\)NO\(_3\) pellets were fully dissolved in purified water and were sprayed to the soil surface monthly in N-added plots, while the control plots received equal volumes of purified water. This primary forest ecosystem was N saturated and could be considered as an N-rich ecosystem as well as tropical forests (Mo \textit{et al.} 2007, Lu \textit{et al.} 2018).

### 2.2. Soil sampling and measurements

In September 2020 (i.e. after 18 successive years of N treatment), samples of 0–10 cm soil were collected from each plot. Three soil cores were collected using a soil auger (3 cm diameter) and mixed into one composite sample in situ. All fresh soil samples were passed through a 2 mm strainer and each soil sample was divided into two soil subsamples, which were stored in a 4 °C refrigerator or air-dried until further processing and analysis.

Soil-derived DOM from the control and N addition plots were extracted with Milli-Q water at a soil/water (m/v) ratio of 1: 40. Briefly, ~5 g of air-dried soil sample was mixed with 200 ml Milli-Q water, and shaken at 150 rpm for 2 h at room temperature. The supernatant was further filtered and then stored for processing analyses. Electrical conductivity (EC) and pH of the soil supernatant were determined using an HQ440d meter (HACA, Loveland, USA). Contents of DOC and dissolved total nitrogen (DTN) were determined using a TOC/TN analyzer (TOC-L CSH/CSN, Shimadzu, Tokyo, Japan). Additionally, SOM was separated by density into heavy and light fractions, and the fraction \(\geq 1.7 \text{ cm}^{-3}\) was collected as heavy fraction, while the fraction \(<1.7 \text{ cm}^{-3}\) was collected as light fraction (Lu \textit{et al.} 2021). Both fractions were dried and ground to determine the OC content. Soil microbial biomass carbon (MBC) was measured using the fumigation extraction method (Niu \textit{et al.} 2021).

### 2.3. Optical characterization and mass spectrometry analysis of soil DOM

To obtain the optical properties of soil DOM, absorption spectra and fluorescence excitation-emission matrix (EEM) spectra of soil supernatants were analyzed using a spectrophotometer (Aqualog®, Kyoto, Horiba). The EEM data was corrected based on background and inner-filter effects and transformed into Raman unit (R.U.). Specific ultraviolet absorbance (UV) at 254 nm, was calculated by dividing the UV absorbance by the DOC content. As indicators for the averaged DOM molecular size, spectral slope ratio (S\(_R\)) was calculated as the slope ratio of UV absorbance at 275–295 nm to UV absorbance at 250–400 nm, while the E2/E3 ratio was calculated by dividing the UV absorbance at 254 nm by the absorbance at 365 nm. Similar to the E2/E3 ratio, the S\(_R\) value was negatively correlated with the averaged DOM molecular size. As an indicator referring to the freshness of the DOM, \(\beta\text{lo}\) ratio was calculated by dividing the maximum emission intensity between 420 and 435 nm with 310 nm excitation into the emission intensity at 380 nm. The fluorescence index (FI) and humification index (HIX) were also calculated, which reflect the DOM source and humification degree, respectively. The FI was calculated as the fluorescent signal ratio of emission wavelength at 470 nm to that at 520 nm at a fixed excitation wavelength of 370 nm, while HIX was calculated as the emission intensity area of 300–345 nm divided by that of 435–480 nm with a fixed excitation wavelength of 254 nm. The maximum fluorescence intensity of the PARAFAC-resolved DOM component \(i\) was recorded as Fmax\(_i\), and its relative abundance was calculated as \(\%\text{Ci} = \text{Fmax}\(_i\)/\(\sum_i\)Fmax\(_i\).

To obtain molecular-level DOM information, soil extracted samples were further analyzed by fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Each composite sample was acidified (pH = 2) with concentrated formic acid for solid-phase-extracted (SPE) with PPL cartridges. After being eluted and diluted by methyl alcohol at room temperature, SPE DOM was further injected into the electrospray ionization source with negative-mode at a flow rate of 2500 \(\mu\text{l}\) h\(^{-1}\). The instrument type was 9.4 T Apex-ultra FT-ICR MS instrument (Bruker Daltonics, Billerica, MA, USA), and the detailed parameters were set according to the method by Wang \textit{et al.} (2020). The raw data were converted to peak lists using Bruker Daltonics (v5.04) software, while Formularity software was used to analyze elemental formula with constraints of C\(_{0–100}\)H\(_{0–200}\)O\(_{0–30}\)N\(_{0–10}\)S\(_{0–2}\), H/C < 2.5, O/C < 1.2, N/C < 0.5, and S/C < 0.2. The double bond equivalents (DBE) and modified aromaticity index (Al\(_{\text{mod}}\)) were calculated to evaluate the unsaturation degree and aromaticity of each formula. Using the intensity weighted methods, we calculated the average molecular weight, H/C, O/C, N/C, DBE, and Al\(_{\text{mod}}\) indices. The ratios of H/C and O/C could be used to describe the degree of hydrogenation and oxygenation, respectively. The percentage of more bioavailable contributions above the molecular lability boundary (\(\%\text{MLB}_i\)) was calculated as the relative abundance of the formula with an H/C > 1.5. Referring to assigned criteria of previous studies (Koch and Dittmar 2006, Wu \textit{et al.} 2018), we defined several biochemical groups as follows: (a) lignin-like
(0.25 < O/C ≤ 0.67); (b) lipid-like (0 ≤ O/C < 0.2, 1.5 ≤ H/C ≤ 2.3, and N/C ≤ 0.04); (c) dissolved black carbon (DBC)-like (AI
mod > 0.67 and C ≥ 15); (d) tannin-like (0.53 ≤ H/C ≤ 1.5); (e) protein-like (0.2 ≤ O/C ≤ 0.52, 1.5 ≤ H/C ≤ 2.2, and 0.178 ≤ N/C ≤ 0.44); (f) carbohydrate-like (0.7 ≤ O/C ≤ 1.1, 1.5 ≤ H/C ≤ 2.4, and N = 0); and (g) amino-sugar-like (0.52 ≤ O/C ≤ 0.7, 1.5 ≤ H/C ≤ 2.2, and 0.07 ≤ N/C ≤ 0.182) compounds. In addition, the carboxylic-rich alicyclic molecule (CRAM)-like compounds were calculated with constraints of 0.3 ≤ DBE:C ≤ 0.68, 0.20 ≤ DBE ≤ 0.95, and 0.77 ≤ DBE-O ≤ 1.75.

2.4. Statistical analyses

One-way ANOVA with Tukey HSD test was used to examine the differences between control and N addition treatments for soil DOM properties and optical parameters, while the non-parametric Kruskal–Wallis with Dunn's test was also used if data could not meet the normality test. To show shifts in optical characteristics of soil DOM at different N addition rates, principal component analysis (PCA) followed by permutational multivariate ANOVA with Euclidean distance were performed using the 'Vegan' and 'FactoMineR' packages. Pearson correlation coefficient was used to illustrate the relationships of these nine DOM optical parameters with principal component axes and soil DOM properties. Planned contrast analysis was further used to test differences between N-added plots and control plots. Statistically significant differences were set with values of p < 0.05, unless otherwise stated. For molecular-level DOM information, there was only one dataset for each treatment. The difference was 'considerable' between N addition and control treatments if their absolute difference value was more than 20%. Statistical analyses were performed in R 4.1.3.

3. Results

3.1. General water properties of soil water extracts

Long-term N addition consistently increased the soil DOC content and changed the DOC/DTN ratio, with an average DOC of 0.45–0.65 mg g⁻¹. N addition also significantly increased the SOC content and MBC/SOC ratio (table 1). No significant effects of decadal N addition were observed on soil EC, soil DTN content, OC content in light fraction (LF-OC), and ratios of DOC/SOC and DON/TN (table 1). Moreover, long-term N addition consistently increased SOC content in heavy fraction (HF-
OC) as compared with control plots, and these effects were significant (p < 0.05) at medium- and high-N addition levels (table 1). However, long-term N addition consistently decreased soil pH value and the LF-OC/HF-OC ratio (table 1).

3.2. Optical characteristics of soil DOM composition

Overall, decadal N addition significantly altered the optical characteristics of soil DOM based on PCA, while medium- and high-N addition had greater influences on indices of SUVA254, Sα, E2/E3, β/α, FI, FmaxC1, and FmaxC2 than indices of HIX and FmaxC3 as displayed by the distribution along PC1 and different correlations of these indices with PC1 and PC2 (figure 1; table 2). Specifically, high-N addition significantly increased values of HIX and SUVA254 by 140.1% and 20.0% as compared to the control, indicating that DOM with N addition treatments had higher humification and aromaticity degree (table 1). In contrast, lower values of Sα, E2/E3, β/α, and FI were observed in N-added plots, which reflected that N addition increased the overall molecular size of soil DOM (reflected by Sα and E2/E3), but decreased the contributions of low-molecular-weight components and fresh microbial matter to DOM (reflected by β/α and FI). Significant negative correlations of DOC, DON, and SUVA254 with Sα, E2/E3, β/α, and FI, as well as HIX with Sα were observed, whereas positive correlations of soil pH with E2/E3, β/α, Sα, and FI were observed (figure 3).

Three PARAFAC components were identified from EEM data as follows: C1, terrestrial-humic-like-I component; C2, terrestrial-humic-like-II component, and C3, protein-like DOM components (figure 2(A)). The relative abundances and absolute contents of these components were represented using %C1-%C3 and Fmax C1-C3, respectively (figures 2(B) and (C)). Medium- and high-N addition treatments generally increased the %C1 and %C2 and decreased the %C3 (figure 3(B)), while FmaxC1 and FmaxC2 were also elevated in N addition plots compared with that in control plots (figure 3(C)). Different from FmaxC1 and FmaxC2, the Fmax 3 were elevated under low- and medium-N addition but were reduced under high-N addition (figure 3(C)). Notably, the overall N addition effects on FmaxC1-C3 were usually significant at p < 0.1, and only medium-N addition effects were significant at p < 0.05 when using multiple comparisons (figure 3(C)). Moreover, Pearson correlation analyses indicated significant negative correlations of FmaxC1 and FmaxC2 with soil pH, E2/E3, β/α, Sα, and FI, as well as positive correlations of FmaxC3 with DON content and EC (figure 3).

3.3. Molecular signature of soil DOM composition by FT-ICR MS analysis

Compared with the control treatment, N addition altered soil DOM composition from a molecular perspective, with values of most molecular signatures decreasing or increasing by 20% and more (table 3).
Table 1. General water and optical properties of soil water extracts.

|                      | Control     | Low-N      | Medium-N   | High-N     | p value in Contrast test |
|----------------------|-------------|------------|------------|------------|--------------------------|
| pH                   | 3.86(0.01)a | 3.78(0.03)ab| 3.66(0.07)b| 3.63(0.03)b| 0.01                     |
| EC (µS cm⁻¹)         | 27.10(0.39)a| 25.84(2.45)a| 33.83(3.69)a| 27.00(1.37)a| 0.25                     |
| DOC (mg g⁻¹)         | 0.45(0.03)a | 0.52(0.06)a | 0.65(0.05)a | 0.56(0.03)a | 0.08                     |
| DTN (mg g⁻¹)         | 0.06(0.00)a | 0.05(0.00)a | 0.08(0.00)a | 0.06(0.00)a | 0.34                     |
| SOC⁺ (mg g⁻¹)        | 30.21(0.94)a| 35.57(2.23)ab| 37.91(2.52)ab| 38.34(2.12)b| 0.06                     |
| MBC/SOC              | 1.58(0.04)a | 1.02(0.07)b | 0.81(0.06)bc| 0.62(0.03)c | 0.001                    |
| DOC/DTN              | 1.51(0.11)a | 1.46(0.10)a | 1.73(0.17)a | 1.49(0.10)a | 0.07                     |
| DOC/SOC (%)          | 1.50(0.12)a | 1.46(0.10)a | 1.74(0.17)a | 1.49(0.10)a | 0.48                     |
| DTN/TN (%)           | 2.72(0.08)a | 1.95(0.14)a | 2.76(0.35)a | 2.09(0.08)a | 0.11                     |
| LF-OC⁺ (mg g⁻¹)      | 7.38(0.47)a | 7.22(0.36)a | 7.12(0.54)a | 6.51(0.33)a | 0.68                     |
| HF-OC⁺ (mg g⁻¹)      | 22.83(1.24)b| 27.84(1.93)ab| 30.79(2.37)a| 31.70(1.43)a| 0.04                     |
| LF-OC⁺/HF-OC⁺ (%)     | 0.32(0.03)a | 0.26(0.01)ab| 0.26(0.01)b | 0.21(0.01)b | 0.01                     |
| SUVA₂₅₄ (L mg C⁻¹ m⁻¹) | 1.37(0.07)ab| 1.26(0.07)b | 1.58(0.09)ab| 1.63(0.06)a | 0.04                     |
| Sₙ                   | 0.80(0.00)a | 0.78(0.01)a | 0.76(0.01)a | 0.75(0.01)a | 0.08                     |
| E₂/E₃                | 5.22(0.06)a | 5.26(0.07)a | 4.79(0.12)b | 4.82(0.02)b | 0.01                     |
| β/α                  | 0.63(0.02)a | 0.58(0.02)a | 0.55(0.05)a | 0.52(0.01)a | 0.21                     |
| FI                   | 1.63(0.02)a | 1.60(0.01)a | 1.55(0.04)a | 1.53(0.01)a | 0.08                     |
| HIX                  | 0.65(0.01)a | 0.67(0.02)ab| 0.69(0.01)ab| 0.83(0.02)b | 0.09                     |

Notes: EC, electrical conductivity; DOC, dissolved organic carbon; DTN, dissolved total nitrogen; SOC, soil organic carbon; MBC, microbial biomass carbon; SUVA₂₅₄, specific ultraviolet absorbance at 254 nm; Sₙ, slope ratio; β/α, freshness index; FI, fluorescence index; HIX, humification index. Data were shown as mean value with SE in parentheses. Lowercase letters indicate significant difference among different treatments at p ≤ 0.1. * data citing from Lu et al. (2021).

Figure 1. Effects of N addition on optical properties of soil DOM. Nine optical properties were used to assess these effects at different N addition levels, and each level was summarized as a single point by PCA (n = 12). Results of permutational multivariate ANOVA test are attached to the lower right corner.
Table 2. Pearson correlations between relative abundance of specific optical properties and PC axes \((n = 12)\).

| SUVA\(_{254}\) | \(S_R\) | \(E2/E3\) | \(\beta/\alpha\) | FI | HIX | FmaxC1 | FmaxC2 | FmaxC3 |
|----------------|--------|----------|----------------|----|-----|--------|--------|--------|
| PC1            | −0.93*** | 0.89***  | 0.93***        | 0.90*** | 0.96*** | −0.49 | −0.96*** | −0.94*** | −0.22 |
| PC2            | −0.07   | 0.15     | −0.10          | −0.08 | −0.02 | −0.86*** | 0.13   | 0.11   | 0.97*** |

Notes: ***Statistically significant at \(p \leq 0.001\).

On average 3683 molecular formulae were identified for each soil sample, but only 1720 could be found in all samples with diversified abundances (table 3). The average molecular weight and abundance of CHON (%) were elevated by medium- and high-N addition, while the average formula was altered by N addition treatments, with an average H/C decreasing by 9.0%, average O/C and average N/C increasing by 18.3% and 26.8%, respectively (table 3). Moreover, the relative abundance of CHO was consistently lower with N addition, while that of CHOS increased in low-N addition plots but decreased in medium- and high-N addition plots (table 3). Low-N addition slightly decreased average \(A_{\text{mod}}\) and DBE values, but medium- and high-N addition increased these values by 34.5% and 32.6%, respectively (table 3). Similar effects were also observed for condensed aromatics, and these effects on average \(A_{\text{mod}}\), DBE, and condensed aromatics indicated that the formation and accumulation of aromatic C and double bonds varied with N addition rates (table 3). The abundance of CRAM was elevated by medium- and high-N addition, while the %MLB\(_L\) decreased in the same N addition plots (table 3). These effects of medium- and high-N addition were contrary to the effects of low-N addition (table 3).

Long-term N addition altered the relative abundances of biochemical components, and these effects varied with N addition dose (figure 4). Specifically, low-N addition decreased the relative abundances of lignin-like, tannin-like, DBC-like, and amino sugar-like components as compared with control plots, which were opposite to the effects of medium- and high-N addition (figure 4). The relative abundances of lipids-like component increased with low-N addition, and decreased with medium- and high-N addition (figure 4). Long-term N addition consistently increased the relative abundances of carbohydrate-like component, and decreased those of protein-like component regardless of the N addition dose (figure 4).
Figure 3. Pearson correlations among optical properties of soil DOM and soil properties. Only significant cases were shown using blue and red circles.

Table 3. Molecular characterization of SPE-isolated soil DOM as determined by FT-ICR MS.

|                      | Control | Low-N | Medium-N | High-N |
|----------------------|---------|-------|----------|--------|
| No. of assigned formulae | 2782    | 2365  | 4226*    | 5902*  |
| Average molecular weight (Da) | 346.3   | 341.9 | 376      | 397.4  |
| Average formula       | C_{18.18}H_{25.41}O_{6.17} | C_{18.39}H_{25.94}O_{5.73} | C_{18.35}H_{23.39}O_{7.90} | C_{18.10}H_{21.06}O_{8.2} |
| Average H/C           | 1.411   | 1.442 | 1.269    | 1.143  |
| Average O/C           | 0.343   | 0.319 | 0.430*   | 0.468* |
| Average N/C           | 0.009   | 0.006* | 0.013*   | 0.014* |
| Average DBE           | 6.536   | 6.247 | 7.960*   | 9.379* |
| Average A_{inod}      | 0.228   | 0.215 | 0.281*   | 0.334* |
| CRAM (%)              | 36.9    | 33.3  | 46.5*    | 52.3*  |
| MLR_{c} (%)           | 44.8    | 50.2  | 30.9*    | 19.1*  |
| CHO (%)               | 84.8    | 79.4  | 83.5     | 77.9   |
| CHON (%)              | 8.6     | 5.7*  | 14.9*    | 16.8*  |
| CHOS (%)              | 6.5     | 14.9* | 16.5*    | 5.3    |
| Condensed aromatics (%) | 2.1     | 1.9   | 2.8*     | 4.2*   |

Notes: * indicate that the differences between N addition treatments and control treatment were 'considerable significant' as their absolute difference value was more than 20%.
4. Discussion

We found a slight increase in the soil DOC content and changes in the DOC/DON ratio, but no obvious changes in the DOC/SOC ratio with N addition (table 1). Two specific mechanisms might explain this point. First, N-induced a decrease in soil pH and the increase in soil iron and aluminum might stabilize more DOC via ligand exchange reactions because of many newly formed mineral colloids with N addition (Lu et al. 2014, Tian and Niu 2015, Kramer and Chadwick 2018). This explanation could be supported by the negative correlation of the soil DOC content with pH and the positive correlation of soil DOC with EC (figure 3). Second, continuous N addition altered the microbial composition, and further altered the contribution of microbial production to DOM via decomposition and the soil physical process of water transfer (Albright et al. 2019, Li et al. 2019, Niu et al. 2021). Interestingly, we did not find increases in the soil DON content or DON/TN ratio after 18 year N addition. At our study site, the soils are highly weathered with high N content, and there are no obvious changes in litter amounts with N addition (Lu et al. 2018). Thus, more N might be lost via leaching or as gas (e.g. N₂O) emission to air (Corre et al. 2010, Lu et al. 2014).

The effects of low-N addition (5 g m⁻² yr⁻¹) on most optical and molecular characteristics of soil DOM were inconsistent with those of medium- and high-N addition (10 and 15 g m⁻² yr⁻¹), indicating that N addition rate was an important moderator. A recent study also reported that realistic rates of N addition differently influence soil C flux rates and C stocks (Wiclots et al. 2022). The reason for no significant effects of low-N addition on these characteristics of soil DOM might be associated with no change in soil acidification status and SOC pool under low-N addition. Another reason might be that plants and soil microorganisms already adapted to the acid soil environment due to long-term acid treatments (Lu et al. 2018). At the same studied site, medium- and high-N addition have stronger influences on soil properties (e.g. soil pH, inorganic N) and soil microbial characteristics, such as microbial biomass and composition (Wang et al. 2018). Moreover, PCA in this study indicated that N addition increased the average molecular weight, and aromaticity of soil DOM, but did not alter the degree of humification, which might be correlated with changes in soil pH.
and microbial characteristics (figure 3, Wang et al 2020). These findings are in line with previous studies on other types of forest ecosystems. For example, in a temperate forest, Wang et al (2020) reported small changes in soil DOC quantity but marked changes in the molecular composition after 22 years of treatment at 10 g N m$^{-2}$ yr$^{-1}$. Smemo et al (2007) reported significant increase in aromatic and soluble polyphenolic content after 10 years treatment at 3 g N m$^{-2}$ yr$^{-1}$.

Different from previous studies, we further observed the significant correlations of HF-OC and DOC content with soil optical properties, indicating that alterations in DOM composition might be closely associated with SOC sequestration. The FT-ICR MS analysis provided more detailed DOM compositional information at the molecular level, and the information was in line with the optical analysis. Importantly, FT-ICR MS analysis further indicates that the recalcitrant and aromatic C content was elevated with N addition (reflected by the increases in CRAM, tannin-like, and condensed aromatics), which was consistent with the increase in HF-OC content. In contrast, the decrease in MLB$_L$ with N addition might indicate that N addition also decreased contents of biolabile compounds of microbe (e.g. microbial muramic acid and polysaccharides) and plant origin (Averill and Waring 2018). The decrease in the MBC/SOC ratio also indicates a relatively lower microbial metabolism of SOC pool under N addition treatments. These changes in the abundance of CRAM and MLB$_L$ reflect that soil microorganisms would selectively utilize soluble C components, leading to the accumulation of recalcitrant C components (Albright et al 2019, Li et al 2019, Wang et al 2019). The point was also supported by the changes in the relative abundances of biochemical groups. In N addition plots, the relative abundances of recalcitrant component (i.e. lignins and tannins) were more than 70% (figure 4), indicating their predominance role in controlling soil DOM pool. Specifically, increase in tannin-like component (highly unsaturated polyphenols) in soil DOM with N addition would suppress microbial activity, which has been observed in this forest and reported in our previous study (Wang et al 2018). Another possibility is that the increases in aromatic DOM (such as tannins) might have precipitated the dissolved proteins, which can accelerate the accumulation of insoluble SOM under long-term N addition (Wang et al 2022). Alterations in molecular-level information with N addition were probably due to N-induced differences in both soil properties and microbial activities as compared to control plots (Averill and Waring 2018, Li et al 2019, Wiclots et al 2022).

The findings must be carefully interpreted due to some potential biases and uncertainties. First, there might be tight correlations between N deposition and soil C retention via DOM pathway due to alterations in molecular-level characteristics of DOM and observed significant correlations of HF-OC with optical properties. However, how soil DOM links soil microbial and physical processes (e.g. formation of mineral colloids) with SOC sequestration remains unclear (Averill and Waring 2018, Kramer and Chadwick 2018). There is no doubt that this mechanism needs to be verified. Second, as the most mobile and reactive C flux, the temporal (season) and spatial (e.g. deeper soil layers) dynamics of soil DOM should be considered. Additionally, at sufficiently low levels of N deposition, there is likely to be no or opposite effects on soil DOM quantity and composition. Thus, N deposition rates should be considered in tropical forest ecosystems, and more studies should be performed to understand the association of soil DOM dynamics with OC cycling using a more combined approach, such as FT-ICR MS and Nuclear Magnetic Resonance spectroscopy.

5. Conclusion

Our results showed that 18 years of N addition significantly altered soil DOM composition and tended to increase the soil DOC content. Both optical and FT-ICR MS analyses jointly indicated that the average molecular weight and aromaticity of DOM were elevated under high N addition rates, and the relatively recalcitrant DOM components, such as lignin-like and tannin-like components, were preferentially elevated. In contrast, the relatively labile soluble DOM components were reduced as indicated by decreased %MLB$_L$. These effects of medium- and high-N addition on the above-mentioned indices were usually inconsistent with those of low-N addition (5 g m$^{-2}$ yr$^{-1}$), indicating that N addition rate was important modulator of changes in soil DOM composition. To our knowledge, this is the first report to uncover the optical and molecular composition of DOM in the primary tropical forests. We suggest that long-term high-N deposition may benefit SOC sequestration through altering soil DOM quantity and composition into recalcitrant and non-bioavailable forms, which merits further research in tropical forests.

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: https://figshare.com/s/2b92f7a5bd727c1ac5d.

Acknowledgments

We thank Miss Xiaoping Pan of South China Botanical Garden, Chinese Academy of Sciences for their help with fieldwork and lab analysis. This project was...
supported by the National Natural Science Foundation of China (41922056, 42192513, and 42122054), and Youth Innovation Promotion Association CAS (Grant Y2019865).

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