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Degradation of terrestrially derived macromolecules in the Amazon River

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Temperate and tropical rivers serve as a significant source of carbon dioxide to the atmosphere[1–4]. However, the source of the organic matter that fuels these globally relevant emissions is uncertain. Lignin and cellulose are the most abundant macromolecules in the terrestrial biosphere[5], but are assumed to resist degradation on release from soils to aquatic settings[6–8]. Here, we present evidence for the degradation of lignin and associated macromolecules in the Amazon River. We monitored the degradation of a vast suite of terrestrially derived macromolecules and their breakdown products in water sampled from the mouth of the river throughout the course of a year, using gas chromatography time-of-flight mass spectrometry. We identified a number of lignin phenols, together with 95 phenolic compounds, largely derived from terrestrial macromolecules. Lignin, together with numerous phenolic compounds, disappeared from our analytical window following several days of incubation at ambient river temperatures, indicative of biological degradation. We estimate that the net rate of degradation observed corresponds to 30–50% of bulk river respiration. Assuming that a significant fraction of these compounds is eventually remineralized to carbon dioxide, we suggest that lignin and other terrestrially derived macromolecules contribute significantly to carbon dioxide outgassing from inland waters.

The immense outgassing of CO_2 from inland waters is now part of the paradigm of the global carbon cycle[2,4]. Globally, inland waters process, transport and bury 2.7 Pg C yr^{-1} (refs 9,10), a flux nearly equivalent to the terrestrial sink for anthropogenic CO_2 of 2.8 Pg C yr^{-1} (refs 9,11). The Amazon River accounts for roughly 20% of the fresh water discharged to the world's oceans, making it an ideal test bed for understanding large-scale biogeochemical processes and fluxes. The Amazon rainforest is responsible for nearly 10% of global primary production, or the fixation of 8.5 Pg C yr^{-1} (refs 12,13). Respiration of contemporary organic matter originating on land and near rivers is the dominant source[14] sustaining a CO_2 supersaturation in the Amazon River that drives an outgassing of 0.5 Pg C yr^{-1} (ref. 2), roughly equivalent to the amount of carbon actually sequestered by the forest[12,13]. Our present understanding of the source, chemical composition and microbial controls of the organic matter pool driving this vast CO_2 outgassing, however, is speculative at best.

As a parcel of organic matter moves through the river continuum, it is degraded by both microbial and photochemical activity. Numerous studies have demonstrated the photo-degradability of chromophoric dissolved organic matter[15] and lignin phenols[16], suggesting that photo-oxidation is an important pathway for organic matter remineralization in inland waters. On the contrary, *in situ* ultraviolet-oxidation experiments performed in the Amazon suggest that photo-oxidation accounts for roughly 0.05% of basin-wide outgassing rates[17]. A parcel of Amazon River water spends little time exposed to ultraviolet radiation in the turbid mainstem because of its rapid velocity, river depth (roughly 60 m at Óbidos, 28 m at Macapá) and low light penetration. Thus, depth-integrated respiration has been suggested to be the primary source of p_{CO_2} in the Amazon River[2].

The organic matter respired in the Amazon has been shown to be relatively young, with a radiocarbon age of less than five years old[14]. This pool of organic matter is thought to be rapidly overturned, whereas terrestrially derived compounds with complex macromolecular structures, such as lignin, have traditionally been assumed to be refractory and implicitly old[6–8]. In the past decade, however, it has been increasingly recognized that recalcitrance is not molecularly intrinsic, per se, but dependent on physiochemical and biological influences[18]. For example, numerous studies have demonstrated the biodegradability of lignin and celluloses in soils[19,20]. If lignin and celluloses are similarly reactive in the aquatic setting, they are a large potential source of bioavailable carbon to the river. The most abundant biochemicals on land are cellulose, hemicellulose and lignin[5]. They represent as much as 80% of the biomass in a typical forest and as much as 60% of the biomass in a typical field (natural or crop)[21,22]. Lignin composes roughly 30% of the organic carbon in the terrestrial biosphere[5]. We assessed the rate of degradation of lignin and other macromolecular compounds as well as bulk respiration and carbon parameters at the Amazon River mouth. Whole water was collected in triplicate 4 l bottles and incubated at ambient river temperature for 5–7 days in the dark without any nutrient amendments. Particulate and pre-filtered dissolved organic matter (DOM) fractions were collected before and after incubation on glass-fibre filters (0.7 μm) and solid-phase extraction cartridges[23], respectively. Lignin and phenolic polymers in the particulate and DOM fractions were analysed by time-of-flight mass spectrometry (GC–ToF–MS) in addition to free monomers in the dissolved phase.

To target a vast suite of the most abundant terrestrial molecules in the river, we quantified all detected compounds containing at least one phenolic ring by GC–ToF–MS. We did this analysis before and after oxidative cleavage with CuO to account for both phenolic monomers and complex polyphenolic macromolecules[24]. We

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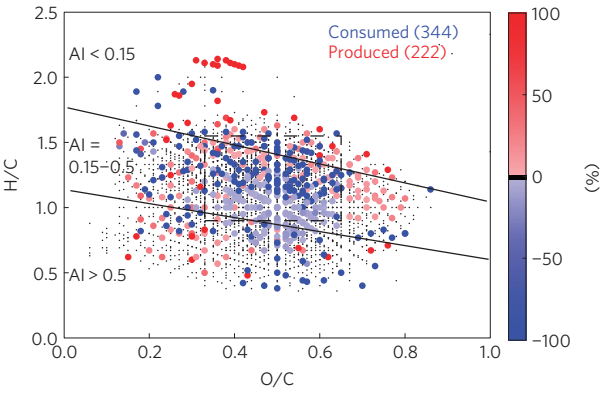

Figure 1 | Van Krevelen diagrams of incubated Amazon River DOM. The relative degradation/production of DOM compounds (based on hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) ratios) after a 10-day incubation period. Of the 2,804 compounds detected (black), 344 were consumed (blue) and 222 were produced (red). Most consumed DOM compounds have element ratios consistent with lignin and other polyphenols (centred rectangle). Compounds with an aromaticity index (AI) > 0.5 consist of aromatic core structures with few side groups, whereas compounds with AI < 0.5 contain more side groups, as typical for lignin and other polyphenols[25].

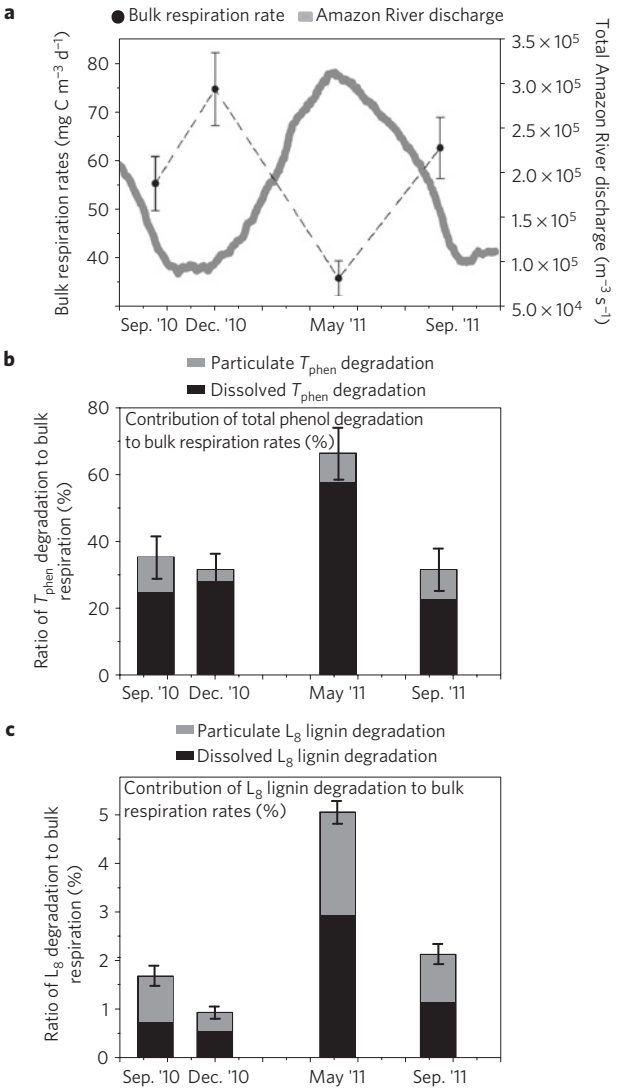

Figure 2 | Bulk and specific respiration rates in the Amazon River. a, Bulk respiration rates (black) and total Amazon River discharge (grey) from September 2010–September 2011. **b**, The relative contribution of dissolved (black) and particulate (grey) total phenolic compound (T_{phen}) degradation to bulk respiration (T_{phen} degradation rate/bulk respiration rate × 100). **c**, The contribution of dissolved (black) and particulate (grey) standard lignin phenols L_8 to bulk respiration.

detected 95 targeted phenolic compounds, which were largely released from lignin or other macromolecules (for example celluloses, tannins and suberins) by our pre-analytic oxidation procedure. Although phenolic structures are ubiquitous, their concentrations in vascular plants are typically orders of magnitude higher than in aquatic primary producers. The abundance of the eight standard lignin phenols (L_8) and our suite of 95 macromolecular derivatives (total phenols or T_{phen}; includes L_8 phenols) was measured before and after incubation to determine net degradation and production rates. Here, a phenol is considered degraded when its phenolic ring is cleaved. L_8 abundance was normalized to a carbon concentration using available standards. T_{phen} abundances were assessed by their relative GC–ToF–MS peak areas. To normalize T_{phen} concentrations and degradation rates from peak area to carbon units, we divided the known L_8 lignin concentrations by the relative proportion of L_8 phenols to the total phenol suite. Error estimations include the uncertainty regarding compound isomerization in this regard. These experiments were performed simultaneously with measurements of bulk respiration and conventional chemical parameters.

In addition to targeting specific compounds as described above, we extended our analytical window by determining the molecular formulae of a very broad range of compounds in DOM using Fourier transform ion cyclotron resonance mass spectrometry (FT–ICR–MS). By using a soft electrospray ionization (ESI) technique, the DOM compounds were ionized as intact moieties. The masses of the individual molecules in the complex DOM cocktail were determined with such accuracy that we were able to determine the molecular formulae of ~2,800 compounds throughout the set of incubations. This approach allowed for the detection of a very broad range of molecular formulae, as opposed to the approach described above. Basic structural features (for example, the density of double bonds, or the presence of aromatic units) can be deduced from molecular formulae alone, even though the exact configuration of these units remains unknown[25]. ESI–FT–ICR results showed significant loss of specific DOM compounds throughout the incubations. Approximately 60% of the compounds whose post-incubation abundance was below detection had molecular formulae consistent with those of lignin and other polyphenols (for example, tannins). Many of these compounds consisted of a phenolic core structure with few side groups (aromaticity index > 0.5) whereas others contained more of such side groups, as typical for lignin and tannin derivatives[25] (Fig. 1). Whereas most polyphenols decreased in abundance, some of these compounds were also produced during the incubations. These produced compounds are probably intermediates in the breakdown pathway (for example, functional group transformations). Consistent with this observation, the targeted GC–ToF–MS approach was used to quantify both the production and degradation of phenolic compounds. The net phenolic degradation rates described here account for this observation and include produced intermediates.

Targeted GC–ToF–MS measurements revealed an annual average net L_8 degradation rate of 0.67 ± 0.08 and 0.57 ± 0.07 mg C m^{-3} d^{-1} for dissolved and particulate L_8 lignin, respectively. The average degradation rate of total phenolic compounds (T_{phen}) in the dissolved and particulate phases was 17.9 ± 3.8 and 4.3 ± 0.9 mg C m^{-3} d^{-1}, respectively. Bulk respiration was, on average, 58.0 ± 5.8 mg C m^{-3} d^{-1}. The rate of T_{phen} degradation is equivalent to approximately 30–50% of bulk respiration rates

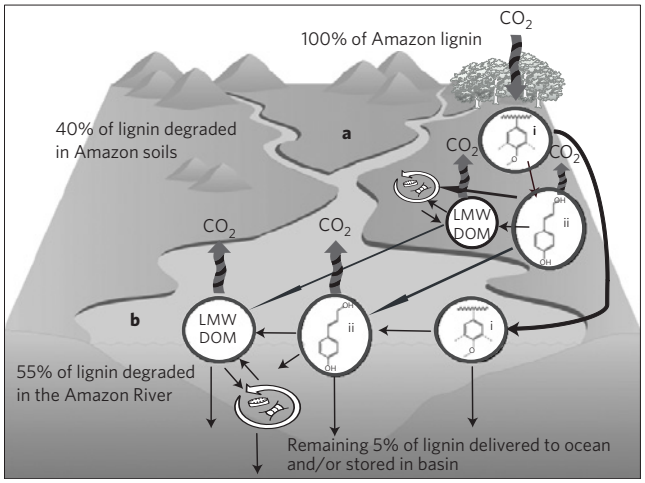

Figure 3 | Fate of lignin in the Amazon River watershed. a, Terrestrial primary production converts atmospheric CO_2 into biomass dominated primarily by lignin, cellulose and hemicellulose compounds[5]. Within soils, large lignin macromolecules (i) are broken down into free lignin monomers (ii). These free lignin monomers are broken down into low-molecular weight (LMW) DOM intermediates. Degradation in soils processes roughly 40% of the lignin sequestered in the terrestrial biosphere into small non-phenolic components[12,13,21,29,30]. These small components are either cycled in soil food webs, remineralized to CO_2, or mobilized into the river with the remaining lignin macromolecules and monomers. **b**, Within the river–ocean continuum, terrestrial macromolecules are continuously broken down into monomers, which are either remineralized directly to CO_2, broken into low-molecular weight (LMW) DOM intermediates, or cycled within the microbial food web, supporting micro and macrofaunal heterotrophic production. 55% of the lignin sequestered on land is degraded (that is, the phenolic structure is broken) in the river continuum[12,13,21,29,30]. The remaining <5% is either stored within the basin or delivered to the ocean, where it is degraded or buried in marine sediments.

in the river, suggesting that lignin and other terrestrially derived macromolecules play a significant role in river productivity. These degradation rates relative to measured concentrations imply that the entire pool of lignin and related phenolic compounds in the Amazon River could completely overturn in only 2–3 weeks.

Although organic matter concentrations and bacterial abundances peaked during high-water conditions in May (Supplementary Table S2), bulk respiration rates were lowest in May and highest in December (Fig. 2), in agreement with previous respiration measurements made in the Amazon showing minimal respiration rates and maximal bacterial growth efficiency during high water[26]. On the contrary, L_8 and T_{phen} degradation rates were loosely correlated with abundance and organic matter inventories, reaching their maximum at high water (Fig. 2). Whereas bulk OC concentrations and bulk respiration rates are not correlated, the rates of L_8 and T_{phen} degradation correspond well with substrate availability throughout the sampling period. Further, the contribution of L_8 and T_{phen} degradation to bulk respiration rates is highest ($73\pm21\%$) in May (Fig. 2). These trends may be a reflection of the efficiency of lignin as a growth substrate relative to other bulk OC components. Efficiency, in this sense, is the proportion of an OC substrate that is fixed as microbial biomass relative to the amount that is respired to CO_2. One explanation for our observed trends is that lignin is used inefficiently (for example, a greater proportion is remineralized to CO_2 than fixed into microbial biomass) relative to much of the bulk OC mobilized at high water. Although bulk OC concentrations increase significantly with increasing discharge, most of these compounds are probably fixed as microbial biomass rather than respired to CO_2 (reflected by low bulk respiration rates and high bacterial abundance), whereas lignin degradation may provide a significant source of the respired CO_2 during high-water conditions. The inherent growth efficiency of different organic matter substrates in the Amazon remains elusive, however, and is a necessary step in resolving the fate of organic matter as it passes along the tropical river continuum.

As lignin concentrations correlate with discharge both on seasonal[27] and hourly to daily[28] timescales in tropical[27] and temperate systems[28] alike, we posit that river systems worldwide provide positive environments for biological macromolecular breakdown. These macromolecular degradation products are probably important fuels for respiration and are either remineralized to CO_2 or recycled among microbial food webs. The remaining balance of organic matter sources that contribute to bulk respiration rates is probably a combination of autochthonous and other terrestrially derived compounds not measured here. We propose that the relative contribution of different organic matter sources to respiration is seasonally dependent; for example, floodplain drainage during falling water delivers a large source of labile algal-derived organic matter to the mainstem. Although microbial degradation probably dominates the breakdown of macromolecules in the Amazon, photochemical reactions probably play a larger role in aquatic ecosystems that are slower moving, less turbid and shallower than the Amazon River mainstem[15,16].

On the basis of our measurements and those made by others[12,13,21,29,30], we have made rough estimations to describe the basin-wide fate of lignin and related macromolecules in the Amazon watershed as they travel from soils, through the river, and into the ocean (Fig. 3). We estimate that approximately 80 Tg C of lignin is fixed in the Amazonian terrestrial biosphere annually. Over the lifetime of those lignin molecules, we estimate that roughly 40% of this lignin is degraded into smaller components in soils, 55% is degraded into smaller components within the river continuum, and the remaining 5% of intact macromolecules are either stored within the river continuum or delivered to the ocean (Fig. 3). We propose that the breakdown of terrestrially derived macromolecules (including lignin, celluloses and hemicelluloses) fuels the small rapidly cycling organic matter pool described as the primary driver for evasive CO_2 gas fluxes in the Amazon[14]. The collective results from this study present strong evidence of the biodegradability of terrestrially derived macromolecules in the aquatic setting and provide a significant step in integrating the emerging paradigm regarding chemical recalcitrance[18] with global carbon budgets.

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References

1. Cole, J. J., Caraco, N. F., Kling, G. W. & Kratz, T. K. Carbon dioxide supersaturation in the surface waters of lakes. *Science* **265,** 1568–1570 (1994).
2. Richey, J. E., Melack, J. M., Aufdenkampe, A. K., Ballester, V. M. & Hess, L. L. Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO_2. *Nature* **416,** 617–620 (2002).
3. Duarte, C. & Prairie, Y. Prevalence of heterotrophy and atmospheric CO_2 emissions from aquatic ecosystems. *Ecosystems* **8,** 862–870 (2005).
4. Butman, D. & Raymond, P. Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geosci.* **4,** 839–842 (2011).
5. Boerjan, W., Ralph, J. & Baucher, M. Lignin biosynthesis. *Ann. Rev. Plant Biol.* **54,** 519–546 (2003).
6. Hedges, J., Clark, W. & Cowie, G. Fluxes and reactivities of organic-matter in a coastal marine bay. *Limnol. Oceanograph.* **33,** 1137–1152 (1988).
7. Gough, M., Fauzi, R., Mantoura, C. & Preston, M. Terrestrial plant biopolymers in marine sediments. *Geochim. Cosmochim. Acta* **57,** 945–964 (1993).
8. Opsahl, S. & Benner, R. Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386,** 480–482 (1997).
9. Battin, T. *et al.* Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geosci.* **1,** 95–100 (2008).
10. Tranvik, L. *et al.* Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanograph.* **54,** 2298–2314 (2009).

11. Canadell, J. *et al*. Factoring out natural and indirect human effects on terrestrial carbon sources and sinks. *Environ. Sci. Policy* **10,** 370–384 (2007).
12. Field, C., Behrenfeld, M., Randerson, J. & Falkowski, P. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* **281,** 237–240 (1998).
13. Malhi, Y. *et al*. Climate change, deforestation, and the fate of the Amazon. *Science* **319,** 169–172 (2008).
14. Mayorga, E. *et al*. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature* **436,** 538–541 (2005).
15. Rodríguez-Zúñiga, U. F. *et al*. Changes in optical properties caused by ultraviolet-irradiation of aquatic humic substances from the Amazon River Basin: Seasonal variability evaluation. *Environ. Sci. Technol.* **42,** 1948–1953 (2008).
16. Spencer, R. G. M. *et al*. Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. *J. Geophys. Res.* **114,** G03010 (2009).
17. Remington, S., Krusche, A. V. & Richey, J. E. Effects of DOM photochemistry on bacterial metabolism and CO_2 evasion during falling water in a humic and a whitewater river in the Brazilian Amazon. *Biogeochemistry* **105,** 185–200 (2011).
18. Schmidt, M. W. I. *et al*. Persistence of soil organic matter as an ecosystem property. *Nature* **478,** 49–56 (2011).
19. Paterson, A. & Lundquist, K. Radical breakdown of lignin. *Nature* **316,** 575–576 (1985).
20. Feng, X. J. *et al*. Increased cuticular carbon sequestration and lignin oxidation in response to soil warming. *Nature Geosci.* **1,** 836–839 (2008).
21. Bose, S. K. *et al*. Lignin content versus syringyl to guaiacyl ratio amongst poplars. *Biores. Technol.* **100,** 1628–1633 (2009).
22. Martens, D., Reedy, T. & Lewis, D. Soil organic carbon content and composition of 130-year crop, pasture and forest land-use managements. *Glob. Change Biol.* **10,** 65–78 (2004).
23. Keil, R. & Neibauer, J. Analysis of cooking spices in natural waters. *Limnol. Oceanograph. Methods* **7,** 848–855 (2009).
24. Goni, M. A. & Montgomery, S. Alkaline CuO oxidation with a microwave digestion system: Lignin analyses of geochemical samples. *Anal. Chem.* **72,** 3116–3121 (2000).
25. Koch, B. P. & Dittmar, T. From mass to structure: An aromaticity index for high-resolution mass data of natural organic matter. *Rapid Commun. Mass Spectrom.* **20,** 926–932 (2006).
26. Benner, R., Opsahl, S., ChinLeo, G., Richey, J. & Forsberg, B. Bacterial carbon metabolism in the Amazon River system. *Limnol. Oceanogr.* **40,** 1262–1270 (1995).
27. Ellis, E. E., Keil, R. G., Ingalls, A. E. & Richey, J. E. Seasonal variability in the sources of particulate organic matter of the Mekong River as discerned by elemental and lignin analyses. *J. Geophys. Res.* **117,** G01038 (2012).
28. Ward, N., Richey, J. & Keil, R. Temporal variation in river nutrient and dissolved lignin phenol concentrations and the impact of storm events on nutrient loading to Hood Canal, WA, USA. *Biogeochemistry* **111,** 629–645 (2012).
29. Hedges, J., Blanchette, R., Weliky, K. & Devol, A. Effects of fungal degradation on the CuO oxidation-products of lignin—a controlled laboratory study. *Geochim. Cosmochim. Acta* **52,** 2717–2726 (1988).
30. Houghton, R., Lawrence, K., Hackler, J. & Brown, The spatial distribution of forest biomass in the Brazilian Amazon: A comparison of estimates. *Glob. Change Biol.* **7,** 731–746 (2001).

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Author contributions

P.M.M. and T.D. performed ESI–FT–ICR–MS analyses and data interpretation. D.C.B. and A.C.C. measured bulk respiration rates and organized Macapá sampling logistics. A.V.K. measured DOC concentrations and oversaw and organized all Amazon River sampling logistics. P.L.Y. measured bacterial abundance and coordinated the River-Ocean Continuum of the Amazon project. J.E.R. calculated discharge rates and managed field logistics. N.D.W. and R.G.K. designed the study, performed all lignin and GC–ToF–MS analyses, and wrote the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.