Leaf wax biomarkers in transit record river catchment composition

Auxiliary Material

1. Methods

1.1. Characterizing catchment hypsometry

River and stream water samples, and fluvial sediment samples, were collected at point locations but integrate a range of elevation and areal extent in the upstream catchments. All or some of the upstream area may contribute material to the samples we collected. Catchment areas upstream of each sampling point were delineated to determine the elevation distributions within each catchment, as well as the median catchment elevations. Catchment areas were determined using a flow routing algorithm in GRASS GIS software, using SRTM-derived digital elevation data with a spatial resolution of 3 arc seconds (~90 m). Results of catchment hypsometric analysis are reported in Table S1 for POM samples and Table S2 for stream waters.

1.2. Water

1.2.1. Stream water sampling: Small streams were collected as representative of local precipitation for measurement of water isotopic composition across the elevation gradient during March 2013 (wet season) and August 2013 (dry season). Sampling locations are reported in Table S2.

1.2.2. Water isotopic analysis: δD values were measured on 8 replicate injections of 0.7 μL water using a Los Gatos Research DLT-1000 liquid water isotope analyzer at the

California Institute of Technology. Replicate measurements yielded a mean precision (1σ) of 0.4‰ (n = 43) and were calibrated using 2 working standards (Maui Water, δD = −10.6‰, and LGR Water # 2, δD = −117.0‰) to the VSMOW–SLAP isotopic scale with accuracy determined to better than 0.2 ‰. δD results are reported in Table S2.

1.3. Biomarkers

1.3.1. River POM sampling: Detailed sampling of the Kosñipata and Madre de Dios Rivers was performed during March 2013 (wet season) and August 2013 (dry season). River water samples were collected across an elevation gradient from 2261−177 masl at the location of sampling, but these sites integrate catchments that extend to above 4km in elevation (Table S1). Sampling locations were selected to span the transition from the Andes to the Amazon lowlands. At the upper sampling locations, the Kosñipata River is turbulent and carries a high, well mixed sediment load in the wet season; here, sampling was achieved from the banks. At the lower stations the river is navigable and samples were collected by boat within the channel where the velocities were highest.

Large volumes of river water (60–180 L) were collected by 10 L bucket; 100% of the sample was transferred into wine bags with a liner of ethylene vinyl alcohol (EVOH). Bags were transported and stored in the dark prior to filtering. Water samples were filtered within 12 hrs at 0.2 μm on polyethersulfone (PES) filters (90 and 147 mm diameter) using pressurized filter units operated by bicycle pump, similar to the methods of *Galy et al.* [2011]. Filters containing the POM were stored in Whirlpak bags under cool conditions during the 2 week fieldwork in remote areas until transport back to the laboratory for refrigeration. In the laboratory, the POM was rinsed off the filter with milliQ water and

subsequently freeze-dried using a Virtis 2k unit. Dry samples were disaggregated and material coarser than 1 mm was removed using a sieve. Coarse material was infrequently recovered and comprised occasional leaf and wood debris. Only one sample (MMD-11), a tributary river, contained a significant portion of >1 mm wood debris, but still it only amounted to 0.8% by weight of the total recovered suspended material. The organic matter content of the sieved suspended sediments constitutes the particulate organic matter (POM 0.2−1000 μm).

1.3.2. River bank and bed sampling: River bed sediments were collected by dredging and bank sediments (fluvial type, not soils) were collected by hand. Neither of these river sediment types contained sufficient plant waxes for isotopic measurement (despite extracting >50 g of dry sediment), suggesting that suspended POM is the primary contributor of leaf waxes to sedimentary archives.

1.3.3. Soil sampling: Soil samples were previously collected from representative 1ha plots within the forested catchment by A. Nottingham and P. Meir (Table S3). Soils were sampled from five systematically distributed 40 x 40 cm sub-plots within the 1 ha sample plot at each study site, then dried and homogenized. Samples were collected from the top of the mineral soil horizons occurring immediately underneath the organic soil horizon. The total thickness of the mineral soil horizons was not measured. In the low elevation rainforest the top of the mineral horizon is found as shallow as 1cm deep in the soil profile while in the high elevation forest the mineral horizon can be as deep as 23cm (Table S3).

1.3.4. Lipid extraction: All sediment samples were extracted under high temperature (100 °C) and pressure (1500 psi) with DCM/MeOH (9:1 v/v) using an Accelerated Solvent

Extraction system (ASE 350®, Dionex). The extract was separated using column chromatography (5 cm x 40 mm Pasteur pipette, NH_2 sepra bulk packing, 60 Å), eluting with 2:1 DCM/isopropanol, followed by 4% HCO_2H in Et_2O, yielding neutral and acid fractions respectively. The acid fraction containing *n*-alkanoic acids was transesterified with 5% HCl and 95% MeOH (of known isotopic composition) at 70 °C for 12 h to yield corresponding fatty acid methyl esters (FAMEs). Excess milliQ water was added to the hydrolyzed products and the lipids were partitioned into hexane, and dried by passing through a column of anhydrous Na_2SO_4. Lipids were further purified using column chromatography (5 cm x 40 mm Pasteur pipette, 5% water-deactivated silica gel, 100–200 mesh), eluting with hexane first, and then with DCM to isolate the pure FAMEs fraction.

1.3.5. Biomarker hydrogen isotopic analyses: Compound specific hydrogen isotopic values were obtained using gas chromatography isotope ratio mass spectrometry (GC-IRMS). We used a Thermo Scientific® Trace gas chromatograph equipped with a Rxi-5ms column (30 m x 0.25 mm, film thickness 1 μm) and a programmable temperature vaporizing (PTV) injector operated in solvent split mode with an evaporation temperature of 150°C to exclude the low boiling point compounds of very high abundance (predominantly C_{16} and C_{18} *n*-alkanoic acids), following *Feakins et al.* [2014]. The GC was connected via a GC Isolink with pyrolysis furnace (at 1400 °C) via a Conflo IV interface to a DeltaV^{Plus} isotope ratio mass spectrometer. To check for linearity in isotopic determination across a range of peak amplitude (1−8 V), the H_3 factor was measured daily and remained close to 4 ppm mV^{-1}. Reference peaks of H_2 were co-injected between *n*-alkanoic acid peaks during the course of a GC-IRMS run; two of these peaks were used for standardization of the isotopic analysis, while the remainders were treated as unknowns to

assess precision. Except for the case of co-elution, precision of these replicates was better than 0.6‰. Samples were interspersed with standard compound mixtures of known isotopic composition. Data were normalized to the VSMOW/SLAP hydrogen isotopic scale by comparing with an external standard containing 15 *n*-alkane compounds (C_{16} to C_{30}) obtained from A. Schimmelmann, Indiana University, Bloomington. The δD values of the external standard mixture span −9 to −254‰. The RMS error determined by replicate measurements of the standard across the course of analyses was 4.2‰. We further monitored for instrument drift by measuring the δD values of a C_{34} *n*-alkanoic acid internal standard co-injected with the sample. The isotopic composition of H added during methylation of FAs as methyl esters was estimated by methylating and analyzing phthalic acid as a dimethyl ester (isotopic standard from A. Schimmelmann, University of Indiana) yielding $\delta D_{methanol} = -198.3‰ \pm 3.9$ ($n = 7$). Correction for H added by methylation was then made by way of mass balance. The results are reported using conventional delta notation (δD‰). Data are reported for the C_{28} *n*-alkanoic acid as it is sufficiently abundant and thought to be derived exclusively form terrestrial leaf waxes [*Kusch et al.*, 2010]. POM samples in Table S1 and soil results are reported in Table S3.

Supplementary Table S1: River POM sampling locations, hypsometric analysis, δD results.

Sample ID	River	Location	Date	Latitude	Longitude	Sed. Load (g/L)	Elevation (km)	Med Elev† (km)	Variance†	δD_{POM}* (‰)	1σ (δD)	δD_{water}** (‰)	$\varepsilon_{wax/water}$
Wet Season													
CMD-6	Kosñipata	Wayqecha	3/12/2013	-13.162811	-71.589136	63.4	2.261	3.242	0.133	-191	2.9	-94	-107
CMD-4	Kosñipata	San Pedro	3/11/2013	-13.161644	-71.588884	6.5	1.377	2.795	0.314	-175	1.8	-85	-99
CMD-9	Kosñipata	Pilcopata	3/13/2013	-12.907491	-71.400879	1.9	0.593	2.245	1.378	-184	3.8	-75	-118
CMD-8	Carbon	Atalaya	3/13/2013	-12.895642	-71.353152	0.6	0.591	0.768	0.015	-171	2.6	-41	-136
CMD-11	Madre de Dios	MLC	3/14/2013	-12.787345	-71.390097	2.6	0.479	1.830	1.141	-194	4.2	-68	-135
CMD-13	Madre de Dios	MLC - Storm	3/15/2013	-12.788753	-71.390375	2.3	0.446	1.830	1.141	-175	1.7	-68	-115
CMD-14	Unknown	MLC-Manu	3/15/2013	-12.670573	-71.293492	2.0	0.407	0.522	0.003	-163	2.5	-36	-132
CMD-15	Madre de Dios	Up+ Manu	3/15/2013	-12.274858	-70.932611	2.6	0.327	1.454	1.195	-177	1.3	-62	-123
CMD-19	Madre de Dios	Up Manu-Storm	3/16/2013	-12.275119	-70.932800	1.6	0.297	1.454	1.195	-161	3.1	-62	-106
CMD-23	Madre de Dios	Up Chiribi	3/16/2013	-12.490537	-70.600230	2.9	0.260	0.471	0.803	-177	2.0	-44	-139
CMD-24	Chiribi	Chiribi	3/16/2013	-12.496893	-70.598998	1.1	0.258	0.369	0.031	-167	4.3	-34	-137
CMD-25	Madre de Dios	Dw++ Chiribi	3/16/2013	-12.545675	-70.521657	1.5	0.255	0.465	0.773	-160	2.7	-44	-119
CMD-30	Los Amigos	CICRA	3/17/2013	-12.577569	-70.074527	0.6	0.227	0.360	0.003	-149	1.9	-32	-121
CMD-29	Madre de Dios	CICRA	3/17/2013	-12.580506	-70.095775	0.2	0.222	0.445	0.686	-163	1.3	-42	-126
CMD-40	Piedras	Pt. Maldonado	3/20/2013	-12.427048	-69.262931	1.7	0.221	0.413	0.007	-160	1.3	-33	-131
CMD-39	Pariamanu	Pt. Maldonado	3/20/2013	-12.429859	-69.276406	0.6	0.218	0.314	0.002	-166	0.9	-31	-139
CMD-33	Madre de Dios	Laberinto	3/18/2013	-12.720328	-69.589414	1.6	0.195	0.605	2.268	-179	0.5	-56	-130
CMD-31	Madre de Dios	Up Inambari	3/18/2013	-12.710840	-69.743153	0.8	0.190	0.419	0.608	-164	4.0	-40	-129
CMD-35	Madre de Dios	Pt. Maldonado	3/19/2013	-12.562655	-69.177003	1.3	0.176	0.451	1.896	-178	1.6	-50	-135
Dry Season													
MMD-2	Kosñipata	San Pedro	8/11/2013	-13.057924	-71.544988	<0.1	1.377	2.795	0.314	-165	2.0	-85	-88
MMD-10	Madre de Dios	MLC	8/14/2013	-12.785203	-71.388184	1.3	0.461	1.830	1.141	-167	2.3	-68	-106
MMD-11	Teparo	Dw MLC	8/14/2013	-12.720018	-71.378024	1.4	0.446	0.564	0.075	-154	8.7	-34	-125
MMD-28	Madre de Dios	CICRA	8/16/2013	-12.579825	-70.096337	1.1	0.211	0.445	0.686	-154	2.1	-42	-117
MMD-34	Piedras	Rio Piedras	8/19/2013	-12.518722	-69.248217	0.1	0.168	0.413	0.007	-146	1.4	-50	-108
MMD-32	Madre de Dios	Pt. Maldonado	8/18/2013	-12.56322	-69.175953	0.3	0.166	0.451	1.896	-152	5.1	-33	-117

† Median elevation for the elevation distribution of the catchment upstream the sampling location.

* δD_{POM} = hydrogen isotope composition or river particulate organic matter (C_{28} *n*-alkanoic acid).

** δD_{water} = estimated value for δD of precipitation at the sample median elevation in the dry season

+ Up = upstream ++ Dw = downstream

Supplementary Table S2: Stream water sampling locations, hypsometric analysis and δD results.

Sample ID	Location (river valley)	Latitude	Longitude	Elevation (km)	Median† Elevation (km)	δD* (‰)	1σ (δD)	δ^{18}O (‰)	1σ (δ^{18}O)
Wet Season									
WP-233	Inambari	-13.64308	-71.10530	4.624	4.720	-145	0.3	-18.7	0.1
WP-232	Inambari	-13.63217	-71.07385	4.366	4.614	-132	0.1	-17.5	0.3
WP-231	Inambari	-13.58482	-70.99729	3.748	4.212	-120	0.8	-16.7	0.1
WP-230	Inambari	-13.58482	-70.99729	3.090	3.822	-110	0.7	-15.4	0.2
WP-228	Inambari	-13.60073	-70.97184	2.650	3.506	-106	0.4	-14.3	0.1
WP-227	Inambari	-13.53295	-70.89756	2.068	3.193	-91	0.5	-13.2	0.1
WP-93	Kosñipata	-13.08953	-71.56399	1.878	2.368	-91	0.5	-13.7	0.2
WP-94	Kosñipata	-13.05412	-71.54554	1.374	1.722	-79	0.8	-12.4	0.0
WP-226	Inambari	-13.44764	-70.90353	1.300	2.563	-76	0.7	-11.5	0.3
WP-95	Kosñipata	-13.03746	-71.51302	1.144	1.470	-67	0.3	-11.4	0.2
WP-101	Alto Madre	-12.90069	-71.36552	0.655	0.793	-69	0.6	-10.6	0.1
WP-102	Alto Madre	-12.90033	-71.36650	0.645	0.639	-65	0.2	-10.2	0.2
WP-103	Alto Madre	-12.90613	-71.37243	0.587	0.772	-64	2.3	-9.4	0.2
WP-105	Alto Madre	-12.90525	-71.38757	0.571	0.664	-66	0.3	-9.5	0.2
WP-225	Inambari	-13.18372	-70.38562	0.410	0.511	-53	0.3	-8.2	0.1
Dry Season									
WP-372	Inambari	-13.63519	-71.08822	4.547	4.683	-134	0.5	-18.3	0.2
WP-371	Inambari	-13.60522	-71.05136	3.941	4.593	-119	0.3	-16.7	0.2
WP-01	Kosñipata	-13.20207	-71.61707	3.509	3.676	-103	0.2	-15.1	0.1
WP-370	Inambari	-13.57515	-71.02935	3.484	3.502	-105	0.5	-14.3	0.3
WP-02	Kosñipata	-13.20518	-71.61352	3.476	3.651	-99	0.4	-14.9	0.2
WP-03	Kosñipata	-13.18539	-71.58909	2.983	3.096	-94	0.4	-13.9	0.7
WP-369	Inambari	-13.59427	-70.97952	2.976	3.465	-102	0.2	-14.4	0.3
WP-05	Kosñipata	-13.16001	-71.60164	2.504	3.215	-91	0.3	-12.8	0.2
WP-368	Inambari	-13.59134	-70.94323	2.457	3.464	-97	0.4	-13.9	0.2
WP-09	Kosñipata	-13.12319	-71.57587	2.144	2.571	-84	0.1	-13.0	0.3
WP-12	Kosñipata	-13.09904	-71.56797	1.976	2.278	-78	0.5	-11.7	0.3
WP-367	Inambari	-13.52594	-70.89607	1.942	3.191	-88	0.4	-12.7	0.1
WP-13	Kosñipata	-13.08740	-71.56375	1.901	2.316	-70	0.2	-10.5	0.4
WP-366	Inambari	-13.49069	-70.89698	1.606	2.251	-81	0.4	-11.9	0.1
WP-17	Kosñipata	-13.04434	-71.53148	1.287	1.594	-62	0.4	-9.4	0.1
WP-365	Inambari	-13.41658	-70.90240	1.256	1.888	-55	0.5	-7.7	0.3
WP-18	Kosñipata	-13.03535	-71.51231	1.118	1.531	-60	0.4	-9.6	0.2
WP-364	Inambari	-13.36737	-70.89830	1.023	1.662	-55	0.2	-8.2	0.1
WP-363	Inambari	-13.31428	-70.82099	0.833	1.313	-48	0.2	-8.0	0.3
WP-362	Inambari	-13.20573	-70.69752	0.626	0.689	-27	0.6	-5.5	0.1
WP-234	Alto Madre	-12.89731	-71.35372	0.614	0.957	-47	0.3	-7.5	0.3
WP-24	Alto Madre	-12.90310	-71.37256	0.550	0.740	-52	0.1	-8.6	0.1
WP-361	Inambari	-13.18774	-70.63807	0.521	0.628	-40	0.4	-6.2	0.3
WP-360	Inambari	-13.18558	-70.38361	0.384	0.511	-37	0.2	-6.3	0.2

† For the elevation distribution of the catchment upstream the sampling location.

* δD = hydrogen isotope composition of stream water samples.

Supplementary Table S3: Soil sampling locations, δD results.

Sample ID	Location	Latitude	Longitude	Elevation (km)	Depth (cm)	δD_{soil}* (‰)	1σ (δD_{soil})	δD_{water}** (‰)	$\varepsilon_{wax/water}$
TC-M	Tres Cruces	-13.123	-71.618	3.644	N/A	-193	0.8	-103	-100
TU1-M	Trocha Union 1	-13.114	-71.607	3.400	14	-181	1.0	-98	-92
TU2-M	Trocha Union 2	-13.111	-71.604	3.200	12	-186	0.5	-94	-102
WAY-M	Wayqecha	-13.190	-71.587	3.025	23	-186	2.7	-90	-105
TU5-M	Trocha Union 5	-13.094	-71.574	2.520	14	-178	0.2	-79	-107
TU7-M	Trocha Union 7	-13.074	-71.559	2.020	17	-188	2.1	-68	-129
SP1-M	San Pedro 1	-13.047	-71.543	1.750	10	-161	2.8	-62	-106
SP2-M	San Pedro 2	-13.049	-71.537	1.500	16	-153	0.7	-57	-102
VC-M	Villa Carmen	-12.866	-71.401	1.000	4	-173	4.6	-46	-133
TP3-M	Tambopata 3	-12.830	-69.271	0.210	3	-155	1.0	-29	-130
TP4-M	Tambopata 4	-12.839	-69.296	0.194	1	-146	4.6	-28	-121

*δD_{soil} = hydrogen isotope composition of plant waxes (C_{28} *n*-alkanoic acid) in soils.

** δD_{water} = estimated value for δD of precipitation at the sample elevation in the dry season