



Cycling of dissolved organic nutrients and indications for nutrient limitations in contrasting Amazon rainforest ecosystems

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Abstract In the nutrient-poor soils of the Amazon rainforest, phosphorus (P) emerges as a critical limiting factor for ecosystem productivity. Despite these limitations, the Amazon exhibits remarkable productivity that is maintained by its efficient nutrient recycling mechanisms. Central to this process is the role of organic matter, particularly its dissolved (DOM) fraction, which serves as a crucial nutrient reservoir for both plants and microorganisms. This study delves into the dynamics of nutrient-containing DOM within the soils of two contrasting rainforest ecosystems: clayey *terra firme* forests, known for their robust

nutrient recycling and presumed P-limitation, and sandy white-sand forests, characterized by reduced nutrient recycling capacity and presumed nitrogen (N)-limitation. Utilizing ultra-high resolution mass spectrometry (HR-MS), we analyzed the molecular composition of dissolved organic nutrient species. We evidenced nutrient limitation applying innovative concepts: (1) assessing nutrient depletion in DOM via nutrient-to-carbon ratios, (2) comparing the composition of nutrient-enriched DOM pools across soil depth profiles to infer microbial nutrient processing, and (3) examining the temporal variability of nutrient-containing DOM as an indicator of nutrient uptake and production. Our results corroborate the

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hypothesis of P-limitation in *terra firme* forests, with significant processing of N-containing DOM also observed, indicating a synergistic demand for both P and N. Surprisingly, white-sand soils exhibited no signs of N-limitation but instead sulfur (S)-limitation, a novel finding for these ecosystems. This study highlights the diversity of potential nutrient limitations in the central Amazon and the importance of the bioavailable “black box” DOM for tropical nutrient cycles.

Keywords Metabolomics · Dissolved organic matter · FT-MS · Orbitrap · Soil fertility

Introduction

The Amazon rainforest plays a critical role in the global carbon cycle and may mitigate negative climatic effects of increasing atmospheric CO₂ levels by increasing productivity (Feldpausch et al. 2016; Girardin et al. 2016; Malhi et al. 2008). It is highly productive and stores large amounts of carbon in biomass and soils (Malhi et al. 2009; Saatchi et al. 2011; Trumbore & Barbosa de Camargo 2009). However, soils in the Amazon are highly weathered and largely infertile (Camenzind et al. 2018; Quesada et al. 2010), with phosphorous (P) being considered the main limiting element for ecosystem productivity (Lugli et al. 2019; Turner et al. 2018; Vitousek 1984). P-limitation likely constrains the productivity response to increasing atmospheric CO₂ concentrations (Martins et al. 2021; Terrer et al. 2019). The Amazon rainforest however is very heterogeneous, and not all rainforest types are hypothesized to be P-limited. Lowland *terra firme* forests constitute the majority of the central Amazon rainforest region. They occur on widespread clayey soils and are hypothesized to be P-limited (Andreae et al. 2015; Cunha et al. 2022; Quesada et al. 2011). *Terra firme*-like forests occur across diverse edaphic conditions, with P-limitations more prevalent in ecosystems with older pedogenesis. Recent evidence strongly supported P-limitation in *terra firme* forests with old and extremely P-depleted soils, while it has been questioned for *terra firme*-like forests on younger soils with higher P availability (Cunha et al. 2022). Although older soils prevail in the central Amazon, *terra firme*-like forests

also occur on younger soils, for which P-limitation remains uncertain.

Less productive white-sand forests occur on strongly leached sandy soils and are potentially more nitrogen (N) limited (Anderson 1981; Herrera & Jordan 1981; Quesada et al. 2011; Zanchi et al. 2015). White-sand soils are highly deficient in most nutrients, but N-limitations have been inferred because white-sand forests are typically more deprived of N than P (Adeney et al. 2016; Matson & Vitousek 1987). In addition to their relative depletion in N, the infertility of these soils may render multiple other elements co-limiting, such as P or sulfur (S). Nutrient dynamics in white-sand forests are understudied compared to other rainforest types, with more direct evidence of nutrient limitations, e.g. from fertilization experiments, being notably absent. Although *terra firme* forests cover more area overall, white-sand forests cover ~5% of the Amazon basin (Adeney et al. 2016; Quesada et al. 2011). To accurately predict the response of the Amazon rainforest to rising CO₂ levels, it is crucial to investigate the nutrient dynamics that govern primary productivity and carbon sequestration in diverse Amazon ecosystems. This includes assessing the extent of P-limitation in *terra firme* forests and the potential N-limitation in white-sand forests. Understanding these nutrient interactions will allow for more precise modeling of carbon cycling processes and ecosystem responses to climate change across the diverse rainforest types of the central Amazon (Fleischer et al. 2019).

Amazonian soils are predominantly characterized by their low levels of accessible inorganic nutrients. This deficiency arises from two main factors: the weaker ability of these soils to retain some mineral nutrients efficiently due to greater leaching losses caused by high precipitation (Gurmesa et al. 2022) (e.g., N, S), and the overall strong retention of nutrients by the high abundance of iron (Fe) and aluminum (Al) oxides that limit P availability (Quesada & Lloyd 2016; Schaap et al. 2021). The low availability of inorganic nutrients favors the recycling of nutrients from organic matter in the Amazon rainforest (Cunha et al. 2022; Lugli et al. 2019). Nutrient recycling occurs to a large degree in the organic and litter layer above the mineral soil, facilitated by extensive root growth and microbial activity (Martins et al. 2021). While this process is not unique to the Amazon rainforest, these ecosystems rely more strongly

on aboveground nutrient recycling due to soil infertility, low-reactivity mineral assemblages in white-sand soils, and lower soil organic carbon contents. White-sand forests have evolved specialized strategies for maximizing aboveground nutrient retention through the development of thick root mats on the soil surface (Adeney et al. 2016; Matson & Vitousek 1987). The dependence on aboveground cycles may reduce soil carbon sequestration and increase ecosystems vulnerability to disturbances like drought or fire. Efficient belowground nutrient retention therefore could be a key aspect in enhancing resilience of these ecosystems (Singh et al. 1989) and may play an important role in the Amazon's response to global change, but efficiencies of belowground cycles are not well characterized for *terra firme* and white-sand forests.

The breakdown of larger polymers releases inorganic nutrients and dissolved organic matter (DOM). DOM is a highly reactive and complex mixture including thousands of N-, S- and P-containing molecules (Gmach et al. 2018), which serve as a critical nutrient reserve. Plant-derived DOM leaching from the litter layer is transported through the soil profile and progressively degraded, mineralized or taken up by microorganisms (Kaiser & Kalbitz 2012; Steinbeiss et al. 2008). These processes will be jointly referred to as *DOM processing* herein (Freeman et al. 2024), which represents a major pathway for resupplying nutrients to plants and soil microorganisms (Schaap et al. 2021). DOM processing is accompanied by a shift in DOM composition from higher abundance of plant-derived products to more microbial compounds (Roth et al. 2019). DOM derived from early plant matter decomposition is characterized by abundant aromatic compounds (Gleixner 2013; Klotzbücher et al. 2013), greater oxygen content and oxidation state (Hertkorn et al. 2016; Simon et al. 2021) and lower saturation (D'Andrilli et al. 2015). In contrast, microorganism-derived DOM is depleted in aromatic compounds (Drake et al. 2019) and is characterized as more aliphatic, saturated, reduced and oxygen-depleted (Benk et al. 2019; Butturini et al. 2020; Riedel et al. 2016). DOM processing and cycling can be investigated with ultra-high resolution mass spectrometry (HR-MS) (Roth et al. 2019).

HR-MS enables the detection and characterization of thousands of mass spectrometric peaks per sample, providing chemical information through

molecular formula assignments (Hawkes & Kew 2020; Hertkorn et al. 2013; Hutchins et al. 2017). Untargeted analysis of DOM using HR-MS offers a promising avenue for understanding DOM sources and changes in their contributions (Drake et al. 2019; Orme et al. 2022; Schroeter et al. 2022). Research on DOM composition in pristine Amazon rainforest soils is scarce, particularly with focus on organic nutrients. The compositional differences between major nutrient pools (N, P, and S) and their variance across contrasting rainforest types remains poorly understood. Knowledge gaps persist in regards to belowground processes, specifically in the transport of organic nutrients through the soil profile and microbial processing of them. Elucidating DOM compositional dynamics can provide insights into ecosystem nutrient demands.

Low nutrient abundance can result in nutrient limitation, which can be reflected in low nutrient-to-carbon ratios of DOM (Cui et al. 2022; Klaus et al. 2016). Furthermore, we hypothesize that limiting nutrients undergo more pronounced DOM processing (Feyissa et al. 2022) because elevated demand drives nutrient mining and preferential processing. This enhanced processing should result in greater compositional shifts of nutrient-DOM during downward transport through the soil. Enhanced DOM processing and nutrient mining can also result in higher temporal variability of nutrient species. Marine ecosystem studies have observed lower temporal variability of dissolved or particulate organic carbon compared to organic N and P (Knudsen-Leerbeck et al. 2017), amino acids (Gaye et al. 2022), or inorganic N and P (Martinez-Garcia et al. 2022). Moreover, higher relative nutrient variability in the biologically active photic zone compared to deeper waters suggests higher variability with higher biological demand (Ramírez et al. 2005). Analogously, in terrestrial settings higher temporal variability of N and S than C has been reported for groundwater (Kwon et al. 2022) and soils (Mobley et al. 2019; Monokrousos et al. 2004). Because organic nutrient concentrations are governed by the release and processing of individual molecules, temporal nutrient variability can be assessed similarly using the molecular composition of DOM. We propose that the dissimilarity in the nutrient-DOM composition between sampling events may serve as an indicator of nutrient variability and limitation also in soils.

This study analyzed the molecular DOM composition of soil porewater from two *terra firme* and two white-sand forests to investigate DOM processing and organic nutrient cycling in contrasting rainforest types. We employed the aforementioned proxies as indicators to diagnose nutrient limitations. Our investigation tested for signs of P-limitation in two *terra firme* forests at different stages of pedogenesis (old and younger) and for N-limitation in two white-sand forests. Additionally, we assessed the relative importance of above- versus belowground processing by comparing nutrient-DOM processing in the soil profile with variability in the 0–5 cm soil layer. We hypothesized that belowground processing would be more pronounced in the clay-rich soils of *terra firme* forests.

Methods

Field sites

Sampling for this study was conducted around the Amazon Tall Tower Observatory (ATTO) in the Utuamã Sustainable Development Reserve, which is located around 150 km northeast of Manaus, Brazil, in the central Amazon rainforest. The region is characterized by a mosaic of valleys and elevated plateaus with distinct types of rainforests and an altitude range of around 35 to 130 m above sea level. The tropical humid climate is marked by a rainy season from November to May with a pronounced peak from February to May, and a drier season from June to October (Andreae et al. 2015).

We investigated four sites belonging to two major rainforest types: *terra firme* forest (plateau and terrace sites) and white-sand forest (*Campina* and *Campinarana* sites). Most of the reserve is covered by upland *terra firme* forests that are highly diverse and productive forests on fine-textured, strongly acidic and clay-rich soils (plateau site; PL). Soils on the plateaus around ATTO are classified as Ferralsols, which are highly weathered and well-drained soils on ancient geological surfaces (Andreae et al. 2015; Chauvel et al. 1987). Another type of *terra firme* forest covers fluvial terraces along rivers (terrace site; TE). The clayey soils of these terraces are classified as Alisols, which represent a more recent pedogenic status with lower clay content than the

Ferralsols (Andreae et al. 2015). White-sand forests are low-diversity, lower-statured forests on coarse-textured sandy soils. Soils in the white-sand forests are classified as Arenosols. They are characterized by very high sand content with high water permeability, low water holding capacity, severe acidity and often very low nutrient contents (Quesada et al. 2011). Both white-sand sites showed thick organic layers and root mats above the mineral soil that are important for nutrient retention and recycling (Stark & Jordan 1978). The *Campina* (CD) and *Campinarana* (CW) sites feature the same specialized vegetation characteristic for white-sand forests, but the CW forest is denser with larger trees.

Sampling

Soil porewater was sampled using sintered glass suction plates (pore size 1–1.6 μm , 1 cm thickness, 12 cm diameter; SPG120 by Meter, Germany) that were installed in all four sites at soil depths of 5, 10, 20 and 30 cm in October 2017 in a fan-like fashion around a central box containing the collection flasks. Thick root mats and organic layers in the two white-sand sites allowed the installation of additional suction plates at 0 cm soil depths directly at the interface between organic layer and mineral soil. The suction plates were connected to glass flasks using PTFE tubes. The sampling bottles were evacuated to an average suction pressure of -150 mbar to collect only freely percolating porewater (Ekberg et al. 2007; Malik & Gleixner 2013; Roth et al. 2019). Sampling was conducted biweekly during the wet seasons in 2019, 2020 and 2021 (October to May). Each site had three independent replicates of the suction plate setup described above.

Soil chemistry: TOC, TN, iron and aluminum contents, soil texture fractions, soil pH and exchangeable cations

Soil samples were taken in November 2017 during the installment of the suction plates. All analyses of soil samples were conducted in the Instituto Nacional de Pesquisas da Amazônia in Manaus, Brazil (INPA). Soil samples were air-dried and sieved (<2 μm) to remove coarse material and roots. Organic carbon and total nitrogen concentrations of soil samples were measured using an automatic C and N analyzer (Vario

Max CN, Elementar Instruments, Germany). Particle size fractions (clay, silt, coarse sand and fine sand; the latter two were summarized as sand) were measured using the pipette method (Gee & Bauder 1986) and are reported in percent. Soil Fe and Al fractions were separated by sequential extraction (van Reeuwijk 2002). Fe and Al concentrations of all extracts were measured using atomic absorption spectrometry (AAS) at INPA (Quesada et al. 2010). Soil pH was determined at a 1:2.5 ratio of soil:liquid using H₂O and 1 M KCl. (Quesada et al. 2010). Exchangeable cations of Ca²⁺, Mg²⁺, K⁺ and Na⁺ were determined using the silver thiourea method (Pleysier & Juo 1980; Quesada et al. 2010).

Soil porewater chemistry: DOC, pH, electrical conductivity

Aliquots of soil porewater samples were measured for dissolved organic carbon (DOC) at INPA (Laboratório de Águas do INPA/ CPRHC). DOC concentrations of water samples were measured using a total organic carbon analyzer (TOC-V_{CPH} model, Shimadzu, Kyoto, Japan) (Monteiro et al. 2014). Before DOM extraction, pH and electrical conductivity (EC) were measured using a Multi 340i probe system (WTW, Weilheim, Germany).

Solid-phase extraction of DOM and Orbitrap MS measurements

The soil porewater from the three independent replicates was collected bi-weekly and mixed equally to obtain a representative sample per site and soil depth. Directly after sampling, water samples were stored frozen at -20 °C at ATTO for up to 1.5 years before performing solid-phase extraction on-site following Dittmar et al (2008). Samples were acidified to pH 2 with ultrapure hydrochloric acid (Rotipuran Supra 30%; HCl) before loading on the solid phase extraction column (Bond Elute PPL, Agilent, Germany). Procedural blanks of acidified ultrapure water (pH 2) were extracted along samples for each campaign. The PPL columns were transported to Germany after drying them on-site. The samples were subsequently eluted with ultrapure methanol, stored frozen at -20 °C and analyzed within three months. DOC concentrations of the solid-phase extracted DOM were measured on a TOC analyzer (vario TOC cube,

Elementar, Germany) (Orme et al. 2022). The extraction efficiency was $72 \pm 22\%$ on a carbon basis. Before measurement, samples were adjusted to a mixture of 1:1 methanol and purified water at a concentration of 20 mg C l⁻¹. We conducted HR-MS measurements at an Orbitrap Elite (Thermo Scientific) as described previously (Schroeter et al. 2022; Simon et al. 2018). Conventional approaches of investigating DOM provide either very specific data (e.g., targeted measurement of amino acids) or broad information (e.g., size-exclusion chromatography separating DOM into a few fractions). HR-MS offers unparalleled resolution of the highly complex composition of DOM and ecosystem-scale dynamics that are reflected therein (Hawkes et al. 2020). In short, the instrument was operated with a continuous flow of H₂O/MeOH 50:50 solvent (20 µl/min) and an ESI (electrospray ionization) needle voltage of 2.65 kV. 100 µl of DOM extract were infused into the HR-MS and spectra were acquired in negative ESI mode. Measurements were conducted for the mass over charge (*m/z*) range of 100–1000 at a nominal resolution of 480,000 (at *m/z* 200) and a maximum ion accumulation time of 100 ms. At least 100 scans per measurement were averaged and combined using the software Thermo Xcalibur (Version 3.0.63). Only peaks above a signal-to-noise ratio of 8 were kept for further analysis.

Data processing

Samples were recalibrated post-measurement using the MFassignR script with 3 ppm error range (Schum et al. 2020) and molecular formulas were assigned using the ICBM-Ocean routine (Merder et al. 2020). Subsequently, all mass spectra were normalized relative to the sum of their peak intensities. We used the following elemental constraints for molecular formula assignment: ¹²C 1–70, ¹H 2–160, ¹⁶O 0–70, ¹⁴N 0–7, ³²S 0–3, ³¹P 0–3 and ± 0.5 ppm tolerance. Formulas containing isotopes, formulas that were assigned more than once, and ambiguous assignments with multiple formula suggestions were removed. Finally, 7188 masses with successful formula assignment were considered for further analysis. Based on the molecular formula data, the following DOM parameters were calculated for each molecular formula: H/C, O/C, N/C, P/C, S/C, N/P, modified aromaticity index (AI_{mod}) (Koch & Dittmar 2006, 2016), and the nominal oxidation state of carbon (NOSC) (Boye et al.

2017). Molecular subgroups were assigned as follows (Riedel et al. 2016): “Aromatic”, “highly unsaturated”, “aliphatic” (Khatami et al. 2019), and “lipid-like” (Rivas-Ubach et al. 2018) (see Supporting Text S1 for more detailed information on data processing).

To compare different pools within DOM, the total set of molecular formulas was separated based on occurrence of N, S and P assignments (Table S2). We classified DOM based on molecular formulas as follows: without N, S and P assignment as “CHO-DOM” (only C, H and O assigned), with N assignment as “N-DOM”, with P assignment as “P-DOM” and with S assignment as “S-DOM”. This approach allowed to compare the dynamics of nutrient and non-nutrient pools separately.

Data analysis

All data analysis and creation of figures were conducted in RStudio 4.2.0. The molecular formula data were analyzed by principal coordinate analysis (PCoA; “cmdscale” function, “stats” 4.2.0) based on Bray–Curtis dissimilarities (“vegdist” function, “vegan” 2.6.2). The intensity-weighted means of DOM parameters were then calculated using the “weighted.mean” function (“stats” package 4.2.0). The averaged DOM parameters were fitted as vectors onto the ordination plots by post-ordination gradient fitting analysis (“envfit” function, “vegan” 2.6.2). The significance level for statistical tests was $\alpha=0.05$, and p-values were adjusted for multiple testing using the Benjamini & Hochberg method (Jafari & Ansari-Pour 2019) (“p.adjust” function, “stats” 4.2.0). We determined molecular formulas with significant association to one rainforest type using two-sided Mann–Whitney U tests based on their relative intensities (“wilcox.test” function, “stats” 4.2.0): First, the association to one rainforest type was tested (*terra firme* or white-sand). Subsequently, the association to one site was tested to determine site-specific markers within the sets of ecosystem markers (PL vs. TE; CD vs. CW). Based on this, ecosystem markers were defined as the formulas that showed significant associations with one ecosystem but were not correlated with site-specific differences. Site-specific markers were defined as formulas that showed further statistical association with one site. Subsequently, intensity-weighted means of the markers were calculated for each sample. These markers were used to differentiate

the characteristic DOM of rainforest types and individual sites.

We further compared the DOM composition from our sites with groundwater data from similar ecosystems in the same broader region north of Manaus (*terra firme* plateau and upland white-sand forests; sampled using piezometers in <2 m soil depths) (Simon et al. 2021). Samples from the study were measured on the same instrument as our samples and raw data were re-processed along with data from our samples by the same routine as described above.

The change in chemical characteristics of DOM with soil depth was quantified using the Bray–Curtis dissimilarity comparing samples from 5 to 30 cm depth, separated per site and sampling event. This was done to estimate the magnitude of DOM processing during soil passage. The Bray–Curtis dissimilarity was chosen to quantify DOM processing because it is a robust and well-established metric to quantify differences in composition between samples. The advantage of the approach lies in analyzing the change of all DOM parameters simultaneously and calculating a single metric that quantifies the overall change. The Bray–Curtis dissimilarity was calculated from intensity-weighted DOM parameters instead of the relative intensities of the molecular formulas. The following intensity-weighted DOM parameters were used: H/C and O/C ratios, NOSC, molecular mass (m/z), and fractions of aromatic [% of ion intensity], lipid-like [% of ion intensity], highly unsaturated [% of ion intensity] and aliphatic formulas [% of ion intensity]. We did not consider N/C, P/C and S/C ratios to ensure comparability with the CHO-DOM group that does not have these elements assigned. All DOM parameters were scaled with min–max scaling to values between 0 and 1 prior to calculating the Bray–Curtis dissimilarity.

The temporal variability of the molecular DOM composition was calculated for the individual DOM pools and used as a proxy for DOM and nutrient-DOM cycling. For this purpose, sampling events were regarded as replicates of the same sample. The Bray–Curtis dissimilarity based on presence-absence data was then calculated for each site and 5 cm soil depth and all temporal replicates were compared in pairwise comparisons. The dissimilarity of all comparisons was used as an estimate for the temporal variability of the composition of respective DOM pools. We chose to assess

nutrient variability through Bray–Curtis dissimilarity of the DOM composition because this allows to investigate the dynamics of nutrients contained in a variety of organic species.

Results

Bulk soil properties and soil porewater chemistry

The *terra firme* and white-sand soils differed strongly in their soil characteristics (Table 1). Clay content in the *terra firme* soils was much higher (PL, 69.4 ± 3.0 and TE, 41.3 ± 2.6) than in white-sand soils (CD, 0.7 ± 0.1 and CW, 1.5 ± 0.1), same as organic carbon and total nitrogen concentration. *Terra firme* soils also showed much higher total extractable Fe and Al (11.7–16.6 and 4.7–7.8 g/kg Fe or Al, respectively) than white-sand soils (<1.5 g/kg of both elements, Table 1). Exchangeable cations were up to 6.5-fold higher in clayey soils than in sandy soils. While they were more depleted in white-sand sites, cation exchange capacities were overall extremely low compared to those of temperate forests (Gruba & Mulder 2015). Soil pH was very low in all sites (range 3.6–4.8 in H₂O) with high amounts of exchangeable hydrogen (range 2.9–4.0 in KCl).

The *terra firme* and white-sand soils were also strongly differentiated by their porewater chemistry (Mann–Whitney U test, $p < 0.05$; Table 1, Fig. S1). DOC concentrations were on average ~sevenfold higher in the white-sand soils compared to *terra firme* soils, which contrasted the results from soil carbon. Soil porewater pH was approximately one pH unit higher in *terra firme* samples whereas electrical conductivity (EC) was three- to four-fold lower. DOC concentrations decreased significantly (Spearman rank correlation, $p < 0.05$) with soil depth in *terra firme* soils (decrease from 13.1 ± 5.6 in 5 cm to 2.0 ± 0.8 in 30 cm) and in the CW site (decrease of 56.8 ± 17.5 to 31.6 ± 5.7). This trend was not found in CD soils where DOC levels remained similar up to 30 cm soil depth. Porewater pH and EC showed significant depth trends in *terra firme* sites with increasing pH and declining EC values, whereas values did not change significantly in white-sand sites.

Table 1 Soil and porewater chemistry for *terra firme* (PL, TE) and white-sand sites (CD, CW)

Site	Soil depth [cm]	Fe _{total} [g/kg]	Al _{total} [g/kg]	Sand [%]	Silt [%]	Clay [%]	Cations [cmolc/kg]	N [wt%]	C _{org} [wt%]	pH (H ₂ O)	pH (KCL)	pH*	EC [μS cm ⁻¹]*	DOC [mg l ⁻¹]*
CD	0–5	1.1	0.4	95.7	3.6	0.7	0.18	0.04	0.66	4.8	3.02	3.7	67.0	102
CD	20–30	1.3	0.6	97.2	2.3	0.5	0.02	0.00	0.12	4.5	3.31	3.8	58.4	40.9
CW	0–5	0.8	0.1	93.5	5.1	1.4	0.07	0.05	0.90	4.1	2.86	3.7	60.0	56.8
CW	20–30	0.8	0.1	95.0	3.4	1.6	0.03	0.03	0.54	4.1	3.21	3.7	76.4	31.6
PL	0–5	16.6	7.8	7.2	18.1	74.8	0.46	0.30	3.98	4.1	3.55	4.4	21.8	6.3
PL	20–30	11.7	6.4	5.6	24.5	69.9	0.07	0.12	1.32	4.4	3.96	4.9	11.9	1.2
TE	0–5	13.3	4.7	43.7	17.7	38.6	0.33	0.23	2.71	3.6	3.32	4.3	33.9	16.5
TE	20–30	15.2	6.3	38.8	16.8	44.5	0.12	0.10	1.31	4.2	3.78	5.0	12.0	2.5

Fe_{total} and *Al_{total}* the sum of pyrophosphate-extractable, oxalic acid-extractable and dithionite-extractable fractions of Fe and Al; *Cations* the sum of exchangeable cations of Ca²⁺, Mg²⁺, K⁺ and Na⁺; *N* soil nitrogen content; *C_{org}* soil organic carbon content; *soil pH (H₂O)* soil pH measured in water, *soil pH (KCl)* soil pH measured in potassium chloride; *EC* electrical conductivity [μS cm⁻¹]; *DOC* dissolved organic carbon [mg C l⁻¹]

*Parameters were measured in soil porewater that was also used for DOM extraction. Soil porewater samples were derived from 5 to 30 cm depth

Proxies for nutrient limitation

In all sites, molecular formulas containing only C, H, and O constituted the largest fraction of ion intensity (>80%) and were assigned in similar numbers in all samples. In contrast, the number of nutrient-containing molecular formulas and nutrient-to-carbon ratios revealed differences between the two rainforest types (Table 2). Differences in N/C ratios between *terra firme* and white-sand sites were not pronounced (Fig. 1A), with slightly higher values in white-sand DOM. P-DOM was strongly depleted in *terra firme* sites compared to that of white-sand sites (Fig. 1B; Table 2), as displayed by much lower P/C ratios. P was also strongly depleted relative to N in *terra firme* DOM, with over factor 5 higher N/P ratios than in white-sand DOM (Fig. 1D). In contrast, S-DOM was considerably depleted in white-sand compared to

terra firme forests, as displayed by much lower S/C ratios (Fig. 1C).

The analysis of the processing of individual DOM pools during soil passage revealed a differential processing (Fig. 2). In the *terra firme* sites, processing was highest for N- and P-DOM (Fig. 2A), with respective means ~40–50% higher than those of CHO-DOM. DOM processing was much lower in S-DOM, with approximately four-fold lower values than for CHO-DOM. In contrast, processing of CHO-, N- and S-DOM was of similar magnitude in white-sand sites (Fig. 2B), and only P-DOM exhibited lower processing magnitudes. In comparison, DOM processing was overall much higher in *terra firme* sites except for S-DOM. Processing of N- and P-DOM was ~3.5 to 4.5 times higher than in white-sand sites, whereas it was only factor 2 for CHO-DOM. For S-DOM a reversed trend was observed,

Table 2 Numbers of formula assignments per DOM pool and nutrient-to-carbon ratios for the *terra firme* forests (PL and TE sites) and the white-sand forests (CD and CW sites)

Rainforest type	CHO formulas (n)	N formulas (n)	S formulas (n)	P formulas (n)	N/C (×1000)	S/C (×1000)	P/C (×1000)
<i>Terra firme</i>	1927 ± 222	1849 ± 152	316 ± 53	72 ± 23	14.2 ± 3	1.08 ± 0.5	0.14 ± 0.1
White-sand	1856 ± 95	848 ± 126	50 ± 14	76 ± 12	14.9 ± 2	0.27 ± 0.2	0.92 ± 0.2

CHO formulas (n) number of molecular formulas per sample that contain only C, H and O assignment; *N formulas* molecular formulas with N assignment; *S formulas* molecular formulas with S assignment; *N/C* intensity-weighted nitrogen-to-carbon ratio; *S/C* intensity-weighted sulfur-to-carbon ratio; *P/C* intensity-weighted phosphorus-to-carbon ratio. Number of samples: white-sand sites 122, *terra firme* sites 79

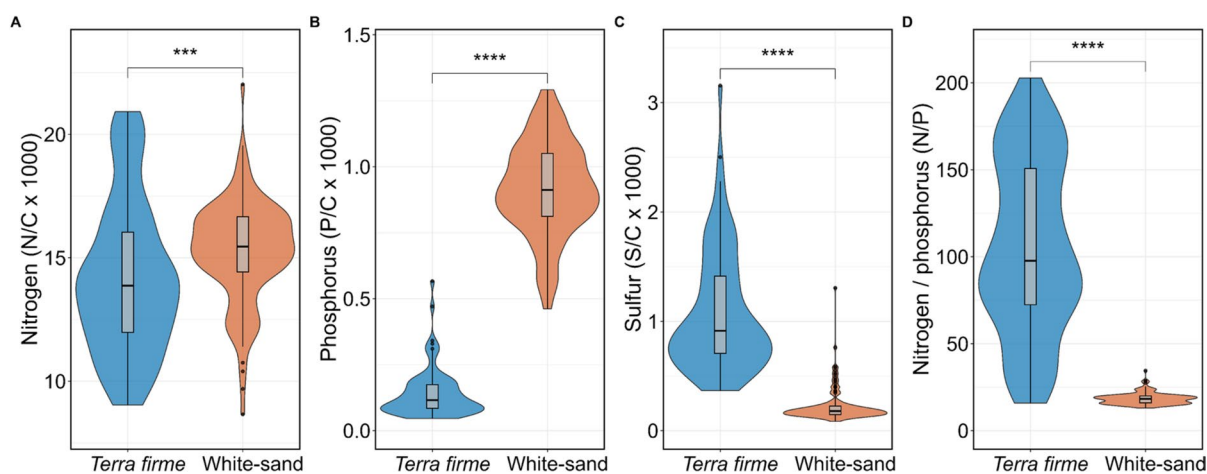


Fig. 1 Intensity-weighted nutrient-to-carbon (C) ratios of DOM for N (nitrogen; **A**), P (phosphorus; **B**), and S (sulfur; **C**), and nitrogen-to-phosphorus ratio (N/P; **D**) displayed for

white-sand and *terra firme* sites. Stars indicate significant differences between white-sand and *terra firme* DOM (Mann–Whitney U test, $p < 0.05$)

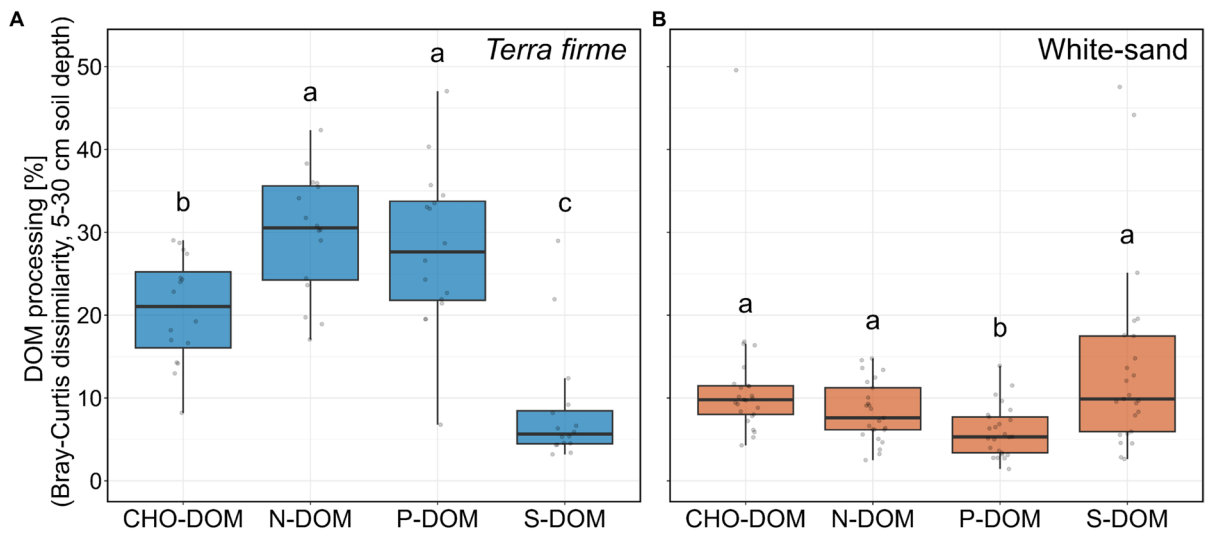


Fig. 2 DOM processing in % between 5 and 30 cm soil depth in *terra firme* (A) and white-sand sites (B) calculated as Bray–Curtis dissimilarity. The Bray–Curtis dissimilarity was used to quantify the difference in chemical characteristics of samples from 5 and 30 cm soil depth, based on nine DOM parameters specified in the methods section. Data are displayed for DOM pools: CHO-DOM (molecular formulas with only C, H

and O assigned), N-DOM (molecular formulas that contain N), P-DOM (molecular formulas that contain P) and S-DOM (molecular formulas that contain S). Letters indicate significant differences between the DOM pools (Kruskal Wallis test, adjustment of p-values by Benjamini & Hochberg method, $p < 0.05$)

where processing was $> 50\%$ higher (Mann–Whitney U, $p < 0.05$) in white-sand sites. Overall, the processing of the DOM pools showed a preferential processing of N- and P-DOM in *terra firme* soils. For white-sand DOM, no preferential processing of individual DOM pools was apparent.

In both rainforest types, the temporal variability of nutrient-containing DOM pools was substantially higher than that of CHO-DOM (Fig. 3). The magnitude of the variability was generally similar in both rainforest types and did not show higher values in the *terra firme* sites as observed for the DOM processing. In *terra firme* forests, the variability was highest for P-DOM, with two-fold higher values than for the second-most variable pool S-DOM (Fig. 3A). The variability of N-DOM was lower but still two-fold higher than that of CHO-DOM. In the white-sand forests, the highest variability was observed for S-DOM, with values approximately 1.7-fold higher than those of N- and P-DOM, which were not statistically different from each other (Fig. 3B). Similar to the *terra firme* sites, variability was the lowest for CHO-DOM with a large three-fold difference compared to N- and P-DOM. The dataset used in this study covered samples collected in the wet seasons of three consecutive

years. Therefore, the variability observed in this study was related to both intra-seasonal variability and variability between years.

DOM processing as reflected in the molecular composition of DOM

The analysis of the DOM composition revealed two main clusters related to white-sand and *terra firme* samples in a principal coordinate analysis (PCoA; Fig. 4A). Samples were separated mainly on the first principal coordinate (PCo1) that explained 85% of variance, showing the dominant effect of differences between clayey and sandy soils. Although clustering together, PL and TE samples were clearly separated (Fig. 4A, blue/ green dots). Site-specific separation was not apparent for the white-sand samples. The separation between *terra firme* and white-sand clusters was linked to significant (Pearson correlation, $p < 0.05$) trends in porewater chemistry and soil parameters such as clay content that followed the separation by rainforest type. In addition to this primary separation, soil depth also showed a significant correlation with the molecular composition, shifting samples both on the first and second (5% of variance)

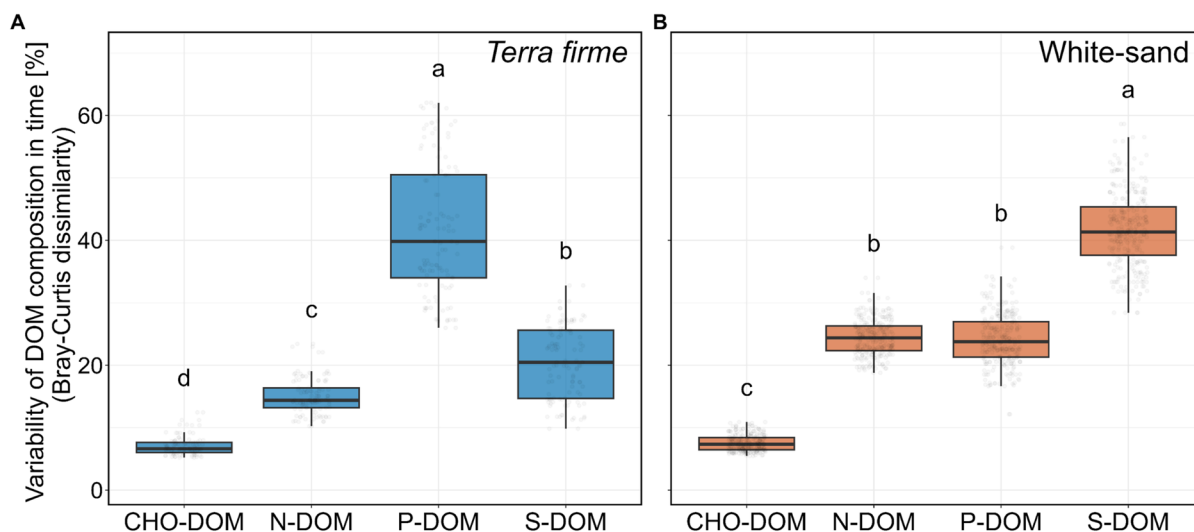


Fig. 3 Temporal variability of the DOM composition in % for 5 cm soil depth in *terra firme* (A) and white-sand sites (B) calculated as Bray–Curtis dissimilarity between all replicate samplings of the same site in 5 cm soil depth compared against each other. The Bray–Curtis dissimilarity was used to quantify the difference in the molecular composition in pair-wise comparisons of repeated sampling events. Data are displayed

for DOM pools: CHO-DOM (molecular formulas with only C, H and O assigned), N-DOM (molecular formulas that contain N), P-DOM (molecular formulas that contain P) and S-DOM (molecular formulas that contain S). Letters indicate significant differences between the DOM pools (Kruskal Wallis test, adjustment of p-values by Benjamini & Hochberg method, $p < 0.05$)

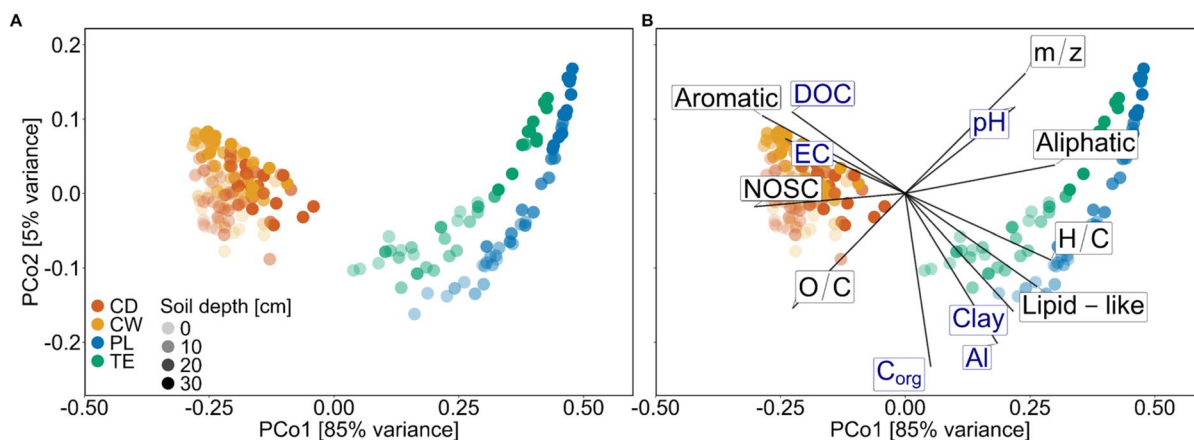


Fig. 4 Ordination plot from a principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity of the molecular DOM composition (A). All masses with molecular formula assignment and their relative intensities were used. Site abbreviations: CD = *Campina* (white-sand), CW = *Campinarana* (white-sand); PL = plateau (old *terra firme*), TE = terrace (young *terra firme*). Soil depth is displayed by continuous alpha shading with lighter shade marking 5 cm, and darker shade marking 30 cm. Intensity-weighted DOM parameters (displayed in black font) and parameters of soil porewater

chemistry and soil (displayed in blue font) were fitted as vectors (B): aromatic (fraction of aromatic formulas in %), NOSC (nominal oxidation state of carbon), O/C ratio, m/z (mass to charge ratio), aliphatic (fraction of aliphatic formulas in %), H/C ratio, lipid-like (fraction of lipid-like formulas in %), (DOC (dissolved organic carbon concentration of soil porewater, mg l^{-1}), EC (electrical conductivity of soil porewater, $\mu\text{S cm}^{-1}$), pH (soil porewater), Clay (clay content of soil in %), Al (Al_{total} , the sum Al fractions in soil, g/kg), C_{org} (organic carbon content of soil, wt%)

coordinates (Fig. 4A). The depth trend was linked to PCo2 and was more apparent in *terra firme* samples (shown by brighter and darker colors in Fig. 4). The separation of the clusters by rainforest type was also reflected in correlations with DOM parameters.

Molecular formulas were separated into unique markers for the individual sites, and shared markers for the rainforest type (*terra firme* / white-sand; Table 3, see Online Resource 2 for list of markers). 1850 formulas were indicative of *terra firme* forests as a whole, differentiating them from white-sand systems. In line with the stronger distinction between the *terra firme* sites apparent in the PCoA, the *terra firme* sites (PL and TE) had more unique markers ($n = 1310$ and 1132 , respectively) than the white-sand sites (CD and CW). 2052 markers were indicative of white-sand forests but only few markers differentiated the sites from each other ($n = 140$ and 175 for CD and CW). Intensity-weighted means of *terra firme* markers were characterized by high molecular mass (m/z) and H/C ratios and low NOSC (Table 3). The fraction of aromatic compounds was extremely low ($< 2\%$), whereas aliphatic and lipid-like compounds were much more abundant. White-sand forest markers were highly aromatic ($74 \pm 4.9\%$) while the non-aromatic compounds were mostly highly unsaturated. White-sand markers further displayed lower molecular mass and H/C ratios and were more oxidized. Lipid-like structures made up less than 0.1% of white-sand markers. Overall, DOM was more aliphatic and lipid-like, and less aromatic in soils with higher clay content. The distinction between the rainforest types observed for the DOM parameters considering all molecular formulas (Fig. 4) was also reflected in each DOM pool separately (Table S2; Figures S2 and S3).

The DOM composition was significantly different between 5 and 30 cm soil depth in all four sites

(Permanova, $p < 0.05$). Almost all investigated DOM parameters were significantly correlated with soil depth (Pearson correlation, $p < 0.05$; Fig. 5), except for the fraction of lipid-like formulas, for which trends were only significant in *terra firme* sites. The DOM composition in both rainforest types shifted in the same direction. m/z and H/C values and the fraction of aliphatic, lipid-like and highly unsaturated compounds increased, whereas the fraction of aromatic compounds and the NOSC and O/C values decreased. Groundwater DOM data from similar *terra firme* plateau and white-sand sites in the same geographic region (Simon et al. 2021) showed a continuation of the depth trends described for topsoil DOM from the sites of this study (Tables S2 and S3). Relative to DOM from 30 cm soil depth of the respective sites, the content of aromatic compounds, the O/C ratio and NOSC values decreased further while the content of aliphatic, lipid-like and highly unsaturated formulas as well as the H/C ratio increased further.

Discussion

Evidence for nutrient limitations in *terra firme* and white-sand forests

We hypothesized P-limitation in *terra firme* forests. In line with our hypothesis, DOM was, on average, more than six-fold depleted in P compared to the white-sand forests based on P/C ratios (Fig. 1A). In addition to low P/C ratios, also high N/P ratios of leaves in tropical trees have been regarded as evidence for tropical forest P-limitation (Vitousek 1984). Similarly, the N/P ratio of DOM was considerably higher in *terra firme* ($N/P = 106 \pm 50.2$) than in white-sand soils ($N/P = 18 \pm 3.8$). This is consistent with the frequently

Table 3 Intensity-weighted means of classes of molecular formulas that correlated significantly with rainforest type (shared markers for white-sand/ *terra firme* sites that were not enriched in one site) and site (site-specific markers that were enriched in one site)

Site	Aromatic [%]	Aliphatic [%]	Lipid-like [%]	m/z [Da]	H/C ratio	O/C ratio	NOSC
CD	47 ± 4.0	1.7 ± 0.8	0.0 ± 0.0	389 ± 9.2	0.90 ± 0.01	0.50 ± 0.02	0.20 ± 0.03
CW	81 ± 1.7	1.2 ± 0.5	0.0 ± 0.0	269 ± 7.6	0.69 ± 0.01	0.66 ± 0.01	0.74 ± 0.02
White-sand	74 ± 4.9	0.8 ± 0.4	0.1 ± 0.0	350 ± 21	0.73 ± 0.02	0.49 ± 0.03	0.39 ± 0.03
PL	0.2 ± 0.1	71 ± 5.2	37 ± 6.1	414 ± 12	1.28 ± 0.02	0.42 ± 0.03	-0.38 ± 0.09
TE	15 ± 8.4	10 ± 2.1	1.5 ± 2.0	450 ± 31	1.04 ± 0.02	0.46 ± 0.05	0.04 ± 0.03
<i>Terra firme</i>	1.9 ± 1.2	33 ± 2.6	5.7 ± 1.1	436 ± 18	1.16 ± 0.01	0.45 ± 0.03	-0.16 ± 0.06

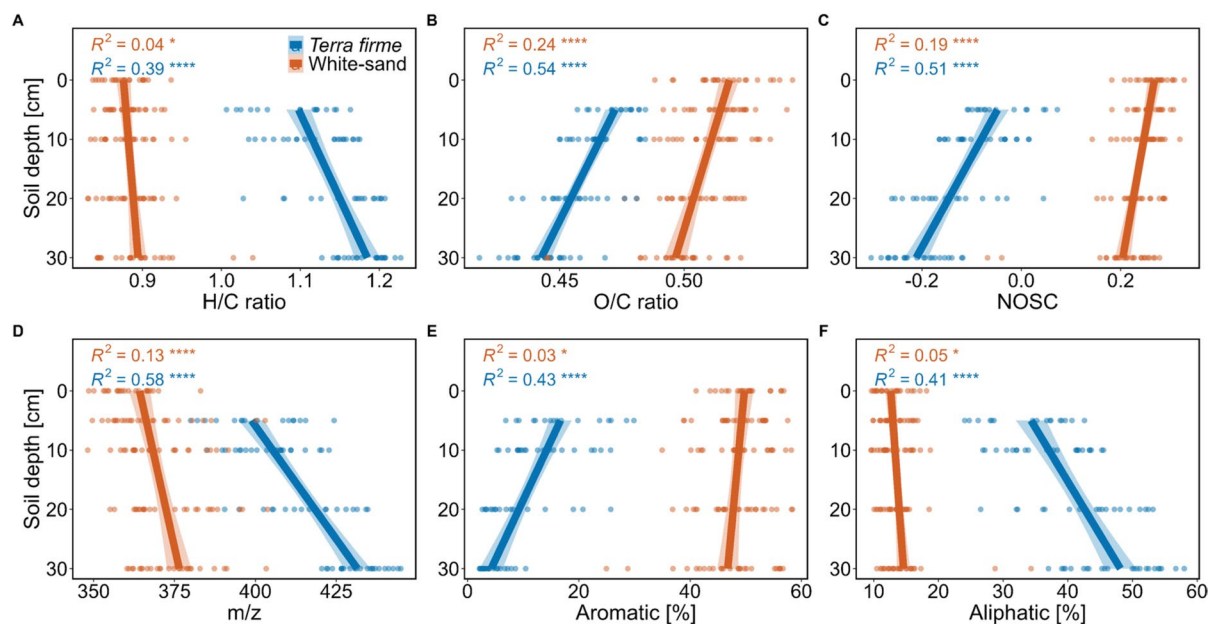


Fig. 5 Pearson correlation of intensity-weighted DOM parameters with soil depth (0/5–30 cm). The following parameters are shown: H/C ratio (A), O/C ratio (B), NOSC (nominal oxidation state of carbon) (C), m/z (mass to charge ratio) (D), aromatic (fraction of aromatic formulas in %) (E), aliphatic (fraction of aliphatic formulas in %) (F). Stars indicate significant correlations (Pearson correlation, $p < 0.05$). Only significant

correlations are displayed as linear regression line. DOM from microbial sources has typically higher H/C values and higher aliphatic content, and lower O/C and NOSC values and aromatic content compared DOM from plant sources. An increase m/z with soil depth was also associated with increasing microbial processing of DOM (Roth et al. 2019)

reported N abundance and P scarcity in tropical forests similar to our *terra firme* sites (Hedin et al. 2009; Reich & Oleksyn 2004). The pronounced P depletion and high N/P ratios of DOM support the proposed P scarcity in the *terra firme* forests investigated.

DOM processing in the soil profile of *terra firme* sites was highest for P- and N-DOM (Fig. 2A). This was accompanied by an increase in the number of P- and N-containing formulas with soil depth, whereas the number of CHO-containing formulas decreased (Mann–Whitney U test, $p < 0.05$). Increasing molecular diversity can result from microbial DOM processing (Chen et al. 2022; Zhao et al. 2019), and implies the synthesis of new compounds that were not present in upper soil layers, likely by microorganisms. The extensive processing of P-DOM is consistent with our hypothesis of P-limitation. However, the high processing of N-DOM was unexpected, although tropical forests have been proposed to cycle large amounts of nitrogen (Brookshire et al. 2012; Hedin et al. 2009). The elevated processing of P- and N-containing pools over CHO- and particularly S-DOM suggests a high

demand for these nutrients by microorganisms or plants in the 5–30 cm mineral soil.

While DOM processing, as investigated in this study, reflected processes in the topsoil from 5 to 30 cm, the temporal variability of the molecular DOM composition in 5 cm soil depth was potentially a result of processes in the organic layer or in the first 5 cm of mineral soil, or both. In the white-sand sites, the temporal variability was extremely similar in soil depths of 5 and 0 cm (Fig. 3A; Fig. S4). Because the 0 cm samples derived DOM directly from the organic layer, this suggests that in white-sand soils the temporal variability of DOM was a signal of processes occurring in the organic layer. For the *terra firme* sites, no samples from 0 cm soil depth were available because they lacked thick root mats. We could therefore not infer whether the variability was created above or within the first 5 cm of mineral soil. In *terra firme* sites, the temporal variability of the DOM composition was also highest for P-DOM, which further supports our hypothesis of P-limitation in these systems.

Overall, all three proxies supported the hypothesized P-limitation in *terra firme* forests. P-limitation is likely for the *terra firme* forest type present at our PL (plateau) site, characterized by ancient highly weathered Ferralsol soils (Cunha et al. 2022; Quesada et al. 2011). It is less expected in forests on river terraces, such as our TE (terrace) site, where Alisol soils reflect a more recent pedogenetic status with often higher P availability (Quesada et al. 2011, 2010). Soil total P contents of forest plots near our PE and TE sites were however highly similar (Andreae et al. 2015) and extremely low in global comparison (He et al. 2021; Turner & Engelbrecht 2010). In tropical soils P availability is not only regulated by overall low P contents but also strong sorption. The TE site features ~50% lower Al oxide content and 25% less Fe and Al bound in crystalline phases, both of which potentially increase P availability due to fewer binding sites. Additionally, its ~40% lower aboveground biomass (Andreae et al. 2015) could decrease ecosystem P demand. Nonetheless, our findings suggest that P-limitations may occur in *terra firme-like* forests on ancient river terraces such as our TE site, despite their younger pedogenesis, potentially higher P availability, and lower aboveground biomass. Previous studies on nutrient limitations have focused on more common rainforest types occurring on different soils (Cunha et al. 2022). While Alisols are not ubiquitous in the Amazon, they may be underrepresented in soil classifications of the Amazon (Quesada et al. 2011). Our findings imply that P-limitation may extend to rainforest types where it is less commonly assumed, which has implications for basin-wide nutrient limitations of the central Amazon.

Terra firme forests are largely not considered N-limited (Brookshire et al. 2012). The observed extensive processing of N-DOM may be caused by a generally high N demand of microbial communities or plants, or due to low N use efficiencies. Alternatively, it could also be linked to elevated production of microbial cell wall components and extracellular enzymes for P acquisition, for example using phosphatases or phytases (Yao et al. 2018) both requiring N. If the *terra firme* sites are in fact P-limited, it is likely that microorganisms and plants will allocate resources preferentially towards P acquisition (Zhang et al. 2017). This implies that any limitation of P is likely to cause elevated enzyme production, which in turn is a significant N investment and automatically

imposes a higher demand for N. Our data showed substantial cycling of organic N in the soil profile, which indicates a potential interlinked demand for N and P to counteract P-limitation. Despite the common assumption of N abundance in tropical forests, the N content in the top 10 cm of *terra firme* soil (~0.22 wt%) was comparable to temperate forests, which typically range between 0.1 and 0.5 wt% (Perakis & Sinkhorn 2011; Prieto-Fernández & Carballas 2000; Spohn & Stendahl 2022). If P is already limiting today, this constraint is likely to intensify with rising atmospheric CO₂ concentrations. Plant nutrient demands are projected to increase in tandem with plant productivity as a response to CO₂ fertilization (Terrer et al. 2019). Alongside increasing severity of potential P-limitations, our results indicate that the increasing P demand may render N co-limiting due to more resource-intensive P acquisition. If demand for both P and N rises, ecosystems are expected to increase both P- and N-mining. P-mining can occur independently of organic matter decomposition through cleavage by extracellular enzymes (Martins et al. 2021), while N-mining requires decomposition. Elevated N-mining might consequently lead to increased decomposition of soil organic matter, which could decrease soil organic carbon stocks. Another effect might be the upregulation of biological N fixation to meet nutrient demands (Moreira et al. 2021).

We hypothesized that N- instead of P-limitation would occur in white-sand forests. The soil N content was more than ten-fold lower than that of *terra firme* soils (Table 1), which suggests a relative scarcity of total N in soils of the investigated white-sand forests. However, the N/C ratios of DOM did not differ strongly between the two rainforest types, and were even higher in white-sand DOM than in the PL site (Fig. 1B). Our hypothesis of N-depletion in white-sand DOM therefore was not confirmed. Unexpectedly, white-sand DOM showed instead a strong depletion in S. Tropical forests are not considered S-limited, but research on S cycling in tropical soils is very scarce. Contrary to our hypothesis, we did not observe preferential processing of N-DOM over other nutrients in the soil profile, at least in terms of signatures reflected in DOM (Fig. 2B). The processing of all DOM pools was considerably lower than that in *terra firme* soils. This suggests a lesser importance of the first 30 cm of mineral soil for DOM and nutrient cycling in white-sand forests in general. In addition,

the temporal variability of DOM was not highest for N-DOM, which contradicted our hypothesis as well. Variability was instead highest for S-DOM, in both 5 and 0 cm soil depths. Our analysis of DOM variability revealed higher variability in low-abundance DOM pools in all sites. This pattern could be expected because higher relative variability occurs more readily for nutrients in low concentration. Nonetheless, ecological meaning can be inferred because high variability of low abundant nutrient pools suggests a rapid and tight nutrient cycle. We caution however that our results could have been affected by analytical insensitivity, where the occurrence of very low abundant compounds might not have been detected by our instrument, thereby increasing the observed variability of these nutrient pools.

The high variability of S in DOM derived directly from the organic layer (0 cm soil depth; Fig. S4) implies extensive cycling of organic S species in the organic layer. Overall, none of the three proxies supported the hypothesized N-limitation in the white-sand forests. The depletion and high temporal variability of S-DOM on the other hand suggest a high demand for S by plants or microorganisms living in the root mats or humus layer. High S demand or any S-limitation have not yet been proposed for these rainforest types. White-sand forests are generally understudied compared to other rainforest types (Adeney et al. 2016), and S has rarely been investigated. To the best of our knowledge, soil S contents have not been reported for white-sand soils to date. There are possible causes for an elevated S demand in white-sand forests. Sandy soils are generally more prone to S deficiency (Narayan et al. 2022). Importantly, atmospheric S deposition has decreased in recent decades due to reduced S emissions from air pollution and coal combustion, and it is generally lower in remote regions (Feinberg et al. 2021). Lower S deposition may thus increase S scarcity also in these systems. The high variability and low abundance of S-DOM could reflect rapid turnover of organic S compounds, suggesting a tightly coupled S cycle where demand closely matches supply. Microbial communities therefore might show specialization for organic S mineralization (Manzoni et al. 2021) or might be structured by their ability to compete for scarce S resources (Cui et al. 2018). A high S demand could furthermore drive plant adaptations towards increased resource allocation to S-acquiring mycorrhizae (Allen

& Shachar-Hill 2009; Vasco-Palacios et al. 2018). Lastly, organic S compounds are also of particular interest because of their connection to S-containing gas species that are emitted from tropical soils and that play an important role in the formation of aerosol particles and act as nuclei for cloud condensation (Jardine et al. 2015; Lehnert et al. 2024). Decomposition of organic S compounds can be an important source of volatile S species, such as dimethyl sulfide, methanethiol and H₂S. We sampled volatile organic compounds in the soil of one white-sand (CW, *Campinarana*) and one *terra firme* site (TE, Terrace) (Supporting Text S3). Volatiles with S and simultaneous S–N assignment were detected in both sites. S-containing species exhibited ~2.5-fold higher intensities in the white-sand site, constituting 2% of all detected volatiles, with CHOS species showing sevenfold higher intensities (Table S4). Soil DOM also contained six oxygen-free CHS compounds (C₁₁H₁₀S₂, C₁₁H₁₂S₂, C₁₂H₁₂S₂, C₁₂H₁₄S₂, C₁₂H₁₄S₂, C₁₃H₁₆S₂, C₁₄H₂₀S₂). Such compounds are more volatile than oxygen-containing species due to their low polarity. Despite a much lower number of S-containing compounds in white-sand than in *terra firme* DOM (50 vs 316 per sample, respectively; Table 2), CHS compounds occurred much more frequently in white-sand samples (108 vs 14 total occurrences, respectively). The higher abundance of S-containing volatiles in white-sand soils support our findings of elevated variability of S-DOM in these forests. Jointly, these findings suggest the possibility of enhanced production of S-containing volatiles in white-sand forests. These compounds are poorly soluble in water and easily degas from soils (Jardine et al. 2015; Van Leerdam et al. 2011). Small quantities of volatile S compounds can have important effects on aerosol and cloud formation (Jardine et al. 2015). Despite the smaller area covered by white-sand forests, S-production in the white-sand soils might therefore affect regional atmospheric chemistry. Overall, our findings warrant further investigation of S dynamics in white-sand forests of the Amazon.

Above- and belowground nutrient cycling

We hypothesized a greater magnitude of belowground DOM and nutrient cycling in the *terra firme* forests. In support of our hypothesis, DOM in the clayey *terra firme* soils exhibited a stronger imprint of microbial

processing already in 5 cm soil depth (Fig. 4) as evidenced by more aliphatic, lipid-like and saturated DOM (Butturini et al. 2020; Li et al. 2021; Riedel et al. 2016; Roth et al. 2019). In contrast, DOM in white-sand soils was dominated by products of early plant matter decomposition, with dominance of aromatic, oxidized and more oxygen-rich compounds (Gleixner 2013; Hertkorn et al. 2016; Klotzbücher et al. 2013; Spencer et al. 2019). This distinction was supported by annotation of molecular formulas in the ChEBI database (Hastings et al. 2016) (Supporting Text S2). The differences were also reflected in the nutrient pools of DOM (Fig. S2 and S3). N-, P- and S-containing DOM consistently showed higher aromaticity and oxygenation in white-sand sites and higher saturation, and aliphatic and lipid-like content in *terra firme* sites. The molecular composition changed remarkably similarly with soil depth in the white-sand and *terra firme* sites. The DOM composition shifted consistently towards higher DOM processing in greater depth, i.e. decreasing aromatic and increasing aliphatic content. The magnitude of DOM processing in the soil profile was two- to three-fold higher than in white-sand soils (Fig. 2), supporting our hypothesis of higher belowground cycling in *terra firme* sites. The DOM composition generally can be influenced by adsorption processes on mineral surfaces (Subdiaga et al. 2020; Zhang et al. 2020). Specific DOM fractions may preferentially bind to sorption sites, leading to compositional shifts that are determined by the mineralogy of the soil. However, the white-sand soils in our study are virtually sorption-free environments containing mostly quartz. If sorption was driving compositional shifts, we would expect qualitatively different trends in the clayey *terra firme* soils with high sorption capacity. The similarity observed in the depth trends of all soil types strongly suggests that the increasing DOM processing imprint in greater soil depth is primarily driven by the biological processes of decomposition, mineralization and assimilation by microorganisms and plants. Additionally, the importance of sorption was recently demonstrated to strongly decrease when mineral surfaces were already preconditioned (Zhu et al. 2023). In our field setting, we assume that a large portion of available mineral surfaces in *terra firme* sites is already preconditioned due to continuous leaching of litter DOM. Characteristics of fine-textured soils, such as longer residence times, likely increase the effect of

biological DOM processing (Simon et al. 2021). In addition to the chemical characteristics of DOM, also the much lower DOC concentrations in *terra firme* sites indicated more intense processing and mineralization of DOM, leading to lower accumulation of DOC in soil solution.

Our findings highlight the importance of mineral soils for nutrient cycling in *terra firme* forests. DOC concentrations decreased strongly from 5 to 30 cm soil depth (Table 1), and plant-derived, N- and P-containing organic nutrients were processed rapidly and broken down or converted into microorganism-derived compounds during soil passage. The first 30 cm of mineral soil appear important for nutrient retention, at least when considering the bioavailable nutrient pool of DOM. A previous study investigated the molecular DOM composition in groundwater from highly similar *terra firme* plateau and white-sand forests in the same region (Simon et al. 2021). Re-processed data from this study were used to compare DOM processing between 30 cm soil depth and groundwaters. Groundwater DOM from a *terra firme* plateau site revealed a continuation of the depth trends observed in our *terra firme* sites, such as an increasing content of aliphatic and lipid-like formulas compared to 30 cm soil depths (Table S3). For example, in P-DOM, the content of aliphatic compounds increased from 35% in 30 cm soil depth to 62% in groundwater DOM. This implies that significant DOM processing persists even in deeper soil horizons below 30 cm depth. *Terra firme* forests however often have very deep soils (> 10 m). Although roots and microbial activity are typically concentrated near the soil surface (Broedel et al. 2017), our findings suggest that deeper soil compartments may contribute substantially to DOM processing and nutrient cycling. While the significance of aboveground nutrient cycling in tropical forests frequently has been emphasized (Cuevas & Medina 1988; Sayer et al. 2024; Vitousek 1984), our results highlight the crucial role of mineral soils and belowground processes in nutrient (re-)cycling within *terra firme* forests.

In contrast, the lower DOM processing in coarse-textured soils suggests that the mineral soil may be of lower importance for nutrient recycling in white-sand forests, confirming our hypothesis of reduced belowground cycling in these systems. Indeed, also nutrient pools in DOM were only weakly processed in the first 30 cm of white-sand soil. This is supported

by the DOM composition of white-sand groundwater (Simon et al. 2021), which was highly similar to DOM leached from the white-sand forest organic layer (0 cm soil depth). For example, the abundance of aromatic compounds was not significantly different between organic layer- and groundwater-derived DOM for N-, S-, and P-containing pools (Kruskal Wallis test, $p > 0.05$). This strongly suggests that the mineral soil is less important for nutrient recycling in white-sand forests than in *terra firme* forests. While the processing of nutrient-DOM in the soil profile was much lower than that in *terra firme* sites, the temporal variability in 0 cm soil depth was of a similar magnitude in both rainforest types (Fig. 3). This suggests that nutrient cycling in white-sand forests was more pronounced in the organic layer than in the mineral soil, and supports the reports that the organic layer and specifically root mats are more important for nutrient retention in these forests, in line with the classic notion of aboveground nutrient recycling (Stark & Jordan 1978; Vitousek 1984). Those organic nutrients that entered the mineral soil were not processed strongly during soil passage compared to the *terra firme* forests. Upland white-sand forests have been shown to export characteristic plant-derived DOM to the aquatic continuum (Simon et al. 2021), and our research suggests that these markers originate from the surface organic layers of white-sand areas, and may thus be directly linked to litter production and recycling. At our white-sand sites, DOC concentrations did not decrease significantly with soil depth (CD, *Campina*) or remained high in 30 cm soil depth ($> 30 \text{ mg C l}^{-1}$; CW, *Campinarana*). In the groundwater of the white-sand site (Simon et al. 2021), DOC concentrations were $> 25 \text{ mg C l}^{-1}$, suggesting that white-sand forests export considerable amounts of DOC to the groundwater and downstream rivers. The low processing of organic nutrient pools during soil passage indicates that organic nutrients were likely not processed efficiently by microbial communities in the mineral soil. Despite indications for higher nutrient cycling in the organic layer, plant-derived organically-bound nutrients could be lost to the groundwater instead of being recycled. Our results support the hypothesis that nutrient retention in white-sand forests occurs primarily above the mineral soil. We provide novel evidence that minimal DOM processing occurs during transport from the organic layer into groundwater. The limited belowground nutrient

processing potentially makes these ecosystems much more vulnerable to disturbances that disrupt the intact aboveground nutrient recycling.

Conclusion

Our study derived three proxies for estimating the nutrient cycling intensity through the DOM pool and indicate potential nutrient limitations: relative nutrient depletion, DOM processing in the soil profile, and temporal variability of the DOM composition. The three proxies supported the hypothesized P-limitation in both *terra firme* forest sites, including one with younger pedogenesis and higher P availability. This implies that P-limitation may extend Amazon rainforests on less infertile soils. As P-limitation on ecosystem productivity is more commonly assumed for ancient soils, this has implications for the distribution of nutrient limitations in the central Amazon and its response to global change. The observed high processing of N-DOM raises the possibility of N co-limitation, further influencing the Amazon's response to global change. Contrary to our hypothesis, N-limitation in white-sand forests was not confirmed. Instead, two proxies unexpectedly indicated elevated demand and cycling of S. These findings highlight the need for a better understanding of the diverse nutrient limitations within the Amazon basin to forecast productivity responses accurately.

Our results further emphasize the important role of the mineral soil for nutrient recycling in *terra firme* but not white-sand soils. Effective belowground processes may alleviate increasing P-limitation to a degree by retaining nutrients efficiently and possibly accessing P reserves present in the soil. Considering the diversity of ecosystems in the Amazon, their individual nutrient dynamics will be key to forecast basin-wide dynamics. The proxies used in this study open avenues to investigate nutrient cycling, which can help understand ecosystem nutrient demands and limitations. HR-MS techniques provide an important analytical means to disentangle the complex composition and dynamics of DOM and the contained organic nutrient species.

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Author contributions Carsten Simon, Gerd Gleixner and Carlos A. Quesada conceived and designed the frame of the study; D. Frederik Lange, Carsten Simon and Gerd Gleixner coordinated the sampling campaigns; Fernanda M. da Luz and Yago R. Santos helped during field work and performed soil and water chemistry measurements; Elaine Pires and Jonismar S. da Silva performed water chemistry measurements; Stefanie Hildmann performed sampling of soil gas, and Stefanie Hildmann and Thorsten Hoffmann helped with measurements and data analysis of soil gas; D. Frederik Lange and Simon A. Schröter analyzed the data. D. Frederik Lange, Carsten Simon, Thorsten Schäfer and Gerd Gleixner interpreted the data; D. Frederik Lange led the writing of the manuscript, and Carsten Simon and Gerd Gleixner contributed to the writing of the first draft; all authors contributed to the drafts and gave final approval for submission.

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Data availability The data generated and analyzed for this study are publicly available on the ‘Environmental Data Initiative’ repository (edirepository.org; <https://doi.org/10.6073/pasta/0f91c0935840c0eaae74d8091a1d5bf1>).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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